



SITC 2017

November 8-12

NATIONAL HARBOR
MARYLAND

Gaylord National Hotel
& Convention Center

Abstracts



Society for Immunotherapy of Cancer

sitcancer.org

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Travel Award Recipients



This image denotes the 2017 Travel Award recipients.

Presidential Travel Award Recipients

See abstracts O1, O15, O26, and O34.

Abstract Travel Award Recipients

See abstracts O8, O9, O18, O23, O27, O28, P41, P93, P130, P157, P160, P166, P191, P195, P296, P321, P355, P379, P387, P394, P418, P458, P459, P478, P482, P484, P492, and P500.

Don't Miss the SITC Oral Poster Sessions!

Beyond regular poster viewing, SITC welcomes the Oral Poster Sessions. These sessions provide select poster presenters with the opportunity to orally share their work with the SITC audience through brief, highlighted presentations. The Oral Poster Sessions will take place in the Prince George's Exhibition Hall DE in the presentation area in the far back right of the hall. The daily schedule of presenters is available in the Exhibition Hall.

Abstract and Poster Information

Publication

Regular abstracts submitted in conjunction with the 32nd Annual Meeting are published in the November 7, 2017 issue of the *Journal for ImmunoTherapy of Cancer* (JITC), SITC's official journal. Late-Breaking abstracts will be published in the December 7, 2017 issue of JITC. All abstracts are available on the SITC website and in this Electronic Abstract Book. An abstract listing is available beginning on page 64 of this program book.

Oral Abstracts

SITC Leadership has selected the highest scoring abstract submissions for 10-minute oral presentations within various meeting sessions. Each oral abstract presentation is followed by a 5-minute question and answer period.

Late-Breaking Abstracts

To fulfill SITC's commitment to the most cutting-edge science, late-breaking abstract submission was offered from August 25-September 14, 2017. Only those who submitted applications by the August 1, 2017 deadline were eligible to submit during this period. The highest scoring submissions were selected for oral presentation during the late-breaking abstract sessions within the 32nd Annual Meeting.

Poster Abstracts

Accepted posters for the 32nd Annual Meeting are on display in the Prince George's Exhibition Hall DE. Posters are available for viewing on Friday and Saturday of the Annual Meeting. For a full listing of displayed posters, please see the listing starting on page 71 or the Electronic Poster Abstract Book. Please see the Poster Abstract Book for a full listing of displayed posters. During the poster display staffing hours listed below, designated posters are staffed by respective authors, allowing for information exchange and interaction between researchers and attendees.

Poster Hall Location

Prince George's Exhibition Hall DE

Poster Hall Hours

Friday, November 10 Noon – 8:00 p.m.
Saturday, November 11 10:00 a.m. – 8:00 p.m.

Poster Presentation Hours

Odd Numbered Posters (Poster Authors are Present)
Friday, November 10 12:30 – 2:00 p.m.
Friday, November 10 6:30 – 8:00 p.m.
Even Numbered Posters (Poster Authors are Present)

Saturday, November 11 12:30 – 2:00 p.m.
Saturday, November 11 6:30 – 8:00 p.m.

Oral Poster Presentation Sessions

SITC welcomes the Oral Poster Presentation Sessions to provide select posters with the opportunity to orally share their work with the SITC audience through brief, highlighted presentations. The Oral Poster Presentation Sessions take place in the Prince George's Exhibition Hall DE during the lunch hours on Friday and Saturday.

A daily schedule of presenters is available in the Exhibition Hall.

Poster Numbers

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Thank you to our 2017 Abstract Reviewers for their time and expertise!

Lisa H. Butterfield, PhD – *University of Pittsburgh*

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P33, P349, P486, P13, P451, P403, P503, P459, P411, P396, P219, P463, P497, P91, P399, P115, P368, P6, P272, P177, P466, P501, P85, P369, P123, P49, P454, P479, P164, P246, P416, P72, P208, P356, P124, P487, P502, P48, P515, P455, P453, P518, P11, P354, P289, P101, P496, P64, P119, P241, P471, P442, P281, P474, P54, P77, P4, P481, P264, P465, P473, P305, P464, P256, P371, P362, P404, P521, P491, P326, P317, P312, P149, P20, P458, P460, P468, P1, P402, P57, P470, P367, P490, P83, P353, P324, P413, P62, P12, P103, P516, P275, P14, P480, P330, P415, P277, P333, P99, P469, P105, P154, P130, P284, P17, P16, P401, P187, P310, P461, P134, P525, P400, P350, P253, P412, P322, P269, P90, P323, P288, P452, P250, P18, P15, P309, P276, P484, P280, P343, P510, P53, P524, P475, P156, P199, P279, P70, P143, P84, P418, P66, P384, P365, P293, P19, P110, P36, P492, P383, P378, P56, P93, P357, P282, P126, P495, P10, P179

Tumor stroma..... P469, P472, P510, P524, P66, P384, P330, P149, P1, P404, P317, P54, P486, P13, P496, P64, P487, P453, P501, P68, P65, P346, P301, P506, P338, P334, P478, P499, P439

Vaccine..... O26, O5, O11, P140, P55, P261, P41, P380, P108, P407, P430, P116, P118, P22, P209, P119, P121, P296, P124, P190, P2, P120, P122, P453, P518, P138, P38, P233, P109, P117, P227, P115, P509, P58, P37, P454, P129, P440, P386, P500, P141, P450, P113, P409, P139, P270, P142, P405, P286, P263, P415, P114, P297, P28, P434, P111, P511, P204, P460, P210, P402, P256, P211, P107, P264, P137, P513, P132, P327, P130, P136, P133, P128, P283, P401, P135, P461, P292, P134, P400, P269, P433, P112, P34, P127, P110, P131, P125, P222, P71, P436, P126

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Biomarkers and Immune Monitoring

O1 Presidential Travel Award Recipient

Identification of unique neoantigen qualities in long-term pancreatic cancer survivors

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Background

Pancreatic adenocarcinoma (PDAC) is a lethal cancer with <7% of patients surviving past 5 years. T cell immunity has been linked to the exceptional outcome of the rare long-term survivors but the antigens are unknown.

Methods

To identify T cell antigens in long-term PDAC survivors, we assembled the largest cohorts of stage-matched short [n=68, median overall survival (OS) 0.8y] and long-term PDAC survivors (n=82, median OS 6y). To enable antigen discovery, we performed a combined analysis of whole exome sequencing, neoantigen prediction, T cell receptor (TCR) Vβ-chain sequencing, 9-color multiplexed immunohistochemistry, and tumor transcriptomic profiling. To assess differential neoantigen immunogenicity, we performed neoantigen fitness modeling integrating clonal genealogy, epitope homology, and T cell receptor affinity. To examine in vivo T cell-neoantigen reactivity, we used functional assays in a subset of very long-term PDAC survivors (n=7, median OS 10.5 years).

Results

We found that tumors of long-term survivors displayed 12-fold greater cytolytic CD3⁺CD8⁺Granzyme-B⁺ cells, with >94% of intratumoral T cell clones unique to tumors and not shared with adjacent normal pancreatic tissue, suggesting intratumoral antigen recognition. Tumors of long-term survivors exhibited greater TCR repertoire diversity compared to tumors of short-term survivors, implying differential antigenic targets. In examining neoantigens as potential T cell targets, we found that patients with both the highest predicted neoantigen number and the greatest CD3⁺CD8⁺ infiltrates together but not either parameter alone, exhibited the longest survival (median OS not reached vs. 0.8y, P=0.004). In investigating possible unique neoantigen qualities in long-term survivors, a neoantigen quality model conferring greater immunogenicity to dominant neoantigens with homology to microbial epitopes identified long-term survivors independent of confounding variables and chemotherapy (Neoantigen Quality^{Hi vs. Low} - median OS 8.6y vs. 0.8y; P=0.002) whereas a model ascribing greater immunogenicity to increasing neoantigen number did not (Neoantigen Quantity^{Hi vs. Low} - median OS 0.8y vs. 1y; P=0.3) (Figure 1). Similarly, neoantigen quality, but not quantity, was independently

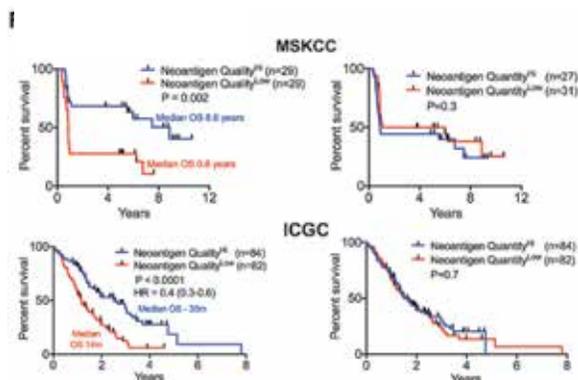
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prognostic of survival in a larger, independent cohort unselected by survival (n =166; Neoantigen Quality^{Hi} vs. Low - median OS 30m vs. 14m; P<0.0001) (Figure 1). Finally, we detected lasting circulating T cell reactivity to both high quality neoantigens and cross-reactive antigens in PDAC survivors, including identical intratumoral clones with specificity to both (n=5 of 7 patients tested).

Conclusions

Our results identify neoantigens with unique qualities as T cell targets in PDAC. More broadly, we identify neoantigen quality as a biomarker for immunogenic tumors that may facilitate rational application of immunotherapies.

Figure 1. Neoantigen quality is prognostic of pancreatic cancer survival



O2

Predictive biomarkers for response to anti-CTLA-4 and anti-PD-1 immunotherapy in melanoma patients

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Background

Immune checkpoint blockade has greatly improved clinical outcomes in melanoma patients. However, there is an urgent need for predictive biomarkers to determine who will likely benefit most from which therapy. To date, most biomarkers of response have been identified in the tumors themselves. Biomarkers that could be assessed from peripheral blood would be even more desirable, because of ease of access and reproducibility of sampling.

Methods

We used mass cytometry (CyTOF), which is a technique similar to flow cytometry, but uses metal-ion tagged antibodies for high-dimensional immune profiling. We performed CyTOF on pre-treatment peripheral blood mononuclear cells from melanoma patients to identify potential biomarkers of response to anti-CTLA-4 or anti-PD-1 immunotherapy.

Results

Our studies revealed that anti-CTLA-4 and anti-PD-1 therapies have distinct sets of candidate biomarkers. Responders to anti-CTLA-4 therapy had higher memory CD4⁺ and CD8⁺ T cells compared to non-responders. Furthermore, responders showed higher frequencies of CD8⁺ effector memory T cells. In anti-PD-1 (but not anti-CTLA-4) treated patients, responders had higher frequencies of CD69 and MIP-1β expressing NK cells. Finally, using multivariate analysis, distinct models for the prediction of response to anti-CTLA-4 and anti-PD-1 were developed.

Conclusions

Our findings point to an important role for memory T cell subsets, as well as NK cells in response to immunotherapy, making them potential predictive biomarker candidates for response to checkpoint blockade.

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O3

Combining immunophenomics with a gene expression panel for improved prostate cancer recurrence prediction

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Background

Multi-omics data provide rich information on different levels such as genome, transcriptome, function or phenotype. Integrating such comprehensive measures offers great potential for identifying improved prognostic and predictive signatures in the context of precision medicine. Here we combine gene expression data with phenomic readouts extracted from immunohistochemically stained sections of resected prostate cancer (PCa) tissue. By integrating both data sets we identify multi-variate signatures with high prognostic value regarding prostate cancer recurrence after radical prostatectomy.

Methods

Data was collected from a cohort of 23 PCa patients (Gleason-score 6-9, pT2, age≤75y). Consecutive FFPE tissue sections were dual-stained for immune-related (CD3/CD8, CD68/CD163) and structural (CD34, CK18/p63) components. Using Tissue Phenomics, we extracted image-based features, e.g., densities, ratios, and distances of positive cells across differently stained sections after co-registration in specific regions-of-interest (tumor, TME, stroma) [1]. Gene data (NanoString Technologies, Seattle, nCounter PanCancer Immune Profiling Panel) was collected from sections containing tumor only and normalized w.r.t. housekeeping genes. We separately selected subsets of image-based and gene-based features by

optimizing a cutoff regarding the uni-variate stratification performance (log-rank test, accuracy) on random data subsets using bootstrapping. Features from both sets were combined by Classification and Regression Trees (depth=2) and the best performing combinations were identified using cross validation. Clinical parameters (age, Gleason-score, PSA blood value) provided no prognostic value as shown by Cox regression analysis.

Results

Several tree models showed improved cross-validated stratification performance compared to all uni-variate stratifications using either feature set (Figure1). We found that (I) a low expression of C4B gene in combination with a low ratio of CD8(+) in stroma to CD3(+)CD8(-) in tumor ($p_{\text{crossVal}} < 0.05$), and (II) a high expression of CD46 gene in combination with a low average distance of CD34(+) microvessels to neighboring CD168(+) M2 macrophages ($p_{\text{crossVal}} < 0.05$) are strong prognostic factors for prostate cancer recurrence.

Conclusions

Our results suggest that an inhibited activity of the innate as well as the adaptive immune system provides prognostic value for prostate cancer recurrence. Both genes C4B and CD46 are related to the complement system where C4B positively correlates with complement activity while CD46 is an inhibiting complement regulator. Limited activation of CD8(+) cytotoxic T cells as well as increased activity of M2 macrophages fostering angiogenesis additionally contribute to the cancer promoting environment. Combining information from genome and phenome therefore helps gaining a broadened picture of factors promoting PCa progression.

References

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O4

Clinical activity of adenosine 2A receptor (A2AR) inhibitor CPI-444 is associated with tumor expression of adenosine pathway genes and tumor immune modulation

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Background

CPI-444, an A2AR antagonist, inhibits suppression of T cell function by adenosine and is active in multiple preclinical models. CPI-444 is being investigated as a single agent and in combination with atezolizumab in an ongoing Phase 1/1b clinical trial in patients with advanced cancers (NCT02655822). Biomarker investigations were conducted to explore immune modulation in serial tumor biopsies and peripheral blood as well associations between adenosine pathway genes and clinical activity.

Methods

Tumor biopsies and blood samples were analyzed in 80 patients with renal cell carcinoma (RCC: n=35), non-small cell lung cancer (NSCLC: n=26), triple negative breast cancer (TNBC: n=17), microsatellite instable colorectal cancer (MSI-CRC n=2) treated with CPI-444 100mg BID as single agent ('SA' n=18) or in combination with atezolizumab ('combo' n=62). Paired tumor biopsies were analyzed: gene expression profiles (Nanostring), CD8, PD-L1 and CD73 (IHC). T cell repertoires were examined by sequencing of the T cell receptor beta chain gene in PBMCs and tumors.

Results

Of 80 evaluable patients, 54 had progressed on prior anti-PD-(L) 1 therapy ('IO-refractory'), 26 were IO-naïve. Most patients were PD-L1 negative in archival tumor (80% TNBC, 91% RCC, 69% NSCLC).

Six objective responses were observed: 2 partial responses (PR) in RCC (1 SA and 1 combo); 2 PRs in NSCLC (both combo); 1 PR MSI-CRC (combo) and 1 PR in TNBC (combo). Notably, 3 PRs were observed in IO-refractory patients (1 RCC, 2 NSCLC) and 2 PRs were PD-L1 negative (1 RCC, 1 NSCLC).

SA and combo treatment significantly increased CD8+ cell infiltration (mean 2-fold; 95%CI 1.18-

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2.91), T cell activation and interferon-gamma pathway gene expression in biopsies as well as T cell clone expansion in both tumor biopsies and periphery. Shared clonotypes expanded in matched post-dose PBMCs and tumor suggesting trafficking of T cells following CPI-444 treatment. This included IO-refractory and PD-L1-negative patients.

Patients with elevated CD73 and expression of adenosine genes in baseline tumor samples experienced significant tumor regression (best % change: high-CD73 -5.6% regression; low-CD73 17.9% growth; p-value=0.03, n=40). In IO-refractory patients, CD73 expression and adenosine pathway genes were higher than IO-naïve patients at baseline suggesting adenosine pathway is a mechanism of immune escape in IO-refractory patients.

Conclusions

CPI-444 anti-tumor activity was associated with immune-modulation of T cells in tumor and periphery, including IO-refractory and PD-L1 negative patients. Adenosine pathway is upregulated in IO-refractory patients and may be associated with clinical response to CPI-444 supporting a role for CPI-444 in treatment of IO-refractory tumors.

Trial Registration

ClinicalTrials.gov Identifier NCT02655822

Cancer Vaccines

O5

A dendritic cell targeting NY-ESO-1 vaccine significantly augments early and durable immune responses in melanoma patients pretreated with human Flt-3 Ligand

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Background

Patients with high-risk melanoma, (AJCC TNM stage II and stage III disease), have a 20-60% recurrence rate with 5-year overall survival (OS) between 45% and 70%. The adjuvant setting is an opportunity to test prevention vaccines that may have efficacy against disease recurrence. Vaccine therapy with CDX-1401 (a fusion protein consisting of human monoclonal IgG1 antibody targeting the dendritic cell (DC) receptor DEC-205 linked to the NY-ESO-1 tumor antigen) can safely lead to humoral and cellular immunity in cancer patients with advanced malignancies that express NY-ESO-1, including melanoma. CDX-301, a recombinant human Flt3 ligand (Flt3L), safely produces increases in DC in humans and may enhance vaccine responses through increased DC number and activity. We evaluated CDX-301 and CDX-1401 combination treatment in a phase II, open-label, multicenter, randomized study of subjects with resected melanoma, to determine whether immune responses to NY-ESO-1 elicited by vaccination with CDX-1401 + poly-ICLC are substantially increased by prior expansion of circulating DC with Flt3L therapy.

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Methods

60 patients with resected melanoma were randomized to two cohorts: Cohort 1 received CDX-301 (Flt3L) pretreatment (25 ug/kg SC x 10 days) in two of four monthly cycles of vaccination with CDX-1401 (1mg IC) + poly-ICLC (2mg SC, days 1 and 2). Cohort 2 received 4 monthly cycles of vaccine with CDX-1401 and poly-ICLC without prior Flt3L. We also assessed immunogenicity to other melanoma-associated antigens and memory viral responses, character of PBMC subsets, and safety, tolerability and clinical efficacy of the regimens.

Results

Both treatments were well tolerated with grade 1-2 AEs of chills, injection site erythema and pain, fever and myalgias most common. A substantial increase of between ~15- to ~200-fold of innate immune cells (DC, monocytes and NK cells) was observed in subjects treated with CDX-301. Further, there was development of higher anti-NY-ESO-1 antibody titers and NY-ESO-1 specific T cells in cohort 1 vs. cohort 2, and evidence of induction of antigen-specific CD8+ T cells in cohort 1. Comparative immune cell gene expression profiling of PBMC are consistent with these differences and reveal a gene signature associated with Flt3L induction of an early and durable immune response.

Conclusions

DC mobilization with vaccines targeting DC is safe and significantly enhances vaccine responses to widely expressed tumor associated antigens.

Trial Registration

ClinicalTrials.gov Identifier NCT02129075

Cellular Metabolism and Antitumor Immunity

O6

Re-educating macrophage through glutamine metabolism-mediated metabolic and epigenetic reprogramming

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Background

The different regulatory mechanisms orchestrating macrophage activation, including signaling cascades and epigenetic programming, are increasingly appreciated. Among these regulatory mechanisms, differences in the bioenergetic demands of M1 and M2 macrophages are emerging as new regulatory circuits that adjust a macrophage's behavior in response to the nutrient state in its habitation and the infected tissues. Glutamine metabolism provides synergistic support for macrophage activation and elicitation of desirable immune responses; however, the underlying mechanisms regulated by glutamine metabolism to orchestrate macrophage activation remain unclear.

Methods

We examine metabolic and epigenetic reprogramming in bone marrow-derived macrophages under M1 and M2 skewing condition. We also examine the impact of glutamine metabolism on supporting endotoxin tolerance format in bone marrow-derive macrophages in vitro and in the in vivo septic shock model. The anti-tumor responses of glutaminase 1 targeting approach was further evaluated in MC-38 and Braf/Pten melanoma-bearing mice in conjunction with or without adoptive tumor-specific T cell transfer.

Results

Here we show that the production of α -ketoglutarate via glutaminolysis is important for alternative (M2) activation of macrophages, including engagement of fatty acid oxidation (FAO) and histone H3K27 demethylase Jmjd3-dependent epigenetic reprogramming of M2 genes. This M2-promoting mechanism is further modulated by a high α -ketoglutarate/succinate ratio, while a low ratio strengthens the pro-inflammatory phenotype

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in classically (M1) activated macrophages. As such, α -ketoglutarate contributes to endotoxin tolerance following M1 activation. This study reveals new mechanistic regulations by which glutamine metabolism tailors the immune responses of macrophages through metabolic and epigenetic reprogramming. We further provide proof-of-concept evidence that targeting glutaminolysis can tailor the immune response of tumor-associated macrophages in vivo, and co-treating glutaminase inhibitor with adoptive tumor-specific T cell transfer can effectively suppress tumor growth.

Conclusions

This study highlights the unrecognized mechanisms controlled by glutaminolysis to fine-tune macrophage activities and advances modulation of glutamine metabolism as an attractive strategy for harnessing macrophage-mediated immune responses. Most importantly, targeting glutaminase is able to re-educate tumor-associated macrophages and provides synergistic anti-tumor responses with adoptive cell transfer therapy. Altogether, our work uncovers new regulatory circuits orchestrated by glutamine metabolism in macrophages and reveals the potential of these mechanisms on harnessing macrophage immune responses.

O7

Early FDG-PET response correlates with dose and efficacy in patients with microsatellite stable mCRC treated with carcinoembryonic antigen T cell bispecific (CEA-TCB) antibody plus atezolizumab

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Background

CEA-TCB (RG7802, RO6958688) is a novel T cell bispecific antibody targeting CEA on tumor cells and CD3 on T cells. An ongoing Phase Ib study (NCT02650713) is exploring the safety, tolerability and efficacy of CEA-TCB in combination with atezolizumab (anti-PD-L1). Here, we report preliminary results of ¹⁸F-fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging as an early pharmacodynamic marker for this novel cancer immunotherapy combination in patients with microsatellite stable (MSS) metastatic colorectal cancer (mCRC).

Methods

In this study, CEA-TCB was given weekly in combination with atezolizumab 1200 mg every 3 weeks in patients with CEA-expressing solid tumors. On-treatment FDG-PET scans were performed at week 4 and compared with baseline. On-treatment changes in maximum standardized uptake value (SUV_{max}), metabolic tumor volume (MTV) and total lesion glycolysis (TLG) were analyzed in up to 10 measurable lesions, identified at baseline by an independent reviewer, in each patient. Exploratory statistical analyses used semiparametric Gaussian regression models and progression-free survival (PFS) was assessed using Cox proportional hazard landmark analyses.

Results

As of March 3, 2017, a total of 35 patients with MSS mCRC were treated with CEA-TCB doses ranging from 5 mg to 160 mg; 17 patients were evaluable for PET image analysis. Of the 15 PET-evaluable patients, 8 (53%) had early (week 4) on-treatment reductions in $SUV_{max} > 15\%$ with CEA-TCB plus atezolizumab. Early decreases in SUV_{max} appeared to correlate with increasing CEA-TCB doses ($P = 0.0081$), with greater and consistent reductions seen at ≥ 80 mg. Partial metabolic response was observed in 6 of 15 patients, with all responders treated at ≥ 80 mg. Decreases in SUV_{max} appeared to be associated with reduction in tumor burden (best

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change from baseline per RECIST v1.1; $P = 0.0061$). Depth of reduction in MTV appeared to be associated with longer PFS ($P = 0.013$). In addition, reductions in MTV and TLG appeared to correlate with decreases in soluble CEA (sCEA) at week 6 (MTV, $P = 0.013$; TLG, $P = 0.034$). Data from an expanded patient population treated with CEA-TCB as monotherapy and in combination with atezolizumab will be presented.

Conclusions

In patients with MSS mCRC, changes in MTV and SUV_{max} correlated with longer PFS along with tumor shrinkage, and decreases in 2 FDG parameters, MTV and TLG, correlated with a decline in soluble tumor marker, sCEA. Thus, early on-treatment changes in FDG-PET can serve as a pharmacodynamic biomarker related to treatment efficacy with CEA-TCB.

Trial Registration

NCT02650713

08 Abstract Travel Award Recipient

Lactic acid as a mediator of metabolic symbiosis between regulatory T cells and the tumor microenvironment

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Background

The tumor microenvironment (TME) presents a major barrier to effective immunotherapy. Metabolically harsh conditions starve infiltrating effector T cells rendering them ineffective. In contrast, regulatory T (T_{reg}) cells, a subset of CD4+ T cells that play a vital role in preventing autoimmunity and maintaining immune homeostasis, thrive in the TME. As a result, the TME

becomes more immunosuppressive. It has been shown that T_{reg} cells have a distinct metabolic profile compared to their effector T cell counterparts. Therefore, we hypothesized that T_{reg} cells thrive because they are metabolically supported by the TME.

Methods

B16, a mouse model of melanoma, was used. From tumor bearing mice conventional effector T cells and T_{reg} cells were transcriptionally and metabolically profiled using flow cytometry, proliferation assays, suppression assays, and isotopic flux analysis. A mouse with a T_{reg} specific deletion of MCT1, a predominant lactate transporter was generated. A pharmacological inhibitor of MCT1 was used to prevent lactate uptake.

Results

Transcriptional and metabolic profiling revealed T_{reg} cells from the tumor upregulate a distinct metabolic profile utilizing the glycolytic end-product, lactic acid. Lactic acid has been known to be immunosuppressive, and indeed, it curbed the proliferation of conventional effector T cells *in vitro*. However, lactic acid had the opposite effect on T_{reg} cells, promoting their proliferation. Isotopic flux analysis revealed lactate is utilized by T_{reg} cells to generate glycolytic intermediates, in part through gluconeogenic pathways. Preventing lactate transport in T_{reg} cells through a conditional knockout of MCT1 resulted in mice with normal immune homeostasis, but superior anti-tumor immunity when implanted with melanoma. Similarly, in wild type tumor bearing mice, preventing lactate uptake through a pharmacological inhibitor resulted in decreased intratumoral T_{reg} proliferation and increased efficacy of checkpoint blockade immunotherapy.

Conclusions

These data suggest that tumors evade the immune system, in part, by metabolically supporting suppressive T_{reg} cells with lactic acid. These data also suggest that targeting lactate metabolism may

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increase the efficacy of checkpoint blockade immunotherapy.

Cellular Therapy Approaches

09 Abstract Travel Award Recipient

Transcriptional approach to understanding the role of tonic signaling and co-stimulation in CAR T cells

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Background

Chimeric antigen receptors (CARs) targeting CD19 have produced impressive outcomes for the treatment of B cell malignancies. Using RNA sequencing, we determined the transcriptional changes downstream of CARs with different co-stimulation domains pre-and post-antigen stimulation.

Methods

T cells were sorted from 3 donors and transduced with one of four CAR constructs. The constructs all contained a CD19-directed scFv fused to one of four different intracellular domains: 4-1BB ζ , CD28 ζ , ζ and $\Delta\zeta$. Cells were expanded with CD3/CD28 beads for 5 days and then rested. On day 12, the T cells were stimulated through their CAR or TCR for 0, 4 or 24 hours. T cells were flow-sorted on viable lymphocytes, CAR+, CD3+ and CD4+ or CD8+ and then sequenced using the SmartSeq2 protocol. Reads were aligned and quantified. Differential gene expression calculated using DESeq2. Significant

differentially expressed (DE) genes had a FDR cutoff of <0.05 .

Results

T cells containing CARs have clearly distinct transcriptional signatures both from each other and from untransduced T cells. We identified a gene signature for tonic signaling of all ζ -containing CAR T cells, which was identified in transduced resting T cells prior to antigen stimulation through the CAR. These genes were DE between untransduced or $\Delta\zeta$ vs 4-1BB ζ , CD28 ζ , ζ CARs. Furthermore, we identified a set of genes that is DE between 41BB ζ and CD28 ζ CAR T cells. Some of these genes such as ENNP2 are DE before antigen stimulation through the CAR, while other genes were significantly DE only after antigen stimulation through the CAR. There was a clear upregulation of HLA Class II genes as well as CIITA, which controls MHCII upregulation, in activated 41BB ζ vs 28 ζ CAR T cells. Across all time points there was an enrichment for human T_H1 signature genes in the 41BB ζ compared to the CD28 ζ CD4+ CAR T cells (p-value <0.0005). Cytokines, IL-15 and IL-21 were both significantly upregulated in 41BB ζ vs CD28 ζ CARs by 24 hours after CAR stimulation.

Conclusions

We have identified a transcriptional signature that reflects tonic signaling in CAR T cells bearing the CD3 ζ signaling domain, independent of the co-stimulation domain. Differences between 41BB ζ and 28 ζ have been previously identified several days following CAR-antigen stimulation. Here we show many of these differences exist before CAR-mediated activation, indicating a transcriptional effect of tonic signaling. We also defined a transcriptional program in 41BB ζ vs CD28 ζ CAR T cells that polarizes CD4+ cells to a more T_H1 like state pre-and post-signaling.

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010

Gamma secretase inhibition increases recognition of multiple myeloma by BCMA-specific chimeric antigen receptor modified T cells

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Background

B cell maturation antigen (BCMA) is expressed on most cases of multiple myeloma (MM) cases and can be targeted by T cells transduced with an anti-BCMA chimeric antigen receptor (CAR). The extracellular portion of BCMA is cleaved by gamma-secretase (γ -secretase), resulting in lower surface expression of BCMA on tumor cells and the presence of soluble BCMA (sBCMA) in the circulation, both of which could hamper BCMA CAR-T cell efficacy and enable escape of BCMA^{low} MM cells. We investigated whether γ -secretase inhibitor (GSI) treatment inhibited BCMA shedding, increased surface BCMA levels, and augmented BCMA-specific CAR-T cell antitumor functionality *in vitro* and *in vivo*.

Methods

BCMA expression on MM cell lines and primary CD138⁺ MM samples was measured by flow cytometry after treatment with the GSI RO4929097. sBCMA levels in patient sera and culture supernatants were measured by ELISA. BCMA-specific CAR-T cells were co-cultured with MM cell lines or primary MM, with or without GSI. Blocking effects of sBCMA on CAR-T cells were measured by titrating recombinant BCMA/Fc into co-cultures. CAR-T cell functionality was measured by cytokine ELISA, CFSE dye dilution, and chromium-release assays. To investigate *in vivo* CAR-T cell efficacy, MM1.R^{ffluc}-tumor bearing NSG mice were treated

with BCMA-specific CAR-T cells alone and in combination with GSI. Tumor burden was monitored by bioluminescence imaging.

Results

BCMA-specific CARs were optimized for functionality by spacer modifications. Patient serum levels of BCMA were found to often exceed 100 ng/mL and concentrations >33 ng/mL inhibited BCMA CAR-T cell function. Treatment with GSI RO4929097 increased BCMA levels on both MM cell lines and primary MM samples in a dose dependent and reversible fashion, and decreased sBCMA shedding. High doses of RO4929097 (>3 mM) inhibited CAR-T cell function, however low doses (0.1-1 mM) were sufficient to upregulate surface BCMA levels on primary MM up to 10-fold, leading to increased recognition by BCMA CAR-T cells. In a preclinical model of myeloma, treatment with RO4929097 increased BCMA on tumor cells in bone marrow and decreased sBCMA in peripheral blood. Treatment of myeloma bearing mice with BCMA CAR-T cells combined with intermittent doses of RO4929097 improved antitumor effects and increased survival as compared to mice that did not receive RO4929097.

Conclusions

Combining GSI with BCMA-specific CAR-T cells improves tumor recognition and therapeutic benefit in a preclinical model of myeloma. The data provide a rationale for translating this combination to the clinic.

Clinical Trials (Completed)

O11

DNA vaccine with PD-1 blockade elicits anti-tumor responses in patients with metastatic, castration-resistant prostate cancer (mCRPC)

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Regular Abstract Oral Presentations

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Background

We have previously investigated a DNA vaccine encoding prostatic acid phosphatase (PAP, pTVG-HP) in two trials of patients with PSA-recurrent prostate cancer. Favorable changes in PSA doubling time were associated with the development of PAP-specific immunity. A randomized phase 2 trial testing this vaccine is currently underway. In preclinical models, we have found that blockade of regulatory receptors, including PD-1, at the time of T cell activation with DNA vaccination produced anti-tumor responses *in vivo*. We have also found that patients immunized with this DNA vaccine developed PD-1-regulated T cells. These findings suggested that combined PD-1 blockade with vaccination might elicit superior anti-tumor responses in patients with prostate cancer.

Methods

A clinical trial was designed to evaluate the immunological and clinical efficacy of pTVG-HP when delivered in combination (over 12 weeks) or in sequence (over 24 weeks) with pembrolizumab, in patients with mCRPC. Correlative studies included effects on serial biopsies, different measures of systemic immunity, and exploratory FLT PET/CT imaging to evaluate proliferative changes in tumors and vaccine-draining lymph nodes.

Results

26 patients were randomized to treatment and evaluable for response. On-treatment adverse events > grade 2 included 1 episode of fatigue, 1 episode of diarrhea, and 1 episode of autoimmune hepatitis. No grade 4 events were observed. 8/13 subjects (61%) treated in combination had any decrease in PSA from baseline, with 4/13 (31%) having a PSA decline >25%. 1/13 (7%) treated in sequence had a decrease in PSA from baseline ($p=0.013$). Several patients treated with the combination experienced decreases in tumor volume by radiographic imaging at 12 weeks. Expansion of peripheral PAP-specific Th1-biased T cells was detected in some patients. Exploratory FLT

PET/CT imaging has demonstrated proliferative responses in metastatic lesions and in vaccine-draining lymph nodes. Preliminary evaluation of biopsy specimens has suggested recruitment of activated T cells.

Conclusions

PD-1 pathway inhibitors have demonstrated little clinical activity to date when used as single agents for treating prostate cancer. Our findings demonstrate that combining this blockade with tumor-targeted T cell activation by a DNA vaccine is safe and can augment tumor-specific T cells, detectable within the peripheral blood and by imaging, and result in objective anti-tumor changes. Based on these results, the randomized portion of this trial was closed to accrual, and an expansion arm has been opened to evaluate the safety and clinical efficacy of combination treatment beyond 12 weeks.

Trial Registration

NCT02499835

O12

Immune and tumor responses to human IL-10 (AM0010, Pegilodecakin) alone or in combination with immune checkpoint blockade

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Regular Abstract Oral Presentations

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Background

IL-10 has anti-inflammatory functions and stimulates the cytotoxicity and proliferation of antigen activated CD8+ T cells. T cell receptor mediated activation of CD8+ T cells elevates IL-10 receptors and PD-1, providing the mechanistic rationale for combining AM0010 and anti-PD1 for the treatment of cancer pts. A phase 1 clinical trial investigated the tolerability and anti-tumor activity of AM0010 alone and in combination with anti-PD1 immune checkpoint inhibitors.

We previously reported partial responses in 4 of 16 RCC pts treated with AM0010 monotherapy (Naing et al JCO 2016) and 4 of 8 RCC pts (50% ORR) and 2 of 5 of NSCLC pts (ORR 40%) treated with AM0010 and pembrolizumab. We enrolled phase 1 cohorts of RCC and NSCLC pts on AM0010 + nivolumab as \geq 2nd line of treatment.

Methods

A total of 34 NSCLC pts. were enrolled on AM0010 (10-20mg/kg QD, SC) and pembrolizumab (2mg/kg, q3wk IV; n=5) or nivolumab (3mg/kg, q2wk IV; n=29). Pts. had a median of 2 prior therapies (PT, range 0-5). 37 RCC patients were enrolled on AM0010 (10-20mg/kg QD, SC) and pembrolizumab (2mg/kg, q3wk IV; n=8) or nivolumab (3mg/kg, q2wk IV; n=29), with a median of 1 PT (1-3). Tumor responses were assessed by irRC. Serum cytokines, T cell activation (FACS) and peripheral T cell clonality (TCR sequencing) were analyzed. (Figure 1)

Results

AM0010 + anti-PD-1 was well tolerated. TrAEs included anemia, thrombocytopenia and fatigue, and were reversible and transient. As of July 30 2017, partial responses (PRs) were observed in 10 of

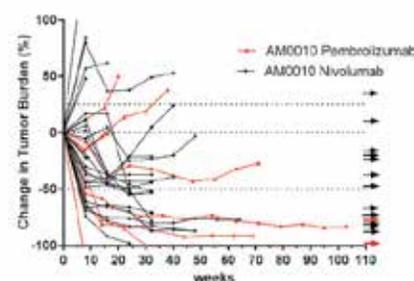
26 evaluable NSCLC pts (38.5%), including 3 (of 11) with PD-L1+ <1% and 4 (of 5) with PD-L1+ >50% cancers. PRs were observed in 14 of 34 evaluable RCC pts (41%). An additional 15 RCC pts had stable disease (44%), 7 of those had a tumor reduction > 30%. The mPFS and mOS has not been reached, the mFU is 8.9 m (range 0.5-26.5). Updated response data including data on delayed responses and the durability of response will be available.

The induction of immune cytokines (IL-18) in the serum, invigoration of CD8+ T cells and the increase of newly expanding T cell clones correlated with objective tumor response to AM0010 monotherapy and AM0010 + anti-PD-1.

Conclusions

AM0010 in combination with anti-PD-1 is well-tolerated in RCC pts, the recommended phase 2 dose is 10ug/kg. The robust efficacy data and the observed CD8 T cell activation is promising and encourages the continued study of AM0010 in combination with nivolumab

Figure 1. RCC - AM0010 + Anti-PD-1



Anti-PD-1 naive RCC were treated with AM0010 in combination with nivolumab (black) or pembrolizumab (red). ORR 41%, DCR 85%

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Clinical Trials (In Progress)

O13

A phase 1 study of TSR-022, an anti-TIM-3 monoclonal antibody, in patients (pts) with advanced solid tumors

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Background

T cell immunoglobulin and mucin-domain containing-3 (TIM-3) is a key immune checkpoint protein that has been implicated in both exhaustion of T effector cells and immune suppression mediated by regulatory T cells and myeloid cells. Up-regulation of TIM-3 expression on PD-1-positive tumor-infiltrating T cells is associated with reduced proliferation and secretion of cytokines important for T cell mediated anti-tumor activity in several cancers. Blocking TIM-3 therefore has the potential to restore tumor recognition by T cells and to increase anti-tumor immunity. TSR-022 is a potent, selective, investigational anti-TIM-3 antibody that is in development for the treatment of solid tumors as

both a monotherapy and in combination with PD-1 blockade.

Methods

TSR-022 is being investigated in a multicenter, open-label, first-in-human phase 1 trial enrolling pts with advanced or metastatic solid tumors who have progressed after, or are intolerant to, available or approved therapies. The primary objective of the current part of this study is to evaluate the safety and tolerability of TSR-022 as a monotherapy and to determine the recommended phase 2 dose of TSR-022 as a single agent. Pts received IV infusion of TSR-022 monotherapy every 14 days in six escalating dose levels.

Results

As of July 19, 2017, 31 pts have been treated with monotherapy: 3 pts at 0.03 mg/kg, 3 pts at 0.1 mg/kg, 3 pts at 0.3 mg/kg, 9 pts at 1 mg/kg (all, 43 days of median follow-up); 7 pts at 3 mg/kg (28 days of median follow-up), and 6 pts at 10 mg/kg (23 days of median follow up). Adverse events that occurred in >15% of patients were fatigue (8 pts, 26%), abdominal pain (7 pts, 23%), nausea (6 pts, 19%); elevated ALT, elevated AST, back pain, constipation, and vomiting was each reported in 5 pts (16%). One dose-limiting toxicity event occurred with TSR-022 monotherapy (immune-related Grade 3 lipase elevation), which did not require treatment modification. No treatment-related serious adverse events were observed. TSR-022 exposure and peripheral receptor occupancy increased in a dose proportional manner from 0.3 to 10 mg/kg. To date, the best response was disease stabilization, observed in patients with various cancer types.

Conclusions

TSR-022 monotherapy was well tolerated across multiple dose levels. Adverse events were manageable and consistent with the safety profiles of other checkpoint inhibitors. Dose-escalation of TSR-022 in combination with an anti-PD-1 antibody is currently ongoing.

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Trial Registration

ClinicalTrials.gov identifier NCT02817633.

O14

Initial results from phase 1 trial of M7824 (MSB0011359C), a bifunctional fusion protein targeting PD-L1 and TGF- β , in patients with NSCLC refractory or resistant to prior anti-PD-1/anti-PD-L1 agents

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Background

The transforming growth factor β (TGF- β) pathway plays an important role in tumor immune escape and may potentially contribute to the failure of programmed death 1/programmed death ligand 1 (PD-1/PD-L1) therapy. M7824 (MSB0011359C) is an innovative first-in-class bifunctional fusion protein composed of a human IgG1 monoclonal antibody against PD-L1 fused with 2 extracellular domains of TGF- β receptor II to function as a TGF- β "trap." We report initial results on the efficacy and tolerability

of M7824 in patients with non-small cell lung cancer (NSCLC) refractory or resistant to prior anti-PD-1/anti-PD-L1 agents.

Methods

NCT02517398 is an ongoing, phase 1, open-label trial. In this expansion cohort, patients with advanced NSCLC refractory (progression after treatment initiation) or with acquired resistance (initial disease control with subsequent relapse) to prior treatment with anti-PD-1/anti-PD-L1 therapy received M7824 1200 mg q2w until confirmed progressive disease, unacceptable toxicity, or trial withdrawal. The primary objective is best overall response (BOR) per RECIST v1.1; secondary objectives include safety/tolerability.

Results

To date, 83 heavily pretreated patients (74.7% received ≥ 3 prior therapies) have received M7824 for a median duration of 8 weeks (range: 2-30 weeks) and 23 patients remain on active treatment with a median follow-up of 22.6 weeks (range, 8-35.3 weeks). Preliminary data show initial clinical activity was observed in 19 patients (disease control rate=22.9%; 2 confirmed partial responses [ongoing at 4.5 and 7.5 months] and 17 patients with stable disease [15 ongoing at 3 months]) based on investigator-assessed BOR. Notably, initial clinical activity was observed both in patients refractory or resistant to prior anti-PD-1/anti-PD-L1 therapy. Fourteen patients (16.9%) experienced grade ≥ 3 treatment-related adverse events (AEs), most commonly rash/pruritus (n=4, 4.8%) and fatigue/asthenia (n=4, 4.8%). Cutaneous lesions occurred in 3 patients (3.6%), including keratoacanthoma and squamous cell carcinoma (similar to other TGF- β -inhibiting agents) and were well managed by surgical excision. One patient discontinued the study due to a treatment-related AE (bullous pemphigoid). One patient died from pneumonia assessed by the investigator as treatment related.

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Conclusions

Based on preliminary data from this still-ongoing trial, treatment with M7824 results in initial clinical activity and a manageable safety profile in heavily pretreated patients with NSCLC refractory or resistant to prior anti-PD-1/anti-PD-L1 agents. Updated data, including results based on biomarkers such as tumor PD-L1 expression and circulating levels of TGF- β 1 and cytokines, will be presented at the meeting.

Trial Registration

NCT02517398

O15 Presidential Travel Award Recipient

Neoadjuvant nivolumab versus combination ipilimumab and nivolumab followed by adjuvant nivolumab in patients with resectable stage III and oligometastatic stage IV melanoma: preliminary findings

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Background

Single agent nivolumab (nivo) and combination ipilimumab (ipi) + nivo are efficacious in advanced melanoma, but have not been extensively evaluated in patients with high-risk resectable disease. The optimal neoadjuvant immunotherapy regimen is

unknown and development of biomarker specific neoadjuvant trials are critical in understanding mechanisms of response, resistance and informing clinical management. We designed a clinical trial to test these regimens in the neoadjuvant setting coupled with longitudinal sample collection designed to identify strategies to further improve outcomes.

Methods

Patients with resectable stage III or oligometastatic stage IV melanoma were randomized to nivo 3 mg/kg IV every 2 weeks for 4 doses or ipi 3 mg/kg + nivo 1 mg/kg IV every 3 weeks for 3 doses prior to surgical resection, with both arms receiving adjuvant nivo 3 mg/kg for 6 months (Figure 1). Responses were measured at time of surgery as defined by RECIST 1.1 and percent tumor viability in surgical specimen. Blood and tumor samples were collected at baseline, on-treatment, and at surgery.

Results

23 out of a planned 40 patients were enrolled. The response rates (RR) were 33% for nivo and 67% for ipi+nivo in evaluable patients (n=18 total, 9 in each arm) (Figure 2). Pathological complete response (pCR) rates were 22% for nivo and 56% for ipi+nivo. Importantly, 22% of patients in nivo arm experienced disease progression and were ineligible for surgical resection, prompting review by the data safety monitoring board and early trial closure. No grade 3 or higher toxicities were seen with nivo compared to 89% with ipi+nivo. All patients on ipi+nivo received surgical resection, but 33% had a surgical delay related to toxicity. RNA-seq analysis of longitudinal tumor samples revealed differential effects of each treatment on the tumor microenvironment as well as between responders (R) and non-responders (NR) within each treatment arm. Immune profiling demonstrated enriched CD8+ T cell infiltrates in on-treatment samples in R versus NR (p=0.04). T cell receptor sequencing showed a lower degree of remodeling from pre- to on-treatment tumor in R vs NR (as measured by Morisita's index, p<0.01).

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Conclusions

Treatment with neoadjuvant nivo or ipi+nivo has activity in resectable melanoma. Ipi+nivo possessed a higher RR but also higher grade III/IV toxicities whereas nivo led to a higher rate of disease progression and resultant unresectable disease. Ongoing correlative studies will provide insights into the mechanisms of response, toxicity and resistance.

Trial Registration

NCT02519322

Figure 1.

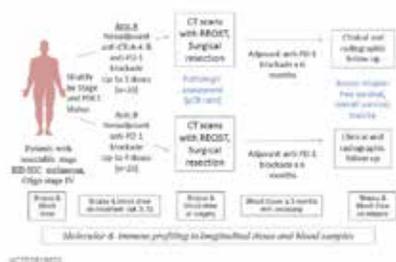
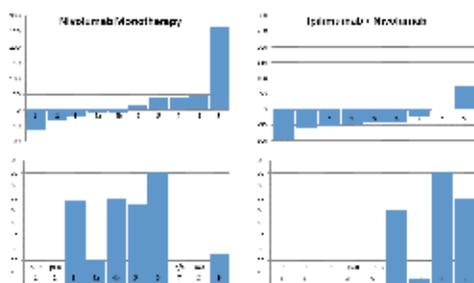


Figure 2.



Combination Therapy (IO/IO, IO/Standard of Care, IO/Other)

O16

A phase 1/2 study of CB-839, a first-in-class glutaminase inhibitor, combined with nivolumab in patients with advanced melanoma (MEL), renal cell carcinoma (RCC),

or non-small cell lung cancer (NSCLC)

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Background

CB-839 is a first-in-class, oral, highly selective inhibitor of glutaminase that targets tumor glutamine metabolism. Competition between tumor cells and immune cells for nutrients, including glutamine, in the tumor microenvironment can create a metabolic checkpoint that induces local immune suppression. CB-839 inhibits tumor glutamine consumption and increases glutamine availability to support T-cell activity. In pre-clinical models, CB-839 increases intra-tumoral glutamine and enhances antitumor activity of PD-1/PD-L1 inhibitors. This study is evaluating the safety and efficacy of CB-839 in combination with the PD-1

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inhibitor, nivolumab, in patients with advanced RCC, MEL, or NSCLC, including cohorts of patients refractory to anti-PD-1/PD-L1.

Methods

In dose escalation, patients received CB-839 600 mg or 800 mg PO BID in combination with standard-dose nivolumab. In expansion, patients are enrolling into one of five cohorts to receive CB-839 800 mg BID with standard-dose nivolumab: Naïve RCC – anti-PD-1/PD-L1 naïve, previously treated with anti-angiogenic therapy; Rescue RCC – on nivolumab with progressive disease (PD) or > 6 month stable disease without response; Other IO RCC – anti-PD-1/PD-L1 in any previous line of therapy with PD and no history of response; Rescue MEL – on anti-PD-1/PD-L1 with PD; Rescue NSCLC – on anti-PD-1/PD-L1 with PD or > 6 month SD without response. Tumor response assessment is every 8 weeks per RECIST 1.1. Tumor biopsy and blood are collected for biomarker analysis. Bristol-Myers Squibb is providing nivolumab for this study.

Results

The study is ongoing and 50 patients have enrolled to date (42 at 800 mg dose). A maximum tolerated dose of CB-839 was not reached. One DLT (Gr3 ALT increased) occurred at the 800 mg dose. The combination has been well tolerated; the most common treatment-related Gr \geq 3 AE has been ALT increased (4%). RECIST 1.1 tumor responses by cohort are shown below (Table 1).

There has been one complete response (CR) and two partial responses (PR) out of 7 response-evaluable MEL patients (ORR 43%). All three responding patients were progressing on anti-PD-1/PD-L1 at study entry and two had progressed on 3 prior immunotherapy regimens. Paired tumor biopsies were available from one MEL responder and analysis showed on-treatment increase in T-effector gene expression including granzyme and perforin.

Conclusions

Treatment with the CB-839+nivolumab is well-tolerated and has clinical activity in anti-PD-1/PD-L1 refractory patients. Notable responses have occurred in MEL patients progressing on nivolumab at study entry and refractory to multiple prior immunotherapy regimens. Updated safety, biomarker and response data will be presented.

Trial Registration

NCT02771626

Table 1.

Cohort	Naïve RCC	Rescue RCC	Other IO RCC	Rescue MEL	Rescue NSCLC
Enrolled	59	9	9	9	4
RECIST 1.1 Response Evaluable	56	5	4	7	4
Progressing on anti-PD-1/PD-L1 at study entry	NA	2 (40%)	NA	7 (100%)	1 (25%)
CR	0 (0%)	0 (0%)	0 (0%)	1 (14%)	0 (0%)
PR	1 (18%)	0 (0%)	0 (0%)	2 (29%)	0 (0%)
SD	9 (16%)	4 (80%)	2 (50%)	1 (14%)	1 (25%)
PD	4 (7%)	1 (20%)	2 (50%)	1 (14%)	1 (25%)
ORR = CR + PR + SD	12 (21%)	4 (80%)	2 (50%)	4 (57%)	1 (25%)

O17

OX40 T-cell costimulatory agonist BMS-986178 alone or in combination with nivolumab in patients with advanced solid tumors: initial phase 1 results

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Background

With the clinical success of checkpoint blockade, focus has shifted to novel approaches to enhance the benefits of immunotherapy. Activation of the costimulatory tumor necrosis factor receptor family member OX40 may lead to T effector cell activation, inhibition of T regulatory cell-mediated suppression, and blockade of T regulatory cell generation. Preclinical data show enhanced antitumor T-cell activity when anti-OX40 is combined with checkpoint inhibitors (eg, anti-PD-1), supporting the potential benefits of this combination to provide deeper, more durable responses than checkpoint blockade alone. BMS-986178 is a fully human IgG1 agonist monoclonal antibody that binds with high affinity to OX40. Here we describe preliminary results of a phase 1/2a study of BMS-986178 ± nivolumab (anti-PD-1) in patients with advanced solid tumors (NCT02737475).

Methods

Patients with advanced/metastatic solid tumors with ≥1 prior therapy were treated in this open-label, dose-escalation and dose-expansion study. During escalation, patients received BMS-986178 (20-320 mg) IV Q2W or BMS-986178 (20-320 mg) + nivolumab 240 mg IV Q2W. Safety, immunogenicity, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity were evaluated. Pharmacodynamic analyses included evaluation of OX40, PD-1, Ki67, FOXP3, and CD8 by immunohistochemistry in tumors and immunophenotyping in peripheral blood mononuclear cells.

Results

As of June 12, 2017, 59 patients were treated with BMS-986178 (n=20) or BMS-986178 + nivolumab

(n=39). Approximately one-third of patients in the monotherapy (30%) and combination (28%) arms had received prior anti-PD-1/PD-L1-based therapy. Most treatment-related AEs were grade 1/2 (Table 1); 1 patient treated with BMS-986178 160 mg + nivolumab had treatment-related grade 3 pneumonitis that led to discontinuation. The maximum tolerated doses of BMS-986178 ± nivolumab have not yet been reached. The pharmacokinetics of BMS-986178 ± nivolumab were linear, and exposure increased proportionally with BMS-986178 doses of 20-320 mg. Preliminary pharmacodynamic activity showed an increase in proliferating T cells and a decrease in FOXP3 cells in tumors with BMS-986178 + nivolumab. Evidence of antitumor activity was observed in several patients treated with the combination, including a patient who had progressed on prior anti-PD-1 therapy; these data will be reported.

Conclusions

BMS-986178, an OX40 agonist, given alone and in combination with nivolumab, was well tolerated; the combination had a safety profile similar to that of nivolumab monotherapy. Treatment with BMS-986178 + nivolumab resulted in pharmacodynamic immune changes in tumors and preliminary antitumor activity. Further evaluation of this combination in patients with advanced solid tumors is ongoing.

Trial Registration

ClinicalTrials.gov, NCT02737475

Table 1. Treatment-related AEs with BMS-986178 and BMS-986178 + nivolumab

Grade	BMS-986178		BMS-986178 + Nivolumab	
	n (%)	n (%)	n (%)	n (%)
Grade 1	1 (5)	1 (5)	1 (3)	1 (3)
Grade 2	1 (5)	1 (5)	1 (3)	1 (3)
Grade 3	0 (0)	0 (0)	1 (3)	0 (0)
Grade 4	0 (0)	0 (0)	0 (0)	0 (0)
Total	2 (10)	2 (10)	3 (8)	1 (3)

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aGrade 3 treatment-related pneumonitis was reported in 1 patient treated with BMS-986178 160 mg + nivolumab 240 mg.

018 Abstract Travel Award Recipient

TLR9 agonist harnesses innate immunity to drive tumor-infiltrating T cell expansion in distant lesions in a phase 1/2 study of intratumoral IMO-2125+ipilimumab in anti-PD1 refractory melanoma patients

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Background

While checkpoint inhibitors (CPI) have transformed melanoma treatment, many patients remain refractory. Pre-clinical models show combination intratumoral IMO-2125 (TLR9 agonist) with anti-CTLA-4 or anti-PD-1 antibody results in improved tumor control compared with either agent alone. We hypothesized that this combination would stimulate local antigen-presenting cells, resulting in activation of anti-tumor T cells and distant responses.

Methods

Adults with anti-PD-1 refractory, unresectable stage III/IV melanoma were enrolled. IMO-2125, escalating from 4 – 32 mg is administered under image guidance, intratumorally on weeks 1, 2, 3, 5, 8, and 11 with standard ipilimumab. Biopsies were obtained in both the injected and distant tumor at

baseline, 1 day and 8 weeks (W8) post injection. Immune analyses included phenotypic, activation, and functional characterization of DC subsets and T cells. T-cell repertoire diversity was evaluated by high-throughput CDR3 sequencing and changes in gene expression signatures were assessed by NanoString.

Results

Biopsies were obtained from 17 of the 18 subjects enrolled on the IMO-2125-ipilimumab arm. Of 15 subjects with post-baseline disease evaluations, 9 (60%) showed shrinkage in uninjected tumors with an ORR of 33% by RECIST 1.1. Fresh tumor biopsies taken 24h post IMO-2125 injection demonstrated induction of an IFN- α -response gene signature (IRF7) in all patients ($p < 0.0001$), as well as, maturation of the myeloid DC1 subset (CD1c⁺CD303⁻) by upregulation of MHC class II (6/12 patients) and upregulation of PD-L1 on malignant cells (10/14 patients). W8 biopsies of the uninjected tumor lesion reveal the induction of Ki67⁺ expression by flow cytometry in effector CD8⁺ T cells in 6 of 7 regressing lesions indicating an abscopal effect of this combination. In 6 patients with both tumor shrinkage and flow cytometry staining of PBMCs, 4 had higher CD8⁺ T-cell proliferation in the tumor than in blood on-treatment. CDR3 sequencing shows that the top 50 clones form a larger proportion of the repertoire at W8 suggesting local proliferation in both lesions, as well as, the specific expansion in the distant lesion of clones shared with the injected site in responders.

Conclusions

Combination IMO-2125 and ipilimumab therapy is a promising new treatment for anti-PD-1 refractory melanoma. We show this treatment strategy to result in a local IFN- α -gene signature coupled with mDC1 maturation. Additionally, the hallmark of tumor shrinkage appears to be the presence of Ki67⁺ CD8⁺ T cell effector cells in the uninjected tumor and expansion of clones shared between both lesions (abscopal effect).

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Trial Registration

NCT02644967

019

ENCORE-601: Phase 1b/2 study of entinostat (ENT) in combination with pembrolizumab (PEMBRO) in patients with non-small cell lung cancer (NSCLC)

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Background

Entinostat is an oral, class I selective histone deacetylase (HDAC) inhibitor that has been shown in pre-clinical models to enhance anti-PD-1 activity through inhibition of immune suppressor cells in the tumor microenvironment. ENCORE 601 is a Phase 1b/2 study evaluating safety and efficacy of ENT plus PEMBRO in NSCLC, melanoma and colorectal cancer patients. We previously reported the results of Phase 1b, which identified ENT 5 mg PO weekly plus PEMBRO 200 mg IV every 3 weeks as the dose to be further explored.

Methods

Efficacy of ENT 5 mg PO weekly plus PEMBRO 200 mg IV q3wks is being assessed in a Simon 2-stage Phase 2 study involving two cohorts of patients with

advanced NSCLC: anti-PD-(L)1-naïve (Cohort 1), and progressed on anti-PD-(L)1 (Cohort 2). The primary endpoint is objective response rate. Based on investigator feedback to incorporate Phase 1b patients dosed at entinostat 5 mg into the Stage 2 go/no go assessment, revised criteria for advancement were ≥ 4 responses observed out of 17 evaluable patients in Cohort 1 and ≥ 3 out of 31 in Cohort 2. Tumor biopsies and blood samples for immune correlates were taken pre- and post-treatment.

Results

Enrollment has been completed for Stage 1 of both cohorts. In Cohort 1, 4 of 17 (24%, 95% CI: 7-50) evaluable patients achieved a partial response (PR; 2 confirmed, 2 unconfirmed). In Cohort 2, 3 of 31 (10%, 95% CI: 2-26) evaluable patients achieved a PR (2 confirmed, 1 unconfirmed). As of the data cutoff, the longest duration of response was 24 weeks (and ongoing). Baseline PD-L1 expression for responders was $<1\%$ (1 patient), 1-49% (2 patients) and not available (1 patient) for Cohort 1, and $<1\%$ (2 patients) and not available (1 patient) for Cohort 2. 31% of patients experienced a Grade 3/4 event deemed related to study drug. The most common of these events included hypophosphatemia and neutropenia in Cohort 1, and fatigue, anemia, and pneumonitis in Cohort 2. 13% of patients discontinued therapy due to an adverse event. Circulating myeloid derived suppressor cells were reduced in both cohorts at Cycle2/Day1 compared to pretreatment. Gene expression analysis of tumor biopsies and association with responses is in progress.

Conclusions

ENT plus PEMBRO demonstrates anti-tumor activity and acceptable safety in patients with NSCLC who are both naïve to and have progressed on prior PD-(L)1 blockade. Based on the responses seen, Cohort 2 has advanced to Stage 2 and is currently enrolling.

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Pivot-02: Preliminary safety, efficacy and biomarker results from the Phase 1/2 study of CD-122-biased agonist NKTR-214 plus nivolumab in patients with locally advanced/metastatic solid tumors

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Background

In patients with advanced solid tumors, NKTR-214, a CD-122-biased agonist, increased newly proliferative CD8+ T cells in the tumor and increased cell surface PD-1 expression, demonstrating a potentially synergistic mechanism with anti-PD-1 therapy. Preliminary safety, efficacy and biomarker results from dose-escalation of the Phase 1/2 study combining NKTR-214 with nivolumab are presented.

Methods

Enrolled patients in dose escalation were checkpoint inhibitor naïve with locally advanced/metastatic melanoma (mM), RCC or

NSCLC. NKTR-214 and nivolumab were administered concurrently by IV infusion in the outpatient setting in Q2W or Q3W dosing schedules. Pre- and on-treatment blood and matched tumor samples were evaluated for changes in immune cell populations, gene expression, and T cell receptor repertoire. Responses were assessed Q8W per RECIST 1.1.

Results

At July 25, 2017, data cutoff, 31 patients were treated across one of 5 dose escalating cohorts of NKTR-214 (@0.003(n=3), @0.006(n=25) or @0.009(n=3) mg/kg) administered with nivolumab given either 240 mg Q2W or 360 mg Q3W. There were no Grade ≥3 treatment-related adverse events (TRAEs) reported at or below the NKTR-214 0.006 mg/kg plus nivolumab cohorts. There were no discontinuations from TRAEs. The most common TRAEs occurring in ≥50% of patients included grade 1-2 fatigue, flu-like symptoms and rash. At the highest dose (NKTR-214 0.009 mg/kg + nivolumab), two patients experienced DLTs including hypotension and metabolic acidosis. DLTs were short-lived and manageable with both patients continuing treatment at lower doses. 16 patients with Stage IV mM or Stage IV RCC were evaluable with ≥1 on treatment scan. In mM, 1 complete response was observed plus 4 partial responses (n=8). In RCC, 4 PRs were observed (n=8). 5/16 patients had SD, 2 patients had PD. In tumor tissue, there was an up to 450-fold increase in tumor-infiltrating CD8+ T cells from baseline with a limited (≤2-fold) increase in T regulatory cells. Infiltrating T cells were newly proliferative with elevated PD-1 expression. In blood, ICOS+ CD8+ T cells increased with treatment representing 13% of total CD8+ T cells. Updated data will be presented.

Conclusions

Combination of NKTR-214 and nivolumab was well-tolerated and manageable in outpatient setting. Nivolumab-related AEs were not increased by NKTR-214. Immune activation was observed in peripheral blood and tumor microenvironment. Preliminary efficacy with mM and RCC show encouraging response rates. A recommended Phase 2 dose

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schedule of Q3W was established (NKTR-214 0.006 mg/kg plus nivolumab 360 mg), and enrollment is underway in expansion cohorts in eight different indications (n=230).

Trial Registration

Trial Registration: NCT02983045

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Pooled 3-year overall survival data from phase II and phase III trials of nivolumab (NIVO) combined with ipilimumab (IPI) in advanced melanoma

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Background

NIVO (anti-PD-1) plus IPI (anti-CTLA-4) improved progression-free survival (PFS) and objective response rate vs IPI alone in the phase II CheckMate 069 and phase III CheckMate 067 trials of treatment-naïve patients with advanced melanoma. Recently, a 2-year overall survival (OS) rate of 64% was reported for patients treated with NIVO+IPI in CheckMate 067. Here, we report 3-year OS, PFS, and safety results in all randomized patients from CheckMate 069.

Methods

Patients were randomized 2:1 to NIVO 1 mg/kg + IPI 3 mg/kg (n=95) or IPI 3 mg/kg + placebo (n=47) Q3W for 4 doses followed by NIVO 3 mg/kg or placebo Q2W, respectively, until disease progression or unacceptable toxicity.

Results

55% of patients in the IPI group crossed over to receive NIVO upon progression. At a median follow-up of 37 months, median OS was not reached (NR) for NIVO+IPI and was 32.9 months for IPI (hazard ratio [HR]: 0.76; P=0.2829), with 3-year OS rates of 57% and 46%, respectively. Median PFS was NR for NIVO+IPI and was 3.0 months for IPI (HR: 0.35; P<0.0001). Median duration of response was NR in both groups. Time to subsequent therapy in the NIVO+IPI and IPI groups was NR and 5.4 months, where 63% (47/75) and 19% (7/37) remain free of subsequent therapy at the 3-year landmark, respectively. The safety profile remained similar to the earlier reports, with grade 3-4 treatment-related adverse events in the NIVO+IPI and IPI groups of 54% and 24%; 38% and 9% discontinued due to any grade adverse events, respectively. Three-year OS rates in patients who discontinued

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due to toxicity were 68% for NIVO+IPI and 25% for IPI; median duration of response was NR in both subgroups.

Conclusions

In summary, NIVO+IPI continues to show a significant improvement in PFS, with consistent safety results, and a favorable 3-year landmark OS rate. A pooled analysis, which will include updated 3-year OS data from CheckMate 067, will be presented as well as outcomes in patients off all therapy.

Trial Registration

NCT01927419 (CheckMate 069), NCT01844505 (CheckMate 067)

O22

Antibody-mediated neutralization of soluble MIC significantly enhances response to CTLA4 blockade and reduces therapy-induced colitis

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Background

Antibody therapy targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA4) elicited survival benefits in cancer patients; however, the overall response rate is limited. In addition, anti-CTLA4 antibody therapy induces a high rate of immune related adverse events (IRAEs). The underlying factors that may influence anti-CTLA4 antibody therapy are not well defined. Ligation of the human activation immune receptor natural killer group 2D (NKG2D) has been implicated in optimizing CD8 T cell co-stimulation and pathogenesis of colitis. Herein we investigated the impact of the human NKG2D ligand MIC, which was broadly expressed by human solid tumors, on the therapeutic outcome of anti-CTLA4 blockade.

Methods

We utilized the well-established MIC^{negative} TRAMP and MIC^{positive} TRAMP/MIC spontaneous pre-clinical tumor models to address our question. We treated cohorts of these animals with anti-CTLA4 antibody or anti-sMIC antibody monotherapy or combination therapy and assessed treatment response and related toxicity. We further corroborate our findings with various indications of sMIC^{negative} and sMIC^{positive} transplantable syngeneic tumor models.

Results

As expected, MIC^{negative} tumors exhibited nominal response to anti-CTLA4 therapy and no response to anti-sMIC therapy. Unexpectedly, animals bearing MIC^{positive} tumors and with elevated serum sMIC (sMIC^{hi}) responded poorly to anti-CTLA4 antibody therapy with significantly shortened survival (Figure 1). Animals with high levels of serum sMIC had increased lung metastasis and developed colitis in response to anti-CTLA4 antibody therapy (Figure 2). Co-administration of a sMIC-neutralizing monoclonal antibody B10G5 with the anti-CTLA4 antibody alleviated colitis in sMIC^{hi} animals and generated a cooperative anti-tumor therapeutic effect by synergistically augmenting innate and adoptive anti-tumor immune responses (Figure 3). Mechanistically, we show that sMIC downmodulation of CD3z on NK and T cells, which ultimately impairs TCR/CD3 signaling and NK cell receptor signaling and potentially CD4 T cell-mediated tolerance. We further demonstrate that the combination of anti-CTLA4 with anti-sMIC antibody cooperatively primes DC activation, overcomes CD8 T cell tolerance, enhances TCR/CD3 signaling capacity in CD8 T cells, and increases T cell clonality or repertoire complexity in tumor infiltrates.

Conclusions

Our findings imply that a new combination therapy could improve the clinical response to anti-CTLA4 antibody therapy. Our findings also suggest that pre-screening cancer patients for serum sMIC may help in selecting candidates who will elicit a better response to anti-CTLA4 antibody therapy.

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Figure 1. High soluble MIC 9sMIC) provokes poor response to anti-CTLA4 therapy

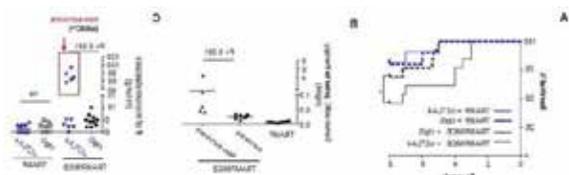


Figure 2. High sMIC induces colitis in response to anti-CTLA4 therapy

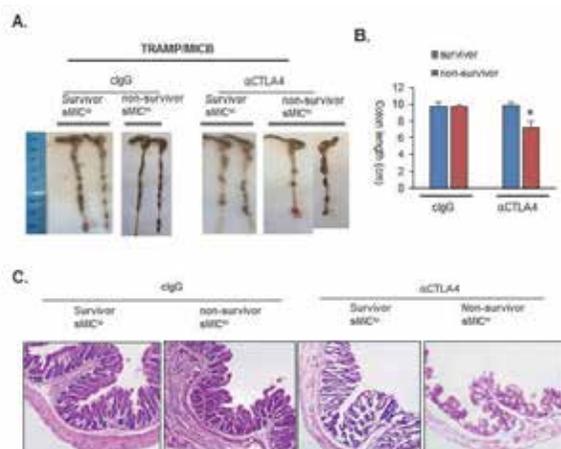


Figure 3. Combination therapy of anti-sMIC (B10G5) and anti-CTLA4 generates synergistic curative effect

Emerging Models and Imaging

023  Abstract Travel Award Recipient

Multiplex three-dimensional optical mapping of tumor immune microenvironment

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Background

The tumor microenvironment is a three-dimensional (3D) system of diverse cellular and non-cellular components whose heterogeneous structure is

typically defined by haphazard growth of cancer cells and a disordered microvasculature. Tumor-immune cell interactions occur within this context, providing a challenge for analysis of the infiltrate and anti-tumor immune responses by two-dimensional (2D) methods such as immunohistochemistry (IHC). Toward overcoming the limitations of 2D methods, we introduce Transparent Tissue Tomography (T3) as a tool for quantitative 3D imaging cytometry of the tumor immune microenvironment.

Methods

For T3 imaging cytometry, tumors were sliced into 400 μm macrosections to facilitate immunofluorescence staining, optical clearing, and confocal microscopic imaging. Macrosections were stained overnight with panels of four to six directly labeled fluorescent primary antibodies then cleared by D-fructose. The full volume of each macrosection was scanned in each channel using a confocal microscope and tumor images were tomographically reconstructed from the macrosection images. The tumor images were segmented to discriminate cell types, map biomarkers, and perform spatial analysis.

Results

As an application of T3 imaging cytometry, we examined the distribution of programmed death-ligand 1 (PD-L1) expression in spontaneous Her2⁺ mammary tumors formed in BALB-NeuT mice. T3 analysis of whole tumors determined PD-L1 expression by tumor cells at the periphery and CD31⁺ vascular endothelium in the core. For the first time, 3D tomographic projection disclosed vascular PD-L1 expression localized between the endothelium and inner layer of smooth muscle cells. In turn, T3 revealed a strong spatial correlation between CD45⁺ immune cell distribution and PD-L1 expression. Applying T3 to pharmacokinetic analysis, we observed uneven accumulation of anti-PD-L1 antibody in perfused regions and lack of delivery to hypoxic domains. In addition, 3D vascular mapping showed that the blood vessels wrapped in smooth muscle cell layers restrained

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anti-PD-L1 antibody extravasation into tumor. Toward translation, T3 was adapted to analyze whole core needle biopsies. Modeling on-treatment biopsy analysis, T3 confirmed a compound effect of radiation and anti-PD-L1 therapy on infiltration of effector cytotoxic lymphocytes such as granzymeB⁺ NK cells and T cells.

Conclusions

By assessing multiple tumor parameters simultaneously at cellular resolution, T3 provides a unique window into the heterogeneity of the tumor immune microenvironment. We anticipate that T3 can be applied broadly to facilitate preclinical studies of tumor biology and therapy. In particular, spatial, multiparameter T3 analysis may serve as a tool to improve diagnostic, prognostic and predictive testing of patient biopsies as part of evaluation for immune checkpoint blockade therapy.

Immune Modulation, Cytokines, and Antibodies

024

First-in-human study with intratumoral administration of a CD40 agonistic antibody: preliminary results with ADC-1013/JNJ-64457107 in advanced solid malignancies

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Background

Agonistic antibodies targeting CD40 activate dendritic cells and can expand and activate tumor

specific T cells. Preclinical studies have demonstrated that intratumoral administration of CD40 agonists has the potential to improve anti-tumor efficacy and reduce immune related adverse events compared to intravenous administration. Herein, we report the first study of a CD40 agonistic antibody administered intratumorally in cancer patients.

Methods

A Phase I, open label, multicentre study was conducted to evaluate the safety, pharmacokinetics and pharmacodynamics of ADC-1013 in patients with advanced solid tumors who had received established treatments. A 3+3 dose escalation was conducted with a 28-day window (bi-weekly dosing). Twenty-three patients were treated with ADC-1013 intratumorally (dosing from 22.5 µg/kg up to 400 µg/kg) or intravenously (dosing at 75 µg/kg). The pharmacodynamic effects observed in the patients were further verified in an hCD40tg mouse model.

Results

Adverse events were primarily fatigue, pyrexia, nausea and vomiting, and were mostly CTCAE Grade 1 or 2 and transient. Two subjects receiving intratumoral bolus injections of ADC-1013 into hepatic lesions at a dose of 400 µg/kg experienced Grade 3 abdominal pain assessed as DLTs. Elevations in cytokine levels (MCP-1, TNFα and IL-6) were observed in patients receiving 200 µg/kg and 400 µg/kg of ADC-1013. The systemic ADC-1013 exposure and cytokine release was more pronounced in patients receiving injections in a hepatic lesion than patients receiving injections in a non-hepatic lesion. Treatment with ADC-1013 resulted in a marked decrease in B cell levels in peripheral blood after 24 h, recovering to baseline levels within 8 days. Remaining B cells significantly increased their expression of the cell surface activation marker CD86 24 h after first dose, returning to baseline within 8 days.

The pharmacodynamic effects were further studied in hCD40tg mice demonstrating activation of

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antigen presenting cells such as dendritic cells and subsequent activation of T cells. Moreover, ADC-1013 treatment in this mouse model acts synergistically with a PD-1 inhibitor.

Conclusions

The results from the first-in-human study of ADC-1013 indicate that intratumoral administration of ADC-1013 into non-hepatic lesions (superficial metastases) is well tolerated at doses up to at least 400 µg/kg, while intratumoral administration of ADC-1013 into hepatic lesions resulted in toxicities associated with cytokine release and Grade 3 adverse events. The pharmacodynamic effects and the preclinical data support further clinical development of ADC-1013 as mono- or combination therapy with e.g. PD-1/PD-L1 targeting antibodies. Janssen is currently developing ADC-1013/JNJ-64457107 for intravenous administration in patients with solid tumors.

025

Phase I trial of IL-15 superagonist ALT-803 (IL-15N72D:IL-15R α Su/IgG1 Fc complex) in advanced solid tumors: tolerability and correlates of activity

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Background

IL-15, a common gamma chain (γ c) cytokine, stimulates expansion and cytotoxic functions of NK and memory CD8+ T cells. Compared to unmodified IL-15, ALT-803 had superior antitumor activity, low toxicity and favorable pharmacokinetics in animal models. These promising data prompted this phase I trial of ALT-803 administered intravenously (i.v.) or subcutaneously (s.c.) to patients with advanced cancers.

Methods

ALT-803 was administered at doses of 0.3-20 mcg/kg weekly i.v. or s.c. for 4 consecutive weeks, every 6 weeks until toxicity or progression. Correlates included pharmacokinetics, immunogenicity, lymphocyte expansion and flow cytometric phenotyping. Clinical endpoints were toxicity and anti tumor activity.

Results

Eleven patients received i.v. ALT-803 (range 0.3 to 6 mcg/kg), and 13 patients received s.c. ALT-803 (range 6 to 20 mcg/kg) in 9 dose cohorts. Ten had melanoma, 7 renal, 3 head and neck, and 4 lung cancer. The most common dose-related toxicity was injection site reaction with s.c. ALT-803; biopsy showed inflammatory infiltrates. Fatigue, fever and nausea were the next most common adverse events. Maximal increases in white blood cell and lymphocyte counts during cycle 1 were modest across all doses, with means of 1.2-fold (1.2-fold at 20 mcg/kg s.c.) and 1.5-fold (1.4-fold at 20 mcg/kg s.c.) over baseline. NK cell expansion typically peaked on cycle 1 day 15 or 29, with a mean maximal 2.7-fold increase across all doses, and 3.3-fold for the highest (20 mcg/kg) s.c. dose cohort. The expansion of circulating CD8+ T cells was modest (mean maximal 1.4-fold increase), but the mean fold increase for the % positivity of activation marker HLA-DR on CD8+ T cells was 2.9-fold across all dose cohorts and 4.7-fold at 20 mcg/kg s.c., typically peaking on cycle 1 day 4. Immunogenicity assays demonstrated no reactivity in patients. Clinical activity was not observed in these heavily-pretreated patients, but combination therapy trials

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are ongoing. Toxicities were mild, non-dose-limiting and similar to IL-2 and other γ c cytokines. One patient experienced symptomatic diffuse cardiomyopathy that resolved slowly after drug discontinuation and corticosteroid treatment.

Conclusions

The IL-15 superagonist ALT-803 was well-tolerated with minimal cytokine toxicities in patients with advanced cancer. Substantial increases in NK cells and rapid activation of CD8+ T cells as evidenced by increased HLA-DR expression are promising immunologic effects, providing a strong rationale for combination anticancer immunotherapy with ALT-803.

Trial Registration

NCT01946789

Mechanisms of Resistance to Immunotherapy

026  Presidential Travel Award Recipient

Metabolic adaptations establish immunotherapy resistance in melanoma

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Background

Despite the success of T cell checkpoint blockade antibodies in treating an array of cancers, the majority of patients still fail to respond to these therapies, or respond transiently and then relapse. The molecular mechanisms which drive lack of

response to checkpoint blockade, whether pre-existing or evolved on therapy, remain unclear.

Methods

To address this critical gap in clinical knowledge, we established a mouse model of melanoma designed to elucidate the molecular mechanisms underlying immunotherapy resistance. Through multiple *in vivo* passages, we selected a B16 melanoma tumor line that evolved complete resistance to combination blockade of CTLA-4, PD-1, and PD-L1, which cures ~80% of mice of the parental tumor. Using gene expression analysis, proteomics, and immunogenomics, we determined the adaptations engaged by this melanoma to become completely immunotherapy resistant. NMR spectroscopy, Seahorse XF Analysis, flow cytometry, confocal microscopy and western blot analysis provided further insight into the mechanisms driving checkpoint blockade resistance.

Results

Acquisition of immunotherapy resistance by these melanomas was driven by coordinate upregulation of the glycolytic and aldose reductase pathways to create a metabolically hostile microenvironment in which T cell function is profoundly suppressed. When re-introduced into the parental tumor, the genes most closely associated with these metabolic adaptations confer enhanced immunotherapy resistance. We have validated upregulation of these pathways in a unique cohort of melanoma patients who failed dual checkpoint blockade. Additionally, we employed MRI imaging to visualize metabolic changes acquired by resistant tumors in live mice. Clinical application of this technique could provide a much-needed non-invasive tool to predict immunotherapeutic sensitivity of patients.

Conclusions

Upregulation of glycolytic metabolism and the aldose reductase pathway by melanoma tumor cells cripples T cells in the microenvironment and confers resistance to checkpoint blockade.

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027  Abstract Travel Award Recipient

Single cell RNA sequencing reveals mechanisms of Merkel Cell Carcinoma escape from intense pressure of T cell immunotherapy

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Background

80% of Merkel cell carcinomas (MCCs) are driven by viral (MCPyV) oncoproteins, rendering them ideal immunotherapy targets. Transfer of MCC-specific T cells alone is insufficient, and half of patients receiving anti-PD1 monotherapy progress. We hypothesized co-transferring MCPyV-specific T cells with checkpoint blockade may enhance efficacy and elucidate interactions between T cells, tumor and the microenvironment. A patient with a dramatic response to T cells plus checkpoint inhibition, whose MCC eventually progressed despite robust T cell persistence and MCPyV expression, was examined by single-cell RNA sequencing (scRNAseq) to identify the mechanisms of response then immunoevasion.

Methods

A 59-year-old man with heavily-pretreated, MCPyV-positive MCC (>5 prior therapies including pembrolizumab) received autologous ex-vivo expanded, HLA B3502-restricted MCPyV-specific CD8+ T cells (CD8+s), with sequential addition of

pembrolizumab and ipilimumab. A 90% tumor reduction was sustained for ~1 year, followed by progression. ScRNAseq was performed on PBMCs and tumor collected before treatment, during response and at progression. Results were compared to flow cytometry and multicolor-IHC.

Results

scRNAseq of PBMCs identified a cluster of intensely-activated CD8+s present exclusively at the time of immunotherapy response. Although flow cytometry detected MCPyV-specific CD8+s, it was unable to discriminate this proliferative subpopulation. Activated CD8+s in blood corresponded with HLA-DR+CD8+ T cell infiltration into the shrinking tumor (including infused cells) and supported a causative role of CD8+s in mediating MCC regression.

scRNAseq of tumor digests was performed to determine the mechanism of acquired immunotherapy resistance. Despite an input of only 10000 cells, the expression profiles of tumor, TIL, macrophages, and fibroblasts could be distinguished. TIL and macrophages expressed transcriptomes that were stable in the pre-immunotherapy and post-resistance samples, suggesting these cells were not mediating the acquired resistance. In contrast, tumor cells displayed strikingly different transcriptional profiles before and after development of resistance. Directed analysis revealed tumor-specific loss of only the targeted HLA-B, but not non-targeted HLA-A, at time of progression. This implies intense immunologic and selective pressure from the transferred HLA-B3502-restricted CD8+s. Unbiased scRNAseq analyses identified significant upregulation of HLA-E, which is an inhibitory signal for NK cells, and helps explain NK avoidance in the context of HLA-loss.

Conclusions

With small cellular inputs, scRNAseq can provide mechanistic insights beyond traditional IHC or flow cytometry, here revealing allele-specific HLA downregulation and HLA-E induction by the escaping tumor. Such insights can guide design of

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improved cellular therapies, in this case suggesting the use of multiple specificities of T cells.

Trial Registration

NCT0175845

O28 Abstract Travel Award Recipient

Functional correlation of increased tumor intrinsic glycolytic activity with resistance to adoptive T cell therapy

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Background

Adoptive T cell therapy (ACT) has produced impressive responses in a subset of patients with advanced malignancies, particularly those with melanoma, where as many as 50% of patients may achieve an objective response. However, these responses tend to be transient and only present in a small portion of patients. Thus, there is an urgent need to understand the resistant mechanisms in non-responders and develop more effective ACT strategies that prevent emergence of resistance or reverse resistance once it occurs. Emerging evidence suggest that tumor intrinsic signaling pathways play a critical role in promoting resistance to T cell-mediated immunotherapy. For example, we recently reported that oncogenic activation of

the PI3K pathway resulting from PTEN loss renders melanoma cells refractory to T cell-mediated therapies and that this could be reverted by targeted inhibition of PI3K signaling. However, work from our laboratory, and others, suggest that melanoma cells may have additional means to circumvent immunotherapy.

Methods

In this study, we employed two independent and unbiased approaches to identify novel molecular determinants of immune resistance. We generated gene expression profiles on PTEN-deficient melanoma cell lines from patients to identify alternative immunosuppressive mechanisms and then extended this finding to include non-small cell lung cancer. Then we utilized a new high-throughput shRNA screening platform developed by our group to functionally interrogate immune resistance in melanoma cells.

Results

Results from both analyses implicated tumor-associated glycolysis as a critical pathway that enables tumor cells to evade T cell-mediated antitumor activity. By using samples from melanoma and non-small cell lung cancer patients, we showed that increased expression of glycolysis-related genes is associated with poor T cell infiltration of tumors. In addition, we found that increased expression of *ALDOA*, which encodes a critical enzyme in the glycolysis pathway, was functionally correlated with reduced sensitivity of tumor cells to T cell-mediated killing. Overexpression of *ALDOA* impaired T cell killing of patient-derived melanoma cells, while inhibiting glycolysis restored T cell-mediated apoptosis of tumor cells. More importantly, when we characterized the patterns of gene expression and generated bioenergetic profiles on tumor samples from two non-overlapping ACT-treated patient cohorts, we discovered that tumor glycolytic activity in patients who experienced disease progression following ACT was significantly higher compared to those patients who were responsive to therapy.

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Conclusions

Taken together, our results demonstrate that tumor glycolytic metabolism is associated with the efficacy of ACT and identify glycolysis as a candidate target for combinatorial therapeutic intervention.

O29

A dual and opposing role for tumor intrinsic type-II interferon sensing in tumor establishment and acquired resistance

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Background

Immunotherapy can augment the host anti-tumor response inducing tumor control and regression. However, in a subset of patients immune selective pressure can drive the outgrowth of resistant tumor cell clones. An understanding of the altered genetic pathways in resistant tumor cell populations is critical to develop therapies to target these tumors.

Methods

To this end, we utilized a whole genome CRISPR library screen to delete genes in B16.SIY cells and selected clones that gained resistance to T cell-mediated killing. One set of mutations was in genes involved in the type-II interferon pathway, namely IFN γ R2 and Jak1. To test the role of these genes in subverting the host immune response we generated B16.SIY cells selectively lacking IFN γ R2 or Jak1.

Results

In contrast to resistance to T cell-mediated killing in vitro, when implanted into mice, these mutant tumors paradoxically were better controlled in vivo. This phenotype was observed with multiple IFN γ R2- and Jak1-deleted B16.SIY lines and using different guide-RNAs, and also with a second tumor cell line MC38. Re-introduction of IFN γ R2 reverted the phenotype and restored tumor growth. Depletion of host CD8⁺ T cells eliminated regression of mutant

tumors, and increased accumulation of antigen-specific CD8⁺ T cells was observed in the tumor microenvironment. It seemed likely that IFN- γ produced by CD8⁺ T cells in the tumor microenvironment might have a dominant negative effect through upregulation of inhibitory factors such as PD-L1 on tumor cells. Indeed, IFN γ R2^{-/-} and Jak1^{-/-} tumor cells expressed markedly reduced PD-L1 and IDO in vivo compared to WT tumors. Further, transduction to express PD-L1 restored the ability of IFN γ R2^{-/-} tumors to subvert the host immune response and grow progressively.

Conclusions

In summary, we find that the type-II signaling in tumor cells in some models can be required for the initiation and stabilization of the suppressive tumor microenvironment. Combined with other published data, our results suggest that whether IFN- γ on tumor cells is predominantly a positive or negative factor for tumor control may depend on whether inhibitory factors like PD-L1 are dominantly expressed and functional through tumor cells versus host immune cells

Microbiome

O30

Diversity and composition of the gut microbiome influences responses to anti-PD1-therapy through beneficial changes in innate and adaptive immunity

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Background

Cancer therapy has benefitted greatly from immune checkpoint inhibitors that target negative regulators of T-cell activation, however responses are variable and not always sustained. Recent evidence in murine models suggests that the gut microbiome can be modulated to enhance responses to immune checkpoint blockade. However, this has not been deeply investigated in patients.

Methods

We collected buccal and stool samples from a cohort of anti-PD-1 treated metastatic melanoma patients (n=112). Patients were classified as either responders (R) or non-responders (NR) based on RECIST criteria (R=CR, PR, or SD>6 months, NR= PD or SD<6 months). 16S rRNA, and whole-genome shotgun sequencing were performed to characterize the diversity, composition and functional capabilities of the microbiome. Immune profiling (via immunohistochemistry and flow cytometry) was performed in available tumors and serum samples at baseline. Fecal microbiota transplants (FMT) from R versus NR patients were performed in germ-free (GF) mice, tumors were implanted and markers of systemic and anti-tumor immunity queried.

Results

We observed significant differences in the diversity and composition of the gut microbiome in R versus NR to anti-PD-1 therapy at baseline with no clear differences in the oral microbiome. Specifically, R had a higher alpha diversity compared to NR (11.2 versus 6.4, $p<0.01$), and an enrichment of the *Faecalibacterium* genus of the Ruminococcaceae family. In contrast, the Bacteroidales order was found to be enriched in NR. High abundance of *Faecalibacterium* was also associated with improved

progression-free survival (HR=2.92. 95% C.I= 1.02 - 12.52) Immune profiling demonstrated significantly increased immune infiltrates in baseline tumor samples of R versus NR ($p=0.04$), with a positive correlation between CD8+ T-cell density in baseline tumor and blood samples and abundance of beneficial Ruminococcaceae bacteria in the gut microbiome ($r^2=0.42$, $p<0.01$). Distinct metabolic signatures were also observed in the gut microbiome with synthetic processes predominating in R and degradative processes predominating in NR. In FMT studies, GF mice transplanted with R-stool demonstrated delayed tumor outgrowth and enhanced responses to anti-PD-L1 therapy ($p<0.01$). Immune profiling in these mice revealed a higher density of innate and adaptive immune infiltrates in the tumor and gut, and upregulation of PD-L1 in the tumor microenvironment.

Conclusions

Diversity and composition of the gut microbiome was associated with differential responses to anti-PD-1 therapy, and enhanced systemic and anti-tumor immunity in metastatic melanoma patients. Our results have far-reaching implications and suggest that modifying the gut microbiome could potentially enhance therapeutic responses to immune checkpoint blockade.

Oncolytic Viruses and Intratumoral Therapies

O31

Preclinical characterization of a novel STING agonist, MK-1454

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Background

Several innate immune danger-sensing pathways have emerged as promising targets for enhancing cancer immunotherapy. In recent years, cyclic dinucleotide agonists of STING (Stimulator of interferon genes) have demonstrated significant anti-tumor efficacy in several mouse syngeneic tumor models and set the stage for STING agonists to be evaluated in human clinical trials.

Methods

Binding of STING agonist MK-1454 to mouse and human STING was evaluated using Biacore. STING activity was monitored in human whole blood and PBMCs, in multiple primary mouse and human cells, cell lines and differentiated myeloid cell subsets using cytokine production and cell activation as readouts. In vivo efficacy studies of intratumorally-dosed MK-1454 were performed in the presence or absence of an anti-PD-1 antibody in MC38, CT26 and B16F10 syngeneic tumor models.

Characterization of MK-1454 activity in primary human tumors was assessed in human histoculture studies. Gene profiling and STING gene signature studies were performed using custom-built Nanostring reagents.

Results

In vitro studies demonstrated potent binding of MK-1454 to human and mouse STING and rapid induction of type-I interferon and proinflammatory cytokine production in human and mouse cell lines and primary cells in a STING-dependent manner. Mechanistic studies were conducted to assess the

effect of STING agonism across multiple myeloid cell subsets including monocytic MDSCs, M2 macrophages and TAMs, which have been previously suggested to represent the suppressive myeloid component of the TME. In addition, we describe important effects of STING agonism on mouse and human T cells and highlight some key species-related differences. Critically, we demonstrate that human primary tumors, stimulated ex vivo with MK-1454, respond robustly as indicated in cytokine production and gene profiling studies. Additional studies using in vitro-stimulated human whole blood were used to define a STING gene signature that could represent a valuable biomarker in the clinic. In multiple mouse syngeneic tumor models, MK-1454 induced strong anti-tumor responses, effecting full tumor eradication upon intratumoral dosing, either as a single agent or in combination with PD-1 blockade, depending on the tumor model and dose level. We also show that MK-1454 induced tumor-specific adaptive immune memory as demonstrated by the lack of tumor growth in tumor re-challenge studies.

Conclusions

MK-1454, a novel STING agonist, induces potent cytokine responses and immune cell activation in vitro and robust anti-tumor activity in vivo. The preclinical data presented here support the ongoing clinical evaluation of MK-1454 in cancer patients both as monotherapy and in combination with Keytruda.

Other

O32

Phase 1 trial of CA-170, a first-in-class, orally available, small molecule immune checkpoint inhibitor (ICI) dually targeting PD-L1 and VISTA, in patients with advanced solid tumors or lymphomas

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Background

Programmed-death 1 (PD-1) and V-domain Ig suppressor of T-cell activation (VISTA) are independent immune checkpoints that negatively regulate T-cell function and are implicated in various malignancies. Pre-clinical studies demonstrated that dual blockade of both checkpoints can be synergistic. CA-170 is a novel

oral small molecule ICI that directly targets both PD-L1/L2 and VISTA and demonstrated significant anti-tumor activity in multiple preclinical animal models.

Methods

Enrollment initially followed accelerated titration and subsequently switched to 3+3 design. Cohorts of selected dose levels were expanded with additional patients. The expansion phase allows for enrollment of patients with selected indications known to be sensitive to ICI. Primary objectives: safety, maximum tolerated dose (MTD) and recommended Phase 2 dose. Secondary objectives: pharmacokinetics (PK) and anti-tumor activity. Exploratory endpoints: biomarkers and pharmacodynamic (PD) effects in peripheral blood and tumor tissues.

Results

A total of 34 patients have been treated across 6 dose levels (50 -800 mg) with 28 evaluable for safety and 4 still ongoing in cycle 1. Enrolled tumor types include lung, ovarian, lymphoma, head and neck, renal cell carcinoma, etc.

No DLTs or \geq grade 3 drug-related adverse events (AEs) have been observed thus far. The most common treatment-emergent AEs (all grades) were fatigue (24%), gastritis (20%), nausea (20%), vomiting (20%), chills (16%), constipation (16%) and headache (16%). These were predominantly grade 1 and self-limiting events.

Seven newly enrolled patients are pending restaging. Nineteen patients were evaluable for anti-tumor activity with 12 showing stable disease and 4 on study for > 6 cycles. An ICI naïve melanoma patient treated at 600 mg had lesion shrinkage by 14% per RECIST and 23% per immune-related response criteria by the end of cycle 2 and is currently ongoing on treatment.

CA-170 exhibits approximately dose proportional plasma exposure with $T_{1/2}$ 5 – 12 hours. Evidence of peripheral T cell activation was observed with an increased proportion of circulating CD8⁺ and CD4⁺ T

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cells expressing activation markers. Additionally, preliminary tumor biopsy data has shown increases in the number of immune cells post-treatment versus pre-treatment.

Conclusions

The data suggest CA-170 has an acceptable safety profile with preliminary signs of anti-tumor activity and peripheral immune modulation and approximately dose proportional PK profile. MTD has not been reached. These data warrant the continued clinical development of CA-170. Dose escalation is ongoing. Expansion cohorts in selected indications are planned.

Trial Registration

Clinical trial identifier: NCT02812875.

PHYSICIAN/NURSE/PHARM: irAE Management: Clinical Care and Best Practices

O33

Variance from evidence-based management of immune-related adverse events among healthcare providers: analysis of an online management decision tool

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Background

Immune checkpoint inhibitors (ICIs) are altering the treatment paradigm in oncology, impacting the care of a rapidly increasing number of patients; but many healthcare providers (HCPs) remain unfamiliar and inexperienced with managing the unique spectrum of immune-related adverse events (irAEs). An online decision support tool was developed to give HCPs easy access to fair-balanced guidance and management algorithms that are patient-specific

and extend beyond standard recommendations found in the respective product inserts [1].

Methods

To use the tool, HCPs entered patient symptoms, organ system affected, and the grade or severity of the event along with their planned management strategy. Subsequently, the HCPs using the tool were provided an expert recommendation for management of that specific irAE. Recommendations in the tool comprised algorithms developed from peer-reviewed publications and the personal clinical experience of Jeffrey S. Weber, MD, PhD. Here, we report a comparison of the intended irAE management of HCPs for 2896 cases entered into the tool from 11/9/16 through 7/21/17 with the expert recommendations for those patient cases.

Results

Cases involving GI symptoms (n = 818; 28% of all cases) were most frequently entered into the tool with renal symptom cases (n = 153; 5% of all cases) having the fewest entries (Table 1). The planned irAE management strategy of HCPs differed from the expert recommendations for 49% of the cases overall with the greatest divergence in neurologic- and endocrine-related cases (56% and 66%, respectively). The proportion of cases in which the planned management strategies of the HCPs differed from the expert recommendations also varied by symptom grade/severity ranging from 84% differing for grade 3 adrenal insufficiency to 21% differing for grade 4 renal toxicity. Overall, the largest proportion of cases with HCP variance from optimal recommended practice occurred with those having intermediate to high symptom severity (grades 2/3). Overall, of 648 HCPs who responded to a survey about the impact of the tool on their practice, 93% indicated that the recommendations provided by the tool either changed or confirmed their management plan.

Conclusions

These data suggest that many HCPs are not optimally managing irAEs associated with ICI use

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and that an online tool can provide a resource to improve patient care and safety. A detailed analysis of the tool, including case entries and planned management vs best practice recommendations for each irAE and grade will be presented.

Table 1

Case Queries Entered by HCPs, n (%)	HCP Planned Management Differed from Tool Recommendation, n(%)
Overall, 2896 (100)	1419 (49)
Gastrointestinal (colitis, diarrhea), 818 (28)	364 (44)
Pulmonary (pneumonitis), 608 (21)	321 (53)
Endocrine, 433 (15)	295 (68)
Hepatic, 416 (14)	162 (39)
Dermatologic (rash), 305 (11)	145 (48)
Neurologic, 163 (6)	91 (56)
Renal (nephritis) 153 (5)	51 (33)

References

1. Clinical Care Options (US). Managing Immune-Related Adverse Events: An Interactive Algorithm Tool [Internet]. Clinical Care Options Oncology (US); 2017 May. Available from: www.clinicaloptions.com/immuneAETool

Tumor Microenvironment (Mechanisms and Therapies)

O34  Presidential Travel Award Recipient

Cross-dressed dendritic cells drive anti-tumor immunity

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Background

Dendritic cells (DCs) play a central role in the orchestration of anti-tumor T cell responses. The presentation of tumor-derived peptide antigens on class I major histocompatibility complex molecules (MHC I) by DCs is essential for the activation of antigen-specific CD8⁺ T cells. Emerging evidence has revealed that migratory Batf3-lineage DCs play a dominant role in the priming of anti-tumor CD8⁺ T cell responses against solid tumors. However, the

cellular mechanisms of tumor antigen presentation by these DCs have not been formally demonstrated. Batf3-lineage DCs are adept at cross-presenting exogenous antigens on MHC I molecules, and it has been presumed that this antigen presentation pathway is critical in anti-tumor immunity.

Methods

To determine the contribution of cancer cell-derived MHC I on antigen-specific CD8⁺ T cell priming, we deleted H2-K^b in the murine acute myeloid leukemia cell line, C1498, using CRISPR/Cas9. Engraftment of K^{b/+} and K^{b/-} C1498 cells into syngeneic C57BL/6 mice, as well as those lacking TAP-1 (a critical component of the classical cross-presentation pathway), and all MHC I (K^b/D^b-/-), allowed us to interrogate the importance of different antigen presentation pathways to the anti-tumor CD8⁺ T cell response.

Results

Consistent with published data, antigen-specific CD8⁺ T cell priming against localized C1498 tumors was entirely dependent upon Batf3-lineage migratory DCs, revealing no role for direct priming by C1498 cells. Surprisingly, the activation of endogenous and adoptively-transferred tumor-specific CD8⁺ T cells was absent in tumor-draining lymph nodes of mice harboring localized C1498 K^{b/-} tumors, suggesting that antigen cross-presentation by DCs was insufficient to activate anti-tumor CD8⁺ T cells. Conversely, cancer-derived MHC I was dispensable for antigen recognition by CD8⁺ T cells in spleens of mice with disseminated leukemia. Further, tumor-derived MHC I was abundantly observed on the surface of DCs isolated from the solid tumor micro-environment, and on migratory DCs in the tumor-draining lymph node. However, no cancer-derived MHC I was found on splenic DCs in leukemia-bearing mice, in which antigen-specific CD8⁺ T cell responses occurred in a TAP-dependent fashion.

Conclusions

Together, these observations support a model in which the CD8⁺ T cell response to an MHC I-

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expressing, localized cancer is driven by Batf3-lineage DCs cross-dressed with intact tumor-derived peptide-MHC I molecules. Furthermore, our data reveal that the processing and presentation of tumor antigens occurs in a fundamentally different manner for solid and hematologic malignancies. These findings could have broad implications both for basic immunology research, and for the development of DC-oriented tumor vaccines.

Late-Breaking Abstract Oral Presentations

Cellular Therapy Approaches

O35

The transcription factor Myb enhances CD8⁺ T cell stemness and polyfunctionality to promote curative antitumor immunity

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Background

Following antigen encounter, CD8⁺ T cells differentiate into effector and memory T cells to mediate pathogen clearance and provide life-long immunity. Although our understanding of the molecular mechanisms regulating CD8⁺ T cell fate has expanded dramatically over recent years, the precise transcriptional programs underlying this process remains incompletely resolved. Myb is a transcription factor known to play a major role in stem cell and progenitor renewal and homeostasis, but its function in mature T cell differentiation is unknown. In this study, we demonstrate the role of Myb in CD8⁺ T cell differentiation and antitumor function.

Methods

We employed CD8⁺ T cells isolated from pmel-1 mice (which recognize the shared melanoma-melanocyte differentiation antigen gp100) carrying loxP-flanked Myb alleles and a fusion of Cre recombinase and the estrogen receptor T2 moiety, which retains Cre in the cytosol until tamoxifen is

administered (pmel-1 Myb^{fl/fl}/Cre-ERT2 cells). Treating these mice with tamoxifen for several days immediately prior to CD8⁺ T cell isolation ensured that pmel-1 Myb^{-/-} T cells had undergone thymic development similar to their ERT2-Cre negative counterparts. pmel-1 Myb^{-/-} or pmel-1 Myb^{+/+} cells were adoptively transferred into wild-type mice infected with a recombinant strain of vaccinia virus encoding gp100 and antigen-specific CD8⁺ T cell expansion and long-term persistence was monitored overtime. Evaluation of tumor treatment efficacy of CD8⁺ T cells was performed in the pmel-1 model of adoptive cell therapy in the treatment of large established B16 melanomas.

Results

We demonstrate that Myb expression is progressively downregulated with T cell differentiation. We found that Myb deficient T cells were more prone to differentiate into short-lived KLRG1^{hi} effector cells resulting in a severe impairment of CD62L^{hi} stem cell-like memory cell formation, indicating that Myb is an essential regulator of T cell stemness. Conversely, enforced expression of Myb enhanced generation of CD62L^{hi} memory cells, T cell polyfunctionality and recall responses, suggesting that these cells might be therapeutically superior for adoptive T cell therapy of tumors. Accordingly, Myb overexpressing T cells mediate enhanced antitumor immunity and promoted curative and long-lasting responses against large established vascularized tumors.

Conclusions

These findings identify Myb as a master regulator of CD8⁺ T cell stemness and highlight the remarkable therapeutic potential of maneuvers aimed at increasing Myb activity in CD8⁺ T cells.

Clinical Trials (Completed)

O36

Late-Breaking Abstract Oral Presentations

First in human study with the CD40 agonistic monoclonal antibody APX005M in subjects with solid tumors

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Background

Immune activating antibodies are being explored as the next generation of immuno-oncology therapeutics. Activation of CD40 can stimulate both innate and adaptive immune responses against cancer, making it an ideal target for the immune activating approach. CD40 engagement with its ligand CD154 leads to antigen presentation, maturation and expression of co-stimulatory molecules and cytokine production by antigen presenting cells (APC), which are requisite for optimal antigen-specific T-cell activation. Apexigen is developing APX005M – a humanized IgG₁ CD40 agonistic antibody that binds with high affinity to human CD40 (K_d=0.12nM) and carries an S267E mutation in the Fc region. APX005M recognizes a unique epitope that overlaps with the CD40 ligand binding sites and uses FcγRIIb to cluster and activate CD40 thus mimicking CD154 engagement.

Methods

In a “first in human” Phase 1 dose escalating clinical trial, APX005M was administered every 21 days at doses ranging from 0.0001 mg/kg to 1 mg/kg to 30 adult subjects with solid tumors. Primary objectives were to evaluate the safety of APX005M, and to determine the maximum tolerated dose (MTD) and the recommended phase 2 dose (RP2D).

Results

APX005M demonstrated a dose-dependent APC activation (increases in expression of CD54, CD70, CD80, CD86, HLA-DR), dose dependent T cell activation and increases in circulating levels of IL-12, INF-γ, TNFα and IL-6. Five subjects had prolonged stable disease. Overall APX005M has been well tolerated; the majority of AEs were mild to moderate in severity, and the majority of serious AEs were considered unrelated to APX005M. The dose limiting toxicity of grade ≥ 3 cytokine release syndrome was observed in subjects receiving doses ≥ 0.6 mg/kg. The maximum administered dose of APX005M was 1 mg/kg. The dose of 0.3 mg/kg of APX005M was selected as the RP2D and represents the dose with maximum pharmacodynamic effects without grade > 2 toxicities. Increases in the dose of APX005M led to approximately dose-proportional increases in maximum serum concentration (C_{max}) and area under the curve (AUC). No accumulation of APX005M was observed with every 21 day dosing.

Conclusions

APX005M produces dose-dependent activation of APCs and T cells at doses that are well tolerated. Toxicities generally associated with the on-target cytokine release are observed at doses above the doses that are required to activate APCs and T-cells. APX005M exhibits a highly differentiated and ideal profile for further clinical development as a single agent or in combination with other treatment modalities including immunomodulatory agents.

Consent

N/A

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Clinical Trials (In Progress)

O37

Nivolumab in mismatch-repair deficient (MMR-d) cancers: NCI-MATCH Trial (Molecular Analysis for Therapy Choice) arm Z1D preliminary results

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Background

The NCI-MATCH (Molecular Analysis for Therapy Choice) trial is the largest national study to date (1173 sites) for patients with relapsed/refractory solid tumors, lymphomas and myelomas, assigning rational targeted therapy based on individual tumor molecular alterations. Patients with mismatch repair-deficiency (MMR-d) may benefit from immune checkpoint inhibitor therapy secondary to increased mutational burden compared to MMR-proficient tumors. The anti-PD-1 inhibitor nivolumab has previously shown antitumor activity

in MMR-d colorectal cancer; we hypothesized that nivolumab would have activity in patients with non-colorectal MMR-d cancers.

Methods

Eligibility for NCI-MATCH included relapsed/refractory cancers, good end-organ function, and ECOG performance status of ≤ 1 . Patients enrolled were screened for molecular alterations by centralized testing on fresh biopsy tissue. MMR-d was defined by loss of nuclear expression of MLH1 or MSH2 by immunohistochemistry. Patients with MMR-d colorectal cancer were excluded. Patients received nivolumab 3 mg/kg q2weeks (28-day cycles) and 480 mg q4weeks past cycle 4. Disease reassessment was performed q2cycles. The primary endpoint of the study was RECIST 1.1 overall response rate (ORR). 35 enrolled patients were planned with the ORR compared against a null value of 5%. If the observed ORR was $\geq 5/31$ (16%), the agent would be considered promising and worthy of further testing. The proposed design had power of 91.8% to find an agent promising assuming true OR rate was 0.25.

Results

4864 enrolled patients had interpretable results for MMR-d. 99 patients were MMR-d, 63 patients were assigned to nivolumab treatment, and 47 patients were treated (35:preplanned and 12:expansion). We report the preliminary results of the first 35 enrolled (70% MLH1 loss, 30% MSH2 loss). Minimum follow-up time for all patients was >6 months, median age was 60 y/o, and median prior therapies was 3. Common histologies included endometrioid endometrial (EEA: 10), prostate (6), and breast (3) cancer. 10 pts remain on treatment; 7 stopped treatment for AEs; 12 for progressive disease. The confirmed ORR was 24% (8/33 patients) with an additional 9/33 (27%) patients with stable disease. Three additional patients had unconfirmed responses [PD at next scan(1), off study prior to reassessment(1), and no follow-up scan yet(1)]. The disease histologies for the PR were prostate(3), EEA(2), breast(1), parathyroid(1), and gallbladder cancer(1). Estimated 6-month PFS was

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43% and median OS has not been reached at this early time-point. Toxicity was predominantly low-grade.

Conclusions

We report the first results of a substudy of the NCI-MATCH trial. Nivolumab has promising activity in MMR-d, non-colorectal cancers.

Trial Registration

NCT02465060

Consent

N/A

O38

Nivolumab + Ipilimumab (N+I) vs Sunitinib (S) for treatment-naïve advanced or metastatic renal cell carcinoma (aRCC): results from CheckMate 214, including overall survival by subgroups

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Background

We report results from the phase III CheckMate 214 study of N+I versus S for treatment-naïve aRCC.

Methods

Adults with clear-cell aRCC, measurable disease, Karnofsky performance status ≥ 70 , and available tumor tissue were eligible. Patients were randomized 1:1 (stratified by IMDC score; region) to N 3 mg/kg + I 1mg/kg every 3 weeks for four doses followed by N 3 mg/kg every 2 weeks, or S 50 mg daily orally for 4 weeks (6-week cycles). Co-primary endpoints were objective response rate (ORR), progression-free survival (PFS) per independent committee (IRRC), and overall survival (OS), all in intermediate- and poor-risk patients. Overall α for treatment effect was 0.05 (allocated as 0.001 ORR, 0.009 PFS, 0.04 OS).

Results

1,096 patients were randomized (N+I: n=550; S: n=546); 425 (N+I) and 422 (S) with

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intermediate/poor risk. With ~17.5 months minimum follow-up, confirmed ORR in intermediate/poor-risk patients was 41.6% (9.4% complete response [CR]) vs 26.5% (1.2% CR) for N+I vs S ($P < 0.0001$); median duration of response was not reached (NR; 95% CI, 21.82-NR) vs 18.2 months (95% CI, 14.82-NR), respectively (Table 1). Median PFS with N+I vs S in intermediate/poor-risk patients was 11.6 vs 8.4 months (hazard ratio [HR] 0.82, $P = 0.0331$, Table 1). At the first prespecified interim OS analysis, the Data Monitoring Committee recommended stopping the study early for statistically significant superiority in OS with N+I vs S (median not reached vs 26.0 months [HR 0.63], $P < 0.0001$, Table 1). ORR favored N+I over S in intermediate/poor-risk patients irrespective of baseline tumor PD-L1 expression, while a PFS benefit with N+I vs S was seen only in patients with PD-L1 $\geq 1\%$. OS favored N+I over S across all prespecified subgroups (data to be presented), including baseline PD-L1 expression status. In all treated patients, drug-related AEs occurred in 509/547 (93% any grade, 46% grade 3-4) with N+I vs 521/535 (97% any grade, 63% grade 3-5) with S, including 22% vs 12% with AEs leading to discontinuation. Death occurred in 159 N+I arm patients (7 [1%] drug-related) and 202 S arm patients (4 [1%] drug-related).

Conclusions

This phase III study showed statistically significant OS benefit, significantly higher ORR, and numerically longer PFS for N+I vs S with a manageable safety profile in intermediate- and poor-risk patients with aRCC, supporting the use of N+I as a new first-line standard-of-care treatment option for these patients. OS benefit with N+I was seen irrespective of baseline PD-L1 status and was observed consistently across other subgroups.

Trial Registration

ClinicalTrials.gov Identifier: NCT02231749

Table 1.

	Intermediate/poor risk					
	N+I	S	N+I	S	N+I	S
	N=426	N=422	N=308	N=298	N=198	N=194
ORR per REC _{1.1} (%) [95% CI]	37 (9%) [31-47]	11 (2%) [0-11]	30 (10%) [7-13]	7 (2%) [1-3]	18 (9%) [6-12]	2 (1%) [0-3]
	$P < 0.0001$		ORR ratio (95% CI) = 1.03 (1.04-1.07)		ORR ratio (95% CI) = 1.02 (1.01-1.03)	
Median PFS per REC _{1.1} months (95% CI)	11.6 (8.7-15.5)	8.4 (7.0-9.8)	11.0 (8.1-13.9)	10.4 (7.5-13.1)	27.0 (14.4-NR)	18.2 (11.1-25.2)
HR (95% CI)	0.82 (0.63-1.05)		1.07 (0.85-1.35)		0.40 (0.27-0.61)	
	$P = 0.0331$		$P = 0.8072$		$P = 0.0001$	
Median OS, months (95% CI)	NR (20.3-NR)	26.0 (22.1-NR)	NR (NE)	NR (NE)	NR (NE)	14.5 (11.5-NE)
HR (95% CI)	0.63 (0.38-1.03)		0.73 (0.50-1.05)		0.45 (0.31-0.63)	
	$P < 0.0001$		$P = 0.0428$		$P = 0.0001$	

ORR, objective response rate; REC_{1.1}, Response Evaluation Criteria in Solid Tumors version 1.1; HR, hazard ratio; CI, confidence interval; NR, not reached; NE, not estimable.

O39

Phase I study of E7046, a novel PGE2 receptor type 4 inhibitor, in patients with advanced solid tumors with high myeloid infiltrate: effects on myeloid- and T-lymphoid cell-mediated immunosuppression

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Background

E7046 is a selective inhibitor of the prostaglandin E₂ (PGE₂) receptor-type-4, EP4, which transduces potent immunosuppressive activity of PGE₂ in both myeloid cells and T-lymphoid cells in the tumor microenvironment. In preclinical studies, E7046 reversed PGE₂-mediated inhibition of monocyte differentiation towards anti-tumorigenic antigen presenting cells and facilitated tumoral recruitment and activation of cytotoxic T-cells. Here, we present initial clinical, pharmacokinetic and pharmacodynamic results from a first-in-human study of single agent E7046 in patients with selected cancer types having high myeloid cell infiltration.

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Methods

E7046 was administered orally, once-daily, in 21-day cycles in sequential dose-escalating cohorts of 6 pts each at 125, 250, 500 and 750mg. Tumor responses were evaluated by irRECIST and metabolic responses by ¹⁸FDG-PET. Modulation of immune response was assessed in pre- and post-treatment tumor biopsies by immunohistochemistry, and in blood samples by TaqMan Low Density Array and Meso Scale Discovery assays. Blood samples were collected for PK analysis.

Results

Thirty patients were treated with no dose-limiting toxicities observed. The most common adverse events were fatigue (37%), diarrhea (33%), and nausea (30%). Grade 3/4 AEs in >1 patient were abdominal pain (3 patients, at 250 mg, 750 mg) and vomiting (2 patients, at 125 mg, 250 mg). Grade 3/4 treatment-related AEs occurred in 4 patients (rash in 2 patients, and diarrhea, allergic reaction, anaphylaxis, hypersensitivity, and hyperuricemia, in 1 patient each). Four patients discontinued treatment due to an AE (bowel obstruction, allergic reaction, abdominal pain, acute renal failure). No objective tumor responses were reported. Duration of treatment of ≥ 20 wks with best response of stable disease (SD) was observed in 5 patients, 3 of these had partial metabolic responses. E7046 exposure was dose-proportional up to the 500 mg dose with a plateau at 750 mg. Elimination half-life (11 hr) justified once-daily dosing. Treatment with E7046 significantly increased tumor CD3⁺ and CD8⁺ T-cell infiltration and expression of the T-effector cell-recruiting chemokine CXCL10 in blood. Gene expression analysis in blood showed modulation of EP4 signaling genes (including IDO1, EOMES, PD-L1). Longer duration of therapy with SD was associated with higher baseline tumor infiltrate of CD8⁺ T-cells and CD163⁺ macrophages.

Conclusions

E7046 demonstrated favorable tolerability profile with preliminary evidence of anti-tumor activity and immune modulation in tumor and peripheral blood. MTD was not reached. Further studies testing E7046 in combination with other agents are planned.

Trial Registration

NCT-02540291

O40

Interim safety analysis of Cancer Immunotherapy Trials Network – 12 (CITN-12): A phase 1 study of Pembrolizumab in patients with HIV and relapsed, refractory or disseminated malignancies

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Background

Anti-PD-1 and anti-PD-L1 antibodies are approved for multiple indications and are becoming mainstays of cancer therapy. However, patients with HIV have been excluded from clinical trials evaluating these agents largely due to safety concerns.

Methods

CITN-12 is a multicenter study of pembrolizumab in patients with HIV and advanced cancers not curable by standard therapies. Three parallel cohorts are accruing based on CD4⁺ counts; 1: 100-199, 2: 200-350, and 3: >350 cells/uL. Additional eligibility criteria: >4 weeks antiretroviral therapy (ART), HIV viral load <200 copies/mL, no uncontrolled infections including hepatitis B and C, ECOG performance status 0-1. Treatment: pembrolizumab 200mg intravenously every 3 weeks for up to 2 years. The primary objective is to assess safety and tolerability by summarizing adverse events (AEs)

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graded by CTCAEv4 and evaluating HIV viral load and CD4+ counts. Immune mediated adverse events are managed using standard guidelines.

We performed an interim analysis of treatment emergent adverse events at least possibly related to pembrolizumab (rTEAEs), serious AEs, and HIV viral load and CD4+ counts on therapy.

Results

17 patients; Cohort 1 (4), Cohort 2 (9), Cohort 3 (4); were accrued starting April 2016 and followed through May 2017. Characteristics: 1 woman, 16 men; median age 56 years (range 43-77); white (13), African American (3), Hispanic (1); HIV viral load <20 copies/mL (94%). Cancers: non-Hodgkin lymphoma (3), Kaposi sarcoma (1), anal cancer (5), head and neck (1), lung (2), bladder (1), hepatocellular (1), pancreatic (1), cholangiocarcinoma (1). Median number prior therapies 1 (range 0-4), prior radiation (71%). Safety observed over a total of 100 cycles, median 4 (range 1-20). 82 rTEAEs were observed and comparable between cohorts. 93% were grade 1-2. Ten primary serious AEs were observed, 2 possibly attributable to pembrolizumab, both occurring in the setting of progressive malignancy. Immune mediated AEs managed with levothyroxine or prednisone included subclinical hypothyroidism 6 (35%), pneumonitis (2) and liver test elevations (2). Median CD4+ counts increased over time, changes did not reach statistical significance. HIV remained suppressed on ART in all patients.

Conclusions

Pembrolizumab has an acceptable safety profile to date in CITN-12. Standard therapy with anti-PD1 is appropriate for FDA-approved indications in patients with HIV. Patients with HIV who meet appropriate immune eligibility criteria for a given cancer should be included in immunotherapy studies. Further evaluation of checkpoint inhibitors in HIV-associated tumors is justified.

Trial Registration

clinicaltrials.gov NCT02595866

Combination Therapy

IO/IO/IO/Standard of Care, IO/Other

O41

Preliminary antitumor and immunomodulatory activity of BMS-986205, an optimized indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, in combination with nivolumab in patients with advanced cancers

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Background

Checkpoint inhibitors have transformed cancer care, but extending those benefits to more patients requires additional approaches. IDO1 allows tumor escape through kynurenine production, which decreases immune cell tumor infiltration/function and increases regulatory T-cell numbers. Anti-PD-1 treatment upregulates IDO1, supporting a rationale for combining nivolumab (anti-PD-1) with an IDO1

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inhibitor. BMS-986205 is a selective, potent, once-daily, oral IDO1 inhibitor with a potentially best-in-class pharmacokinetic/pharmacodynamic/safety profile in combination with nivolumab that was previously disclosed (NCT02658890) [1]. Here we present updated safety and preliminary efficacy and pharmacodynamic data.

Methods

Dose-escalation methods were previously described [1]. During cohort expansion in this phase 1/2a open-label study, patients with advanced cancers were treated with BMS-986205 100 or 200 mg orally once daily + nivolumab 240 mg IV Q2W or 480 mg IV Q4W. Objectives included safety, preliminary antitumor activity, and pharmacodynamics (including immunomodulatory assays).

Results

As of the July 20, 2017, data cutoff, safety data were available for 216 patients across the study. Maximum tolerated dose during escalation was 200 mg; at 400 mg, 2/4 patients experienced dose-limiting toxicities (grade 3 AST/ALT increased; grade 2 anemia, fatigue). Treatment-related AEs occurred in 47% of patients (11% grade 3/4), and 4 patients (2%) discontinued due to study drug toxicity; the safety profile was generally consistent with that previously reported for nivolumab monotherapy. In the bladder cancer cohort, among 15 heavily pretreated patients (39% received ≥ 2 prior regimens), 5 partial responses (PRs), 3 stable disease (SD), and 6 progressive disease (PD, including a patient with prior anti-PD-[L]1 therapy) were reported, with 1 death prior to assessment. In the cervical cancer cohort, among 17 heavily pretreated patients (52% received ≥ 2 prior regimens), 3 PRs, 5 SD, and 7 PD were reported, with 2 deaths prior to assessment. Within 39 paired pre- vs on-treatment tumor samples across various tumor types, BMS-986205 plus nivolumab decreased kynurenine and increased the percentage of proliferating CD8⁺ T cells.

Conclusions

BMS-986205 plus nivolumab was well tolerated, increased proliferating CD8⁺ T cells in tumors, and demonstrated preliminary antitumor activity. Updated efficacy, safety, and pharmacodynamic data will be presented.

Trial Registration

ClinicalTrials.gov, NCT02658890

Consent

Not applicable

References

1. Siu L, et al. AACR 2017, abstract CT116.

O42

First-in-human phase 1 dose escalation and expansion of a novel combination, anti-CSF-1 receptor (cabiralizumab) plus anti-PD-1 (nivolumab), in patients with advanced solid tumors

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Background

Resistance to immunotherapy may be related to activity of several immunosuppressive cell types. Depletion of tumor-associated macrophages (TAMs) may promote a pro-inflammatory state, increasing antitumor T-cell responses. Cabiralizumab, a humanized IgG4 monoclonal antibody, binds to CSF-1 receptor and blocks cytokine signaling that is needed for TAM activation and survival, leading to TAM depletion. The combination of cabiralizumab plus anti-PD-1 may work synergistically by modifying the immunosuppressive tumor environment while simultaneously suppressing the PD-1 checkpoint pathway. This is the first clinical disclosure of safety, pharmacokinetics, and pharmacodynamics of this novel combination, along with preliminary evidence of antitumor activity in pancreatic cancer (NCT02526017).

Methods

In phase 1a dose escalation, patients with advanced solid tumors were treated with cabiralizumab 1, 2, 4, and 6 mg/kg alone or combined with nivolumab 3 mg/kg, both given IV Q2W, in a 3+3+3 design.

Results

As of August 1, 2017, 205 patients were treated with the combination. Most received cabiralizumab 4 mg/kg Q2W plus nivolumab 3 mg/kg Q2W. Cabiralizumab, alone or with nivolumab, demonstrated target-mediated clearance and dose-dependent increase in exposure, and pharmacodynamic activity as evidenced by reduced circulating CD14⁺ CD16⁺ nonclassical monocytes. Grade 3–5 treatment-related AEs (TRAEs) attributed to cabiralizumab occurred in 43% of patients, with 13% of patients discontinuing due to AEs. Elevations in creatinine phosphokinase (14%) and AST (5%) were among the most common grade 3 TRAEs but were

secondary to cabiralizumab's depletion of macrophages, which would otherwise metabolize these enzymes, and were reversible without significant clinical sequelae. Among the cohort of prior chemotherapy-treated and immunotherapy-naïve patients with pancreatic cancer, 31 were efficacy evaluable. There were 3 confirmed partial responses in microsatellite-stable patients (293, 275+, and 168+ days on study) and 1 prolonged stable disease (182 days); 1 patient treated beyond progressive disease experienced >40% reduction in baseline target lesions (247 days on study). The 6-month disease control rate was 13%, and objective response rate was 10%. Studies in a larger pancreatic cohort and other tumor types are ongoing, and preliminary translational biomarker data will be presented.

Conclusions

Cabiralizumab plus nivolumab, a mechanistically novel immunotherapy combination, demonstrated a tolerable safety profile across several cohorts and promising preliminary antitumor activity in pancreatic cancer. These results also show a potential immunotherapeutic strategy to treat patients with tumors resistant to anti-PD-1 blockade.

Trial Registration

NCT02526017

Tumor Microenvironment (Mechanisms and Therapies)

O43

Monotherapy dose escalation clinical and translational data from first-in-human study in advanced solid tumors of IPI-549, an oral, selective, PI3K-gamma inhibitor targeting tumor macrophages

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Background

IPI-549 is a potential first-in-class, oral, selective PI3K-gamma inhibitor being developed as an immunology therapeutic in multiple cancer indications. Preclinical research demonstrated that IPI-549 results in transcriptional reprogramming M2, pro-tumor macrophages to the M1, anti-tumor phenotype. In preclinical tumor models, IPI-549 was active as a monotherapy and was able to overcome checkpoint inhibitor (CPI) resistance in CPI-insensitive models. These preclinical data provide a strong rationale for the ongoing Phase 1/1b study.

Methods

This study (NCT02637531) is being conducted to evaluate the safety, tolerability, pharmacodynamics and pharmacokinetics to determine the recommended dose and activity of IPI-549 as monotherapy and in combination with nivolumab in patients with advanced solid tumors. The study design includes four parts: 1) monotherapy dose escalation 2) combination dose escalation of IPI-549 with nivolumab 3) monotherapy expansion, and 4) combination expansion in specific tumor types with de novo or acquired resistance to checkpoint inhibitors. Pre- and on-treatment blood samples are being obtained in all patients to perform flow cytometry, gene expression, and serum cytokine and chemokine analysis to better

understand the biological effect of IPI-549 on immune cells and to identify correlations with any clinical response. Pre- and on-treatment biopsies are being mandated in the expansion cohorts to evaluate the effect of IPI-549 on the tumor microenvironment.

Results

A total of 19 patients have been enrolled (18 evaluable) in the monotherapy dose escalation phase (10, 15, 20, 30, 40, 60 mg qd). No DLTs, or drug related SAEs have been observed. The majority of treatment-emergent adverse events were low grade (grade 1-2). The most common (≥ 2 patients) drug related treatment-emergent adverse events are alanine aminotransferase increase, rash maculo-papular, white blood cell count decrease, and headache. Durable clinical benefit has been observed, with 8 patients able to remain on treatment ≥ 16 weeks, including 2 patients on study for ≥ 52 weeks. The PK profile of IPI-549 has favorable characteristics including dose proportionality. PD analysis demonstrates full and sustained suppression of PI3K- γ at 60 mg qd. Translational studies performed on peripheral blood demonstrated increased activation of circulating myeloid cells in patient subsets, as well as, evidence of interferon-gamma mediated immune stimulation after IPI-549 treatment. Detailed PK, PD, translational, safety, and efficacy data will be presented.

Conclusions

The monotherapy dose escalation has completed enrollment, demonstrating favorable tolerability, evidence of immune modulation, and PK/PD defining 60 mg qd as the monotherapy expansion dose. The monotherapy expansion phase in solid tumors is actively enrolling.

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Biomarkers and Immune Monitoring

P1

Characterization of the tumor microenvironment and immune profile in non-small cell lung cancer

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Background

Immunotherapies with programmed death receptor-1/ programmed death-ligand 1 (PD-1/PD-L1)-blocking antibodies have shown encouraging results in patients with advanced NSCLC, HNSCC, RCC, Melanoma, Hodgkin Lymphoma and other cancer types. Despite a subset of patients exhibiting durable and long lasting benefit, the majority of patients show no signs of efficacy. The identification of the right patient population is often challenging, and although there is an incremental understanding of tumor characteristics and biomarkers bearing limited predictive value, such as PD-L1, there is a clear need for an accelerated development of better predictive biomarkers. This knowledge may permit the guidance of therapy-relevant decisions and potentially increase the clinical benefit of CPI-related anti-cancer interventions.

Methods

To broaden our understanding of the spatial expression of relevant biomarkers in the tumor microenvironment and gain deeper insights into immune-stimulatory mechanisms, we performed histopathological analysis, image analysis, and gene expression analysis in a complementary approach. In thirty (n=30) NSCLC tissues the expression patterns of pre-selected biomarkers (CD8 and PD-L1) were conventionally evaluated by a board-

certified pathologist and the obtained H-scores were compared with Image Analysis (IA)-derived i-scores. Subsequently, gene expression data for all 30 NSCLC samples were generated in order to complement the histopathological evaluations with mRNA expression patterns.

Results

Computer-based image analysis algorithms for the quantification of CD8 and PD-L1 have been established showing a very high accuracy and concordance compared to conventional histopathological evaluations performed by trained pathologists. The complementary analysis based on image analysis, conventional histopathological evaluation, and gene expression analysis revealed excellent/good agreement. Based on the attained knowledge from the multi-modal approach the image analysis tools were further optimized. Following optimization, preliminary data has demonstrated that the optimized image analysis algorithms and the application of virtual multiplexing approaches permits characterization of samples into four types of tumor microenvironment *sensu* Teng et al [1]. Subsequently, a further seventy (n=70) NSCLC samples will be interrogated using the optimized image analysis tools and correlated, where applicable, with gene expression analysis.

Conclusions

The insights gained during this complementary analysis will help us to design and implement advanced biomarker investigations in future clinical trials to support the co-development of valuable biomarkers in the field of cancer immunotherapy. The application of advanced image analysis and virtual multiplexing approaches may be suitable vehicles to better characterize the tumor microenvironment, honing precision medicine within the immune-oncology field.

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References

1. Teng, et al. Classifying cancers based on T cell infiltration and PD-L1. *Cancer Res.* 2015; 75: 2139-45.

P2

ADXS-PSA immunotherapy increases the magnitude and quality of prostate cancer antigen-specific T cell responses in patients with metastatic castration-resistant prostate cancer

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Background

Active immunotherapies, such as ADXS-PSA, are designed to generate tumor antigen-specific T cell effectors that recognize and kill tumor cells. ADXS-PSA, a highly attenuated *Listeria monocytogenes*-based immunotherapy that targets prostate-specific antigen (PSA), is currently being evaluated as a treatment for metastatic castration-resistant prostate cancer (mCRPC) in the phase 1/2 KEYNOTE-046 trial as a monotherapy (Part A) and in combination with KEYTRUDA[®] (Part B). Because tumor antigen-specific T cell responses may be linked to the clinical efficacy of active immunotherapies, we quantified the frequency of functional prostate cancer antigen-specific T cells in

the peripheral blood of ADXS-PSA-treated mCRPC patients by ELISpot analysis.

Methods

ELISpot assays were performed on peripheral blood mononuclear cells (PBMCs) isolated at multiple time points from 9 mCRPC patients who received 3 doses of ADXS-PSA monotherapy in Part A, the ADXS-PSA dose-determining stage, of the KEYNOTE-046 trial (NCT02325557). In addition to peptides from PSA, CD4⁺ and CD8⁺ T cell reactivity was assayed to peptides from prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), prostate stem cell antigen (PSCA), and prostein to determine the extent of antigen cascade/spreading after ADXS-PSA treatment. Secretion of IFN γ , TNF α , and the cytolytic granule granzyme B were assayed simultaneously to examine the quality (i.e. degree of multi-functionality) of the T cell responses.

Results

During a 9-week treatment course, which included 3 ADXS-PSA doses, 7/9 patients exhibited increases in the magnitude of the PSA-reactive T cell response, including a patient with undetectable PSA-reactive T cells at baseline. Notably, during the same time course, increases above baseline in the frequency of T cells reactive to one or more of the other prostate cancer antigens was observed in all 9 patients. In addition to increases in the frequency of prostate cancer antigen-specific T cells, all 9 patients exhibited increases in the quality of T cells reactive to at least 1 of the 5 prostate cancer antigens, indicating that ADXS-PSA induces multifunctional T cell responses to a broad range of prostate cancer antigens.

Conclusions

These results demonstrate that ADXS-PSA monotherapy increases the magnitude and quality of T cell responses specific not only to PSA, its target antigen, but also to other prostate cancer antigens, which is indicative of antigen cascade/spreading. Patient enrollment is ongoing for Part B, in combination with KEYTRUDA[®].

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Trial Registration

ClinicalTrials.gov Identifier NCT02325557

P3

Persistence of 6-thioguanine-resistant T cell clones in a melanoma patient with durable antitumor response following treatment with immune checkpoint blockade

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Background

Ex vivo selection of T cells resistant to 6-thioguanine can be used to identify T cells with *in vivo* mutation in the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) gene. We hypothesize that *in vivo* *HPRT*-mutant T cells (MT) will enrich for *in vivo* proliferating T cells and provide a predictive biomarker for melanoma patients treated with immune checkpoint blockade.

Methods

This study examined clonotypic diversity and T cell persistence in peripheral blood mononuclear cells (PBMC) freshly cryopreserved (T0) and in PBMC expanded as mass cultures with (MT) or without (wild-type (WT)) 6-thioguanine selection in a metastatic melanoma patient who achieved a durable antitumor response following treatment with intratumoral α -gal glycolipid injections (IT-AG) followed by Ipilimumab (Ipi). IT-AG (0.1 mg/injection every 4 weeks for 2 doses) was initially administered, and Ipi (3 mg/kg (capped at 125 kg)/dose every 3 weeks for 4 doses) was then administered 4 weeks later at a time of disease progression. Blood samples were obtained before and 4-weeks after IT-AG as well as 1-, 4-, and 13-

months post-Ipi. TCR beta chain (TRB) repertoire of T0, MT and WT were examined via 5' RACE and Illumina MiSeq sequencing. TRB repertoire of T cells from a tumor sample obtained ~20 months prior to IT-AG (T-T0) was also evaluated. TRB were collapsed to identical nucleotide sequences to enumerate T cell clones.

Results

Expanded or activated T cell clones, defined by sequences with identical TRB, were detected in T-T0 as well as in T0, WT, and MT at each of the 5 time points. The range of TRB sequences was as follows: T0: 47,602-58,588 (median 54,284); WT: 49,799-58,896 (median 52,277); MT: 35,527-58,217 (median 49,035), and 51,713 in T-T0. The range of distinct TRB amino acid sequences was as follows: T0: 4,682-9,644 (median 5,993); WT: 2,083-4,573 (median 2,702); MT: 84-182 (median 154), and 1850 in T-T0. Clonotypic diversity, measured as the number of unique TRB nucleotide sequences, was substantially reduced in MT compared to either WT or T0 at each time point. Numerous TRB matches were observed between MT and WT and T0, as well as between T-T0 and MT, WT and T0. The patient achieved a durable antitumor response, and several TRB persisted in this patient over a four-year span. Some TRB from this study matched TRB from the literature with defined melanoma specificity.

Conclusions

MT from melanoma patients are enriched for proliferating and/or activated T cells and merit additional study as a biomarker of response to immunotherapy.

P4

The prognostic and predictive roles of a CD8/PD-L1 signature in cancer

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Background

Predictive signatures to a-PD1 and a-PD-L1 immunotherapies are active research areas. We previously showed that non-small cell lung cancer (NSCLC) patients with high densities of CD8(+) and PD-L1(+) cells have improved outcome compared to those with high CD8(+) or PD-L1(+) cell densities alone [1]. The applicability of this signature to other indications such as urothelial carcinoma (UC) is uncertain. The potential prognostic contribution of this signature is also unknown.

Methods

The predictive potential of the CD8/PD-L1 signature in UC, was tested in baseline tumor samples obtained from 43 durvalumab-treated patients in a phase 1/2 clinical trial (NCT01693562).

To assess the prognostic significance in NSCLC, we analyzed surgically-resected tumors of 134 NSCLC patients that later received chemotherapy (non-IO). Tumors were immunostained for CD8 (SP239) and PD-L1 (SP263) using single stains (UC) or a dual immunohistochemistry (IHC) assay (NSCLC). Densities of CD8(+) and PD-L1(+) cells from IHC stained digitized slides were calculated by the Definiens Tissue Phenomics Platform for a total of 183 patient samples.

Results

UC patients with CD8/PD-L1(+) profiles showed improved survival compared to those with CD8/PD-L1(-) profiles ($p < 0.06$), which may help to identify patients with beneficial outcome under α -PD-L1 treatment.

Further, non-IO treated NSCLC patients with CD8/PD-L1(+) profiles did not show improved OS over those with CD8/PD-L1(-) profiles, suggesting that the CD8/PD-L1 signature is predictive of therapeutic response to α -PD-L1 in NSCLC patients (Table 1).

Conclusions

Our results demonstrate the value of profiling the tumor and its immune-contexture by image analysis for discovery of prognostic and predictive signatures in NSCLC and UC.

Table 1. Clinical Outcomes

Patient subset	Cohort	Treatment	n	Median OS months (95% CI)	pOS
PD-L1/CD8(+) Prevalence = 50%, cutoff = 4.82*10 ⁶ cells/mm ²	UC	IO	22	20.1(8.3-31)	0.06
PD-L1/CD8(-)	UC	IO	21	2.0(1.8-3.1)	
PD-L1/CD8(+) Prevalence = 56%, cutoff = 4.85*10 ⁶ cells/mm ²	NSCLC	non-IO	46	51(50-100)	0.48
PD-L1/CD8(-)	NSCLC	non-IO	88	52(50-67)	

Trial Registration

ClinicalTrials.gov Identifier NCT01693562

References

1. Althammer S, et al. Combinatorial CD8+ and PD-L1+ cell densities correlate with response and improved survival in non-small cell lung cancer (NSCLC) patients treated with durvalumab. *Immunother Cancer*. 2016; 4(Suppl 2):91

P5

Analytical comparison of methods used to assess mismatch repair deficiency

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Background

Deficiency in mismatch repair occurs in approximately 15-20% of sporadic colorectal cancer, and to a lesser degree in a variety of other solid tumors. Mismatch repair deficiency (dMMR) has been associated with disease prognosis and recently has also been linked with potential response to the

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checkpoint inhibitors pembrolizumab and nivolumab. Defects in the mismatch repair process can be identified by a number of laboratory procedures including immunohistochemical (IHC) methods to assess expression of the mismatch repair proteins (MLH1, MSH2, MSH6 and PMS 2), or by using PCR methods to assess replication fidelity of specific microsatellite sequences in the genome. Tumor samples are indicated as dMMR when one or more the MMR proteins are not expressed, or if the tumor tissue has a high level of microsatellite instability (MSI-H).

Methods

In this study we compared the performance of the IHC and PCR methods, to identify colon cancer tumors that demonstrate dMMR. A series of archival formalin-fixed paraffin-embedded samples were used in this comparison study. The IHC staining was performed on the Ventana Benchmark XT system with standard detection chemistry. The specific monoclonal antibody clones were for MLH1 (G168-228, Cell Marque), MSH2 (G219-1129, Cell Marque), MSH6 (44, Ventana) and PMS2 (EPR3947, Cell Marque). For the MSI PCR assay the following microsatellites were evaluated BAT-25, BAT-26, TGF β R2, D5S346, D17S250 and D2S123. The panel contains both dinucleotide and mononucleotide repeat sequences, and includes the loci recommended by the NCI. PCR fragments were detected by capillary electrophoresis and fragment analysis was used to determine MSI status. If > 2 loci exhibited evidence of replication defects the sample was considered MSI-H.

Results

In this cohort approximately 40% of the samples showed absence of tumor staining in either the MLH1/PMS2 or MSH2/MSH6 pathways. All cases demonstrating the MSI-H phenotype also demonstrated lack of expression of one or more MMR proteins. The most common pattern of expression was the loss of MLH1 and/or PMS2, which has been reported as being the most common phenotype in sporadic tumors. For the

cases with MMR protein expression, all were considered MSI-stable by PCR.

Conclusions

This comparison study shows an equivalent performance of the two commonly used methods for the detection of mismatch repair deficiency. These data are relevant for selecting the appropriate methodology to assess dMMR when considering the use of checkpoint inhibitors for the treatment of solid tumors.

P6

Characterization of AB928, a dual adenosine A_{2a}R/A_{2b}R antagonist that retains potency under conditions of high albumin and high receptor activation

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Background

In many tumors, extracellular adenosine contributes to an immunosuppressed micro-environment (TME) via activation of the A_{2a} receptor, expressed on lymphocytes, and the A_{2b} receptor, expressed on myeloid cells. Relative to other tissues like the brain, adenosine concentrations in the TME are much higher. Tumors also contain higher levels (~3%) of albumin, to which many drugs bind non-specifically. These factors adversely affect anti-tumor efficacy of drugs previously designed as CNS drugs.

AB928 is a novel, selective, and highly potent small-molecule dual antagonist of A_{2a}R and A_{2b}R designed to minimize potency loss associated with non-specific albumin binding. Consequently, AB928 blocks the activation of the A_{2a}R and A_{2b}R receptors at high adenosine agonist concentrations, even in the presence of high levels of albumin.

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Methods

The activation of A_{2a}R and A_{2b}R (G_s-coupled receptors) increases intracellular levels of cAMP and subsequent phosphorylation of the CREB protein, which can be used to determine receptor occupancy/activation. To assess the potency of AB928 under conditions with high non-specific protein binding and adenosine receptor signaling, 5mM of synthetic adenosine agonist (NECA) was used to stimulate CREB phosphorylation in human whole blood (WB). Flow cytometry was used to quantify AB928-mediated inhibition of CREB phosphorylation by NECA on CD8⁺ and CD4⁺ T cells, NK and B cells.

Results

AB928 inhibits NECA-induced phosphorylation of CREB with a potency of 88 nM (n=25) and is more potent than other adenosine receptor antagonists currently in clinical development (Table 1). Consistent with A_{2b}R inhibition, AB928 inhibits CREB phosphorylation in monocytes. In the mouse, inhibition of CREB phosphorylation was evaluated using *ex-vivo* WB assays, allowing for correlation of compound plasma levels with inhibition of phosphorylated CREB, confirming 90%+ target inhibition between dose intervals.

Conclusions

In conclusion, AB928 was designed as a best-in-class dual antagonist of A_{2a}R and A_{2b}R with optimal properties for effective shut-down of the effects of adenosine in the TME. AB928 is expected to enter clinical trials in late 2017.

Table 1. Inhibition by clinical stage adenosine receptor antagonists of NECA (5 μM)-induced CREB phosphorylation in CD8⁺ T cells (human WB)

Compound	IC ₅₀ in human WB (nM)
AB928	88 (n=25)
CPI-444	12,667 (n=2)
AZD4635	2,600 (n=2)
PBF-509	8,030 (n=2)

P7

Immuno-Oncology companion diagnostics development: a complex systems approach

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Background

Cancer immunotherapy is one of the most important medical advances of our time, and the first approach with the potential to generate long-lasting regressions for all types and stages of cancer. Development of companion diagnostics for emerging immunotherapies is more complicated because they are not dependent on driver mutations in the drug target. Consequently, we need to develop new strategies for the development of immunotherapies.

Methods

One emerging model is to review multiple immune checkpoints and other factors within the tumor that may be contributing to immune exclusion or susceptibility to immune attack. By getting a multifactorial assessment of what is happening within the tumor, investigators should be better able to derive indicators to rationally deliver individualized therapy, either monotherapy or combinatorial immunotherapy. During this review, we will give some examples of how complex systems approach is supporting the development of new biomarkers and potentially companion and complimentary diagnostic tests.

Results

We will review some examples of the complex systems approach such as: a cytokine profile determined by data-mining analysis to set into clusters non-small-cell lung cancer patients according to prognosis; a multiscale computational model for spatio-temporal tumor immune response and an IFN-g gene expression signature to correlate with clinical outcomes in PD-L1-treated advanced non-small-cell lung cancer and urothelial carcinoma patients.

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Conclusions

- The field of immune-biomarkers aims to characterize this ongoing interaction between the immune system and cancer
- The interplay of these individual elements determines the balance of immune activation versus suppression
- As components and regulators of the immune response, multiple immune-biomarkers, include cell surface proteins, secreted proteins or peptides, and tumor-infiltrating immune cells
- We need to redouble our efforts to be more sophisticated in our use of the powerful technologies at our disposal
- Combining drugs based on increasingly well-understood molecular interactions and attacking complementary cancer hallmarks or distinct cell populations in heterogeneous tumors, is now imperative
- As a result, in the near future, we will develop multiple biomarkers on different samples using different platforms which will result in an integrative lab tests and serial testing which will provide added clinical but, also epidemiological value.
- These next generation of immuno-oncology biomarkers will achieve different questions related with the outcome such as the treatment response, survival and toxicity, must probably at the same time

P8

T cell response profiling in colorectal carcinoma patients reveals an enrichment in responses to specific tumor-associated antigens

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Background

In colorectal cancer (CRC) the stage of the tumor at the time of diagnosis is considered to be the most important predictor of survival. Several non-invasive diagnostic tests for CRC are in development most focusing on detection of soluble macromolecules or circulating tumor cells. Here, we are exploiting the ATLAS™ technology, with the ability to identify an individual's T cell recall responses to putative antigens, for profiling tumor-associated antigen (TAA)-specific T cell responses in CRC patients.

Methods

T cell responses to 23 common TAAs previously implicated in CRC were profiled using the ATLAS technology. Libraries composed of full-length open reading frames expressed in *E. coli* alone or together with listerolysin O, thereby targeting antigen presentation to the MHC II or I pathways, respectively, were generated. Peripheral blood mononuclear cells (PBMC) were isolated from 50+ subjects who were healthy or suffering from different stages of CRC or adenomatous polyps. Peripheral CD4⁺ and CD8⁺ T cells were non-specifically expanded using anti-CD3/CD28 beads and CD14⁺ monocytes differentiated into monocyte-derived dendritic cells (MDDCs). *E. coli* libraries were co-incubated with MDDCs for antigen processing and presentation before autologous T cells were added and incubated for 21h. Recall T cell responses were measured through detection of interferon- γ and TNF- α secretion using a Meso Scale Discovery assay. Normalized cytokine concentrations that differed by >3 median absolute deviations from the median concentration secreted in response to non-immunogenic control clones were considered antigen-specific T cell responses.

Results

Several TAAs were identified for which specific T cell responses were detected in CRC patients of all stages. The breadth of response varied between individuals, but a strong enrichment of recall

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responses was observed to a subset of TAAs in CRC patients, to which T cell responses were not detected in healthy individuals. These responses could be attributed to both CD4⁺ and CD8⁺ T cell subsets and appeared to be Th1-polarized based on the predominant secretion of IFN- γ .

Conclusions

ATLAS is a highly suitable platform to profile TAA-specific T cell responses in CRC patients despite its classification generally as a tumor type with relatively low immunogenicity. The potential emergence of a specific T cell response profile to a subset of TAAs may open the possibility for the development of a blood-based assay to support early detection and diagnosis of CRC. We are currently investigating the utility of the identified immune signature in detection of pre-malignant stages and whether it is associated with progression to malignancy.

P9

Using iPair-TCR™ and iPair-TCR+™ to track tumor infiltrating lymphocytes from peripheral blood in a longitudinal breast cancer patient study

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Background

Historical tissue samples are typically stored in such a way that make it difficult to access single cell TCR or BCR receptor pairing sequence information, such as samples stored as FFPE slides or tissue stored in Trizol or RNAlater. However, interrogating the bulk TCR or BCR repertoire of these samples is possible by extracting total RNA from such tissue slices or appropriately stored tissue. However, the information regarding the cognate pairing of the alpha and beta chains is lost. We wanted to see if it

was possible to identify the most likely paired alpha-beta receptor sequences which likely match the historical tumor infiltrating lymphocyte TCR beta repertoire using circulating PBMCs.

Methods

As part of a longitudinal study, we sequenced the human TCR beta CDR3 fragments from a patient's breast cancer tissue six years ago. The patient's peripheral blood TCR beta repertoire has been examined every 6-9 months since surgical resection by amplifying CD14-CD8-CD4⁺ T cells and various sorted T cell subsets using arm-PCR. Single cells from this patient were recently amplified using iPair-TCR™ technology directly from the sorted cells. Identified TCR-beta CDR3 regions were used to compare to the longitudinal data sets.

Results

Three matching TCR beta sequences to bulk TIL data were identified from 104 sorted single cells. Two of these were private (meaning not shared with any of our database of 75 million CDR3 beta sequences), and one of these contained a paired alpha chain. The third sequence was considered a very public CDR3, meaning that it is present in many of the TCR beta repertoires analyzed within our database.

Conclusions

Further single cell experiments are being performed to identify more potential pairs matching the TIL data, including additional detailed phenotyping of the circulating lymphocytes. The ability to match paired receptor chains to archived data contained within banked tissue is promising and has important implications for therapeutic development.

P10

Epithelial-mesenchymal transition (EMT) signature was inversely associated with activated CD8 infiltration in non-small cell lung cancer (NSCLC)

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Background

EMT is a dynamic process in which epithelial cells acquire traits of mesenchymal cells. It has been known to drive metastasis and drug resistance during progression of cancer, while it is also associated with poor prognosis in cancers such as NSCLC. Though it has been suggested that EMT may contribute to immune escape, a clear relationship is yet to be established [1].

Methods

Gene expression data was obtained from the TCGA database, which contained mRNA-seq expression data of lung adenocarcinoma (ADCC, n=515) and squamous cell carcinoma (SqCC, n=501) patients. The mRNA z-score of 812 immune metagene signatures from previous studies were evaluated using Gene Set Enrichment Analysis (GSEA) [2]. Any immune cell types with a false discovery rate (q-value) $\leq 10\%$ were considered as positive infiltrations, and 31 distinct immune cells were analyzed in each tumor sample. The immune landscape of EMT in NSCLC was studied by calculating EMT scores by subtracting the average expression level of 'epithelial' genes (n=5) from 'mesenchymal' genes (n=24). The tumor samples were subsequently classified as EMT-high (EMT scores \geq highest 1/3) or EMT-low groups (EMT scores \leq lowest 1/3).

Results

There was a significantly lower infiltration of activated CD8 cells in EMT-high groups compared to EMT-low groups in both ADCC and SqCC (p<0.001, 15.7%; 37.2%, p<0.001, 15.5%, 40.5% respectively), as well as decreased infiltration of activated CD4 cells (p=0.161, 25.0%; 31.2%; p<0.001, 26.8%; 48.2%) (Figure 1). Effector memory CD4 cells showed significantly lower infiltration in both EMT-

high groups of NSCLC (p<0.001, 3.5%; 16.8%, p<0.001, 3.6%; 16.7%). EMT-scores showed an inverse relationship with infiltrating immune cells, most notably by activated CD8 cells. Increased expression of mesenchymal genes (VIM, ZEB1, ITGB6, etc.) in both ADCC and SqCC resulted in significantly lower infiltration of activated CD4/CD8 cells. However, increased expression of epithelial genes (COL5A1, TJP1) also resulted in significantly lower infiltration of CD8 cells in NSCLC.

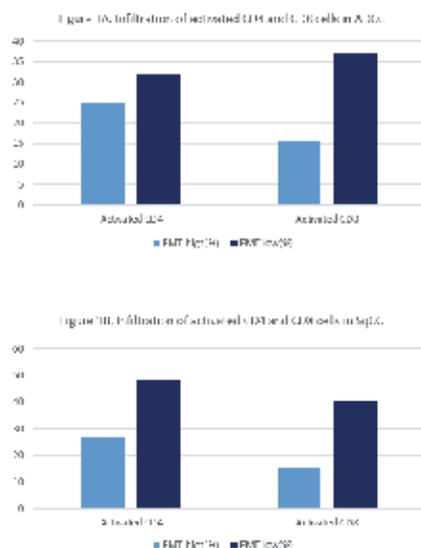
A significant positive correlation between immunosuppressive cytokine, IL-10 expression and EMT was found in both NSCLCs (p<0.001). Other cytokines with immunosuppressive functions that showed a significant positive correlation with EMT among others were TGF- β and ADCC. EMT-score, or infiltration of CD8 cells among EMT-high/low groups did not show any significant differences in overall survival (OS).

Conclusions

We report for the first time, the association between EMT-score and decreased infiltration of activated CD8 cells in both NSCLCs. This may be related to increased expression of immunosuppressive cytokines such as IL-10 or TGF- β . Markers of immune exclusion such as EMT scores may be potential biomarkers that predict resistance to immunotherapy.

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Figure 1.



References

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P11

WNT/β-catenin pathway is inversely correlated with activated CD8 T cell infiltration in non-small cell lung cancer (NSCLC)

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Background

It is well known that the WNT/β-catenin pathway promotes cell proliferation which is associated with tumorigenesis. While many mechanisms of tumorigenesis have recently been elucidated, one which has particularly garnered attention is the immune escape mechanism [1]. The correlation

between WNT/β-catenin pathway and immune escape has been investigated in several cancers including melanoma. However the relationship in lung cancer is still largely unknown.

Methods

In this study, TCGA data of 515 samples of lung adenocarcinoma (ADCC) and 501 samples of squamous cell lung carcinoma (SqCC) were utilized for analysis of immune landscape and gene expression scores. 17 promotor genes of WNT/β-catenin pathway (CTNNB1, DVL1, EP300, LRP6, etc.) and 8 suppressor genes of WNT/β-catenin pathway (AMER1, APC, AXIN1, etc.) were utilized for the analysis. The expression score of WNT/β-catenin pathway was calculated by subtracting the average of suppressor gene mRNA expression from the average of promotor gene mRNA expression. The tumor samples were then divided into 'low WNT/β-catenin expression group'(WNT/β-catenin pathway expression scores≤lowest1/3), and 'high WNT/β-catenin expression group'(WNT/β-catenin pathway expression scores≥highest1/3) according to their level of gene expression scores. For immune landscape, Gene Set Enrichment Analysis (GSEA) was utilized to analyze the mRNA z-score of 812 immune metagene signatures from previous studies [2]. Positive infiltration was considered as any immune cell type with a false discovery rate (q-value) equal to or less than 10% and 31 types of immune cells were analyzed for each tumor sample.

Results

We found that in both ADCC and SqCC, the high WNT/β-catenin score group showed lower infiltration of activated CD8 T cell (p=0.006, <0.001 respectively) (Figure 1). We also found a negative correlation between WNT/β-catenin pathway and the expression of immune stimulatory cytokines such as IL-2, IFN-gamma in ADCC (p=0.003, 0.029 respectively), whereas SqCC showed a negative correlation between WNT/β-catenin pathway and the expression of the immune stimulatory cytokines such as IL-2, IFN-gamma and IL-12(p=0.008, 0.041, 0.018 respectively). The expression of immune suppressive cytokine such as TGF-β showed positive

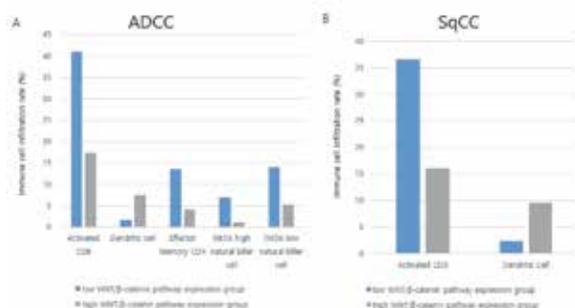
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correlation with WNT/ β -catenin pathway ($p=0.011$ for ADCC, <0.001 for SqCC).

Conclusions

We report for the first time that immune exclusion by WNT/ β -catenin pathway may also occur in NSCLC. The WNT/ β -catenin pathway is associated with lower immune stimulatory cytokine secretion, which leads to lower infiltration of activated CD8 T cell. Such findings suggest that WNT/ β -catenin pathway may serve as a potential biomarker for predicting resistance to immunotherapy.

Figure 1. Immune landscape of ADCC and SqCC



References

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P12

Precision immune monitoring: new 30-parameter flow cytometry uniquely and comprehensively defines immune checkpoint expression and T cell phenotypes in tumor tissue

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Background

The cellular processes that govern T cell activity at the tumor-immune interface involve many different proteins. To better understand these processes, and identify biomarkers of efficacy and toxicity, comprehensive analysis will be needed, through the measurement of as many T cell related proteins as possible. In this abstract, we introduce state-of-the-art, 30-parameter flow cytometry technology for use in immunotherapy research and immune monitoring, and describe the first studies applying this technology to studies of peripheral blood and tumor tissue.

Methods

We developed the first 30-parameter flow cytometry panel focused on the expression of checkpoint molecules. The panel includes CD45, CD3, CD4, and CD8 (to identify major T cell lineages); CD45RO, CCR7, CD27, CD28, CD57, CD95, and CD127 (markers of T cell differentiation); PD1, ICOS, CTLA4, LAG3, TIM3, TIGIT, VISTA, GITR, 41BB, and 2B4 (immune checkpoint molecules, involved in T cell exhaustion); CXCR3, CXCR6, and CD103 (trafficking markers); CD25, CD69, and HLA-DR (immune activation markers); and a viability dye (to exclude dead cells from analysis). We also developed a 30-parameter panel that quantifies major cell lineages (T, B, NK, myeloid, dendritic) and their subsets (naive, central memory, effector, plasma, etc.). These panels were applied to peripheral blood and resected tumor tissue from glioblastoma multiformae (GBM) patients, in order to comprehensively define the types of cells present in patient peripheral blood and tumors.

Results

We could clearly identify populations of cells expressing and not expressing each marker. We developed new technical approaches to ensuring robust data analysis, and novel bioinformatics approaches that are easy to understand and accessible to researchers. This will allow broader adoption of this technology. Analysis of GBM tumor tissue revealed about 10 unique subsets of cells, some of which are not found in peripheral blood.

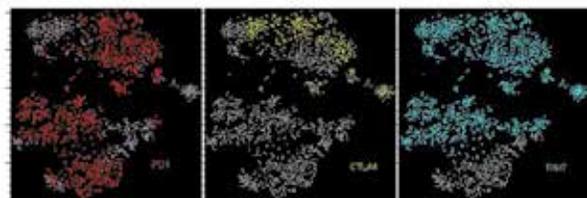
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Notably, we could easily identify cell types expressing checkpoint molecules that are current therapeutic targets, and define cells expressing combinations of those targets - or importantly - none of those targets (Figure 1). Further analysis allowed comprehensive characterization of these cells, to identify their differentiation status, activation status, and trafficking markers.

Conclusions

The technology and approach we have developed will be broadly applicable to phenotyping and monitoring immunotherapy in cancer patients. The technical platform has a number of advantages over other approaches (such as mass cytometry and RNA sequencing), and can be a central technology in precision oncology efforts, which will depend on complete and comprehensive characterization of cells.

Figure 1. T-sne analysis of T cells from GBM tumors



T-sne Analysis of GBM Tumor T-cells

P13

Pre-existing T cell immunity, mutational burden and stromal TGF- β signaling drives clinical responses to atezolizumab in locally advanced or metastatic urothelial carcinoma (mUC)

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Background

Checkpoint inhibitor blockade can result in robust and durable anti-tumor responses in many cancers. However, only a subset of patients experience long-term clinical remissions. Identifying determinants of response to cancer immunotherapy is critical for extending therapeutic benefit to more patients. Atezolizumab (anti-PD-L1) was approved in the US for the treatment of mUC based on the single-arm Phase II study IMvigor210 (NCT02108652). Here, we examined the biology underlying primary immune escape and responsiveness to anti-PD-L1 in patients from IMvigor210.

Methods

Cisplatin-ineligible patients previously untreated for mUC or patients who progressed post-platinum therapy received atezolizumab (1200 mg IV q3w). In both cohorts, RECIST v1.1 objective response was a primary endpoint evaluated in all patients and in PD-L1 expression subgroups on tumor-infiltrating immune cells (IC; VENTANA SP142 IHC assay). Exploratory analyzes in evaluable pre-treatment tissues included CD8 IHC, whole-transcriptome RNA sequencing, gene set enrichment analyzes and Lund subtyping. A FoundationOne panel was used to estimate tumor mutation burden (TMB), and whole-exome sequencing data were used for neoantigen prediction. EMT6-grafted BALB/c mice treated with anti-TGF- β and/or anti-PD-L1 antibodies were evaluated for tumor growth inhibition and

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characterization of tumor microenvironment phenotypes.

Results

PD-L1 IC expression and clinical response were positively associated with a CD8+ T-effector expression signature ($P < 0.001$ and $P = 0.0087$, respectively); patients with complete responses had higher T-effector signatures than did partial or non-responders ($P = 0.002$). Response was also strongly associated with high tumor mutation and neoantigen burden ($P < 0.001$ each). Likewise, response was positively associated with the genomically unstable molecular subtype ($P < 0.001$) and pathways involved in proliferation and DNA damage response. Further, non-responsiveness correlated with expression of a 2-gene TGF- β signature ($P < 0.001$), particularly in patients with CD8+ T cells predominantly in the collagen-rich matrix surrounding tumors (immune-excluded tumor phenotype; $P < 0.001$). Addition of anti-TGF- β to anti-PD-L1 in the mouse promoted T cell localization to immune-excluded tumors and reduced tumor growth *in vivo*.

Conclusions

Pre-existing T cell immunity and TMB are associated with response to atezolizumab in mUC, whereas TGF- β signaling in the stroma is a negative indicator of response, especially in immune-excluded tumors, a common phenotype of mUC. Integration of these 3 independent biological features provides a strong basis for understanding clinical outcomes in this setting and suggests that TGF- β can interact with the tumor microenvironment to restrain responsiveness to anti-PD-L1 and anti-tumor immunity.

Trial Registration

ClinicalTrials.gov Identifier NCT02108652

P14

Comprehensive characterization of solid tumor immune profiles for precision immunotherapy

using Immune Report Card

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Background

Monoclonal antibodies directed at the inhibitory immune receptors have emerged as successful treatment options for numerous tumor types using both mono and combination modalities. However, responses are not universal and better predictive biomarkers are needed to support precision immunotherapy. We present the findings from patients tested by Immune Report Card (IRC), a validated clinical assay that measures immunotherapeutic targets and immune response cycle markers to provide a complete profile for appropriate selection of immunotherapy.

Methods

300 FFPE cancer samples from patients with diverse histologies were evaluated by IRC using 5 test modes. RNA-seq measures transcript levels of genes related to T cell receptor signaling and tumor infiltrating lymphocytes, DNA-seq is used to estimate mutational burden, MSI-PCR to assess microsatellite instability, Fluorescent *in situ* hybridization (FISH) to detect PD-L1/2 copy number gain, and FDA-approved Immunohistochemistry to measure PD-L1 protein expression and pattern of expression for PD-L1, CD3, and CD8. Results are interpreted and summarized in an integrated report that comprehensively characterizes the tumor microenvironment to assess likelihood of response.

Results

At least one over expressed marker associated with either an FDA approved checkpoint inhibitor or combination immunotherapy clinical trial was identified for more than 80% of patients. Among PD-L1 IHC negative patients, the majority

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overexpressed at least one target with therapies in clinical development. Frequently overexpressed genes with targets in clinical development were identified for anti-inflammatory response (TGFB1, IL10), myeloid suppression (CSF1R), checkpoint blockade (PD-L1, LAG3, TIM3), T cell primed (GITR, OX40, CD40, CD137, ICOS), metabolic immune escape (IDO1) and pro-inflammatory response (IL1B, TNF) phenotypes. Conversely, tumors with low overall immune related expression (i.e. immune deserts), often exhibited high expression of a single marker with a pertinent clinical trial therapeutic opportunity.

Conclusions

Each of the 5 IRC test modes is necessary for determining the overall likelihood of patient response to FDA approved checkpoint inhibitors. Comprehensive profiling by IRC allows for the identification of patients with over expression of markers with therapeutic targets in clinical development, providing options for patients who are PD-L1 IHC negative. The results also highlight immune phenotypes with therapeutic targets beyond checkpoint blockade, suggesting a complex tumor microenvironment in many tumors. As immune therapy moves from treatment of last resort to first and second line treatment, IRC can provide actionable results for the total tumor immune microenvironment.

P15

Overexpression of immunotherapeutic targets in the immune desert phenotype

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Background

Immunotherapy using checkpoint blockade with monoclonal antibodies has gained increasingly high

importance in treatment for cancer patients. However, a large proportion of cancer patients do not highly express primary cancer immune biomarkers such as PD-1/PD-L1, MSI, and mutational burden, which have been associated with response. Here we present immune-related expression signatures for patients that present with an immune desert phenotype, distinguished by lack of CD8 positive T cells and characteristic T cell receptor signaling expression levels. As part of our clinical immune cell analysis assay, Immune Report Card (IRC), we identified secondary immune biomarkers that are singularly expressed in the otherwise non-inflamed tumor microenvironment and are potential clinical immunotherapy targets.

Methods

167 formalin-fixed, paraffin-embedded (FFPE) cancer samples of diverse histologies were evaluated by the RNA-seq component of IRC to measure transcript levels of genes related to T cell receptor signaling and tumor infiltrating lymphocytes. Resultant data was QC filtered, normalized and ranked based on an assorted reference population of various tumor types. Gene signatures were determined using these ranked expression values with a rank value > 85th percentile considered high. Tumors are also defined as inflamed or non-inflamed based upon RNA-seq analysis of CD8, wherein, Tumors in upper 50th percentile of rank for CD8 are considered inflamed, while those in the lower 50th percentile are considered non-inflamed.

Results

The immune desert phenotype represented 28% (n=47) of the tested samples. 95% (n=45) tumor were non-inflamed (CD8 Rank < 50). 51% (n=24) of all samples did not express any gene at a high level. However, 19% (n=9) of the immune desert phenotype samples have potential for monotherapy targets and another 19% (n=9) have underlying biology indication for combination therapy based on the high expression of a singular immune-related gene.

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Conclusions

Immune Report Card allows for the profiling of the tumor immune microenvironment to delineate underlying immune biology of solid tumor samples. With a significant number of non-inflamed tumors lacking high expression of any immune biomarker, IRC results suggests that an underlying biological immune ignorance state exists in the tumor microenvironment of many patients. However, IRC could identify so-called “oasis” targets that could be potentially targeted with mono or combination immune therapy in the immune desert phenotype. With the ever-increasing numbers of FDA-approved therapies and clinical trials, IRC offers a robust tool to identify patients that might benefit from these options.

P16

The immune activated phenotype: secondary immunotherapeutic targets in the primary biomarker negative inflamed tumor

Sean Glenn¹, Jeffrey Conroy¹, Sarabjot Pabla¹, Ji He¹, Blake Burgher¹, Vincent Giamo¹, Jonathan Andreas¹, Mark Gardner¹, Antonios Papanicolau-Sengos¹, Mary Nesline¹, Carl Morrison¹

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Background

Immune checkpoint inhibitors are now used to treat many different types of cancer, with some patients demonstrating durable clinical responses. As expected, a significant number of responders express high levels of primary cancer immune biomarkers such as PD-1/PD-L1, MSI, or mutational burden. Here we present immune-related expression signatures for patients with an immune activated phenotype that overexpress several pro- and anti-inflammatory genes with or without primary biomarker detection. These immune signatures were identified as part of Immune Report Card (IRC), a comprehensive molecular and immunological assay that uses five testing modes to

detect several known markers of the host anticancer immune response.

Methods

167 formalin-fixed, paraffin-embedded (FFPE) cancer samples of diverse histologies were evaluated by IRC to measure transcript levels of genes related to T cell receptor signaling and tumor infiltrating lymphocytes (RNA-seq) and mutational burden (DNA-seq). Resultant data was QC filtered, normalized and ranked based on an assorted reference population of various tumor types. Gene signatures and mutational burden were determined using these ranked values with a rank value > 85th percentile considered high. Tumors are also defined as inflamed or non-inflamed based upon RNA-seq analysis of CD8. Tumors in upper 50th percentile of rank for CD8 are considered inflamed, while those in the lower 50th percentile are considered non-inflamed. RNA-seq analysis of CD8 had been previously calibrated against quantitative image analysis using the Aperio platform.

Results

The immune activated phenotype represented 20% (n=35) of the tested samples, of which 32 were inflamed (91%). 31% (n=11) of these samples had co-expression of the primary immune biomarker PD-L1 or demonstrated a high mutational burden. However, 66% (n=23) of the immune activated tumors were primary biomarker negative but instead expressed multiple secondary immune biomarkers that are potential checkpoint inhibitor therapy targets.

Conclusions

Immune Report Card profiles the tumor immune microenvironment to outline the immune biology of tumor samples. IRC is not only able to identify samples with highly expressed primary immune biomarkers such as PD-L1, MSI, or mutational burden, but using RNA-seq can also identify secondary biomarkers in many samples. These secondary biomarkers shed light on the underlying biological immune-state of the tumor microenvironment, potentially identifying additional

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mono or combination immunotherapies in PD-L1 negative inflamed tumors. With the ever-increasing numbers of FDA-approved therapies and clinical trials, IRC offers a robust tool to identify patients that might benefit from these options.

P17

The immune-excluded phenotype beyond colorectal cancer

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Background

Absence of tumor infiltrating lymphocytes in the tumor proper, referred to as the immune-excluded phenotype, has been directly described in the peer-reviewed literature for colorectal cancer, but indirectly for melanoma and other solid tumors in the field of immunotherapy discussions. As part of our clinical immune cell analysis using a New York State CLEP approved assay, Immune Report Card (IRC), we routinely perform CD8 and CD3 immunohistochemistry (IHC) to determine the infiltrating versus the non-infiltrating (excluded) phenotype for all tumor types.

Methods

For all tumor types tested including carcinoma, sarcoma, and melanoma the immune-excluded phenotype is defined as a restriction of more than 95% of all CD8+ T cells present in a tumor tissue section to the periphery or interstitial stromal areas and not actively invading nest or groups of neoplastic cells. Tumors are also defined as inflamed or non-inflamed based upon RNA-seq analysis of CD8 from the same tissue section and comparison to a reference population of several hundred prior analyzed samples. Tumors in upper 50th percentile of rank for CD8 gene expression are

considered inflamed, while those in the lower 50th percentile are considered non-inflamed. RNA-seq analysis of CD8 had been previously calibrated against quantitative image analysis using the Aperio platform. A total of 100 consecutive samples tested, excluding colorectal cancer, were evaluated for the excluded infiltrate phenotype.

Results

The immune-excluded phenotype was identified in all tumor types with the most frequent association seen in lung cancer and the least frequent being melanoma. The immune-excluded phenotype was more common in non-inflamed tumors with a direct correlation to CD8 rank, but was also seen in some moderate to highly inflamed tumors. The most common histological pattern of the immune-excluded phenotype was restriction of CD8+ T cells to the interstitial stromal areas.

Conclusions

The immune-excluded phenotype does occur in tumor types other than colorectal cancer. Providing a precisely defined nomenclature and method of testing for the immune-excluded phenotype in inflamed and non-inflamed tumors is important for both clinical and research purposes.

P18

Withdrawn

P19

Genomic determinants of response to pembrolizumab in gastric cancer

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Background

The mutation load of somatic non-synonymous variants (ML) is associated with response to anti CTLA-4 and PD-1/PD-L1 immunotherapies in select tumors, likely because neoepitope formation is not subject to central immune tolerance. An IFN γ gene expression profile (GEP) characteristic of a tumor T cell inflamed environment is also related to response to anti PD-1/-L1 therapy. ML and GEP have been reported as predictive in a pan-tumor setting and in HNSCC. This study evaluated relationships between ML and response, independent predictive value of ML, GEP and PD-L1 IHC in patients with gastric cancer treated with pembrolizumab monotherapy, and the association of response with TCGA molecular subtypes (MSI, EBV, CIN, and GS).

Methods

Whole exome sequencing (WES) and GEP were assessed in FFPE tumor specimens from previously treated patients with gastric cancer from KEYNOTE 059 cohort 1. ML, neoantigen load (NL), EBV status, and clonality were assessed by state-of-the-art WES analytics. GEP score is a weighted sum of normalized expression values of 18 genes. Molecular subtypes were determined using DNA genomic features. Statistical testing of ML and response, ML and GEP or PD-L1 relationship in EBV-negative patients, and association with molecular subtypes were pre-specified.

Results

85/259 patients (33%) had WES results, 76 of which had GEP results and 84 of which had PD-L1 results. There were 7 responders (CR or PR) and 78 non-responders. Five patients were identified as EBV-positive (EBV+), 3 as MSI-H, 45 as CIN, and 32 as GS. All MSI-H patients and no EBV+ patients were responders. For non-MSI-H responders (n=4), response was not significantly associated with the remaining TCGA subtypes EBV, CIN and GS. In EBV-negative (EBV-) patients, association between ML and objective response (OR) was statistically significant (p= 7E-4; AUROC 0.83). ML was not significantly correlated with either GEP or PD-L1. In a joint model, ML was significantly associated with

response after adjusting for GEP (p=4E-3) or PD-L1 (p=2E-3) in EBV- patients. NL and clonality-weighted ML were also significantly associated with response in EBV- patients (p=2E-3 and 8E-4, respectively).

Conclusions

As reported in other indications, ML and GEP or PD-L1 were independently predictive of response to pembrolizumab monotherapy in gastric cancer. This suggests that ML and GEP or PD-L1 may help characterize responses to anti PD-1 therapies and novel treatment regimens in gastric cancer.

Trial Registration

ClinicalTrials.gov Identifier NCT02335411

P20

Distinct tumor immune profiles in non-small cell lung cancer (NSCLC) revealed by automated quantitative digital image analyzes of immune biomarker densities and spatial relationships

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Background

The tumor microenvironment comprises complex interactions between infiltrating immune cells and tumor cells. Understanding these interactions may provide valuable insights into mechanisms of response and resistance, and aid clinical decisions. In the present study, immune cell densities and PD-L1 expression in NSCLC samples were quantified to determine immune status of each sample prior to conducting sophisticated spatial analyzes to establish the proximity and ratio of cytotoxic T cells and regulatory T cells to tumor expression of PD-L1.

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Methods

Serial sections of formalin-fixed paraffin-embedded NSCLC samples (n=9; Tissue Solutions, Glasgow, UK) were stained immunohistochemically using well validated assays for CD3, CD8, Foxp3 and PD-L1. Whole slide digital images were generated and viable tumor regions annotated by a clinical pathologist. The immune profile of each sample was established by applying digital image analysis tools available in the Indica Halo™ platform to determine immune cell densities and PD-L1 expression. Selected images were subsequently registered and spatial analyzes between immune components performed.

Results

Tumor membrane PD-L1 scored by a pathologist ranged from 0% to 90% positivity with variable intensity of staining. Digital image analysis of PD-L1 using a membrane algorithm coupled with a classifier tuned to analyze tumor only delivered comparable PD-L1 positivity. Immune cell densities ranged from minimal infiltration up to 2315, 1512 and 340 cells/mm² for CD3, CD8 and Foxp3, respectively. Variable CD8:Foxp3 ratios were observed ranging from 1.3 to 16.5, and no direct relationship between immune cell infiltration and PD-L1 expression was evident. Spatial analyzes of the proximity of CD8 and Foxp3 to PD-L1 revealed samples (n=2) exhibiting 15-20% of cytotoxic T cells within 5µm of PD-L1 with few adjacent Foxp3+ cells (CD8:Foxp3 ratio >10.0). In contrast, we identified samples (n=2) where CD8+ T cells were within close proximity (5µm) of the immunosuppressive influences of both PD-L1 and Foxp3+ regulatory T cells (CD8:Foxp3 ratio <2.6).

Conclusions

We have demonstrated using next generation digital image analysis tools that the potential immunosuppressive influences within close proximity of cytotoxic T cells within a tumor can be mapped. This approach has potential to identify tumors where cytotoxic T cells are in close proximity to PD-L1 and those where cytotoxic T cells may be in the vicinity of additional immunosuppressive

influences, thus identifying patients most likely to benefit from different immunotherapies.

P21

Correlation of inflammatory biomarkers and patient-reported outcomes in patients with urothelial carcinoma treated with durvalumab

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Background

A correlation between changes in inflammatory/cachexia biomarkers, tumor shrinkage, and overall survival (OS) has been demonstrated in patients with urothelial carcinoma (UC) receiving durvalumab [1]. The current analysis assessed the relationship between inflammatory/cachexia biomarkers and patient-reported outcomes (PROs), and differences in PROs between responders versus non-responders in patients with UC treated with durvalumab.

Methods

In a phase 1/2, dose-escalation study of durvalumab, Functional Assessment of Cancer Therapy—Bladder Cancer (FACT-BL) and the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30) were administered before other procedures at screening, day 1 of doses 1 (d1; baseline assessment), 3 (d29), 4 (d43), 5 (d57), 7 (d85), 9 (d113), and every 8 weeks thereafter. For 126 patients, the association between PRO scores and maximum percent changes in tumor size, albumin level, neutrophil/lymphocyte ratio (NLR), and

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durvalumab clearance from baseline was assessed by Spearman's correlation analysis.

Results

Pretreatment FACT-BL scores were similar for responders and non-responders, with patients reporting relatively high health-related quality of life (QoL) at baseline. Compared with non-responders, clinical tumor responders reported statistically significant improvement in FACT-BL total scores (mean change of 17.1 vs -4.13, $P=0.0005$), FACT-BL bladder cancer-specific symptoms (mean change of 5.8 vs -0.56, $P=0.0047$), and FACT-BL Trial Outcome Index (mean change of 14.3 vs -1.9, $P=0.0005$) at Day 113. Sustained improvement in PROs was observed in 43% of the responders compared with 12%–18% of non-responders. Fifty percent of patients with a clinical tumor response also reported a clinically meaningful change in UC symptoms at Day 113 using a threshold based on minimum important difference (MID) defined as $\frac{1}{2}$ baseline standard deviation, which was sustained over time (≥ 2 consecutive visits with MID improvement vs baseline). Clinical tumor responders also showed higher mean scores and improvement from baseline in EORTC QLQ-C30 functional and global health status/QoL scales.

FACT-BL scores and EORTC QLQ-C30 functioning improvement were correlated significantly with decreased tumor size, increased albumin, decreased NLR, and decreased durvalumab clearance (Table).

Conclusions

PROs were correlated significantly with inflammatory/cachexia biomarkers and durvalumab clearance in advanced UC; additionally, there was a strong correlation between clinical tumor response and FACT-BL and EORTC QLQ-C30 scores.

Trial Registration

ClinicalTrials.gov Identifier NCT01693562

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P22

Up-regulation of a T and NK cell gene signature in peripheral blood is associated with mRNA-based immunotherapy in lung and prostate cancer

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Background

CV9201 and CV9104 represent novel mRNA-based cancer immunotherapeutics targeting multiple tumor antigens associated with either non-small cell lung cancer (NSCLC) or prostate cancer (PC), respectively. We sought to comprehensively profile transcriptional changes in peripheral blood of cancer patients after repeated mRNA treatment.

Methods

Whole-genome transcriptome profiling was performed in blood samples from 46 patients with localized, intermediate or high risk PC and samples from 22 stage IV NSCLC patients before and after repeated intradermal immunizations with protamine-formulated mRNA. The 46 study participants with PC included a cohort of 16 untreated control subjects. Expression data was analyzed by gene set enrichment analysis and modular approaches using blood transcriptional modules. Phenotypic leukocyte analyzes were performed by flow cytometry.

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Results

Peripheral blood samples post mRNA treatment were characterized by an enrichment of various modules consistent with a T and/or NK cell profile in NSCLC as well as PC patients. Notably, this up-regulation of effector lymphocyte genes was completely absent in the PC control subjects. We observed an enrichment of a myeloid cell signature at baseline compared to samples derived after mRNA immunotherapy. The T and NK cell gene signature and the myeloid gene signature were inversely correlated and non-overlapping. Furthermore, correlation analyzes between gene expression data with findings from the phenotypic analyzes, suggest changes in the cellular composition of peripheral blood cells after treatment.

Conclusions

The consistent transcriptional up-regulation of T and NK cell modules in post treatment samples in different cancers and the absence of it in the untreated control cohort suggest an association with intradermal mRNA immunotherapy including its potential immunostimulatory effects.

P23

Deep molecular and immune-infiltrate stratification of cancer identifies mechanistic subtypes and predicts response to checkpoint inhibition

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Background

Checkpoint inhibitors are revolutionizing cancer therapy but identifying patients which are likely to achieve clinical benefit remains a major challenge. While relatively simple predictors such as PD-L1 expression or tumor mutation load have shown promise in predicting responders in specific cancer types, they fail to explain or predict response for

many patients, suggesting that a more comprehensive understating of biology underlying tumor and immune system interactions and immunotherapy response is needed.

Methods

We developed an integrated machine-learning system that stratifies cancers using multimodal omics data and associated profiles of immune infiltration and immune pathway activity. This framework holistically determines the underlying pathway-level architecture of genomic and epigenetic alterations in each cancer using a new Model-based Network-informed Stratification approach (MNS, [1] for early version). It further uses a multiscale pathway mapping approach [2, 3] to predict subtype-specific immune-infiltrates, activation of key immune pathways, and, ultimately, response to checkpoint inhibition.

Results

The new framework is used to interrogate the tumor-immune system in colorectal and lung adenocarcinomas. Stratification of 533 TCGA lung adenocarcinomas into four subtypes (LUAD_MNS4) allows for reanalysis of pembrolizumab trial data [4] by matching the trial patients to the LUAD_MNS4 to infer immune infiltration and pathway activation signatures for each patient and predict patient response to pembrolizumab (Figure 1). In colorectal cancer, a total of six subtypes (COADREAD_MNS6) are identified along with subtype-specific profiles of pathway mutations and copy-number alterations. Two subtypes are hypermutated and one (COADREAD_MNS6_S4) is associated with high predicted CD8 T cell infiltration. The system also uncovers subtypes S1 and S6 which link WNT/ β -catenin pathway mutations with immunologically cold tumors, suggesting the possibility of a similar mechanism for immune evasion as recently discovered in specific subtypes of melanoma [5].

Conclusions

Comprehensive systems-level interrogation of tumor genomic architecture and microenvironment may improve our understanding of tumor-immune

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biology and allow for more accurate patient stratification for immunotherapy.

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P24

Systematic literature review of PD-L1 assays, their scoring algorithms and validation metrics

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Background

Programmed cell death protein 1 (PD-1) is a cell-surface receptor expressed on T cells. The interaction between PD-1 and its ligand, programmed death ligand 1 (PD-L1), down-regulates immune responses. Many cancer types express PD-L1 and, via the PD-1/PD-L1 interaction, evade immune destruction. Antibodies targeting this interaction are approved treatments for several cancers with a variety of PD-L1 tests available to

measure PD-L1 expression. A systematic literature review was conducted to evaluate these commercially available PD-L1 assays, their scoring algorithms and validation data in bladder/urothelial, lung, gastric, or ovarian cancer.

Methods

A search was conducted in Medline and EMBASE and supplemented by abstracts from recent oncology conferences (January 2013 to November 2016). Studies meeting pre-defined criteria (such as cancer types of interest, with validation test data) were extracted and key trends summarized.

Results

26 primary studies were identified from a total of 950 records. All the included studies reported data relating to PD-L1 assays in lung cancer using immunohistochemistry (IHC) testing; one study also reported on bladder/urothelial cancer. Significant heterogeneity was reported among the available tests for PD-L1; for example, cut-offs used by test scoring algorithms to define PD-L1 positivity ranged from $\geq 1\%$ to $\geq 50\%$. Studies also differed as to whether they evaluated tumor cells only and/or tumor-infiltrating immune cells. However, all assays developed within clinical-trial settings met recognized acceptance criteria of $\geq 90\%$ inter-reader concordance. In head-to-head comparisons, there was poor concordance between PD-L1 assays developed outside of clinical trials compared with companion diagnostics developed within clinical trial-settings.

Conclusions

Published data on PD-L1 testing relates predominantly to IHC assays in lung cancer. Review of this evidence suggests the need to develop standardized testing methods, or, minimally, some common approach for test scoring processes. The current variability of such testing could be a source of confusion among treating physicians and might complicate the uptake of effective treatments.

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P25

A systematic literature review (SLR) of the predictive value of programmed death ligand 1 (PD-L1) tests on clinical outcomes

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Background

Promising trial results have led to FDA approval of several drugs that target programmed cell death protein 1 (PD-1) and its ligand (PD-L1). However, the predictive value of PD-L1 expression on clinical outcomes is unclear. Therefore, an SLR was conducted to evaluate evidence on the predictive value of PD-L1 expression in bladder, gastric, lung, and ovarian cancers.

Methods

The SLR included a search of Medline and EMBASE databases from 1/1/2010 to 15/9/2016 using keywords for PD-L1 paired with terms related to predictive value and relevant abstracts from recent oncology conferences. Studies were selected using pre-defined criteria and then qualitatively summarized.

Results

The SLR identified 29 primary studies. There was limited evidence on bladder (n=4), gastric (n=1), and ovarian (n=1) cancer. Bladder cancer studies showed a trend towards an association between response outcomes and PD-L1 expression, while results were more variable for survival outcomes. Most studies (n=23) were related to lung cancer and were inconsistent. Data for pembrolizumab was most supportive, showing PD-L1 expression is associated with increased probability of response across treatment-naïve, previously-treated, and

advanced or metastatic patient subgroups. For the other therapies there was variation across subgroups. Evidence on the predictive value of combinations of biomarkers was limited to two durvalumab studies, which found improved outcomes when PD-L1 was detected along with additional biomarkers.

Conclusions

Evidence on the value of PD-L1 as a predictive biomarker is currently heterogeneous and relates mostly to lung cancer. While some studies suggest a correlation between PD-L1 expression and clinical outcomes, the SLR indicated that PD-L1 expression alone may not identify those likely to respond to treatment. Factors such as level of expression, cell phenotype, and variability in testing methods are possible reasons for these differences. Ambiguity in the validity of PD-L1 tests adds complexity regarding its use as a standalone biomarker. Further research is needed to explore the relationship between biomarker expression, cancer phenotypes, and treatment outcomes.

P26

Certain KIR/KIR ligand genotypes influence patient response to immunotherapy in neuroblastoma patient

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Background

In 2010, a phase III randomized trial (ANBL0032; NCT00026312) conducted by the Children's Oncology Group (COG), found that patients treated with an immunotherapy regimen of dinutuximab, GM-CSF, IL-2 and isotretinoin had improved event-free survival (EFS) and overall survival (OS) compared to treatment with isotretinoin alone. Dinutuximab (monoclonal/chimeric anti-GD2 antibody) acts in part via engaging NK cells in antibody-dependent cellular cytotoxicity (ADCC). Killer Immunoglobulin-like Receptors (KIR) are a family of receptors expressed by NK cells that can influence their function. The genotypic profiles of inhibitory KIR/KIR-ligands have previously been shown to influence the response of neuroblastoma patients to immunotherapy. We investigated whether EFS and OS were associated with KIR/KIR-ligand genotypes and this immunotherapy regimen.

Methods

Of the 226 pts randomized in ANBL0032, 174 pts had DNA available to genotype for correlations with outcome (isotretinoin alone: n=86; Immunotherapy: n=88; >5yr follow-up if no event). KIR gene status was determined via SYBR green melt curve analysis; KIR-ligands were determined by PCR-SSP reactions using the KIR HLA Ligand SSP typing kit (Olerup). Algorithms were used to assess associations of inhibitory KIRs with their respective KIR-ligands and clinical outcome. Log-rank tests and Cox proportional hazards regression models were used to compare EFS/OS by genotype group; adjustment was made for non-proportional hazards as needed using time-dependent covariates.

Results

In this randomized trial of neuroblastoma patients, we found that certain KIR/KIR-ligand genotypes were associated with significantly improved outcome for patients receiving the COG immunotherapy regimen vs. those that received isotretinoin alone; whereas the patients with the

complementary KIR/KIR-ligand genotypes had no significant difference in EFS and OS regardless of whether they received immunotherapy vs. isotretinoin alone.

Conclusions

For this study, certain KIR/KIR-ligand genotypes, but not others, are associated with improved outcome for patients treated with immunotherapy compared to isotretinoin alone. As validation of these KIR/KIR-ligand findings has not yet been performed in other studies comparing patients receiving immunotherapy to those not receiving immunotherapy, further investigation is required. However, if validated, KIR/KIR-ligand genotyping could potentially be used as a predictive biomarker, allowing for administration of this COG immunotherapy regimen to those that might best benefit. Enhancements to anti-GD2 mAb-based therapy, based on preclinical and early clinical data, are being evaluated in efforts to improve its efficacy. Further studies of KIR/KIR-ligand associations with outcome in subsequent trials of immunotherapeutic regimens for children with neuroblastoma will be needed to determine the potential clinical utility of these KIR/KIR-ligand findings.

P27

Optimized workflow improves the characterization of tumor-infiltrating T cells

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Background

Immunotherapy has proven clinical efficacy and tremendous potential in multiple cancers. Syngeneic mouse tumor models represent the gold standard to analyze effects of immunotherapy, as they possess a fully competent immune repertoire.

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However, the amount and composition of tumor-infiltrating leukocytes (TILs) is highly variable, complicating the analysis of individual subpopulations. In particular, small subpopulations might escape analysis as they could get lost in the background noise. When working with large cohort sizes, even immunophenotyping of TILs by flow cytometry is time consuming and data processing highly work intensive. Therefore, pre-enrichment of TILs is highly desirable to increase the sensitivity of analysis and save time and effort during flow cytometry.

Methods

We have established workflows combining tissue dissociation, T cell isolation and phenotyping. Tumor dissociation was automated using the gentleMACS™ Octo Dissociator and optimized for epitope preservation. Moreover, isolation of tumor infiltrating T cells was improved by developing new CD4-, CD8- and Pan-T cell specific enrichment reagent for magnetic cell sorting, based on MACS® Technology, direct from dissociated tumor tissue. Finally, we used optimized panels of recombinant REAfinity fluorescently-labelled antibodies to phenotypically characterize tumor infiltrating T cells.

Results

Our workflows were validated in B16.F10, B16-OVA, CT26 and 4T1 mouse tumor models. Optimized tumor dissociation was essential for isolation of specific sub-populations of tumor-specific T cells. Tumor-infiltrating T cells were isolated to purities above 80% and yields ranging from 60-95%. Time of downstream analysis was reduced up to 50-fold while enhancing the detection and phenotypic characterization of T cell subpopulations within the tumor.

Conclusions

Standardized processing of tumor samples and magnetic isolation of tumor infiltrating T cells greatly reduces time and cost of downstream analysis while significantly increasing reproducibility and the quality of data obtained from TIL analysis.

P28

Profiling of T cell responses to tumor-associated antigens in lung cancer patients treated with checkpoint inhibitors

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Background

While the recent approval of checkpoint inhibitor therapies (CPIs) has transformed the therapeutic landscape of advanced lung cancer, the early identification of patients who will benefit from CPIs requires improvement. In addition to neoantigen-specific responses, the success of CPI therapies can in part be attributed to reinvigoration of T cells specific for tumor-associated antigens (TAAs), providing an opportunity to explore using TAA-specific T cell response profiles for patient stratification. Here we utilized ATLAS™, a high-throughput antigen discovery platform that can profile autologous CD4⁺ and CD8⁺ T cell recall responses to putative antigens, to profile TAA-specific T cell responses in NSCLC.

Methods

Based on literature review, 77 common antigens were selected to be cloned as full-length proteins in *E. coli*, either alone or co-expressed with listeriolysin O to facilitate MHC II and I presentation, respectively. PBMCs from patients with stage III or IV lung cancer, primarily NSCLC, post-checkpoint inhibitor treatment were analyzed. CD4⁺ and CD8⁺ T cells were isolated and non-specifically expanded. Autologous CD14⁺ monocytes were differentiated into monocyte-derived dendritic cells and co-incubated in an ordered array with the bacterial libraries for antigen processing and presentation. T cells were added and IFN- γ and TNF- α secretion was

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quantified in the supernatant after 21h, using a custom Meso Scale Discovery kit. Significant antigen-specific recall responses were defined as greater than 3 median absolute deviations from the median response to negative control clones.

Results

ATLAS identified broad TAA-specific T cell responses across the cohort of lung cancer patients, with a greater breadth of CD8⁺ than CD4⁺ response, and little overlap between subsets. In addition to T cell activation, antigen-specific inhibition of T cell activity was also observed. T cell response profiles were consistent over at least three weeks in patients for which longitudinal samples were available.

Conclusions

ATLAS is a valuable tool to profile tumor-associated T cell responses in advanced lung cancer non-invasively, providing the opportunity for cohort analysis to identify blood-based signatures that correlate with disease subtype, stage, or responder status to CPI. The consistency of observations over time supports the reproducibility of the platform, validating the utility of ATLAS in anti-tumor response profiling. Future prospective studies will compare T cell response profiles prior to and after CPI therapy to define signatures predictive of clinical response.

P29

Incorporating premixed staining cocktail for improving the workflow for whole blood immunophenotyping

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Background

Whole blood immunophenotyping is indispensable for monitoring immune responses to cancer immunotherapy. We have successfully introduced an automated liquid handler equipped with a two-dimensional barcode reader for the cocktail preparation that enabled us to prepare antibody cocktails for multicolor flow cytometry for more than 800 fresh whole blood patient samples over last two years. Although it is reliable, our current workflow takes up 2-3 hours to prepare cocktails and is not easily scalable to meet an increasing demand. As a solution, we evaluated the compatibility of custom-made dried cocktail reagents.

Methods

As brilliant violet dyes are not compatible with the dried reagent format, we have re-established the staining panels for T cells, B cells, NK cells, monocytes and dendritic cells with other fluorochromes. Stained cells are analyzed by CytoFlex (Beckman Coulter).

We will evaluate custom-made dried antibody cocktails based on our revised immunophenotyping panels, focusing on the comparative analysis between liquid and dried cocktails for ease of use and the quality and reproducibility of staining.

Results

We have successfully reduced from the current 13-color panels to 10-color ones without compromising the ability to detect immune subsets and their activation status by omitting redundant staining. The comparative study between liquid and dried cocktails is underway.

Conclusions

The dried format allows multicolor antibodies to be dried within the analytical tubes, facilitating their ease of use. Unlike liquid cocktails, dried cocktails require no refrigeration, titration, or manipulation before using. Therefore, it not only saves time but also reduces potential errors and obviates the need to manage individual antibodies. With a shelf life of

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at least 12 months, the reagents also offer potential savings in reagent costs by reducing waste due to expiration or tandem breakdown in standard liquid formulation.

P30

Mass spectrometry-based test predicts outcome on anti-PD-1 therapy for patients with advanced non-small cell lung cancer, including those with brain metastases

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Background

Checkpoint inhibition has become a standard of care therapy for advanced non-small cell lung cancer (NSCLC), but only a minority of patients achieves a durable response. PD-L1 expression is often used to identify patients likely to have good outcomes. However, patients with low PD-L1 expression can have long-term treatment benefit and this biomarker is dynamic and requires tumor tissue. A less invasive approach that could provide predictive information regarding treatment outcomes could be useful.

Methods

Pre-treatment blood samples were collected from patients with advanced NSCLC. Cohort A (n=98) received nivolumab, 3mg/kg every 2 weeks in a compassionate use program, 59% (32%) in 2nd (3rd) line. Patients in cohort B (n=32) had at least one

new or progressing brain metastasis and were treated on a clinical trial with pembrolizumab 10mg/kg every 2 weeks. Spectra were generated from all samples using a sensitive method of MALDI mass spectrometry and processed to render them comparable. Spectra from cohort A were combined with clinical data using modern machine learning methods to create a test able to identify patients likely to have better or worse overall survival (OS), as assessed by reliable test set evaluation methods. The locked test was applied to cohort B, blinded to all clinical data.

Results

Using mass spectral features associated with acute response and wound healing, we produced a test able to identify patients likely to have better or worse OS (HR=0.41, log-rank p=0.005) and progression-free survival (PFS) (HR=0.56, log-rank p=0.022) within cohort A (Figure 1). Test classification was an independent predictor of OS and PFS in multivariate analysis, with 1 year survival of 66% in the good outcome group and 36% in the poor outcome group. Thirty-four percent of patients were classified to the good outcome group. In cohort B, 25% classified to the good outcome group, which had 1 year survival of 88%, compared to 24% in the poor outcome group (Figure 2). The HR for OS between groups was 0.36 (log-rank p=0.062).

Conclusions

We developed a blood-based test able to identify patients with advanced NSCLC likely to have better or worse survival with anti-PD-1 therapy. It showed promise in a validation cohort of patients with NSCLC with progressing brain metastases. Further validation of the test in additional, larger cohorts is planned and necessary to assess clinical utility.

Trial Registration

Clinical Trials.gov Identifier NCT02085070

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P31

Proteomic biomarker analysis of metastatic melanoma patients treated with anti-PD-1 checkpoint blockade

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Background

Biomarkers identifying who will derive benefit from anti-PD-1 therapy from pre-treatment parameters would further clinical understanding of PD-1 blockade and help development of alternative treatments for patients unlikely to benefit. We evaluate the performance of a serum proteomic test, BDX008, in metastatic melanoma patients treated with anti-PD-1 agents and investigate the role of BRAF mutation status.

BDX008, a pre-treatment test associated with acute phase reactants, wound healing and complement activation, stratifies patients into two groups, BDX008+ and BDX008-, with better and worse outcomes on immunotherapy. Following development and preliminary validation [1], it has been applied to independent cohorts in melanoma [2] and lung cancer [3].

Methods

Pre-treatment serum samples were available from 71 patients (under IRB); 70 pre-treated with ipilimumab. BRAF mutations were identified in 25 patients; 39 were wild type (WT). BDX008 results were generated by Biodesix blinded to clinical data.

Results

Overall, BDX008+ patients had significantly better OS (HR=0.40 (95%CI:0.21-0.76), p=0.005) and PFS

(HR=0.54 (95%CI:0.32-0.93), p=0.027) than BDX008- patients. BDX008 was a significant predictor of disease control (p=0.002) and trended to significance for response (p=0.056). Outcomes were numerically inferior for patients with BRAF mutations (median OS/PFS: 160/85 days for BRAF mutations; 479/181 days for BRAF WT respectively). BDX008+ patients had superior OS (HR=0.20 (95%CI:0.07-0.59), p=0.004) and PFS (HR=0.45 (95%CI:0.21-0.99), p=0.046) in the BRAF WT subgroup, but not in the BRAF mutation subgroup (OS: HR=0.62 (95%CI:0.24-1.59), p=0.318; PFS: HR=0.57 (95%CI:0.24-1.40), p=0.223), possibly due to smaller sample size.

Conclusions

BDX008 stratified patients into groups with better and worse PFS and OS. The difference between BDX008+ and BDX008- groups was consistent across BRAF status, but did not reach statistical significance in smaller subgroups. Patients with BRAF mutations may have worse outcomes than those with BRAF WT with anti-PD-1 agents, possibly due to prior targeted therapies, and more previous lines of therapy overall. BDX008 as a possible predictive biomarker warrants further prospective evaluation

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P32

Differential association of myeloid cell and IFN- γ associated proteins with clinical response to durvalumab treatment in urothelial bladder cancer

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Background

Durvalumab, a human monoclonal antibody that binds PD-L1, was recently granted accelerated approval for the treatment of patients with locally advanced or metastatic urothelial carcinoma (UC) who have disease progression during or following platinum-containing chemotherapy. While PD-L1 expression in tumor or infiltrating immune cells has been valuable in the prediction of clinical responses, new biomarkers and prognostic factors should be investigated to support precision medicine approaches.

Methods

In a phase 1/2 trial, durvalumab was administered intravenously at 10 mg/kg to 182 patients with UC Q2W for up to 12 months. PD-L1 positivity was defined as $\geq 25\%$ expression in tumor cells or tumor-infiltrating immune cells. Ninety-three serum proteins with inflammatory, growth factor, or metabolic functions were measured by multiplex immunoassay in samples from 158 patients prior to durvalumab treatment.

Results

Univariate analysis identified IL8, CRP, and IL6 to be the most significant prognostic factors among all measured proteins for overall survival (OS) of UC patients receiving durvalumab (log-rank $P < 0.00001$).

Their baseline concentrations were significantly higher in progressive disease (PD) compared with stable disease (SD), complete or partial responders (Kruskal rank sum $P < 0.01$). Fast progressors ($n=36$) who received only 1-2 doses of durvalumab demonstrated higher baseline levels of the 3 proteins versus progressors who stayed on treatment for >2 doses of durvalumab ($n=60$; Mann-Whitney $P < 0.001$). Similarly, higher macrophage colony-stimulating factor (M-CSF) levels were associated with shorter OS and PD. In contrast, higher IFN γ -inducible proteins, CXCL9 and CXCL10, were associated with longer OS, along with TNF-related weak inducer of apoptosis (TWEAK). Using Cox stepwise proportional hazards modeling, we evaluated the impact of protein markers, PD-L1 status, and various clinicopathological factors on OS for 120 patients with complete data for all variables measured. IL8 levels above median showed the highest hazard ratio (HR=5, $P < 0.001$), while CRP and IL6 were no longer associated with OS when the effects of other factors were taken into account. CXCL9 and TWEAK levels below median had HRs of 3.4 ($P < 0.001$) and 2.9 ($P = 0.002$), respectively, in the multivariate model, which also included low albumin levels (HR=4, $P < 0.001$), liver metastasis (HR=3, $P = 0.001$), and low PD-L1 (HR=2, $P = 0.03$).

Conclusions

Our results demonstrate the association of high serum concentrations of myeloid cell-associated proteins and low concentrations of IFN γ -inducible proteins with shorter OS in UC patients receiving durvalumab. Myeloid-associated proteins may identify patients with myeloid cell-regulated resistance mechanisms to immune checkpoint blockers, which may be further explored as predictive biomarkers for combination therapy with macrophage-targeting agents.

Trial Registration

ClinicalTrials.gov Identifier NCT01693562

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P33

The presence of effector immune cells in human CD200 positive tumor samples supports the CD200 immune checkpoint as a novel therapeutic target

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Background

CD200 (OX-2) is an immune checkpoint protein expressed by a number of immune cells (e.g. B-cells, T cells, macrophages), and non-immune cells, (e.g. endothelial cells, neurons). CD200 binds to its receptor (CD200R) expressed on antigen-presenting cells and T cells, and is believed to play an important role in normal immune homeostasis.

Overexpression of CD200 by tumor cells implicates CD200 in tumor-mediated immunosuppression and regulation of anti-tumor activity. Samalizumab is a fully humanized therapeutic monoclonal antibody against CD200 and its binding to CD200 disrupts the immunosuppressive CD200-CD200R interaction.

Samalizumab is currently under investigation in two Phase 1 trials in patients with acute myelogenous leukemia and solid tumors (NCT02987504 and NCT03013998).

Recent evidence suggests that the presence of immune effector cells within tumors is critical for clinical response to immune checkpoint therapy. Similarly, the expression of the immune modulatory target within the tumor may correlate with response to therapy. The purpose of this study was to identify tumor types which may be most sensitive to samalizumab, based on CD200 expression and the presence of immune effector cell populations within the tumor.

Methods

Publically available tumor gene expression data was mined to evaluate CD200 expression in multiple tumor types and to develop an immune cell gene signature that may correlate with response to samalizumab. To confirm the gene expression data, we analyzed a series of adult and pediatric tumor sections by immunohistochemistry for expression of CD200 and the infiltration of immune cells.

Results

Gene expression values for CD200 were described as the percentage of samples with expression higher than the median for the set of all tumors. A “samalizumab competent” gene signature was developed from genes which co-correlate with CD200R expression across tumor types, and includes markers of T cells and macrophages; this signature was used to identify tumor types which harbor CD200R-expressing immune infiltrates that may mediate sensitivity to samalizumab. CD200 gene expression and gene signature scores were calculated for all tumor samples and expressed as the percentage of tumor samples which express the signature at a higher level than the overall median. Immunohistochemistry and gene expression data were highly concordant.

Conclusions

We explored both CD200 expression and the presence of CD200R+ immune infiltrates in multiple tumor types. These data point to a role for CD200 signaling in certain tumors, and the potential for anti-tumor activity of samalizumab.

P34

Pre-existing anti-therapeutic antibodies against Fc-region determinants shared by rituximab and hu14.18K322A are associated with outcome in a phase I trial of hu14.18K322A

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Background

Tumor-reactive monoclonal antibody (mAb) therapy has been successful in a growing number of clinical settings, including the use of anti-GD2 mAb as a treatment component for high-risk neuroblastoma. Following treatment with a therapeutic mAb, patients may generate an endogenous antibody response against that mAb. Depending upon the strength and specificity of this antibody against the mAb, this anti-therapeutic antibody response can potentially augment the clearance, or neutralize the molecular functions, of the mAb. For chimeric or humanized therapeutic mAbs, these neutralizing antibodies are often directed against the hypervariable, antigen-binding region (idiotype), of the mAb's Fab components. Patient derived antibody responses directed against non-variable sites on chimeric, humanized, or fully-human mAbs have been less well studied. We describe the detection of Pre-treatment Anti-Therapeutic Antibodies (PATA) in the sera of patients with neuroblastoma who were treated in a Phase I study of the anti-GD2 mAb hu14.18K322A. We sought to serologically characterize these PATA and evaluate for any clinical correlations.

Methods

Enzyme-linked immunosorbent assays (ELISA) were performed on pre- and post-treatment patient sera to detect the presence of anti-therapeutic antibodies. Sera from PATA+ patients were further

characterized in ELISA for reactivity against other known antibodies. The association between the PATA data and clinical outcome data were assessed.

Results

Among samples from 38 patients obtained prior to mAb therapy, 9 (24%) patients demonstrated serological recognition of hu14.18K322A. Unlike the sera from patients who generate post-treatment neutralizing antibody responses, PATA sera did not diminish mAb detection in circulation or inhibit its antigen binding ability in vitro. In 5 of 5 PATA+ patients, IgM is a component of PATA reactivity. Sera from 8 of 9 PATA+ patients demonstrated preferential recognition of the Fc-portions of 14.18K322A and rituximab (both IgG1) as compared with the Fab portions of these mAbs. PATA recognized 14G2a and mouse IgG2a isotype mAbs, but did not recognize mouse IgG1 isotype or the fully human panitumumab (IgG2) mAb. Of the 38 treated patients, only 4 (all PATA+) demonstrated no disease progression for >2.5 years without receiving further therapy ($p=0.002$; Fisher's exact test).

Conclusions

This study demonstrates an association between the presence of pre-existing antibodies against a tumor-reactive mAb and a favorable outcome. This association may suggest that PATA are playing a role in augmenting mAb-based anti-tumor effects. Further screening for the presence of non-neutralizing antibodies, analyzes of their clinical correlates, identification of their immunologic targets, and potential anti-tumor mechanism(s) are warranted.

P35

Withdrawn

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P36

Computational tissue analysis–based quantification of tumor-infiltrating leukocytes using morphometrics in immunohistochemistry stained NSCLC samples

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Background

Quantification of tumor-infiltrating lymphocytes (TILs) is predictive of patient response to immunotherapy regimens. Because of the role of anti-PD-L1 and anti-PD-1 treatments in promoting tumor-specific cytotoxic T cell responses, the identification and quantification of TILs and PD-L1–positive tumor cells by immunohistochemistry (IHC) may provide important information for disease prognosis. Using Computational Tissue Analysis (cTA™)–based morphometric features instead of an IHC-based lymphocyte assay enables a simpler workflow for TIL assessment and can be combined with additional IHC assays, enabling the identification and classification of immune cell subsets within the complex tumor microenvironment.

Methods

Non–small cell lung cancer (NSCLC) samples were stained with IHC assays for CD45, CD3, CD8, and PD-L1. Serial sections were stained with appropriate isotype-negative controls. cTA™ tools were used to determine the morphometric parameters that identified hematoxylin-stained leukocytes on isotype-stained slides and, once developed, to quantify the total immune cell population in tumor nests and surrounding stroma. The same morphometric ruleset was used to quantify total leukocytes in CD45-, CD3-, CD8-, and PD-L1–stained tissues. The results of this analysis were then compared with the results of the biomarker-specific

assay to evaluate the frequency of biomarker-positive TILs in the context of total infiltrating leukocytes quantified by the cTA™ ruleset.

Results

Morphometric parameters were established that determined the number of hematoxylin-stained leukocytes correlated with the number of leukocytes identified by CD45 in serial sections. The relative populations of CD3-positive and CD8-positive TILs were consistent with available literature findings. The cTA™ morphometric ruleset separated PD-L1–positive leukocytes from PD-L1–positive tumor staining in the tumor nests and surrounding stromal tissue.

Conclusions

Methods were developed that use morphometric features to identify the total number of infiltrating immune cells in tumor tissues stained with hematoxylin and eosin or by IHC biomarker assays. These methods provide an additional dimension of data without requiring additional biomarker staining. In some IHC assays, like anti-PD-L1 assays, both the immune infiltrate and tumor cells may stain positive, and the accuracy and precision of quantifying one population by visual pathology is hindered by the staining of the second population. The cTA™-based methods can be used to aid pathologists in the interpretation of these types of biomarker assays in which both tumor cells and immune infiltrate may stain positive

P37

Combining *in situ* vaccination with checkpoint blockade enhances an endogenous anti-tumor B-cell response resulting in tumor-specific humoral memory

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Background

In a murine model of 5-week established (~200mm³) melanoma, we previously reported that combined treatment with radiation and intratumor (IT) injection of anti-GD2 hu14.18-IL2 immunocytokine (IC) results in an *in situ* vaccine effect, rendering most mice disease-free and eliciting tumor-specific T cell-dependent immunologic memory. In the treatment of larger 7-week (500 mm³) melanomas, combining this *in situ* vaccine with anti-CTLA-4 resulted in greater complete tumor regression (73%) and enhanced survival compared to dual combinations of radiation, IT-IC, and anti-CTLA-4. All mice (17/17) rendered disease-free by this triple combination therapy exhibited immunologic memory. Here, we evaluate whether this combination may trigger an endogenous anti-tumor antibody response.

Methods

GD2⁺ B78 melanoma cells were injected subcutaneously on the flank of C57BL/6 mice. After 5 weeks, B78 tumors were treated with 12Gy, IT injection of hu14.18-IL2 on days 6-10 after radiation, and IP injection of anti-CTLA-4 on days 3, 6, and 9 after radiation. Blood was drawn from mice via facial vein bleeds prior to treatment, at 10 day intervals thereafter to day 50, and from disease-free animals >90 days after radiation. Serum from these samples was tested by flow cytometry against the closely related GD2⁻ B16 melanoma (parental to B78) and the unrelated GD2⁺ Panc02-GD2 pancreatic tumor lines for the presence of tumor-specific IgM and IgG antibodies. Functional capacity of tumor-specific serum was tested using Complement-Dependent Cytotoxicity (CDC) assays.

Results

A tumor-specific endogenous IgG response was observed against GD2⁻ B16 melanoma in untreated

tumor-bearing, untreated mice, and levels of this tumor-specific IgG declined over the first ~20 days following combined treatment with 12Gy + IT-IC + anti-CTLA-4. Beginning 20-30 days after this treatment, a tumor-specific IgM response against GD2⁻ B16 melanoma was identified in most mice. T and this briefly peaked and then declined by day 50. A renewed tumor-specific IgG response was observed in serum from mice rendered disease-free with combined *in situ* vaccine and anti-CTLA-4, and the level of this tumor-specific IgG increased modestly following subcutaneous re-challenge of these mice with B78 cells.

Conclusions

In this preclinical melanoma model, combined treatment with 12Gy + IT-IC + anti-CTLA-4 augments an endogenous antibody response to the B78 tumor, resulting in a memory humoral response in animals rendered disease-free. This may suggest an opportunity to improve this treatment regimen by using the endogenous anti-tumor B cell response as a biomarker of adaptive anti-tumor immunity.

P38

A strategy to assess contributions of individual agents in combination immunotherapy trials

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Background

A recent FDA workshop sought discussion on how to demonstrate the contribution of each individual

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agent in combination immunotherapy trials. Here we outline a strategy of coordinated assessment of B and T cell response kinetics to a complex cancer vaccine as a possible model for such an evaluation. This strategy was applied to assess patients receiving DPV-001 DRibbles[®], a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and > 200 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. Based on preclinical studies where anti-OX40 significantly ($P < 0.05$) improved survival and apparent cures, a clinical trial of DPV-001 plus anti-OX40 is planned and will use the proposed strategy to monitor the impact of this combination for cancer immunotherapy.

Methods

Patients received induction cyclophosphamide followed by 7 vaccines at 3-week intervals. The first vaccine was given intranodally; subsequent vaccines intradermally. Patients were randomized to receive DRibble alone (A), or with imiquimod (B) or GM-CSF (C). Thirteen pts were enrolled (Arm A: 5; B: 4; C: 4). PBMCs and serum were collected at baseline and at each vaccination to assess changes in antibodies (Ab) (Protoarray, microsphere affinity proteomics (MAP)), cytokines (Quanterix), PBMCs (flow cytometry) and TCR repertoires (Adaptive immunoSEQ).

Results

IgG levels to 5000 proteins was assessed thrice prior to vaccination and at 3-week intervals. For some antigens, IgG responses peaked and then returned to baseline with new Ab responses developing or being augmented at each time point. In others, Ab responses were maintained at multiple time points. Ab responses were detected against proteins whose genes were commonly upregulated in NSCLC, in some cases this upregulation was associated with significantly reduced survival (TCGA). Evaluation of CD4 and CD8 T cell clones by TCRseq identified significantly ($p = 0.002$) increased clonal expansion

compared to normal controls ($n = 3$). Similar to Ab responses, T cell clonal expansion exhibited expansion followed by apparent contraction.

Conclusions

This monitoring strategy identified that continued vaccination was associated with induction of new IgG Ab responses, inferring that new CD4 T cell responses were developing with repeated vaccination. Expansion and apparent contraction of CD4 and CD8 T cell clones, consistent with basic immunological principles, provides insights into combination immunotherapy strategies that might be used to augment response as well as a method to monitor for that effect.

Trial Registration

ClinicalTrials.gov Identifier NCT01909752

P39

Use of *ex vivo* histoculture to identify potential predictive biomarkers for the ICOS agonist antibody, JTX-2011

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Background

ICOS (Inducible T cell CO-Stimulator) is a co-stimulatory molecule expressed primarily on T lymphocytes. Clinical and preclinical data suggest that ICOS plays an important role in the immune response to cancer. Therefore, we generated JTX-2011, an ICOS agonist antibody currently in clinical development in advanced solid tumors in the ICONIC trial. In preclinical studies, single agent efficacy correlates with the percentage of ICOS-expressing T cells within the tumor. Thus, ICOS expression is being used as a biomarker to enrich for patients in Phase 2 of the ICONIC trial. Building

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on our biomarker-driven strategy, we have explored additional potential predictive biomarkers using *ex vivo* tumor histoculture which allows for *in vivo*-like analysis of therapies using patient intact tumor tissue. Herein, we report on the results of such analysis, including assessment of the induction of an IFN-gamma gene signature.

Methods

Tumor processing: Fresh human tumor samples were obtained post-surgery through the Cooperative Human Tissue Network. A section of each tumor was cut and fixed for IHC. 300 mM slices of remaining tumor were placed in a 6-well plate. Treatments were added into the medium and plates were incubated at 37°C. Tumor slices were stored in RNAlater after incubation.

RNA extraction and QC: Tumor slices were lysed using Qiagen's TissueLyser processor and FFPE samples were deparaffinized. RNA was extracted from FFPE and fresh tumor samples, quantified using Qubit, and QC'd using AATI's Fragment Analyzer.

Gene expression: Gene expression for interferon signature and other genes of interest were performed using Taqman qPCR probes or NanoString nCounter using the Human Immunology V2 panel.

IHC: ICOS (Spring SP98) and PD-L1 (CST E1L3N) levels were assessed by IHC.

Results

Treatment of NSCLC and HNSCC histoculture samples with either JTX-2011 or nivolumab, alone and in combination, led to induction of an IFN-gamma gene signature in a subset of the treated samples. This induction correlated with baseline ICOS expression for JTX-2011-treated samples, and with PD-L1 expression for nivolumab-treated samples. Additionally, expression of an ICOS RNA gene signature correlated with IFN-gamma induction in JTX-2011-treated samples.

Conclusions

Ex vivo histoculture is a robust tool that can be used to assess candidate predictive biomarkers, identify novel potential biomarkers, and interrogate post-dose responses to novel therapeutics. As part of the Jounce Translational Platform, we have used it to build on our JTX-2011 biomarker-driven strategy as we continue to generate new hypotheses to be tested in the context of the ICONIC trial.

P40

Deep proteomic and transcriptomic analysis of sorted T cells with a simple, integrated workflow

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Background

In addition to the long-standing efforts to understand and manipulate the immune system in the treatment of autoimmune diseases, the immune system is increasingly becoming a direct target for cancer therapeutics. These cancer immunotherapy efforts require deep biological characterization to better understand and characterize multiple rare cell populations, necessitating next-generation methods to expand or augment current flow cytometry methods. Recent advances in flow and mass cytometry have greatly expanded the number of cell parameters that can be interrogated resulting in an improved understanding of Immune system heterogeneity. These technologies, however, remain limited in the number and types of analytes that can be examined in a single sample.

Methods

We utilized a novel workflow integrating flow cytometry cell sorting with the nCounter® Vantage 3D™ RNA:Protein Immune Cell Profiling Assay to deeply characterize multiple flow-sorted immune cell populations. The NanoString nCounter platform

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enables the highly multiplexed digital analysis of both RNA and protein from a single biological specimen for multiple research applications. This workflow enabled us to interrogate 30 cell surface proteins and 770 immune-related RNA starting with cells in suspension. We used the unsupervised machine learning algorithms viSNE and SPADE on the Cytobank informatics platform to characterize the expression signatures across all measured proteins and RNA in each of the sorted cell populations.

Results

PBMC were co-stained with both fluorescently-labeled and NanoString DNA barcoded antibodies followed by the isolation of different T cell populations by standard flow cytometry. Using our novel workflow, we subsequently analyzed 30 proteins and 770 RNA from each sorted population with no additional staining. Demonstrating the value of this workflow in analyzing potentially rare cell populations, cells were titrated to determine the sensitivity of the workflow, producing concordant protein data from 5000 cells down to only 500 cells.

Conclusions

High-plex RNA data was obtained without the requirement for additional molecular biology methods, such as RNA purification or sequencing library construction, reducing potential for technical biases. Streamlined data analysis and visualization was accomplished by combining Cytobank with nSolver™ software packages to rapidly analyze this high-plex data. This experiment illustrates '3D Flow™' methodology, which is ideally suited for incorporation into cell sorting workflows, simultaneously producing high-plex, multiomic data from multiple rare and potentially clinically-relevant cell populations.

P41 Abstract Travel Award Recipient

IgG antibodies correspond with T cell responses to tumor neoantigens

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Background

Herein we report a survey of humoral and cellular immunity to tumor neoantigens which demonstrates interrelationships between IgG antibody and T cell responses specific to individual tumor peptides. Although a high background of preexisting autoantibody signals to thousands of normal proteins are frequently observed in IgG biomarker surveys, little work has been done to determine whether such antibodies might represent a broader immunologic history that enhances CD8+ T cell responses or adapts in concert with them. Improved understanding of any antigen-specific relationships between IgG and T cells could lead to improved immune monitoring for cancer patients and a deeper understanding of what features define clinically-relevant tumor antigens.

Methods

We sought to screen for IgG antibodies to peptides centered at single nucleotide variant and wild-type versions of known mutation sites in our 4T1 vaccine model, and hypothesized that patterns in these antibody profiles would relate to vaccine-induced T cell responses to those same mutation sites. We vaccinated female BALB/c mice with an autophagosome vaccine derived from 4T1 mammary carcinoma cells, then screened serum for IgG binding to a custom array of single nucleotide

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variant and paired wild-type peptides from those 4T1 cells.

Results

Vaccine-induced IgG signal changes correlated to antigens with stronger-predicted MHCI binding domains, indicating an overlap between the short peptide antigens recognized by antibody and CD8+ T cells. Interestingly, these IgG signal changes occurred on top of a background landscape of preexisting IgG signals. To determine whether these observations related to T cell immunity, we stimulated CD8+ T cells from naïve and vaccinated animals with a diverse selection of the top-predicted MHCI binding minimal 8-11mer peptides matching the mutation sites profiled on the antibody array. Similar to the antibody data, vaccine-induced CD8+ recognition of 4T1 cells was most improved by tumor peptides with stronger-predicted MHCI affinity. In addition, minimal peptides from Wdr33:H13Y, the mutation site garnering the strongest preexisting and post-vaccination IgG signals, also generated the largest post-vaccination IFN γ responses in multiple independent T cell experiments.

Conclusions

These results demonstrate that preexisting and post-vaccine serum antibody signals share important features with antitumor T cell responses. This could have important implications toward understanding why natural surveillance and clinical therapies either succeed or fail in creating immune responses to individual tumor antigens.

P42

The checkpoint inhibitor TTI-621 (SIRP α Fc) stimulates innate and adaptive immune responses in patients with hematologic and solid tumor malignancies

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Background

CD47 is an immune checkpoint that binds to SIRP α and delivers an anti-phagocytic (“do not eat”) signal, suppressing macrophage phagocytosis. Tumor cells frequently over-express CD47 to evade macrophage-mediated destruction. TTI-621 (SIRP α Fc) is an immune checkpoint inhibitor that promotes phagocytosis of tumor cells by blocking CD47 and engaging activating FcRs on macrophages. TTI-621 may stimulate T cell responses through enhanced antigen presentation and alteration of the tumor microenvironment. Two Phase 1 studies are underway evaluating the safety and tolerability of TTI-621 following intravenous delivery in subjects with hematologic malignancies (Study TTI-621-01; NCT02663518) and intratumoral injection in subjects with solid tumors and mycosis fungoides (Study TTI-621-02; NCT02890368). We report ongoing pharmacodynamic assessments from these trials.

Methods

In Study TTI-621-01, subjects with relapsed/refractory hematologic malignancies received weekly IV infusions of TTI-621 at 0.2 mg/kg as monotherapy or 0.1 mg/kg in combination with

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Rituximab. At the investigator's discretion, the protocol allows dose intensification in 0.1 mg/kg increments to a maximum of 0.5 mg/kg. Serial blood draws were obtained to assess receptor occupancy, cytokine/chemokine levels, T cell repertoire and immune phenotype. In Study TTI-621-02, TTI-621 was administered intratumorally starting at 1 mg/injection up to a maximum of 10 mg/injection to subjects with percutaneously accessible solid tumors and mycosis fungoides. Peripheral cytokines/chemokines, immune phenotype and gene expression were assessed in serial blood draws. Biopsies were collected to assess the impact of TTI-621 on the tumor microenvironment using gene expression, T cell repertoire, and multi-spectral imaging analyzes. Reported data are current as of July 26, 2017.

Results

Subjects receiving IV TTI-621 had increases following infusion in cytokines including MIP-1a, MIP-1b, TNF- α , IL-6, and IL-8 with median peak concentrations of 917.7, 1644.2, 392.2, 262.2, and 400.4 pg/ml at Week 1 and 168.9, 176.4, 38.0, 17.9 pg/mL at Week 6, respectively. NanoString analysis of peripheral blood and tumor tissue pre- and post-intratumoral injection of TTI-621 indicated activation of the innate and complement systems, including modulation of IFN-stimulated genes, such as *IFI27*. Increases in CD8+ and NK cells were observed in the peripheral blood of subjects. An induction of peripheral T cell clonality was observed in the majority of subjects exhibiting objective responses following IV TTI-621.

Conclusions

Pharmacodynamic analysis demonstrated that TTI-621 stimulates the innate and adaptive immune responses in relapsed/refractory cancer patients. Importantly, changes in the TCR repertoire correlated with objective responses, suggesting that innate checkpoint inhibition with TTI-621 is capable of driving an anti-tumor T cell response.

P43

Assessment of pharmacodynamic effects of immuno-oncology agents in cynomolgus monkeys using high-content gene expression profiling

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Background

Non-human primate studies serve as valuable tools for preclinical safety and pharmacokinetic (PK) evaluation; however, their utility in Pharmacodynamic (PD) assessment of immunomodulatory agents in immuno-oncology is limited. Here, we assessed PD effects of immunomodulatory agents in Cynomolgus monkeys (*Macaca fascicularis*) using a high-content gene expression platform (nCounter Non-Human Primate (NHP) Immunology Panel, NanoString) that covers 20 immunologically relevant pathways.

Methods

NHP nCounter codeset used in the study, covers 770 genes and spans around 20 immune-related signaling pathways including but not limited to toll-like receptor, interferon signaling, innate and adaptive immune response. Blood was collected from naïve Cynomolgus monkeys, before and at various time points after intravenous administration of experimental immunotherapies. Based on previously generated Quantigene Plex gene expression data from four different studies, nCounter analysis was focused on select time points/samples along with respective controls. Two different sample types, purified RNA from

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peripheral blood cells and whole blood lysates, were also evaluated.

Results

nSolver software (NanoString) was used for quality control and normalization of the data. The positive control scaling factor was within the recommended range of 0.3-3, indicating that there were no platform-associated sources of variation. Due to the variability in the negative controls background subtraction was excluded from analysis. Expression levels of housekeeping genes (HKGs) were similar across samples, and all 16 HKGs were included in the analysis. Approximately 300-350 genes were below lower limit of detection under conditions tested and were removed from analysis. The nCounter assay demonstrated comparable performance on purified total RNA and whole blood lysates. Depending on the immunomodulatory agent studied, the number of differentially expressed genes (pre- vs post-dose) ranged from 3 to 76. Principal Component Analysis revealed that gene expression levels post-dose segregated from those of pre-dose. Most of the gene expression changes observed were indicative of peripheral innate immune response exemplified by upregulation of genes attributable to monocytes/macrophages and/or granulocytes.

Conclusions

Taken together, the data indicate that the nCounter gene expression platform was capable of detecting immune-related PD effects of immunomodulatory agents in Cynomolgus monkeys using both RNA and whole blood lysates. High-content molecular platforms such as nCounter, can significantly enhance PD assessment and broaden understanding of immune-related changes in NHP studies which may facilitate informative decision making and PK/PD modeling in clinical trials

P44

Patient selection strategies and pharmacodynamic assays for CCR4 antagonists

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Background

Regulatory T cell (T_{reg})-mediated suppression of effector T cells in the tumor microenvironment (TME) can diminish anti-tumor immune responses. The CCR4 receptor can mediate recruitment and accumulation of T_{reg} in the TME making it an ideal target for improving anti-tumor immune responses. We have previously reported on the development of potent and selective CCR4 antagonists. As these move towards the clinic, it is important to have a strategy for selecting patients most likely to respond to this therapy and to measure CCR4 engagement with our inhibitors in these patients once clinical trials begin.

Methods

Data from The Cancer Genome Atlas (TCGA) was mined for expression of relevant genes and signatures. Tumors were classified based on their inferred levels of immune infiltrate, and signatures for T_{reg} and CD8⁺ T cells. RNAScope® (RNA *in situ* hybridization) was used on more than 400 tumor biopsy cores to confirm expression levels and localization of key genes. For receptor occupancy, whole blood from healthy donors or cancer patients was incubated with CCR4 antagonist prior to addition of fluorescently-labeled hCCR4 ligand, hCCL22 (A647-hCCL22). At 37°C and in the absence of a CCR4 antagonist, A647-hCCL22 stimulates receptor internalization and accumulation of fluorescence signal. Cells were then antibody-stained and interrogated by flow cytometry to measure CCR4-inhibition in T_{reg}.

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Results

Many tumor types show elevated expression of the CCR4 ligands, CCL17 and CCL22, and increases in T_{reg} and CD8 signatures, with head and neck (HN), stomach, lung, and breast cancer types showing strong enrichment. Subtype analysis shows further immune signature enrichment in subtypes such as Triple Negative Breast and EBV⁺ stomach cancers. RNAScope analysis supports these observations, with lung and HN, in particular, showing strong clustering of T_{reg} and CD8 cells. In the receptor occupancy assay, our CCR4 inhibitors demonstrate dose-dependent reductions in A647-hCCL22-induced internalization. This assay is highly reproducible within donors, on different days, and between operators. This signal stability makes it a robust PD assay for our First-in-Human trials.

Conclusions

Based on RNA expression and *in situ* hybridization analysis, we hypothesize that patients with a variety of tumors including types of breast, lung and HN cancers, would be good candidates for treatment with CCR4 antagonists. Once these patients are enrolled in clinical trials, robust assays to monitor receptor occupancy and establish pharmacokinetic-pharmacodynamic relationships are ready for deployment.

P45

The inhibitory checkpoint molecule NKG2A is upregulated on tumor infiltrating NK cells and CD8 T cells in human head and neck tumors

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Background

Immunotherapy has revolutionized cancer therapy by targeting checkpoint molecules found on endogenous immune cells. Presently, the most commonly targeted checkpoint molecules are CTLA-

4 and PD-1, which results in response rates of approximately 25% in head and neck squamous cell carcinomas (HNSCC). To improve treatment, additional immune checkpoint molecules expressed in the tumor microenvironment must be identified. Natural Killer Group 2 A (NKG2A) is an inhibitory receptor found on NK cells and CD8 T cells, the receptor for which is HLA-E, a non-classical MHC molecule often overexpressed in solid tumors. This study aimed to identify if NKG2A is expressed on tumor infiltrating NK cells and CD8 T cells from human HNSCC tumors as it is a potential therapeutic target.

Methods

Fresh human HNSCC tumors were digested with miltenyi human tumor dissociation kit and Gentle Macs machine following manufactures instructions. Cells were then stained for surface phenotyping or frozen for functional assays.

Results

We analyzed and compared tumor infiltrating NK cells and CD8 T cells from HNSCC patients with matched PBMC by flow cytometry for the expression of activating and inhibitory receptors. We found a unique population of effector memory PD-1⁺ NKG2A⁺ CD8 T cells which was absent from the blood. NKG2A⁺ PD-1⁺ CD8 T cells expressed higher levels of CTLA-4 and LAG3 as well as produced lower IFN γ than NKG2A⁻ PD-1⁺ CD8 T cells. Interestingly, NKG2A⁺ PD-1⁺ CD8 T cells expressed higher levels of both Perforin and Granzyme B, suggesting that these cells are cytotoxically potent. We found a similar upregulation of NKG2A in the NK cell population. NK cells from the primary tumor, but not the blood of HNSCC patients, significantly increased expression of inhibitory KIR2DL4, PD-1 and NKG2A. In addition, we determined that the ligand for NKG2A, HLA-E, was abundantly expressed on CD45⁺ monocytes and T cells, but not on CD45⁻ cells in the tumor.

Conclusions

We believe that this study provides the first characterization of human tumor-infiltrating

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NKG2A+ PD-1+ CD8 T cells. We have shown that NKG2A+ CD8 T cells express the highest levels of cytotoxic markers, suggesting an enhanced capacity to kill tumor cells, yet also the highest levels of checkpoint molecule expression. As we have also shown that NKG2A is upregulated in tumor infiltrating NK cells, we believe this makes NKG2A an ideal therapeutic target to improve anti-tumor cytotoxic responses. Ongoing studies are underway to determine the effect of inhibiting NKG2A on tumor immune responses.

P46

The impact of anti-PD-1 treatment on the immune cells and their correlation with the circulating tumor cells in patients with non-small cell lung cancer

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Background

The major immune-check point, programmed cell death-1 (PD-1), plays a pivotal role in tumor immune escape. The interaction of PD-1 with its ligand (PD-L1) results in T cells exhaustion, and the blockade of this interaction can partially restore T cell function. Antibodies targeting PD-1 and PD-L1 have been approved for treatment of advanced Non-Small Cell Lung Cancer (NSCLC). In this pilot study, we aimed to investigate the effect of anti-PD-1 treatment on the frequencies of circulating PD-1⁺ and PD-L1⁺ immune cells (ICs), as well as their correlation with circulating tumor cells (CTCs) in NSCLC patients.

Methods

Peripheral blood samples were collected from 24 advanced NSCLC patients before and after 1 and 3 cycles of anti-PD-1 treatment. Flow cytometry and immunocytochemistry were used to quantify the changes of the frequencies of PD-1- and PD-L1-expressing ICs and CTCs, respectively.

Results

At baseline, CTCs and PD-L1⁺CTCs were negatively correlated with PD-1⁻CD8⁺ ($p=0.03$ and $p<0.0001$, respectively). Moreover, PD-1⁻CD4⁺T had a reverse correlation with PD-L1⁺CTCs ($p=0.04$), whereas PD-1⁺CD4⁺T cells were positively correlated with PD-L1⁺CTCs ($p<0.0001$). A significant decrease in PD-1⁺CD4⁺ and PD-1⁺CD8⁺ T cells was observed after 3 administrations of anti-PD-1 antibody ($p=0.01$ and $p=0.05$, respectively). In contrast, PD-1⁻CD4⁺ ($p=0.05$) and PD-1⁻CD8⁺ ($p=0.05$) levels were increased in response to anti-PD-1 therapy. After one dose of anti-PD-1 therapy, these increased levels of PD-1⁻CD8⁺ and PD-1⁻CD4⁺T cells were associated with reduced levels of CTCs ($p<0.0001$). Most importantly, PD-L1⁺CTCs were reversely correlated with PD-1⁻CD8⁺ ($p<0.0001$) and PD-1⁻CD4⁺T cells ($p<0.0001$) following 3 doses of treatment. Patients, with non-progressive disease after 3 doses of treatment, had reduced percentages of PD-1⁺CD4⁺ ($p=0.05$) and PD-1⁺CD8⁺ T cells ($p=0.002$), whereas the percentages of PD-1⁻CD4⁺ ($p=0.04$) and PD-1⁻CD8⁺ ($p=0.004$) T cells were increased compared to baseline. Finally, the levels of CD4⁺PD-L1⁺ Tregs and PD-L1⁺G-MDSCs (granulocytic-MDSC) were decreased after 1 dose of anti-PD-1 therapy ($p=0.01$ and $p=0.02$, respectively). No correlation was observed between CTCs and immunosuppressive cells at any time point.

Conclusions

These data indicate that anti-PD-1 therapy seems to exert an effect on circulating immune cells and provides evidence for their possible interaction with CTCs via the PD-1/PD-L1 axis, in NSCLC patients. These findings may pave the way for additional studies in a larger cohort in order to document its impact in NSCLC patients.

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P47

Gene expression analysis of IL-13R α 2 in human adrenocortical carcinoma correlates with poor survival

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Background

Adrenocortical carcinoma (ACC) is a rare disease where cancer cells develop in the outer layer of the adrenal gland. Because of unreliable early detection, the five year survival rate of subjects with ACC is only 65%. In a variety of cancers including glioblastoma, ovarian, and pancreatic cancers, expression of the interleukin-13 receptor α 2 (IL-13R α 2) has been shown to be a prognostic biomarker and potential target for immunotherapy. Using RNA sequencing data from the Genomic Data Commons (GDC), we analyzed the association between IL-13R α 2 gene expression levels with duration of survival and disease outcome in the ACC patients.

Methods

ACC datasets were accessed from the GDC [<https://portal.gdc.cancer.gov/legacy-archive>] in January 2017. Seventy-nine samples, 48 female and 31 male, were categorized and merged using JMP Genomics. Samples were then evenly distributed into three groups based on low, medium or high levels of IL-13R α 2 expression. Statistical analyzes were performed to compare survival, hormone levels, and tumor reoccurrence with IL-13R α 2 expression.

Results

Subjects with high IL-13R α 2 expressing tumors (n = 27) showed a statistically significant lower survival rate (56%) compared to patients with low IL-13R α 2 expressing tumors (88%) (n = 26) (p= .0135). Subjects with high serum hormone levels (cortisol, estrogen, androgen) exhibited a worse outcome;

high IL-13R α 2 expression was associated with a greater incidence of high serum hormone level and poorer survival compared to low IL-13R α 2 expressing subjects. Subjects with new tumor events (n = 35) such as reoccurrence or metastasis exhibited a worse survival rate and high IL-13R α 2 expression showed a higher rate of new tumor events when compared to low expression levels (p= .0042). Individuals with new tumor events exhibited a lower rate of survival, whereas individuals with high IL-13R α 2 expression had a low rate of survival regardless of whether a new tumor event occurred or not. Subjects with metastatic tumors (n = 17) had a lower survival rate compared to those without metastatic tumors, but no correlation with survival for subjects with medium or high expression levels of IL-13R α 2 was observed.

Conclusions

IL-13R α 2 expression levels were a major determinant of outcome in ACC subjects; high IL-13R α 2 levels associated with a lower rate of survival, higher incidence rate and worse outcome with excess hormone production, and more new tumor events. Additionally, subjects with higher expression levels of IL-13R α 2 exhibited a low survival rate regardless of new tumor events or metastases. These results indicate that IL-13R α 2 may be a prognostic biomarker in subjects with ACC.

P48

Different impact of immune cell infiltration and HLA class I expression in lymph node vs. cutaneous/subcutaneous metastases as predictive markers in melanoma patients treated with ipilimumab

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Background

Immunomodulatory antibodies targeting immune checkpoints are gaining ground in cancer treatment, with simultaneous efforts to search for clinically usable predictive markers which could help making treatment decisions. The aim of our study was to explore tumor-infiltrating immune cells and HLA class I expression as potential biomarkers of response to ipilimumab and survival in patients with metastatic melanoma.

Methods

In pretreatment surgical tissue samples (52 lymph node and 34 cutaneous or subcutaneous metastases from 30 patients), intratumoral immune cell infiltration was determined using antibodies against a panel of 11 markers (CD4, CD8, CD45RO, CD20, CD134, CD137, FOXP3, PD-1, NKp46, CD16, CD68). Tumor infiltration by each cell type was evaluated with regard to response to treatment and patients' survival. Expression level of HLA class I antigens and its correlation with immune cell densities as well as with response to ipilimumab and disease outcome was also examined.

Results

Immune cell infiltration in lymph node metastases was markedly higher compared to skin or subcutaneous ones in the case of the majority of markers studied (9 of 11). Evaluated separately in the two locations, in the group of lymphoid metastases significantly larger amount of CD4⁺, CD8⁺, FOXP3⁺, CD134⁺ lymphocytes, CD20⁺ B cells and NKp46⁺ NK cells was found in responders compared to nonresponders. The most pronounced differences were seen in the case of FOXP3 and

CD8, which also showed association with patients' survival. Infiltration level of CD45RO⁺, PD-1⁺, CD16⁺ and CD68⁺ cells correlated with survival but not with treatment response. On the other hand, analyzing subcutaneous/cutaneous metastases revealed significant associations with clinical response or survival only in the case of CD16⁺ and CD68⁺ cells. Furthermore, HLA class I antigen expression level of lymphoid metastases, but not that of skin or subcutaneous ones, showed correlation with T cell density and with patients' survival.

Conclusions

Our results indicate different predictive impact of immune cell infiltration and HLA class I expression in lymphoid vs. non-lymphoid metastases of melanoma patients treated with ipilimumab. This finding points to the potential importance of analyzing metastases of different locations separately in order to reveal all existing predictive associations, part of which could be obscured in the case of joint evaluation because of high variation in immune cell prevalence.

P49

Characterization of immune checkpoint marker expression and infiltrating lymphoid and myeloid immune cells in the tumor microenvironment by RNA in situ hybridization

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Background

Interactions between tumor cells and surrounding immune cells in the tumor microenvironment (TME) play a key role in tumor progression and treatment response with accumulating evidence indicating a crucial role for tumor infiltrating immune cells. Although infiltrating T cells have been correlated with improved clinical outcome, tumor infiltrated lymphocytes are ineffective in eradicating tumors

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due to their inhibition by immune checkpoint molecules. Cancer immunotherapy blocking the PD-1/PD-L1 immune checkpoint pathway is an established treatment with long-lasting clinical benefits. However, the majority of patients are resistant or relapse after initial response, showing the need for understanding resistance mechanisms and stratifying patients using predictive biomarkers. Also, immunosuppressive factors in the local TME contribute to tumor evasion by recruiting and modulating regulatory T cells and myeloid-derived cells.

Methods

In this study, we used RNAscope® ISH to evaluate *in situ* expression profiles of therapeutic checkpoint targets in the TME of 30 NSCLC and 30 ovarian cancer archived FFPE tissue samples. In addition, immune infiltration of lymphoid and myeloid cells in the TME was investigated.

Results

Specific checkpoint target molecules were visualized in a highly specific and sensitive manner in individual cells within tissue morphological context. Multiple checkpoint molecules, including PD1, PD-L1, TIM3, and CTLA-4, were detected in the same immune environment, especially in highly inflamed tumors. Unexpectedly, we observed tumor cell-intrinsic expression of TIM3, LAG3, PD-L2, and GITR in a subset of samples. Furthermore, PD1 was often co-expressed with other therapeutic checkpoint targets, including LAG3, TIM3, and TIGIT, in the same infiltrating immune cells. Expression patterns of key functional markers FOXP3, IFN γ , CXCL10, and CCL22 combined with cell lineage markers CD4, CD8, CD68, and CD163 demonstrated infiltration of lymphoid and myeloid cells across samples. Recruitment of CXCL10-expressing immune cells in local tumor regions was visualized. The presence of regulatory T cells (FOXP3+CD4+) was evaluated in relation to CCL22 expression in immunosuppressive local environment. IFN γ positive CD8 cells were rarely detected.

Conclusions

These findings highlight the utility of RNAscope® ISH in understanding how cancer cells evade the host immune surveillance and ultimately develop resistance against checkpoint blockades. This approach visualizes states of lymphoid and myeloid cells present in the TME by detecting key functional molecules, such as cytokines and chemokines, in addition to immune co-inhibitory and lineage molecules. Considering the highly plastic immune environment with a spectrum of maturation and polarization, characterizing the immunomodulatory phenotype by RNAscope® ISH could offer fundamental insights in contributing factors in immunosuppressive environment.

P50

Application of the immunoscore as prognostic biomarkers in patients with epithelial ovarian cancer

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Background

The analysis of single parameters alone may not provide sufficient insights about complex immune system–tumor interactions. This study is to validate the immune contexture as prognostic biomarkers in high-grade serous ovarian cancer (HGS-OC) and to find new era of immunoscore in HGS-OC.

Methods

We collected FFPE samples from 187 patients with HSOC and produced TMA samples. We accomplished the OPAL multiplex IHC assay for the quantitative analysis of immune markers, including CD4, CD8, CD20, FoxP3, PD-L1, and CK. Multiplex Biomarker Imaging and inForm® Image Analysis Software was used.

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Results

FIGO stage III and IV patients were 84.5% (158/187). The optimal debulking surgery was done in 66.8% (125/187). The 3-year disease-free survival and 5-year overall survival were 35.1% and 50.0%, respectively. Any single marker was not related to the survival including CD8, FoxP3, and PD-L1. However, high CD8:FoxP3 and CD8:PD-L1 ratios were correlated with the good survival. In cox regression model, the risk factors for HGS-OC survival were FIGO stage (HR 1.784, 95% CI: 1.295-2.457, $p < 0.001$) and platinum resistance (HR 4.257, 95% CI: 2.753-6.582, $p < 0.001$). Additionally, CD8:PD-L1 ratio was a favorable prognostic factor (HR 0.621, 95% CI: 0.042-0.917, $p = 0.017$).

Conclusions

These findings indicate that, although any single immune marker is not related to the survival, CD8:FoxP3 and CD8:PD-L1 ratios provide the positive correlation with the prognosis in HGS-OC. Especially, CD8:PD-L1 ratio is prognostic biomarker which is comparable to clinical biomarkers. The next study for immunoscore is necessary to define immunoscore in ovarian cancer.

P51

FGFR2b expression and baseline immune signature to guide FPA144 development in urothelial cancer

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Background

Five Prime Therapeutics, Inc. has developed an FGFR2b-specific humanized monoclonal antibody, FPA144, to treat cancer patients with overexpression of the FGFR2b receptor. FPA144 blocks ligand binding and has been glycoengineered for enhanced antibody-dependent cell-mediated

cytotoxicity. Our preclinical models showed FPA144 reprograms the tumor microenvironment by recruiting natural killer cells to the tumor, upregulating PD-L1 expression, and enhancing T cell infiltration [1]. FPA144 is currently in clinical development in gastric and urothelial cancers (UC). FPA144 completed dose escalation in patients with solid tumors and was well tolerated with no dose-limiting toxicity. During dose escalation, a patient with metastatic UC treated with FPA144 at 3 mg/kg exhibited a complete response [2]. Currently, UC patients have enrolled in the dose expansion portion of the trial. We characterized baseline immune cells in the tumor microenvironment and their relationship with FGFR2b in UC patients to guide potential development of FPA144 in combination with other therapies.

Methods

We selected a cohort of archival primary UC formalin-fixed paraffin-embedded whole tumor sections (n=34) with known FGFR2b expression and performed fluorescent IHC on these sections using the proprietary FPR2-D mouse monoclonal antibody, which is specific for FGFR2b. We also performed multi-panel multiplex immunofluorescent assays to quantify immune cell compositions in sequential sections of UC samples.

Results

FGFR2b H scores positively correlate with tumor-infiltrating CD163, PD-L1/CD8 and PD-L1/CD68 cells. From baseline immune compositions and FGFR2b expression level, we clustered cases into three distinct groups: 1) Immune desert (low CD8 T cells, tumor-associated macrophages (TAM), and PD-L1 expression in the whole tumor) with low or negative FGFR2b; 2) Immune excluded (high CD8 T cells, TAM, and PD-L1 expression in tumor-associated stroma) with low to moderate FGFR2b; 3) Inflamed (high CD8 T cells, TAM, and PD-L1 expression in the whole tumor) with moderate to high FGFR2b.

Conclusions

The UC cases with high FGFR2b expression have higher tumor-infiltrating T cells, TAM and PD-L1

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expression compared to FGFR2b low or negative tumors. Profiling FGFR2b expression and baseline tumor associated immune cells could help FPA144 development strategy in UC patients. We are currently exploring the utility of combining FPA144 with PD-1/PD-L1 blockade in clinic.

Trial Registration

ClinicalTrial.gov Identifier NCT02318329

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P52

Partially exhausted T lymphocyte directed neoadjuvant immunotherapy in unresectable Stage III melanoma

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Background

Programmed death 1 (PD-1) inhibition activates partially exhausted cytotoxic T lymphocytes (peCTL) and induces tumor regression. We have previously demonstrated that the peCTL frequency predicts response to anti-PD-1 monotherapy and combination CTLA-4/PD-1 blockade in metastatic melanoma. However, the utility of this assay in the neoadjuvant setting has not been established. In the current study, 15 patients with unresectable Stage III Melanoma were assessed for response to

neoadjuvant anti-PD-1 or anti-PD-1/CTLA-4 inhibition as assigned by peCTL frequency.

Methods

Pretreatment tumor samples from 15 patients with locally advanced melanoma underwent multiparameter flow cytometric analysis. Patients received neoadjuvant anti-PD-1 monotherapy or combination therapy and best overall response to therapy (BOR) as evaluated by Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) and irAE incidence and severity by NCI Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0) were tabulated by clinicians blinded to immunophenotypic analysis. Patients with low peCTL burden (<20%) received anti-PD-1/CTLA-4 inhibitors whereas those with high peCTL (>20%) were assigned to anti-PD-1 monotherapy. Patients with documented follow up history and evaluable immunophenotype were included in efficacy and safety analysis.

Results

Of 82 patients screened, 15 met criteria for further analysis. Patients were 60% male with mean age of 58 years. Twelve patients had Stage III disease with LDH<ULN, and 13 out of 15 patients were BRAF WT. Prior therapy included CTLA-4 monotherapy in 4 of 12 patients, with only 1 patient with prior PD-1 inhibitor exposure or intralesional therapy. Eleven patients received anti-PD-1 monotherapy and 4 received CTLA-4/PD-1 blockade and were evaluable for BOR at time of analysis. Eleven out of 15 patients achieved complete response, with two partial responses, one patient exhibiting stable disease and one patient with progressive disease per RECIST v1.1 criteria. Of the 9 patients that underwent surgery, 6 attained complete pathologic response. Relapse free survival was 26 months and all patients remained alive at time of analysis. Four cases of disease relapse occurred, without correlation with peCTL frequency. Treatment was well tolerated, with grade 1/2 diarrhea, pruritus, arthralgia, and hypothyroidism most frequently reported. Grade 3/4 adverse effects included two isolated cases of gastritis and anaphylaxis.

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Conclusions

Our results suggest the efficacy of peCTL directed neoadjuvant treatment in unresectable Stage III melanoma with the large proportion of patients achieving complete treatment response with acceptable toxicity profile. These promising data from this exploratory analysis merit further investigation with a larger cohort validation study.

Trial Registration

UCSF IRB Protocol 138510

P53

The crosstalk between PD-1, CD137 and OX40 in head and neck cancer

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Background

Head and neck squamous cell carcinoma (HNSCC) is one of the malignant diseases with highly immunosuppressive tumor microenvironment, which limits the efficacy of cancer therapies. Tumor infiltrating lymphocytes (TIL) express immune checkpoint receptors in the microenvironment, which can be divided into two groups, inhibitory receptors, such as Programmed Death 1 (PD-1), and costimulatory receptors, such as CD137 and OX40. However, the T cell in TIL exhibit dysfunctional status and the inhibitory receptors has more dominant effect than the costimulatory receptor. The aim of this study was to investigate whether there is crosstalk between PD-1, CD137 and OX40 pathways in TIL expressing multiple immune checkpoint receptors. [1, 2, 3, 4, 5]

Methods

We used PD-L1 (the ligand of PD-1) beads alone or in combination with CD137 or OX40 agonists to treat TIL T cell for 48 hour. Also we used anti-CD3/CD28 beads treated PBL to mimic the TIL environment.

Results

TIL T cells treated with anti-CD3/CD28/PD-L1 beads expressed less CD137 and OX40, and produced less IFN-g than control-treated TIL T cells. Activated PBL T cells treated with anti-CD3/CD28/PD-L1 beads showed similar results as TIL. CD137 or OX40 stimulation could partially reverse this effect.

Conclusions

The result suggest the potential for intracellular signaling cross-talk. We are investigating the pathways is involved in the PD-1, CD137 and OX40 crosstalk.

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P54

Multiplex IHC immuno-oncology panel for standardized profiling of the immune status based on spatial and functional characterization of the tumor microenvironment

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Background

Characterization of the immune status of patients will become increasingly important to personalize cancer treatments [1]. As the number of treatment options is expected to dramatically increase in the next years, standardized information that guides treatment decision is highly needed. Therefore, we developed a multiplex Immuno-Oncology (IO) panel to profile the immune status of patient tumors into biologically meaningful and clinically actionable categories which support therapy suggestions.

Methods

Three chromogenic IHC assays were developed and optimized for image analysis (PD-L1/CD68/CD3, PD1/FoxP3/CD8, and Granzyme B) using brown (DAB), red and green chromogens for several indications. Definiens' Cognition Network Technology[®] was used for spatially resolved image analysis of biomarker expression levels combining rule-based object hierarchy detection with machine-learning strategies. Verifications strategies have been developed in preparation for the application in prospective stratification studies. Marker-positive cells were detected and classified as PD-L1+ tumor cells, (exhausted) CD8+ T cells, CD3+ T cells, regulatory T cells, and macrophages. Global and local densities were computed within regions annotated as Tumor Center and Invasive Margin as well as within automatically detected tumor epithelium (TE) and non-epithelial regions (NE).

Results

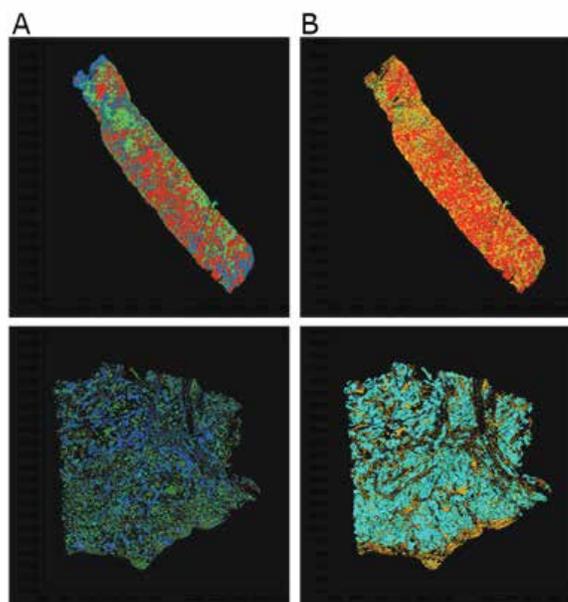
Based on the measured densities, NSCLC patients were grouped into one of six possible immune status categories and biomarker profiles have been computed. Additionally, heterogeneity between

patients within one category is discussed against the backdrop of therapy suggestions and applicability in further indications. The intra-tumoral biomarker heterogeneity is visualized with cell co-occurrence heat maps and regional context maps, providing insights on inhibition status, effector status, and checkpoint blockade (Figure 1).

Conclusions

Our IO Panel serves as a ready-to-use approach for standardized immune profiling that quantifies the set of seven and subsequently more biomarkers reproducibly on a continuous scale. Definiens' Tissue Phenomics[®] technology combined with Mosaic's multiplex IHC assays provides next generation spatial and functional resolution of the tumor microenvironment. In the future, we envision applications that facilitate the identification of novel prognostic and predictive signatures, with the goal to support stratification and guide eligibility of patients for a broad range of therapeutics.

Figure 1. Regional context maps displaying potential checkpoint blockade (A) and PD-L1 landscape (B).



Selected maps are shown for two immune status categories belonging to either hot tumors (top) or cold tumors (bottom). A: Light blue: PD-L1 negative

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T cells, Darker blue: PD-L1 negative T cells (NE), Light Green: Macrophages (TE), Dark Green: Macrophages (NE), Orange: PD-L1 positive T cells, Red: PD-L1 positive tumor cells. B: Red/Light Blue: PD-L1 positive/negative tumor cells, Orange: Macrophages, Green: T cells.

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P55

Non-fucosylated anti-CTLA-4 antibody enhances vaccine-induced T cell responses in a non-human primate pharmacodynamic vaccine model

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Background

Ipilimumab, which blocks the CTLA-4 pathway, is an effective immunotherapy treatment for melanoma and in combination with nivolumab for other malignancies. To improve the potency of this immune checkpoint inhibitor, the Fc region of ipilimumab was altered to enhance antibody-dependent cellular cytotoxicity (ADCC) activity and thereby the potential for increased T-regulatory cell depletion. Here, we assessed the ability of a non-fucosylated form of ipilimumab (anti-CTLA-4-NF) to modulate T cell activity in a non-human primate pharmacodynamics (PD) vaccine model.

Methods

Mauritian cynomolgus macaques (MCMs) were preselected for the high frequency allele *Mafa-A1063* and vaccinated intramuscularly with 2 non-replicating adenovirus serotype 5 (Ad5) viral constructs, encoding simian immunodeficiency virus (SIV) Gag or Nef proteins. Following vaccination, the

animals received a single dose of either a vehicle control, ipilimumab, or anti-CTLA-4-NF. Ipilimumab and anti-CTLA-4-NF were administered intravenously at either 1 or 10 mg/kg. Peripheral blood was sampled pre-treatment and then longitudinally over 7 weeks. Vaccine-elicited T cell responses and major T cell subsets were measured by flow cytometry, including the use of peptide-loaded MHC class I tetramers, and IFN-gamma ELISPOT assays.

Results

A single dose of ipilimumab or anti-CTLA-4-NF resulted in augmentation of antigen-specific CD8⁺ T cell responses at early (2-3 weeks post-vaccination) and later time points (>6 weeks). The resulting peripheral immune responses included T cells directed against the SIV protein inserts as well as against the Ad5 viral vector itself. Both treatments also enhanced circulating CD8⁺ and CD4⁺ T cell proliferation as measured by intracellular Ki-67 expression. Anti-CTLA-4-NF demonstrated enhancement of the vaccine-induced T cell responses over that observed in ipilimumab-treated MCMs. This included increased CD8⁺ CD3⁺ tetramer⁺ frequencies of 2 immunodominant SIV-specific T cell responses by flow cytometry (at 3 weeks post-vaccination: Nef RM9 median, 4.16% with anti-CTLA-4-NF vs 1.95% with ipilimumab treatment; Gag GW9 median, 3.08% with anti-CTLA-4-NF vs 1.17% with ipilimumab treatment).

Conclusions

Using this non-human primate PD vaccine model, we observed that immune checkpoint blockade with anti-CTLA-4 augments T cell activation and proliferation in circulating blood. These results suggest that, in addition to the use of non-human primates in toxicology models, the evaluation of antigen-specific T cell responses in MCMs provides meaningful preclinical pharmacological assessment of human drug candidates that target immune modulation. The improved T cell activity observed in these studies also lend support for the use of anti-CTLA-4 antibodies for vaccine enhancement,

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broadening the potential utility of next-generation anti-CTLA-4 antibodies beyond cancer therapy.

P56

Widespread human T cell receptor variable gene polymorphism revealed by long amplicon TCRB repertoire sequencing: Implications for the prediction and interpretation of immunotherapy outcome

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Background

Human T cell antigen receptors play a critical role in protective adaptive immune responses to infectious disease and cancer, but are also implicated in autoimmune disease and the emergence of severe immune-mediated adverse events during immunotherapy. The antigen specificity of the T cell receptor is determined in part by the sequence of the CDR and Framework regions encoded by the TCRB variable gene. Previous studies of population sequencing data indicate that current antigen receptor allele databases, such as IMGT, fail to capture a significant portion of human variation, though interpretation of this data is challenging. Here we sought to use long amplicon multiplex sequencing of rearranged TCRB receptors to validate putative novel human variable gene alleles previously recovered from 1000 genomes data.

Methods

TCRB rearrangements were amplified from cDNA template from 85 Caucasians undergoing treatment for melanoma using Ion AmpliSeq™-based multiplex

Framework 1 and Constant gene primers to produce ~330bp amplicons. Samples were sequenced in multiplex using the Ion 530™ chip to produce ~1.5M raw reads per sample. Raw data was uploaded to Ion Reporter™ for clonotyping and identification of rearrangements containing variable gene sequences absent from the IMGT database. Putatively novel sequences were compared with those reported in the Lym1k database of alleles recovered from 1000 genomes sequence data.

Results

We identified 15 variants of variable gene alleles, absent from the IMGT database, which result in amino acid changes to the CDR or Framework regions of the T cell receptor. Typically, a single individual was found to be heterozygous for a novel variant, though we note two instances of novel alleles that are found in multiple individuals within this cohort. We also find evidence for novel variable gene alleles that are absent from the Lym1k database, potentially due to challenges in inferring receptor alleles from short-read population sequencing studies.

Conclusions

We find evidence for significant human diversity in TCRB variable gene alleles beyond what is currently represented in the IMGT database. TCRB sequencing using multiplex Framework 1 and Constant gene targeting primers is ideally suited for studying the role of T cell antigen receptor diversity in autoimmune disease and the emergence of immune-mediated adverse events during immunotherapy.

P57

Insights into the tumor microenvironment and therapeutic T cell manufacture revealed by long amplicon immune repertoire sequencing

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Background

TCRB immune repertoire analysis by next-generation sequencing is emerging as a valuable tool for research studies of the tumor microenvironment and potential immune responses to cancer immunotherapy. Here we describe a multiplex PCR-based TCRB sequencing assay that leverages Ion AmpliSeq™ library construction chemistry and the long read capability of the Ion S5™ 530 chip to provide coverage of all three CDR domains of the human TCR beta chain. We demonstrate use of the assay to evaluate tumor-infiltrating T cell repertoire features and monitor manufacture of therapeutic T cells.

Methods

To evaluate assay accuracy we sequenced libraries derived from 30 well-studied T cell lymphoma rearrangements, then compared our results with those reported by another commercially available immune repertoire sequencing technology. We next used the assay to profile tumor infiltrating T cell repertoires for a cohort of 19 individuals with non-small cell lung cancer. We correlate repertoire features with gene expression profiling data. We then harnessed the long read capability of the assay to profile T cells at various stages of the therapeutic T cell manufacturing process.

Results

Long read TCRB sequencing of 30 reference rearrangements yielded strong linearity in detection of clonal frequencies in reference spike-in experiments. We demonstrate the quantitative nature of the assay by studying populations of counted T cells. Profiling of T cell repertoires in non-small cell lung cancer samples revealed a positive correlation between the number of clones detected and CCR7, CXCR5 and CD3 expression. T cell

Evenness (i.e. normalized Shannon Entropy) correlated most strongly with myeloid-specific genes and markers for T cell exhaustion and was anti-correlated with IFNG expression. Sequencing of therapeutic T cells during manufacture revealed a steady increase in the evenness of clone sizes during in vitro expansion and allowed for quantification of clonal expansion.

Conclusions

These results demonstrate: 1) The accuracy and versatility of immune repertoire sequencing using Ion AmpliSeq™ library construction 2) The benefit of combining targeted gene expression and repertoire profiling for studies of the tumor microenvironment 3) The utility of repertoire sequencing covering all CDR regions in monitoring the manufacture of therapeutic T cells.

P58

Public NY-ESO-1 specific TCRs as novel biomarkers for immune monitoring of NY-ESO-1 positive cancer patients

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Background

T cell clonotypes with shared T cell receptors (public TCRs [pTCR]) are involved in the immune response to chronic viral infections, however, their role in immune responses to cancer is largely unknown. We evaluated the association of NY-ESO-1 specific pTCR sequences and survival in solid tumor patients

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treated with LV305 or CMB305, which are active immunotherapies based on the dendritic cell targeting lentiviral vector platform ZVex[®], expressing the cancer-testis antigen NY-ESO-1.

Methods

Peripheral blood mononuclear cells (PBMC) were collected before and after patients with NY-ESO-1 positive solid tumors, including soft tissue sarcomas, received therapy with LV305 or CMB305 (n=64). PBMC were subjected to deep sequencing to study the repertoire of the TCRVB-CDR3 region.

Results

The TCR-VB CDR3 amino acid sequences of three NY-ESO-1 specific pTCR clones obtained through *in vitro* culture from a LV305 patient with a near complete response were fully conserved in 41/56 (73.2%) of LV305/CMB305 patients and 54% of 539 healthy blood donors. Induction of NY-ESO-1 pTCR on LV305 or CMB305 therapy (baseline negative to positive, or doubling of frequency) was observed in 31% of patients and was associated with a trend towards better overall survival (data as of ASCO 2017). Querying TCR databases from multiple published clinical trials revealed NY-ESO-1 pTCR sequences in blood of patients with melanoma (6/13), renal cancer (1/3), and glioblastoma (6/13), and with a lower incidence in tumor biopsies.

Conclusions

We have identified NY-ESO-1 specific public TCRs in the PBMC of cancer patients undergoing active immunotherapy, as well as in healthy blood donors. In patients, the induction of pTCR appeared to be associated with better survival, whereas their presence in healthy blood donors may indicate frequent low-level baseline T cell immunity against this cancer testis antigen. pTCR should be investigated as a prognostic or predictive biomarker of cancer immunotherapies targeting NY-ESO-1, and possibly other cancer-testis antigens.

P59

Multi-color flow cytometry comparative analysis with DA-Cell(TM) centrifugation-less washing

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Background

Sample preparation for multi-color flow cytometry analysis requires staining of suspension cells with a mix of fluorescently labelled antibodies. This preparation requires the need to centrifuge and pack cells multiple times in order to wash away the excess of fluorescent antibody unbound to cells. Here, we explore a novel way to stain cell suspension by using wall less DropArray and Laminar flow wash properties of DA-Cell.

Using PBMC samples stained with various multi-color panels of 6, 11 and 21 antibodies, standard centrifugation or DA-Cell wash method was performed before acquisition with flow cytometry. We then subjected multi-parametric acquired flow data to unbiased clustering analysis with Cytokit Bioconductor package.

Clustering Analysis and flow cytometry raw acquisition revealed improved segregation of surface markers intensity and cell population when DA-Cell wash method was used as compared to standard centrifugation method. Human annotation of multiple immune cell population type was also facilitated with DA-Cell wash method as compared to standard centrifugation wash method based on stochastic non-linear embedding (T-SNE) clustering map results. DA-Cell method led in unbiased analysis to identification of CD4 CD57 cells and switch memory B cells absent from standard centrifugation wash method.

The superior separation and identification of cell population with DA-Cell offers a new perspective to study various rare immune cell populations.

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Methods

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Conclusions

The superior separation and identification of cell population with DA-Cell offers a new perspective to study various rare immune cell populations.

Consent to publish

Consent granted.

P60

Comparative study of flow cytometry analysis between conventional centrifuge method and centrifuge-less DA-Cell™ method from the perspective of cell retention

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Background

The preparation of cell suspension samples for flow cytometry commonly involves staining of cells with a specific fluorescent marker for detection of a cell population or a cell phenotype. For over half a century this standard preparation has required the need to centrifuge and pack cells at 500g for multiple times in order to wash away the excess of fluorescent marker unbound to cells. This “worldwide standard” remains however, inherently loses a significant portion of the cell population, particularly when cells are fixed and permeabilized. The biggest challenge imposed by the loss of cells is a well-known belief and intuition that the loss of cells varies depending on cell types and processing. The biggest challenge imposed by the loss of cells is a well-known belief and intuition that the loss of cells varies depending on cell types and processing.

Methods

Here, we present a new convenient methodology to wash suspension cells based on unique laminar flow properties of DropArray plate technology DA-Cell. This technology offers after multiple wash more than 95 % retention of millions of suspension cells and uses natural gravity without cells packing. The cell retention of 95 % can further increase when incubation time of cells and reagents increases by 10 – 20 min as the slight increase of the incubation time helps precipitating cells onto a plate surface by gravity. As an automated method DA-Cell wash is performed conveniently and consistently in 2-4 minutes as compared to a common 20-40 minutes requirement with a centrifugation wash based method.

Results

Application of this new technology on various sample preparation for flow cytometry analysis showed improvements in cell viability, segregation of cell population and detection of rare immune cell population when compared to conventional sample preparation. Various examples of multi-color flow cytometry analysis will be presented to

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demonstrate the performance of this new technology.

Conclusions

With minimal hands on time, DA-Cell technology enables researchers to conduct complex multi-parametric flow cytometry cell assays without the cellular stress or long time associated with centrifugation based procedures.

Consent to publish

Consent granted.

P61

Cellular and genomic disease signature of peripheral blood mononuclear cells in patients with malignant pleural mesothelioma

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Background

Recent data on the incidence malignant pleural mesothelioma (MPM) and the continued large-scale use of asbestos throughout the developing world portends an epidemic of asbestos-related disease. MPM is an aggressive and fatal cancer with few treatment options. Recent advances in large scale genomic and high throughput cellular analyzes now provide the tools to more easily attain markers of disease status and potential responsiveness to immunotherapeutics.

Methods

Here we present pre-treatment cellular and genomic biomarker data on a cohort of chemotherapy-naïve MPM patients, and demographically matched healthy donors (HD). MPM patients were enrolled in a Phase 1b study utilizing CRS-207, a live, attenuated *Listeria*

monocytogenes strain engineered to express the tumor-associated antigen, mesothelin. Four different multi-color flow cytometry panels were used to provide resolution on major immune cell populations of T cells, $\gamma\delta$ T cells, B cells, dendritic cells, monocytes, and natural killer cells. Together, these panels provided deeper resolution on 39 distinct subpopulations of major immune cell subsets. RNA from these cells was used to perform multiplex gene expression analysis on 770 genes using the Nanostring nCounter PanCancer Pathway Panel.

Results

FACS analysis yielded numerous subpopulations with statistically significant differences between MPM patients and healthy controls. Differences in immune populations were analyzed by median and significant findings included populations of CD4⁺ T cells, CD8⁺ T cells, B cells, classical monocytes, and monocytic myeloid derived suppressor cells. Class comparison and hierarchical clustering of gene expression data revealed genomic markers that were significantly expressed in MPM compared to healthy controls. Immune subset deconvolution of gene expression data provided similar findings as FACS analysis and corroborated this disease signature across experimental platforms.

Conclusions

Understanding a patient's biological disease signature can aide in diagnosis, as well as in making informed choices about therapies amidst the complex and broadening immunotherapeutic landscape. Until recently, existing biomarker data in MPM has been limited to a small number of serological markers and limited immune analysis. Here, we present the first comprehensive report of a MPM disease signature from the cellular and genomic perspectives. Correlation of patient baseline disease signatures with treatment outcome may yield biomarkers predictive of treatment efficacy. Predictive signatures are being investigated in the on-going Phase 1b study of CRS-207 and chemotherapy, as well as in the Phase 2 study of

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CRS-207 with pembrolizumab in MPM patients who failed prior treatment.

Trial Registration

ClinicalTrials.gov Identifier NCT01675765; NCT03175172

P62

Circulating T cell subpopulations correlate with immune inflammatory signatures at the tumor site in melanoma and non-squamous non-small cell lung cancer

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Background

Checkpoint inhibitors have transformed cancer therapy, although outcomes can be variable. Response to anti-PD1 therapies depends in part on tumor type and characteristics, particularly its mutation burden, as well as pre-existing antitumor immunity. These parameters may constitute the basis for patient stratification strategies for several immuno-oncology therapies. This analysis aimed to characterize the relationship between the local immune environment at the tumor site and peripheral immunity in melanoma and non-squamous non-small cell lung cancer.

Methods

Melanoma (n=42) and non-squamous non-small cell lung (n=40) FFPE tumor biopsies and peripheral blood mononuclear cells (PBMC) were provided by the Moffitt Cancer Center. All patients were appropriately consented through Total Cancer Care (TCC) protocol. None of these patients had received treatment with checkpoint inhibitors. For local immune responses, we used gene expression data

in FFPE tissues to calculate inflammatory signature scores in the tumors [1]. For systemic responses, we performed flow cytometry for T cell subpopulations in PBMC. Control PBMC (n=27) were obtained from the BMS employee volunteer blood donation program.

Results

We observed that there are distinct circulating T cell maturation patterns that distinguish between control samples and those belonging to patients with the studied tumors. We detected particular circulating T cell profiles in patients with melanoma and non-squamous lung cancer who have an inflammatory milieu in the tumor as determined by inflammatory signature score.

Conclusions

These findings represent progress in the characterization of peripheral immunity, as it relates to the inflammatory immune status in the tumor. The results also provide potential approaches to measure the immune response which are more easily accessible compared to tumor biopsies.

References

1. Spranger, Bao, Gajewski. Melanoma-intrinsic beta-catenin signaling prevents anti-tumor immunity. *Nature*. 2015;523: 231-235.

P63

Measurement of the immune-suppressor nitric oxide within immune cell subsets in patients receiving ipilimumab with a peptide vaccine

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Background

Biomarkers are unavailable to accurately predict clinical response to checkpoint blockade therapy. We employed the SPADE (spanning-tree progression analysis of density-normalized events) algorithm to precisely cluster immune cell phenotypes. Because phenotypic identification is laborious (Qiu et al., 2011, *Nature Biotechnology*, 29(10):887-891), we developed a novel algorithm to address this challenge. We explored whether nitric oxide levels are differentially expressed in distinct immune cell subsets before and after checkpoint blockade therapy, which may serve as a reliable biomarker.

Methods

Patients with resected stage III/IV melanoma were treated with ipilimumab plus a peptide vaccine. Pre and post treatment peripheral blood mononuclear cells (PBMCs) at week 13 were available for analyzes 44 patients pre-treatment samples, 35 patients had both pre/post treatment samples; Sarnaik et al., 2011, 17(4):896-906). Two flow cytometry panels (100,000 live cell events; LSR II flow cytometer) were constructed to compare PBMCs collected pre and post ipilimumab/vaccine treatment (Myeloid panel: nitric oxide stain (DAF-FM), HLADR, CD33, CD11b, CD14, CD15, and CD11c; Lymphoid panel: DAF-FM, CD3, CD4, CD8, CD25, CD127, CD56, CD19, and CD11c). Controls included: flow cytometric compensation beads to establish robust compensation matrices, fluorescence minus one controls to set negative and positive gates, isotype controls to control for patient variations, and a live/dead marker. SPADE was utilized to cluster and differentiate cell populations based on marker expression, and then a novel algorithm, MPAT-R (multi-parameter phenotyping analysis tool in R) was developed to quickly determine cell phenotypes in the SPADE tree.

Results

By the MPAT-R algorithm, 58/200 lymphoid and 116/200 myeloid phenotypes were differentially expressed after ipilimumab/vaccine treatment (Wilcoxon signed-rank test, False Discovery Rate (FDR) <0.05). Ten myeloid and 7 lymphoid

phenotypes post-treatment trended with relapse-free survival (RFS) at one year ($p < 0.05$, FDR >0.05, Wilcoxon rank-sum test). Two lymphoid and myeloid populations pre-treatment trended with RFS at one year (Cox regression, Wilcoxon rank-sum test, $p < 0.05$, FDR >0.05). Two interesting populations associated with increased RFS were: a) Monocytic myeloid cells without NO (HLADR⁺CD33^{+/low}CD11b⁺CD14^{+/low}DAF-FM^{neg}) and b) naïve/central memory T cells containing intermediate levels of NO (CD8⁺CD127⁺CD25⁻DAF-FM^{+/low}).

Conclusions

To our knowledge, this is the first application of SPADE to pre/post clinical immunotherapy PBMC samples. The MPAT-R algorithm efficiently detected immune cell phenotypes associated with increased RFS as potential biomarkers for immune checkpoint blockade therapy with ipilimumab. Future studies will focus on the potential immunosuppressive role of NO identified in precise immune cell subsets both in the circulation and in the tumor microenvironment.

P64

Analytical validation of digital spatial profiling - a novel approach for multiplexed characterization of protein distribution and abundance in FFPE tissue sections

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Background

Characterization of the spatial distribution and abundance of key proteins within tissues enables a better understanding of biological systems in many research areas, including immunology and oncology. However, it has proven difficult to perform such studies in a highly-multiplexed manner on FFPE tissue sections. To address this unmet need, we

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have developed a novel imaging platform, Digital Spatial Profiling (DSP), designed to simultaneously analyze 10's to 100's of proteins from discrete regions by detecting oligos conjugated to antibodies that can be released via a UV-cleavable linker. Here we describe the validation of the oligo-conjugated antibodies used with this new approach to highly multiplexed protein analysis.

Methods

Various antibodies targeting important immunology proteins (CD3, CD4, CD8, CD45, PD1, PD-L1, etc.) were tested for specificity and sensitivity. Immunohistochemistry was performed on FFPE human tissues including tonsil and tumor samples, as well as human cell line pellets to evaluate binding specificity in both unconjugated and oligo-conjugated antibodies. The sensitivity and dynamic range of oligo-conjugated antibodies were tested using FFPE cell pellets with target-specific positive and negative cells at different ratios (0:100, 5:95, 20:80, 50:50, 100:0) and specific limits-of-detection (LODs) were determined. An interaction screen was performed to evaluate potential deleterious effects of multiplexing antibodies. Finally, a human tissue microarray (TMA) containing normal and cancer tissues was employed to assess assay robustness.

Results

Immunohistochemical analysis of antibodies integrated into the cocktail displayed indistinguishable staining patterns on control tissues and cell lines for both unconjugated and oligo-conjugated antibodies. Mixed-proportion cell pellet assays revealed strong correlations between observed counts above background and positive cell numbers in a region of interest, allowing us to accurately calculate LODs. For example, CD3 displayed a LOD of 4% when assayed using a cell pellet mixture containing increasing numbers of CD3⁺ CCRF-CEM cells with CD3⁻ HEK293T cells. Antibody interaction studies showed similar count values for antibodies alone or in combination ($R^2 > 0.8$). Finally, TMA hierarchical clustering analysis demonstrated expected patterns for immune cells

and tumor cells across normal and disease tissue types.

Conclusions

These results demonstrate that indexing oligo conjugation does not interfere with antibody specificity and that these conjugated antibodies are robust reagents for quantification of protein abundance. Continued work on the DSP platform will expand the library of antibodies accessible for profiling.

P65

Spatially-resolved, multiplexed digital characterization of protein abundance in FFPE tissue sections: application in preclinical mouse models

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Background

Characterization of the abundance, distribution, and colocalization of key immunoregulatory proteins within the tumor microenvironment is necessary for a thorough understanding of tumor immune responses. Historically, immunohistochemistry and immunofluorescence have been used to assess spatial heterogeneity of proteins in tissue slices, but these techniques are of limited utility due to the challenge of measuring multiple targets in parallel. We recently developed a platform to enable spatially-resolved protein detection with the potential to simultaneously quantify up to 800 targets from a single formalin-fixed paraffin-embedded (FFPE) sample slide, termed Digital Spatial Profiling (DSP). To demonstrate preclinical applications of DSP, we have developed an assay to detect and quantify ~20 key immuno-oncology (IO) targets in mouse FFPE tissue sections.

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Methods

DSP uses DNA oligo tags covalently linked to detection reagents (primary antibodies) via a UV photocleavable linker to identify targets in situ and enable quantitation via the standard nCounter[®] technology. A slide-mounted FFPE tissue section is incubated with a cocktail of oligo-labeled primary antibodies, and a serial section is stained with low-plex visible/fluorescent probes (e.g. nuclear staining probes, or select antibody pairs such as anti-CD3) to generate an image of the FFPE tissue slice morphology. Regions of interest (ROI) in the tissue/tumor are then identified and sequentially illuminated with UV light to release the DNA-oligos. Following UV illumination, the photocleaved oligos are released into the aqueous layer above the tissue slice, collected via microcapillary aspiration, and stored in an individual well of a microtiter plate. Oligos are then hybridized to nCounter optical barcodes to permit ex-situ digital counting of as many as 800 different analytes localized within a single ROI, which can be referenced using image capture software.

Results

We demonstrate preclinical applications of this technology by characterization of a panel of immune proteins on mouse FFPE tumor and normal tissue sections. We demonstrate that this approach enables protein detection at single cell resolution, and enables simultaneous multiplexed detection of AKT, B7-H3, Beta-2M, Beta-Catenin, CD3, CD4, CD8, CD45, CD68, PD-1, PD-L1, GZMB, Ki67, pan-cytokeratin, Stat3 and additional key IO targets.

Conclusions

The ability to measure DNA, RNA, and protein at up-to 800-plex from single slices of FFPE tissue may improve the early evaluation of drug targets with high-resolution spatial information, enable the discovery of key immune biomarkers in mouse tissue (tumors and inflammation et al), and accelerate the preclinical development of immunotherapies.

P66

Digital spatial profiling platform allows both spatially-resolved, multiplexed measurement of solid tumor and immune-associated protein distribution and abundance using a single FFPE tissue section

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Background

Spatial characterization of immune activations and tumor proteins within tumor tissues enables a better understanding of immunology and oncology. However, it has proven difficult to perform such studies in a highly multiplexed manner using limited samples. To address this unmet need, we have developed an imaging and tissue-sampling platform designed to simultaneously analyze hundreds of tumor and immune proteins in a single FFPE tissue section with spatial resolution. Using this approach, we demonstrate that a single FFPE tissue section is enough to obtain multi-dimension data sets, such as solid tumor protein expression, immune activity, and cancer pathways with an emphasis on markers important for cancer immunotherapy.

Methods

We developed a novel optical-barcode based microscope that can spatially resolve up to 800 different proteins or RNAs on a fixed tissue (Digital Spatial Profiling, DSP). DSP probes are not multiplex-limited by spectral resolution. Instead, colors are determined using barcode indexing oligos that are conjugated to antibodies or nucleic-acid probes via UV-cleavable linkers. The UV-cleavage is precisely controlled by a programmable digital micromirror device that can illuminate discrete regions ranging from entire tissue microenvironments to single-cell

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and subcellular regions. Following spatially-defined UV light exposure, released indexing oligos are siphoned off the tissue surface via a microcapillary tip. For quantification of signal, the UV-cleaved oligos from spatially-resolved regions of interest (ROIs) are hybridized to NanoString barcodes, providing digital counts of the protein or RNA targets in each ROI using standard NanoString nCounter® instruments.

Results

Using this novel approach, we demonstrate multiplexed detection from discrete micro regions within a tumor enables systematic interrogation of tumor protein expression and immune activity inner or outer regions of tumors. In addition, the whole tissue analysis allows us to characterize and link global distribution of tumor protein expression, immune activity and cancer pathways.

Conclusions

The simplicity of the DSP platform allows high-resolution, high-multiplexed, spatially-resolved tumor and immune protein characterization in any laboratory capable of performing immunohistochemistry procedures. DSP platform provides a potential method that can bridge the gap between translational research discovery and clinical applications.

P67

Standardization of tumor infiltrating lymphocytes isolation for flow cytometric analysis

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Background

Cancer immunotherapy has become a mainstay of cancer treatment. These agents can target the tumor/lymphocyte interaction in the tumor microenvironment. Therefore, analysis of tumor

infiltrating lymphocytes (TIL) needs to be part of comprehensive immunomonitoring in patients receiving immunotherapy. There are two major approaches to isolate TIL, enzymatic digestion and mechanical dissociation. While enzymatic digestion may give better yields, it potentially destroys epitopes. In contrast, mechanical digestion may preserve epitopes, but can suffer from poor yields and reduced viability of TIL. As standardization of TIL isolation for flow cytometry is key to accurate and precise phenotyping of TIL populations, we compared these different approaches for cell yield, epitope preservation and viability.

Methods

Three different isolation techniques were tested: 1) Miltenyi tumor dissociation kit, 2) enzymatic digest, and 3) mechanical isolation.

Results

The Milenyi protocol and lab prepared enzymatic digests delivered higher yield of TIL from tumor, but proteases in the enzyme mix cleaved proteins and chemokine receptors on the cell surface of T cells. Mechanical dissociation preserved the integrity of the surface antigens and chemokine receptors, but negatively impacted the ability to breakdown the tumor matrix and release TIL populations into a homogenous single cell suspension. A combination of mechanical dissociation through sieves and 15 minutes enzymatic digest buffered with HSA at room temperature yielded high recovery of Tregs, retention of surface markers and chemokine receptors and good viability.

Conclusions

The combination of enzymatic digestion and mechanical dissociation provided the best result. Due to small tumor size, frequently <1g, and high heterogeneity from tumor to tumor, it is challenging to validate TIL isolation methods for reproducibility and precision. Therefore, we are evaluating a surrogate system using mouse tumors, which allows us to directly compare methods and test proficiency of technologists.

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P68

Immunogenomic evolution and atypical response to atezolizumab in a patient with metastatic triple-negative breast cancer (mTNBC)

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Background

mTNBC has poor prognosis and few treatment options. Atezolizumab (anti-PD-L1) demonstrated clinical activity in mTNBC, which has been linked to high levels of tumor-infiltrating lymphocytes (TILs) and PD-L1 expression. We describe the immunologic and genomic evolution of TNBC across sequential therapies in a patient with a 31-year history of TNBC and complete response (CR) to atezolizumab.

Methods

A 48-year-old woman enrolled in the Phase Ia atezolizumab study (PCD4989g) on March 5, 2013. She presented in 1986 with early TNBC and had surgery and radiotherapy (XRT). Two locoregional recurrences were managed with surgery and adjuvant chemotherapy. In 2009, she was diagnosed with mTNBC and was sequentially treated with capecitabine, gemcitabine-carboplatin-iniparib (GCI), XRT and an experimental vaccine. She then received atezolizumab for 1 year with partial response (PR), pseudoprogression and reversion to PR. After 1 year off-treatment, she progressed and reinitiated atezolizumab, achieving a CR. Ten tumor tissues obtained between 2008-2015 were assessed

by immunohistochemistry, RNA-seq (Illumina) and DNA-seq (FoundationOne).

Results

Tumor-immune microenvironment (TiME) biomarkers, including CD8, TILs and PD-L1, increased after capecitabine exposure, remaining high after GCI and XRT and through pseudoprogression on atezolizumab. At relapse post-atezolizumab, expression of TiME biomarkers decreased, particularly PD-L1. Immune-related RNA signatures (T and B cells, cytotoxicity and antigen presentation) confirmed these findings. Angiogenesis signature varied over time and was highest at early time points, lower post-capecitabine, higher before GCI, lowest at pseudoprogression and increased at relapse post-atezolizumab. Proliferation signatures were highest in early tumors, decreasing over time. TNBC subtyping confirmed evolution of TiME from luminal androgen receptor (LAR) to basal-like immune suppressed (BLIS), then to basal-like immune active (BLIA) post-capecitabine and through pseudoprogression, with reversion to BLIS at relapse post-atezolizumab. Genomic profiling suggested possible driver mutations in *RB1* and *TP53*. *MYC* amplification was lost over time, possibly associated with decay of the proliferation signature. The incidence of subclonal somatic mutations peaked post-XRT and were undetectable post-atezolizumab. Likewise, tumor mutational burden was highest post-XRT and lowest at pseudoprogression.

Conclusions

This case report describes the evolution of tumor immunity and molecular subtypes with sequential therapies over time in a patient with TNBC and CR to atezolizumab. Immune, stromal and genomic biomarkers of TiME demonstrated temporal plasticity across multiple therapies, including atezolizumab. These findings suggest that TiME is pliable and may be strategically manipulated to maximize response to immunotherapy.

Trial Registration

ClinicalTrials.gov Identifier NCT01375842

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Consent to publish

Written informed consent was obtained from the patient for this publication. A copy of written consent is available upon request.

P69

A pan-cancer diagnostic, prognostic and targetable biomarker pipeline defining the failed immune-response

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Background

Tumor infiltrating lymphocytes (TILs) represent positive prognostic and predictive indicators of patient outcomes. Their signature genes are, however, still under tight control by the overwhelming tumor microenvironment. Therefore, despite many of these genes reflecting effector T cell subsets, their totalities actually belong to a failed immune response (FIR) against cancer. This was best first exemplified by association of renal cell carcinoma (RCC) TILs with poor prognosis, yet was later also validated to be a reversible TIL condition by use of immune-checkpoint modulators. Since circulating T cells can share TIL tumor-reactivity and neoantigen-specificity, we used profiles of circulating RCC CD8⁺ and CD19⁺ lymphocytes and their paired TILs and normal tissue infiltrating immune cell (TIIC) counterparts as a model of a FIR signature. Commonalties of this signature were observed in several other solid cancers and represents a diagnostic, prognostic, and targetable biomarker model pipeline defining novel and feasible immunotherapeutic targets.

Methods

A rapid extraction protocol isolated RNA from CD8⁺ effector and CD19⁺ antigen presenting cells (APC) isolated from paired TILs, TIICs and PBMCs, and matched normal donor PBMCs. Microarray analysis interrogating the expression of all genes and RNA isoforms, was followed by bioinformatics probing an additional 500 RCC validation cohort. This provided prognostic linkage to biomarker expression, revealing novel antagonistic and agonistic targets, which were refined using clinical datasets from 11,000 additional patients with lung, breast, gastric, and ovarian cancers. Feasible novel immune-promoting targets exclusively expressed by immune cells, and in the context of cancer, included receptors and plasma membrane-associated proteins, or those having pre-existing targeting small molecules. These were further investigated for protein-protein interaction (PPI) and associated pathways, coexpression, and comprehensive literature reviews establishing relevant immune-cell function.

Results

Validation of original hits from the discovery cohort provided strong evidence of which cancers were most related at the TIL level, and PPI revealed that pan-cancer targets were functionally associated in novel pathways ($p = 1.85e-10$). Coexpression dynamics reflected key proteins responsible for APC:effector TIL communication, and results also showed that lung and breast cancer TILs and circulating PBMCs were most similar to other cancers, and that a FIR is skewed towards a diminished expression of genes otherwise expressed by the healthy population.

Conclusions

We present a strict, comprehensive pipeline defining a pan-cancer targetable immune-signature having many pre-existing and tested targeting small-molecules, in an effort to develop more feasible and broad reaching diagnostics and precision medicine to counter cancer, foiled by the surge of bystander TIL-signatures.

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Halioseek™, a dual CD8 and PD-L1 IVD assay to improve NSCLC patients stratification

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Background

Immune checkpoint inhibitors (ICI) improved non-small cell lung cancer (NSCLC) survival in 20% of the patients. Their administration is decided based on the expression level of PD-L1 on tumor cells despite limited predictive value of PD-L1 alone. We hypothesized that ICI action depends on both PD-L1 expression and on the presence of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment.

We developed Halioseek™ according to IVD regulatory processes, a standardized PD-L1/CD8 dual-staining assay. In addition to PD-L1 detection, critical information is provided on TILs through the detection of CD8+ cells on the same tissue section. Halioseek™ includes a Digital Pathology (DP) analysis module to determine CD8+ cell density and a proximity index between CD8+ and PD-L1+ cells. Here we show the main analytical performance of Halioseek™ and concordance with two PD-L1 IVD assays.

Methods

Samples are double-stained with anti-PD-L1 (clone HDX3) and anti-CD8 (clone HDX1) antibodies, respectively revealed with DAB and fast-red substrates. The assay is fully automated on the Benchmark XT platform (Ventana). Digital images of stained slides are obtained using a whole slide scanner (Hamamatsu). Percentage of PD-L1 positive tumor cells (PD-L1+ TC) is estimated by a

pathologist; DP analysis is performed using Halioseek™ DP module.

Staining accuracy of PD-L1 by HDX3 antibody was evaluated by comparison with SP263 IVD assay (Ventana) and 22C3 IVD assay (Dako) on 100+ NSCLC including resections and biopsies. Overall, Positive percent and negative percent agreements were assessed at 1%, 5%, 10% and 50% of PD-L1+ TC corresponding to published clinical cut-offs.

The precision of PD-L1 assay was evaluated in terms of repeatability, inter-primary antibody batches (3 lots), inter-revelation kit batches (3 lots) and inter-instruments (2 stainers) using 332 slides from 11 independent NSCLC samples covering the range of the assay.

Results

- PD-L1 staining accuracy: overall agreements with SP263 were above 90% and with 22C3 above 85%.
- PD-L1 precision: none of the tested parameters had a significant impact on results showing the robustness of the assay.
- CD8+ cell density: distribution spanned between 30 and 1200 cells/mm² ; global CV was below 13%.

Conclusions

Halioseek™ is a new robust IVD assay leveraging the advantages of DP to combine TILs and PD-L1 quantification within the tumor microenvironment. Halioseek™ could have a higher predictive performance than existing IVD tests and could fill a major gap in the management of ICI administration. As next step we intend to investigate predictive performance of the assay on ICI treated patient's samples.

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Peptide-induced whole blood gene expression analysis for peptide-specific T cell response

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Background

Peptide-based cancer vaccine is a promising approach in cancer immunotherapy. To monitor the efficacy of therapy, a simple tool to monitor specific T cell response to peptide vaccine is in need.

Although MHC multimer and Elispot have been used frequently in various vaccine studies, the former requires specialized reagents and instrument and the latter requires long incubation and imaging instrument.

Methods

Whole blood *ex vivo* peptide stimulation assay was developed to investigate peptide-specific T cell responses. A 60 μ L heparin blood was incubated in triplicate with class I peptide for 4 hours at 37°C to form a complex among major histocompatibility complex (MHC) – peptide – T cell receptor (TCR). Following the incubation, mRNA was isolated and quantified by real-time RT-qPCR. Fold increase of mRNA was calculated against solvent control in triplicate.

Results

We identified that *IFNG*, *CXCL10* and *GMCSF* mRNA were significantly induced (fold increase >2, p value <0.05) by CMV peptide in 4, 4 and 2 out of the 8 healthy donors, respectively. IFN-gamma Elispot assay confirmed at least 7 of the subjects were positive following 2-week *in vitro* sensitization. On the other hand, these genes were not induced by HIV peptide, which was corroborated by Elispot with *in vitro* sensitization. To compare the assay performance with Elispot, 0 to 300 cells of CMV-peptide specific cytotoxic T-lymphocyte (CTL) were

spiked in 1×10^5 cells of PBMC and assayed by both methods. The limit of detection was less than 3 to 10 cells for both assays and a linear correlation between mRNA quantity and Elispot count was obtained (Pearson correlation; *IFNG*, -0.942 (p = 4.9×10^{-6}), *CXCL10*, -0.690 (p = 1.3×10^{-2}), and *GMCSF* -0.888 (p = 1.1×10^{-4})). In a study with 12 samples of whole blood blindly spiked with peptide-specific CTL at 2.5 to 12.5×10^3 cells / mL, the assay sensitivity and specificity of each mRNA were 17% and 83% for *IFNG*, 100% and 100% for *CXCL10*, and 100% and 33% for *GMCSF*. These data suggest that these markers especially *CXCL10* could be a useful marker to monitor peptide-specific T cell response.

Conclusions

The whole blood mRNA assay developed in this study is useful to measure peptide-specific T cell responses, which could be used to monitor the efficacy of peptide vaccine therapy.

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Analysis of biomarkers from a cohort of advanced melanoma patients previously exposed to immune checkpoint inhibition treated with entinostat (ENT) and pembrolizumab (PEMBRO)

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Background

Treatment options following immune checkpoint inhibition remain an area of active investigation across multiple cancer indications, including advanced melanoma. Results from the ongoing ENCORE 601 study demonstrated that ENT, a class I selective HDAC inhibitor, combined with PEMBRO elicited a 31% response rate in a cohort of 13 patients with melanoma progressing on or after a PD-1 blocking antibody. Assessment of blood and tissue-based biomarkers may be useful to understand how patients who respond to ENT plus PEMBRO differ from those who do not.

Methods

ENCORE 601 employs a Simon 2-stage design to assess activity of ENT 5mg PO weekly combined with PEMBRO 200 mg IV every 3 weeks across 4 cohorts: 1) advanced anti-PD-(L)1-naïve NSCLC; 2) advanced NSCLC progressed on anti-PD-(L)1; 3) advanced melanoma progressed on anti-PD-(L)1; 4) advanced anti-PD-(L)1-naïve microsatellite stable colorectal cancer. Pre-treatment and Cycle 2 Day 15 (C2D15) biomarker analysis included gene expression by Nanostring Pan-Cancer Immune Profiling Panel, PD-L1 expression by immunohistochemistry, and levels of CD8+ T cells and LOX1+CD15+ MDSCs by immunofluorescence staining on paraffin embedded human tissue sections. Phenotypic evaluation of immune cell subsets was conducted in peripheral blood samples collected pre-treatment, C2D1 and C2D15.

Results

All patients enrolled in Stage 1 of the melanoma cohort (n=13) received a prior anti-PD-1 therapy with eight also receiving prior ipilimumab, and two receiving prior BRAF inhibitors. Four patients (31%) had a partial response (PR; 3 confirmed, 1 unconfirmed), and four additional patients (31%) had stable disease. Pre-and post-treatment tissue samples were evaluated for PD-L1 expression (n=10 pre; 7 post), gene expression (n=7 pre; 7 post), CD8+

T cells (n=9 pre; 9 post) and MDSCs (n=9 pre; 9 post). In 3 of the 7 patients whose tissue samples were analyzed for gene expression (2 with confirmed PRs, one with progressive disease), non-inflamed tumors at pre-treatment were converted to inflamed. Increases in both T cells and MDSCs were observed only in those tissue samples converted from non-inflamed to inflamed. Overall, regardless of clinical outcome, we noted a decrease in MDSCs (-35.7%; n=9) and an increase in CD8+ T cells (47.4%; n=9) between pre-and post-treatment biopsies.

Conclusions

ENT plus PEMBRO shows promising activity with an overall response rate of 31% observed in Stage 1. Enrollment to Stage 2 continues. Preliminary biomarker analysis supports our hypothesis that the addition of ENT restores inflammation in the tumor microenvironment necessary for successful re-treatment with an anti-PD-(L)1 blocker.

P73

First-in-human neoadjuvant study of the immunogenomic impact of the oral IDO inhibitor epacadostat (INCB024360) on the tumor microenvironment of advanced ovarian cancer

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Background

The tryptophan catabolizing enzyme indole-amine 2,3 dioxygenase 1 (IDO1) has been identified as a potent immunosuppressive mechanism that fundamentally alters the ovarian tumor microenvironment (TME) and effector T cell function in ovarian cancer. Epacadostat is a novel oral IDO1 inhibitor that suppresses systemic tryptophan catabolism and is currently being evaluated in ongoing clinical trials. Although the pharmacodynamics (PD) effects of epacadostat in reducing kynurenine concentration in plasma has been demonstrated in pre-clinical and clinical studies, it is currently unknown whether the PD effects will occur at the TME and alter it to become more immunogenic.

Methods

Seventeen patients with newly diagnosed stage III or IV ovarian cancer underwent pre-treatment tumor biopsy and paracentesis. Subjects were scheduled to receive 600mg epacadostat twice daily for 2 weeks, followed by standard tumor debulking surgery. Peripheral blood and tumor specimens were utilized to assess the phenotype and functional status of multiple immune populations in the TME, define changes in the tumor genomic profile and gene expression by whole-exome sequencing, Nanostring, and RNAseq, TCR clonal evolution, and impact on humoral immune responses to cancer-testis antigens.

Results

Expression profiling identified an upregulated IFN γ induced gene signature in a subset of post-treatment tumor specimens, indicative of reactivation of IFN γ signaling in the TME. Likewise, an increase in CD8⁺ T cells was detected in some, but not all subjects. Of note, IFN γ signaling and increases in CD8⁺ frequency was not consistently observed in all subjects. Evidence of reduced IDO1 enzyme activity, determined by Kyn:Trp ratio, was observed not only in plasma but also in ascites fluid for a subset of patients (5 of 7 evaluable ascites fluid specimens). Although IDO1 activity was blocked, IDO1 expression within the TME was not

affected by epacadostat. Mitochondrial spare respiratory capacity of T cells in peripheral blood increased in 8 of 12 patients after treatment. Additional immunogenomic analyzes are underway to identify molecular and metabolic pathways impacted by epacadostat along with determining the clonal evolution of T cells with reactivity to mutational antigens.

Conclusions

This is the first demonstration of the effect on tumor tissue of inhibition of IDO1 enzyme activity. IDO1 inhibition by epacadostat resulted in an increase in CD8⁺ T cell and IFN γ gene signatures in the TME. Additional multi-dimensional immunogenomic data from on-going analyzes will be presented.

Trial Registration

ClinicalTrials.gov Identifier NCT02042430

P74

Increasing the levels of anti-beta glucan antibodies by administration of intravenous immunoglobulin (IVIG) induces immunopharmacodynamic (IPD) responses of a novel immunotherapeutic Imprime PGG

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Background

There is a critical need for rational combination immunotherapies that have mechanism-driven predictive biomarkers. Imprime PGG (Imprime), is a novel, intravenously (i.v.) administered innate immunomodulator currently in clinical development as a combination therapy with checkpoint inhibitors

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in biomarker-selected patients. Imprime is a soluble β -glucan PAMP that requires immune complex formation with serum anti-beta glucan antibodies (ABA) for its functionality. *Ex vivo* human studies, a healthy volunteer phase I trial and retrospective analyzes of clinical studies have demonstrated that IPD changes and clinical responses mediated by Imprime correlated with serum ABA levels. *Ex vivo* studies have also shown that innate immune functionality in subjects with lower ABA values can be restored by supplementation with purified ABA or ABA-containing IVIG. Herein we present a case study of a cancer patient with low ABA levels demonstrating enhanced Imprime-induced PD responses post IVIG administration.

Methods

A 54-year-old female with metastatic colorectal adeno-carcinoma was offered immunotherapy with bevacizumab, cetuximab and Imprime as part of a compassionate use program after she could not tolerate first line therapy with FOLFOX and bevacizumab. The patient was dosed in 4 week cycles for 12 cycles. Imprime and cetuximab were administered i.v. weekly. Bevacizumab was administered every 4 weeks from cycle 2 through 8. Evaluation of serum ABA levels from cycles 1-6 confirmed low values in this patient. To boost ABA levels, IVIG was added to dosing beginning at cycle 6. ABA, complement, and cytokine levels in serum (ELISA and Luminex), Imprime binding and immune cell phenotyping (flow cytometry) were measured prior to and within 30 minutes after Imprime administration.

Results

Compared to the weeks prior to adding IVIG to the dosing regimen, IVIG infusion resulted in increased serum ABA levels at the End of Infusion (EOI), which then dropped to the baseline levels in the subsequent weeks. Concomitant with the ABA increase, serum C5a levels peaked at the EOI. Furthermore, serum chemokines such as IL-8 also increased at EOI with the most pronounced change observed during cycles 7 and 8. Increased ABA levels also correlated with significant Imprime

binding on neutrophils and monocytes. Importantly, minimal PD changes were observed with Imprime dosing alone in the cycles prior to IVIG administration. Disease remained stable for 10 months.

Conclusions

These human data provide the first evidence of rescue of Imprime-driven PD responses in a cancer patient by supplementation of ABA, a crucial prerequisite for the therapeutic activity of Imprime.

P75

iSEND is an algorithmic model specific for advanced non-small cell lung cancer patients treated with PD-1 / PD-L1 inhibitors

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Background

We have shown that the iSEND model may be predictive of clinical outcomes for advanced NSCLC (aNSCLC) patients treated with nivolumab but not with chemotherapy or TKI.^[1] However, little is known about its potential performance for other advanced solid cancer patients treated with PD-1/PD-L1 inhibitors (PD-1/PD-L1i).

Methods

We evaluated the clinical outcomes of 370 solid cancer patients who received PD-1/PD-L1i and compared the performance of iSEND in aNSCLC (n=203) and other advanced solid cancers (n=167). Patients were either treated with nivolumab (n=237), pembrolizumab (n=109), or atezolizumab (n=24). As described in our previous reports, the iSEND model (immunotherapy, Sex, ECOG [Performance status], NLR [Neutrophil-to-Lymphocyte Ratio] & DNLR [Delta NLR = NLR after

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treatment - pretreatment NLR]) was developed. We stratified each treatment group by iSEND and compared progression free survivals (PFS) and clinical benefit rates (CBR) at 12+/-2 weeks in the iSEND Good, Intermediate, and Poor groups.

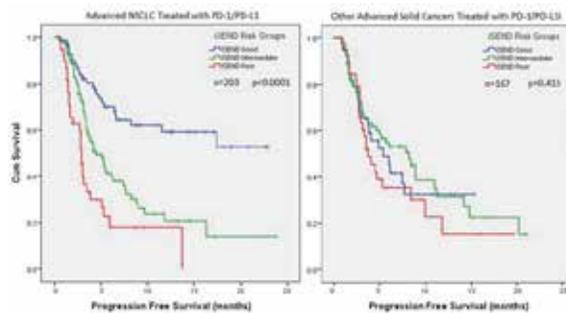
Results

Median follow-up was 9.1 (95% CI: 8.0-10.2). In the aNSCLC, The 3-, 6-, 9-, and 12-months PFS rates were 83%, 70%, 62%, and 58% in the iSEND Good group, 71%, 40%, 29% and 20% in Intermediate group, and 60%, 18%, 18%, and 18% in Poor group, respectively ($p < 0.0001$). Median PFS was unreached in iSEND Good, 4.2 months; 95% CI [2.8-5.6] in iSEND Intermediate, and 2.9 months; 95% CI [2.7-3.1] in iSEND Poor groups. (Figure 1). In contrast in other advanced solid cancers, median PFSs were 5.4, 8.2, and 3.7 months in iSEND Good, Intermediate, and Poor groups, respectively ($p = 0.415$). The area under the curves (AUC) of the iSEND score for CBR at 12+/-2 weeks for aNSCLC patients treated with PD-1/PD-L1i was 0.720, (95% CI: 0.648-0.793, $p < 0.0001$). The AUCs of iSEND for CBR in other advanced solid cancers was not significant (Figure 1).

Conclusions

In our single-institution retrospective cohort, the iSEND model showed a predictive potential specifically for advanced NSCLC patients treated with PD-1/PD-L1i but not for other advanced solid cancers treated with PD-1/PD-L1i.

Figure 1. Kaplan-Meier curves for PFS in aNSCLC vs. other advanced solid cancers treated with PD-1/PD-L1i.



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P76

Usefulness of automated multidimensional flow cytometry analyzes for monitoring the status of T and APC compartments in breast cancer patients under neoadjuvant chemotherapy

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Background

Immune monitoring systems based on flow cytometry (FC) are usually analyzed by manual strategies of gating based on two-dimensional plots. Recently the forthcoming of new technologies such as CyTOF and advances of multi-parametric FC analyzes demand new approaches to analyze massive immune related data. New algorithms like SPADE, FLOCK, tSNE, and more recently CITRUS, have provided new tools for automated multidimensional FC analysis. These tools have been

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used mainly for the cellular hierarchy analysis of bone marrow derived cells and in leukemia patients but to a lesser extent for immune-monitoring of patients under anti-tumor therapy. In this work we explore the usefulness of these automated systems for the immune-monitoring of breast cancer patients under neo-adjuvant chemotherapy.

Methods

We used data from an *in vitro* system to monitor by FC various immunological readouts in T and APCs in PBMCs from breast cancer patients (BCPs) before, and after three cycles of neo-adjuvant anti-tumor therapy with Doxorubicin and Cyclophosphamide (A/C). We applied different automated algorithms including SPADE, FLOCK and CITRUS to process FC data on the status of T and APC compartments in these patients.

Results

Using CITRUS, we detected *ex vivo* alteration in the frequency of Dendritic Cells (DCs) with a plasmacytoid phenotype in BCPs patients before treatment that was recovered to the levels observed in healthy donors after three cycles of A/C chemotherapy. Regarding T cells, we confirmed that before therapy T cells in BCPs exhibited marked unresponsiveness to a polyclonal stimulus evidenced by a deficient TCR internalization and low expression of CD25, CD69, and CD154 that was also recovered in BC patients after chemotherapy.

Conclusions

Our results let us to argue that automated systems are useful tools for the immune-monitoring by FC of BCPs under chemotherapy and for the analysis of cell populations with a complex immune-phenotype not analyzable through manual gating.

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The novel IL-2 cytokine immune agonist NKTR-214 harnesses the adaptive and innate immune system for the treatment of solid cancers

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Background

The novel, CD-122 biased PEGylated IL-2 cytokine immune agonist NKTR-214 harnesses the potent immune stimulatory benefits of the IL-2 pathway to maximize anti-tumor responses, and minimize unwanted biological effects. NKTR-214 has been safely administered in the outpatient setting, with low-grade hypotension as the most clinically significant adverse event. NKTR-214 robustly increases expression of activation and proliferation markers on immune cells in blood and tumor. Evidence of clinical activity has been observed in IO naïve and relapsed/refractory patients. Here we describe the phenotype of proliferating T cells in the blood and present an in-depth characterization of tumor-infiltrating immune cells using gene expression analysis and TCR profiling.

Methods

Pre- and on-treatment blood and tumor biopsies from patients receiving 0.003 to 0.009 mg/kg NKTR-214 monotherapy were analyzed. Blood samples (n=22 pts) were analyzed with 16-color multi-parameter flow-cytometry. Tumor biopsies (n=10 pts) were analyzed for gene expression using the Nanostring PanCancer immune panel. Differentially expressed genes in pre- and on-treatment samples were identified and mapped to public databases for pathway enrichment analysis. Tumor T cell receptor (TCR) sequencing was conducted at Adaptive Biotechnologies, and analyzed using the R package LymphoSeq.

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Results

In blood samples, we observed increased expression of activation markers (ICOS and OX40) as well as co-inhibitory receptors (PD-1, CTLA-4, TIM-3 and Lag-3) in both CD4⁺ and CD8⁺ T cells proliferating in response to NKTR-214. Differentially expressed genes (n=54) mapped to several innate and adaptive immune system pathways, including NK cell mediated cytotoxicity, IL-2, IL-12, and TCR signaling pathways. Pairwise comparison of matched baseline and on-treatment tumor biopsies confirmed increased expression of genes associated with T cell signaling (ie. *CD3G/D/E*, *CD247*, *CD8A* and *CD8B*) and function (ie. *PRF1*, *GPLY*, *GZM*, and *IFNG*), as well as upregulation of several immune checkpoint and costimulatory genes (ie. *PD1*, *PDL1*, *LAG3*, *IDO*, and *CTLA4*). Gene expression changes were observed at all dose levels and across tumor types studied. Tumor TCR sequencing indicates changes in clonality with a trend towards increased frequency of specific clones on-treatment.

Conclusions

A single dose of NKTR-214 modulates both the innate and adaptive immune system. Changes in clonality and increased frequency of specific clones suggest efficient remodeling of the T cell repertoire. Upregulation of checkpoint and costimulatory genes provides insights for potential combination therapies. Based on the favorable safety profile and strong correlative biomarker data, a phase 1/2 trial combining NKTR-214 and nivolumab is currently enrolling.

P78

Immune, molecular and T cell repertoire landscape of 235 resected non-small cell lung cancers, paired normal lung and peripheral blood mononuclear cells

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Background

Non-small cell lung cancer (NSCLC) is characterized by a high mutational load. Accordingly, it is also among the tumor types that respond to immune checkpoint blockade, presumably mediated by the anti-tumor T cell response. However, the lung is continuously exposed to the outside environment, which may result in a continuous state of inflammation against outside pathogens rather than tumor cells. Therefore, further investigation into the T cell repertoire and T cell phenotypes across normal lung and tumor is warranted.

Methods

We performed T cell receptor (TCR) sequencing and whole exome sequencing (WES) on PBMC, normal lung, and tumor from 235 NSCLC patients. Of these patients, 96 were also subjected to WES and TCR sequencing. We further performed Cytometry by Time-of-Flight (CyTOF) on 10 NSCLC tumors and paired normal lung tissues to phenotype immune and T cell subsets.

Results

Comparison of the T cell repertoire showed 9% (from 4% to 15%) of T cell clones were shared

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between normal lung and paired tumor. Furthermore, among the top 100 clones identified in the tumor, on average 57 (from 0 to 95) were shared with paired normal lung tissue. Interestingly, T cell clonality was higher in the normal lung in 89% of patients suggesting potential differences in the immune response and immunogenicity. A substantial number of somatic mutations were also identified not only in NSCLC tumors (average 566; from 147 to 2819), but also in all morphologically normal lung tissues (average 156; from 50 to 2481). CyTOF demonstrated striking differences in the immune infiltrate between normal lung and tumor, namely a unique GTR+ T cell subset (0.96%) which was entirely restricted to the normal lung. Conversely, increases in regulatory T cell frequency (CD4+FoxP3+) were observed in the tumor (10.4% vs 1.7% in normal lung), further highlighting the differences in T cell phenotype and response across normal lung and tumor.

Conclusions

These results suggest that a substantial proportion of infiltrating T cells in NSCLC tumors may be residential T cells associated with response to environmental factors. However, normal lung and NSCLC tumors carry T cells of distinct phenotypes including increases in immunosuppressive T cells within the tumor which may further highlight the differences in the anti-tumor immune response.

P79

T cell inflamed gene expression profile (GEP) analysis of pembrolizumab- and ipilimumab-treated patients with advanced melanoma in the multicenter, randomized, open-label phase 3 KEYNOTE-006 study

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Background

The independently validated T cell-inflamed GEP has been consistently associated with response to the anti-programmed death 1 (PD-1) antibody pembrolizumab. We analyzed gene expression in baseline tumor samples from the KEYNOTE-006 study of pembrolizumab versus the anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibody ipilimumab to determine the relative predictive effects of the T cell-inflamed GEP on clinical outcomes in advanced melanoma.

Methods

Patients were randomized to pembrolizumab 10 mg/kg every 2 or 3 weeks or 4 doses of ipilimumab 3 mg/kg every 3 weeks. Total RNA from baseline tumor samples was isolated from 4- μ m formalin-fixed, paraffin-embedded tissue sections and analyzed on the NanoString nCounter gene expression platform. Overall survival (OS) was the prespecified primary end point at final analysis; progression-free survival (PFS) and best objective response (BOR) were secondary end points. For efficacy comparison of pembrolizumab versus

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ipilimumab across T cell–inflamed GEP levels, the scores were categorized into tertiles (low: $GEP < -0.28$; middle: $-0.28 < GEP < 0.07$; high: $GEP > 0.07$), as prespecified in the statistical analysis plan, and Cox proportional hazards modeling was used to compare the treatment effect of the pooled pembrolizumab arms versus ipilimumab within each subgroup. Data cutoff for clinical analyzes was December 3, 2015.

Results

Of 834 enrolled patients, gene expression data were available for 427 (309 pembrolizumab; 118 ipilimumab) with OS/PFS data and for 375 (269; 106) with BOR data. Baseline characteristics were similar between the GEP and overall patient populations. Association of the T cell–inflamed GEP with OS was confirmed within each treatment group ($P < 0.0001$ pembrolizumab; $P = 0.0127$ ipilimumab). Evaluation of the between treatment group effect on OS was significant in all tertile subgroups (hazard ratio [HR]=0.61, $P = 0.0282$ [low]; HR=0.60, $P = 0.0224$ [middle]; and HR=0.44, $P = 0.0042$ [high]) but was most pronounced in the high group. Qualitatively similar results were observed for PFS. Although the T cell–inflamed GEP was not associated with BOR among ipilimumab-treated patients (area under the receiver operating characteristic [AUROC]=0.511; $P = 0.4723$), it was associated with BOR among pembrolizumab-treated patients (AUROC=0.696; $P < 0.0001$). Association between the T cell–inflamed GEP and tumor PD-L1 expression by immunohistochemistry and the relationship with clinical outcomes will be presented.

Conclusions

T cell–inflamed phenotype as measured by the GEP shows a differential association with OS for pembrolizumab versus ipilimumab, which may be explained by the ability of the GEP score to predict BOR in the tumor microenvironment with anti-PD-1, but not anti-CTLA-4, therapy.

Trial Registration

ClinicalTrials.gov Identifier NCT01866319

P80

Testing systems immune monitoring by mass cytometry for clinical correlative research

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Background

Systems immune monitoring during cancer treatment can track therapy response and reveal biomarkers [1]. In metastatic melanoma, this approach has implicated proliferating T cell subsets as a cellular effector mechanism for checkpoint inhibitors [2, 3]. Aims here were: 1) develop a robust cancer immune monitoring panel for multi-center clinical correlative research conducted by a core, and 2) generate pilot data to train and test computational tools employing machine learning algorithms.

Methods

Peripheral blood mononuclear cells (PBMC) were collected with informed consent from melanoma patients with local Institutional Review Board (IRB) approval and in accordance with the Declaration of Helsinki (N = 10 samples from 5 patients). For each patient, PBMC were collected before and approximately 3 weeks post-treatment with anti-PD1 immunotherapy. A commercially available antibody kit, Fluidigm's Complete Human T cell Immuno-Oncology panel kit, was chosen to focus on T cell subsets, memory, activation, and immunology proteins. Also tested was the addition of proliferation marker Ki67 and cell identity markers CD19 and CD14. Data were collected using a Helios mass cytometer, normalized using normalization beads, and analyzed with viSNE, Cytobank, and MEM [4] following established methods [5].

Results

Individual patient immunophenotypes were consistent pre- and post- anti-PD-1 therapy. Addition of markers to the commercial antibody sets was straightforward. CD19 and CD14 proved

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unnecessary for automatic identification of cell subsets by viSNE and MEM. Ki67 enabled detection of T cell proliferation. Because Ki67 is less abundant in cells, mass cytometry panel design should be used to detect Ki67 in a channel with high sensitivity.

Conclusions

Use of mass cytometry and commercially available metal conjugated antibodies provided a robust method for systems immune monitoring in cancer therapy compatible with correlative research in larger clinical studies.

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P81

The immunomodulatory effects of cancer therapy on IFN-gamma responses in the periphery

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Background

The immune system is recognized as an important ally in the fight against cancer. Indeed patients' survival outcome can be predicted by their immune response bias at the tumor site. With the ever expanding landscape of cancer therapy options, including immunotherapeutic agents, it has become critical to determine how patients' immune responses are affected by treatment. In light of the challenges of repeatedly accessing tumor tissue, investigating immune responses in the periphery assumes increased importance.

Methods

IMAGE-1 is a randomised open-label, phase II, first-line, proof of concept study (NCT01303172), investigating the combination of IMM-101, an immunomodulatory treatment comprising of heat-killed whole cell *Mycobacterium obuense* (NCTC13365), with Gemcitabine for the treatment of advanced pancreatic cancer. Treatment with IMM-101+Gemcitabine increased median survival to 7.0 months in metastatic pancreatic cancer patients compared to 4.4 months following treatment with Gemcitabine alone (1). We measured levels of IFN-gamma, a critical cytokine for anti-tumor responses, in patients' serum samples. To overcome technical difficulties associated with measuring serum IFN-gamma, we used an immunoassay (LLOQ=9.5fg/ml) developed by Myriad RBM for the Quanterix Simoa™ platform. This approach provides ultra-sensitive measurement of biomarkers, achieving orders-of-magnitude greater sensitivity than conventional immunoassay platforms.

Results

We report that peripheral IFN-gamma levels increased following initiation of treatment (Gemcitabine±IMM-101). In the initial 50 serum samples analyzed, there was a statistically significant increase in IFN-gamma levels from time 0 (screening/randomization; 0.12pg/ml, 95% CI 0.084, 0.18pg/ml) to week 13 (0.41pg/ml, 95% CI 0.26, 0.66pg/ml), when patients had been scheduled to receive 3 cycles of Gemcitabine (1000 mg/m² for 3 consecutive weeks out of 4) ± IMM-101 (1mg,

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intradermally on 6 occasions). Our previous data show that IMM-101 activates primary murine and human dendritic cells, and in murine models instigates IFN-gamma production by a variety of innate and adaptive immune cells. We investigated whether patients that received IMM-101 in addition to Gemcitabine had increased serum IFN-gamma levels. Preliminary data suggest that patients treated with Gemcitabine+IMM-101 (n=15) had higher IFN-gamma secretion at week 13 (0.57pg/ml, 95% CI 0.33, 0.98pg/ml) than patients (n=9) treated with Gemcitabine alone (0.24pg/ml, 95% CI 0.099, 0.58pg/ml).

Conclusions

We are in the process of confirm these findings in further serum samples from IMAGE-1 patients and addressing the specific cellular sources of IFN-gamma in murine experimental models.

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P82

Potential biomarkers in mRCC patients treated with nivolumab

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Background

Checkpoint inhibitors produce meaningful survival benefit in mRCC patients (pts). Predictive biomarkers of response to anti-PD1 therapy are still under evaluation in clinical trials. Prospectively we

explored biomarkers that might have potential predictive and prognostic value in mRCC pts on nivolumab therapy.

Methods

Patients with mRCC who had 1 or more prior lines of therapy received nivolumab at 3 mg/kg q2w (n=23) from 2015 to 2017. Blood samples were collected at baseline and 2 mo *after* treatment start. Serum levels of TGF- β 1, IL-17A, and soluble PD-1 (sPD-1) were measured by ELISA. Pre-treatment specimens from primary tumors (n=17) were evaluated for PD-L1 and FOXP3 expression on tumor-infiltrating immune cells (IC) using the IHC assay (SP142; EP340).

Results

With a median follow-up of 10 mo, PFS was 4 mo (95% CI: 1,37-10,04) and 1-year PFS was 17%, median overall survival was not reached. The optimum diagnostic cut-off value (mean + 2SD) for TGF- β 1 was 20 ng/mL (sensitivity 43,75%, specificity 100%), for IL-17A was 0,5pg/mL (sensitivity 40%, specificity 88,23%), for sPD-1 was 0,45 ng/mL (sensitivity 40%, specificity 88,8%). Higher TGF- β 1 level at baseline was associated with shorter PFS (6 vs 1,5 mo; p=0,03). Higher baseline IL-17A (p=0,0174) and sPD-1(p=0,039) levels were significantly associated with higher ORR (21,7%). Using the $\geq 0,5\%$ and $\geq 1\%$ cut-off for PDL-1 expression , 11/17 (64%) and 8/17 (47%) pts were considered PD-L1 positive respectively. When a cut-off of $\geq 0,5\%$ was used clinical effect (tumor shrinkage $>10\%$) was higher in the PD-L1-positive group than in the PD-L1-negative group (54,5% vs 33,3%). Using the $\geq 10\%$ cut-off for FOXP3, 11/17 pts were considered positive (64%). Clinical effect was 45% in the FOXP3-positive group and 50% in the FOXP3-negative group. Expression of PD-L1 and FOXP3 on IC didn't not have a significant effect on PFS (p $>0,05$).

Conclusions

Our results demonstrated improved survival benefit in patients with lower levels TGF- β 1 (<20ng/mL). Elevated baseline levels of IL-17A (>0,5pg/mL) and

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sPD-1 (>0,45ng/mL) were significantly associated with ORR. PD-L1 and FOXP3 IC expression have no predictive or prognostic value for pre-treated RCC pts receiving nivolumab therapy.

P83

Characterizing cancers by prevalence on the PD-L1/CD8 axis

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Background

Checkpoint inhibitor-based immunotherapies are successful across various cancers, particularly, type I cancers, characterized by tumoral PD-L1 expression and by a repertoire of tumor-infiltrating lymphocytes, respond well to therapies targeting PD1/PD-L1 [1]. While it has been shown that combinatorial CD8(+) and PD-L1(+) cell densities provide predictive value for durvalumab in NSCLC [2], we present in this study profiling results for multiple indications. The findings are set into the context of tumor mutation burden (TMB) which has been proposed as additional predictor for response to immunotherapies [3].

Methods

Image analysis (Definiens, Munich, Germany) detected PD-L1(+) tumor and CD8(+) immune cells in pathologist annotated tumor center in serial, IHC-stained tissue sections (Ventana SP263 and SP239 resp.) from multiple cancer indications. Automatic image alignment enabled the computation of tile-based (size=64µm) cell coverage statistics: percentage of tiles with at least one PD-L1(+) (CD8(+) resp.) cell, and co-occurrence C-score [4] of tiles containing at least one PD-L1(+) and one CD8(+) cell. The indications were ranked per TMB as published in [3], where the authors used

comprehensive genomic profiling using the FoundationOne assay (Cambridge MA, USA).

Results

In Figure 1, PD-L1(+) and CD8(+) low and high patients were defined by median cut-points (1% PD-L1(+) tiles, 36% CD8(+) tiles). The population prevalences in the PD-L1(+)^{High}CD8(+)^{High} group correlate with mutational burden (Spearman's $r=0.96$, $p<0.01$), with the notable exception of BLAD (similar prevalence reported in [5]). PANC prevalence dominates the PD-L1(+)^{Low}CD8(+)^{High} group ($>2\sigma$). The CD8(+)^{Low} groups are heterogenous in prevalences.

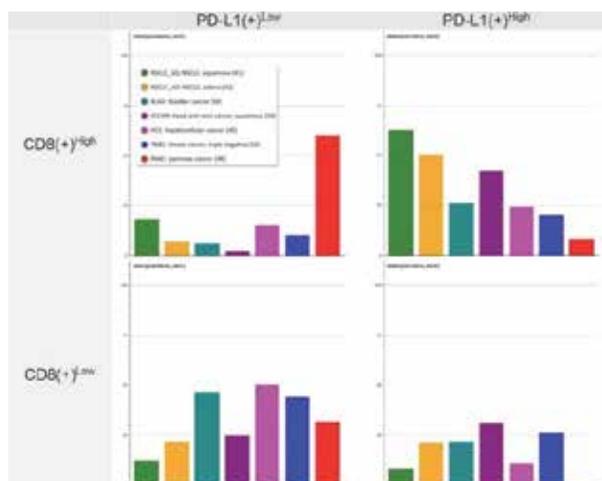
Figure 2 shows the mean PD-L1(+) and CD8(+) C-score in all cancers of this study. Mutation-high NSCLC is characterized by high C-scores, whereas mutation-low PANC is characterized by low C-scores, suggesting dysfunctional CD8(+) cells or tumor specific mutations suppressing response to IFN γ .

Conclusions

This study presents an automated profiling method for cancers, which may provide clinical decision support for which type of tumor a certain type of immunotherapy works best. While our findings are consistent with population statistics on tumor mutational burden, they highlight the relevance of spatial analysis in tissue for a deepened understanding of the mechanisms of diseases.

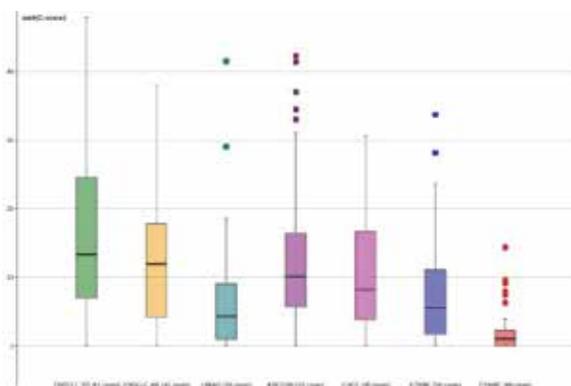
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Figure 1. Cancer prevalences in the PD-L1(+) and CD8(+) low and high groups



Prevalences of seven cancer indications (see inset, with number of patients), grouped by classification into PD-L1(+) and CD8(+) low and high tile percentage tumors. The indications were sorted with respect to decreasing TMB as defined in [3].

Figure 2. Mean of C-scores



Mean of C-scores (x-axis) for seven cancer indications (y-axis), whereas the indications are sorted by tumor mutational burden (TMB). The ranking of indications in C-scores correlates well with TMB (Spearman's $r=0.96$, $p<0.01$).

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P84

Relevance of the microenvironment and MHC class I mediated immune escape mechanisms of tumors for anti-tumoral immune responses

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Background

Abnormalities of classical and non-classical MHC class I antigens were frequently found in tumor cells of distinct origin resulting in evasion of tumor cells from T cell-mediated immune surveillance. The underlying molecular mechanisms of these alterations are complex and include transcriptional, epigenetic and posttranscriptional control of MHC class I antigens and components of the antigen processing machinery (APM). Furthermore, the escape of tumor cells from immune surveillance is also linked to changes in the cellular composition of the tumor microenvironment and peripheral blood mononuclear cells.

Methods

Tumor cells were analyzed for the expression of HLA class I APM components by qPCR, Western blot analysis and/or flow cytometry. Immunohistochemistry and multispectral imaging was employed to determine the frequency, localization and topology of suppressor and effector immune cells.

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Results

While impaired expression of classical MHC class I and HLA-G is often due to transcriptional downregulation, alterations in the histone acetylation and methylation appear not to be so frequent. Defects in the IFN signal transduction, oxidative status of MHC class I antigens as well as altered expression of immune modulatory microRNAs and RNA-binding proteins affects the expression of MHC class I APM and/or HLA-G. In addition, the extracellular matrix protein biglycan has been shown to increase MHC class I surface expression, which was associated with enhanced immune cell responses and reduced expression of members of the TGF- β pathway. However, not only tumor-, but also therapy-induced effects are responsible for shaping MHC class I antigen expression. Furthermore, high levels of immune suppressive cells in the tumor were associated with a worse prognosis, but in particular the distance of immune cells to each other, and to tumor cells has prognostic value. This could be further extended by combining the immune cell infiltration with MHC class I APM component expression. In addition, downregulation of MHC class I APM component expression has been shown to occur could upon adoptive cell therapy.

Conclusions

Thus, these data suggest that the molecular make up of tumor cells might shape the immune response, which might serve as biomarkers for prediction of immune-based therapies. In conclusion, a better understanding of the immune evasion mechanisms is still required for improving T cell-based, individualized immunotherapies, and appears to be of importance in the development of resistances to these therapies, but opens also new venues for therapeutic intervention.

P85

HAAH and MMP9 are complementary cancer biomarkers and potentially enhanced predictors of metastasis

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Background

When used together, complementary biomarkers associated with mediators of cancer cell mobility and invasiveness should be useful early disease and patient outcome predictors. The present investigation is an initial quantitative assessment of HAAH (aspartyl (asparaginy) beta hydroxylase) and MMP9 (matrix metalloproteinase 9) in serum/serum exosomes from cancer patients to evaluate their concerted role in metastasis.

Cancer-specific cell surface HAAH functions by enzymatically modifying a number of motif-restricted protein targets including Notch. It thereby indirectly triggers events leading to metastasis. MMP9 is a well-known predictor of metastasis due to its inherent effect on the process of proteolytically-assisted tumor cell escape, albeit not completely useful as a cancer biomarker on its own. We propose here that up-regulated HAAH is a prerequisite for metastasis and that in turn MMP9 is an enabler of this process.

Methods

We detect serum and exosomal HAAH by a simultaneous-homologous ELISA format using an in house manufactured reagent kit comprising pre-coated microplates and pre-formulated reagents. Serum and exosomal MMP9 was detected with a commercial reagent kit ELISA (Abcam). Exosomes were prepared using a 50% polyethylene glycol 6000/ 0.5 M NaCl solution added to serum, centrifugation, and reconstitution. CEA positive cancer and healthy serum samples were commercially obtained (Complex Antibodies) or through off site collaborators.

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Results

Similar recovery of HAAH (58.3%) and MMP9 (54.0%) from exosomes compared to the source CEA positive cancer serum samples was observed, suggesting exosome co-localization. Further, both biomarkers were quantified in a larger investigation of samples obtained from high risk volunteers that potentially could have cancer. In this ongoing field study of 48 accumulated serum samples, 9 were positive for HAAH. Of these 9 samples 6 were positive for MMP9. Therefore at least three subjects had elevated HAAH that was not paralleled by increased MMP9 levels.

Conclusions

While HAAH and MMP9 are both expected to be closely associated with metastatic activity of cancer cells, both co-localize in cancer derived exosomes, and both appear to be regulated by the same transcription factor(s), their expression in serum samples are mostly coincident but sometimes may differ. This may explain differences in metastatic potential. These studies are ongoing and focused upon determining whether using both biomarkers could lead to a more accurate prediction of metastatic potential.

P86

Altered immune cell repertoire and activity after checkpoint blockade immunotherapy with nivolumab in renal cell carcinoma

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Background

Programmed cell death protein-1 (PD-1) and its ligand (PD-L1) provide tumor cells with mechanisms to escape from immune surveillance. Antibodies directed against these checkpoint molecules have been developed and successfully employed for the treatment of solid tumors including renal cell carcinoma (RCC). Despite promising clinical benefits, only 20 - 30% of tumor patients respond to these therapies. In order to search for predictive or prognostic biomarkers the immune cell repertoire in the peripheral blood of RCC patients was determined prior and during anti-PD-1 therapy with the PD-L1 antibody nivolumab.

Methods

We included 43 patients who had an indication for second line therapy for clear cell RCC with nivolumab. Blood specimen and tumor samples were collected at baseline and three months post-Nivolumab onset and at a clinical event such as progression or remission. Cell differentiations were performed as well as using multicolor flow cytometry and nanostring technology. In addition, tumor biopsies obtained before treatment were analyzed by multispectral imaging (MSI).

Results

Peripheral blood and tumor lesions of seven RCC patients have been analyzed in detail: 1/7 RCC patients had a stable disease (14%), 3/7 (43%) patients a partial or complete remission. One RCC patient had serious adverse effects upon treatment resulting in discontinuation of therapy, while two other RCC patients had a progressive disease and therefore were shifted to chemotherapy. Immunohistochemical evaluation by MSI indicated a higher frequency of Foxp3+ cells in the three progressive RCC patients in the stroma of the tumor, and particularly in the central core, whereas lower levels of CD163+ macrophage were present in the core of the tumor. Increased levels of CD4+ Foxp3+ regulatory T cells (Treg) were also present in the peripheral blood of progressive RCC patients. During the therapy, variation in the Treg frequency of peripheral blood did not correlate with clinical

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outcome of patients, whereas a reduction in HLA-DR+ antigen presenting cells did. Nanostring analyzes highlighted enhanced levels of interleukin (IL)-2 transcript in the peripheral blood of the two progressive RCC patients, in contrast to the diminished IL-2 mRNA levels in the three responders.

Conclusions

These data suggest that the evaluation of a larger number of RCC patients combining these three different techniques might allow identifying a set of reliable markers that predict responsiveness to nivolumab therapy in these patients.

P87

Results of epigenetic-based quantitative PCR assisted immune cell counting analysis in bavituximab SUNRISE trial subgroup

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Background

SUNRISE (NCT01999673), a global, double-blind, randomized Phase III trial of docetaxel plus bavituximab (D+B) or docetaxel plus placebo (D+P) in previously treated non-squamous non-small cell lung cancer (NSCLC), demonstrated similar overall survival (OS) in the intent-to-treat population (n = 597). In the subgroup of 93 patients who received subsequent immune checkpoint inhibitors (ICI), median overall survival (mOS) was not reached (95% CI, 15.2-NA) in the D+B group (n=46) and was 12.6 months (95% CI, 10.4-17.8) for patients in the D+P group (n=47) (HR for death, 0.46; P = 0.006). Epigenetic-based quantitative real-time PCR assisted cell counting (qPACC) of immune cells in

blood was used to potentially identify predictive biomarkers.

Methods

DNA was isolated from peripheral blood mononuclear cells (PBMC) from randomized patients and treated with bisulfite leading to conversion of de-methylated cytosine (epigenetically active) residues into uracil, but left methylated ones unaffected. The de-methylation status of DNA regions, previously identified of being specific to respective immune cell phenotypes sorted by FACS: CD3+, CD4+, CD8+, TFH, TH17, PD1, Foxp3, naïve CD8, MDSC, CD14+, NK56+, B-cells, GNLY, and CCR6+, was quantitated by qPACC. The number of de-methylated gene copies per biomarker was quantitated and translated into % total of cells in the sample. Each biomarker was classified as high or low based on the median value across all patients and correlated to OS. Hazard ratios (HR) and confidence intervals (CI) were estimated using a Cox proportional-hazards model.

Results

Pre-treatment (pre-tx) samples were evaluable by qPACC for 62 (32 D+B, 30 D+P) out of the 93 patients who received ICI as next line therapy after the SUNRISE assigned treatment. High pre-tx (\geq median) levels correlated with statistically significant OS benefit favoring D+B for the following biomarkers: CD3+ (HR=0.37, p=0.023), CD4+ (HR=0.32, p=0.012), CD8+ (HR=0.42, p=0.036), PD-1 (HR=0.33, p=0.017), GNLY (HR=0.25, p=0.011), FoxP3 (HR=0.34, p=0.032), naïve CD8+ (HR=0.32, p=0.034), B-cells (HR=0.24, p=0.007), MDSC (HR=0.33, p=0.037) NK56+ (HR=0.29, p=0.026). Low (<) pre-tx levels of THF correlated with OS favoring D+B (HR=0.34, p=0.033). No OS difference was observed for pre-tx high or low levels for TH17, CCR6, or CD14+.

Conclusions

High pre-tx levels (\geq median) of circulating immune cells including T cells, B-Cells, MDSCs, NK cells correlated with significant improvement of OS in patients who received D+B then ICI compared to

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D+P then ICI in the SUNRISE trial. These results support further investigation of these markers in future bavituximab clinical trials.

P88

CD96, a new candidate for checkpoint blockade in human hepatocellular carcinoma

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Background

It was reported that murine CD96 functions as a checkpoint in natural killer (NK) cell functional exhaustion. To date, blocking CD96 has not been demonstrated to be of substantial benefit in patients with cancer. Here, we demonstrated that human CD96 works as a checkpoint in hepatocellular carcinoma (HCC) patients.

Methods

In this study, we analyzed CD96 expression and the related dysfunction of NK cells located in intra- or peritumor regions of liver tissue samples from HCC patients, in addition to analyzing disease outcomes.

Results

Through the use of paired peritumoral tissues (PT) and IT from 236 HCC patients, we found that increased expression of CD96 on NK cells in intratumoral but not peritumoral regions, along with increased expression of its ligand CD155 and a poor prognosis. Then we investigated the expressions of CD226, TIGIT and CD96 (Immunoglobulin-like family members and share the common ligand CD155) in 54 fresh tumor tissues from HCC patients and 20 normal livers from health controls. We observed increased CD96 and decreased TIGIT expressions in NK cells from IT of

HCC patients, and an increased number of CD96⁺ NK cells in HCC patients is associated with poor clinical characteristics and prognosis.

Human CD96⁺ NK cells from health controls exhibited functional exhaustion, showing decreased IFN- γ and TNF- α productions, impaired cytotoxicity in response to *in vitro* stimulation. CD96 blockade can enhance NK cytotoxicity on CD155 expressing target cells, and play a protective role against tumor through boosting NK cell immune response. Global transcriptomic analysis of sorted CD96⁺ and CD96⁻ hepatic NK cells further demonstrated exhausted features of CD96⁺ NK cells comparing to CD96⁻ NK cells.

Further, we demonstrated that high levels of TGF- β in HCC patients induced this increased expression of CD96 in NK cells. Blocking TGF- β specifically inhibited CD96 expression in NK cells. In addition, we compared other two receptors, CD226 and TIGIT, which share common ligand CD155 with CD96, and found CD96 play a more important role in NK exhaustion.

Conclusions

Overall, these data demonstrate that targeting CD96 is a potential approach for cancer immunotherapy.

P89

Plasma-derived exosomes carrying CTLA-4, PD-1 and PD-L1 in head and neck squamous cell carcinoma patients treated with immunotherapy is associated with disease outcome

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Background

Exosomes, the smallest subset of extracellular vesicles, present in body fluids serve as a cellular communication network. In the tumor microenvironment, exosomes carry messages from the tumor to local and distant cells and are also potential biomarkers of tumor progression. Exosomes express immunomodulatory molecules and shape antitumor immunity.

Methods

The role of exosomes as circulating biomarkers of response to immunotherapy was evaluated by monitoring changes in the protein cargo of exosomes isolated from plasma of patients with head and neck squamous cell carcinoma (HNSCC) treated with a combination of Cetuximab, Ipilimumab and radiotherapy. We retrospectively evaluated changes in the exosome protein cargo at multiple time points during therapy to assess the correlation of these changes with clinical outcome. Patients (n=18) with previously untreated advanced HNSCC enrolled in the phase I clinical trial (NCT01935921) donated plasma specimens at baseline, during (week 5, week 14), after (6 months) and long-term after immunotherapy. Exosomes were isolated from plasma by size exclusion chromatography. The protein content of exosomes in fraction #4 (30-150nm vesicles) was measured. Immunocapture with biotinylated anti-CD3 mAb was used to separate T cell-derived from non-T cell-derived exosomes. CD3+ exosomes bound to streptavidin-labeled beads were harvested. The uncaptured exosomes, derived from CD3- cells, were bound to beads using biotinylated anti-CD63 mAb. Flow cytometry-based antigen detection on bead-bound exosomes was performed using labeled Abs specific for CTLA-4, PD-1 and PD-L1. Ratios of positive exosomes/total plasma exosomes were assessed for correlation with patients' recurrence status at last follow-up (median of 14 months).

Results

Increases in the protein levels of total exosomes during therapy associated with disease recurrence. Decreases in frequency of CTLA-4+ exosomes in

CD3+ and CD3- fractions or of PD-1 in the CD3+ fraction occurred as early as 5 weeks into therapy. Patients segregated into two groups based on increasing vs. decreasing PD-L1+ exosomes/total exosomes. 4 patients with increasing CTLA-4+ and PD-L1+ exosomes during therapy had recurrence and died of disease. The 5th patient with this "unfavorable" exosome profile remains disease free (NED). 13 patients who had "favorable" profiles (decreased CTLA-4+ and PDL1+ exosomes) have not recurred.

Conclusions

Changes in the ratio of exosomes carrying checkpoint proteins/total exosomes during immunotherapy associated with oncologic outcome. The checkpoint molecules content of T cell-derived vs non-T cell-derived exosomes defined "favorable" and "unfavorable" immune profiles that associated with the patients' responses to therapy. Plasma exosomes in cancer patients emerge as early markers of response to immune therapies and outcome.

P90

RNA-based immune gene set signatures demonstrate immune modulation by RXDX-106, a novel TAM (TYRO3, AXL, MER) family small molecule kinase inhibitor

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Background

The TAM family of receptor tyrosine kinases, including TYRO3, AXL, and MER, is pivotal in regulating the immune system. In the tumor microenvironment, TAMs promote anti-inflammatory/pro-tumorigenic activities. RXDX-106

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is a potent and selective TAM inhibitor in preclinical development, shown to have immunomodulatory effects. We herein illustrate that (1) MC38, a syngeneic mouse model of colorectal cancer, is sensitive to RXDX-106, and (2) using RNA sequencing, we demonstrate RXDX-106 treatment results in the recruitment of various immune cell subtypes to the tumor microenvironment. Flow cytometry analysis supports key RNA sequencing findings.

Methods

MC38 tumor-bearing C57BL/6 mice were treated with and without RXDX-106 for 7 days. Tumor tissue was collected for cellular and molecular characterization to measure immune modulation, via flow cytometry and total RNA sequencing respectively. Single sample gene set enrichment analysis (ssGSEA) was applied to RNA sequencing data to calculate normalized enrichment scores (NES) for 15 adaptive and 13 innate immune cell types, based on previously published gene sets. RNA-sequencing significance levels are presented as false discovery rates (FDR) to correct for multiple hypothesis testing. Significance of flow cytometry data are indicated by probability (P) values calculated using the two-tailed T-test.

Results

MC38 tumors. A high proportion (66%, 33 of the top 50) of individual genes differentially expressed between treated and untreated mice have immunomodulatory roles. Furthermore, ssGSEA suggested a significant enrichment (FDR<0.01) of T cell subtypes, including T follicular helper (Tfh) cells (FDR=0.0005), Type 1 T helper (Th1) cells (FDR=0.0005), and central memory CD8+ T cells (FDR=0.0008) with RXDX-106 treatment. flow cytometry data revealed a significant increase in overall tumor infiltrating lymphocyte (TIL) recruitment (P<0.0001), as well as a non-significant trend towards increased CD8+ T cell infiltration in RXDX-106 treated mice. Innate immune cells, such as plasmacytoid dendritic cells (pDCs; FDR=0.0005) and macrophages (FDR=0.0005), also had significantly elevated NES values in treated mice. A

negative correlation ($r=-0.7$) between macrophage NES and M2 secreted *VEGFA* expression was observed and may suggest infiltrating macrophages are polarized to the pro-inflammatory/anti-tumorigenic M1 phenotype upon treatment with RXDX-106. Flow cytometry data supported the observed increase in tumor associated macrophages (P=0.0008), and demonstrated M1 polarization by a highly significant (P<0.0001) increase in M1/M2 ratio with RXDX-106 treatment.

Conclusions

We have demonstrated the application of an RNA sequencing based approach to detect immunomodulatory effects and provided evidence supporting immunomodulatory activity of RXDX-106 *in vivo*.

P91

A harmonized evaluation system for expression of HLA class I antigens in formalin-fixed paraffin embedded cancer tissues

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Background

Expression of human leukocyte antigen class I antigens (HLA-I) on tumor cells is crucial for target recognition of cytotoxic T cells (CTLs). Therefore, it should be an important biomarker for CTL-based cancer immunotherapy to evaluate the expression levels of HLA-I on tumor cells. A monoclonal anti-human HLA-I antibody, EMR8-5 is the first reliable antibody that is suitable for immunohistochemistry (IHC) of formalin-fixed paraffin embedded (FFPE) tissues [1]The aim of this study is to establish the

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harmonized protocol to evaluate expression levels of HLA-I on cancer cells in FFPE tumor tissues.

Methods

First of all, we determined a standardized method of staining, and proposed tentative criteria to evaluate expression levels of HLA-I in tissue sections; (+++) as $\geq 90\%$ of cancer cells are strongly positive, in which the staining density is equal or stronger than that in lymphocytes or endothelial cells, (++) as $< 90\%$ and $\geq 50\%$ of cancer cells are strongly positive, (+) as $< 50\%$ and $> 10\%$ of cancer cells are strongly positive or $> 10\%$ of cancer cells are weakly positive, in which the staining density is weaker than that in lymphocytes or endothelial cells, and (-) as $\leq 10\%$ of cancer cells are positive. Reference IHC photo atlas was made for each type of cancer. After IHC, the microscopic images were converted into digital photo files, and the immunolabeled cells were analyzed on the standardized calibrated monitors (ColorEdge CG2420, EIZO, Japan). Then we examined inter-observer reproducibility of the criteria by multi-institutional study using each 20 cases of 7 cancers (colorectal, breast, prostate, pancreatic, ovarian, lung, and kidney cancers), which had been surgically resected in National Cancer Center Hospital, Tokyo.

Results

IHC with anti-pan HLA-I antibody was performed using automated IHC slide staining system. Expression profiles of HLA-I were characteristic in each cancer type. We made reference photo atlas of IHC to harmonize the evaluation for each type of cancer. According to the reference atlas, four certified pathologists scored the expression levels of HLA-I independently. Inter-observer reproducibility for each cancer was within moderate to substantial agreement levels (Fleiss's kappa values were within 0.5 to 0.7).

Conclusions

Expression of HLA class I antigens can be evaluated with an acceptable reproducibility in several cancers using FFPE tissues.

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P92

Early biomarker correlates of severe neurologic events and cytokine release syndrome in ZUMA-1, a multicenter trial evaluating axicabtagene ciloleucel in refractory aggressive non-hodgkin lymphoma

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Background

Axi-cel, an autologous anti-CD19 chimeric antigen receptor (CAR) T cell therapy, demonstrated an 82% objective response rate including 54% complete remissions in refractory aggressive NHL patients.¹ NE and CRS are associated with CAR T cell therapy.¹ Post-treatment levels of serum analytes, measured after the onset of clinical toxicities, were associated

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with severe NE and CRS.² Lymphodepleting conditioning-related (day 0) and early post-CAR T cell treatment (day 1) biomarkers associated with NE and CRS may provide important mechanistic insight and inform toxicity management.

Methods

Serum samples were obtained pre and post axi-cel infusion, from 101 refractory aggressive NHL patients in the Phase 2 ZUMA-1 (NCT02348216) trial [1]. A panel of 44 analytes representing major categories of immune function were measured as described before. [2] *Post hoc* statistical analyzes evaluated associations of analyte levels on day 0 and day 1, in patients with/without grade ≥ 3 NE/CRS. Univariate Wilcoxon two-sample tests were applied and adjusted for multiplicity, to identify important co-variates. Multivariate machine learning (random forest analysis [3]) was conducted to rank the most influential co-variates.

Results

Grade ≥ 3 NE were observed in 28/101 (28%) patients. Those developing grade ≥ 3 NE had increased IL-15 and decreased perforin ($P=0.0006$ and $P=0.0011$, respectively) at Day 0, and increased IL-15, MCP-1 and IL-6 ($P=0.0021$, 0.0037 and 0.0085, respectively) and decreased perforin ($P=0.0025$) at Day 1. Multivariate analysis identified identical analytes as the univariate analysis and ranked the importance of analytes relative to each other. Grade ≥ 3 CRS was observed in 13/101 (13%) patients. Those developing grade ≥ 3 CRS had no statistically significant differences in analyte levels at Day 0, and increased IL-15 at Day 1 (adjusted $P=0.026$).

Conclusions

These analyzes indicate IL-15 in NE/CRS pathogenesis post- anti-CD19 CAR T cells treatment, consistent with previous reports [4, 5, 6]. Association between early serum markers (day 0 and 1) and grade ≥ 3 NE may support development of clinically useful prevention and treatment algorithms.

Trial Registration

ClinicalTrials.gov Identifier NCT02348216

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P93 Abstract Travel Award Recipient

Small molecule binders of Ly6K inhibit tumorigenic growth and induce immunity via TGFb/Stat1 and PDL1 pathway

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Background

We have previously shown that members of the Ly6 gene family, namely Ly6E, Ly6D, Ly6H and Ly6K are expressed in multiple types of solid human cancers and that the increased expression of these genes is associated with poor outcome [1]. We also have found that Ly6K and Ly6E are required for in vivo tumor growth and anti-tumor immune response [2]. Ly6K is an attractive target due to its cancer cell-specific expression; normal organ expression of Ly6K is limited to testis.

Methods

We used surface plasmon resonance technology to identify two small molecule binders of Ly6K. These

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molecules showed strong binding to Ly6K (kd range 1-2mM) but not to Ly6E and Ly6D.

Results

Combined small molecule treatment led to reduced TGF β signaling and reduced PDL1 expression in MDA-MB-231 cells. Homology modeling showed that the two small molecules bind to the N-terminus and C-terminus of the mature Ly6K protein, respectively. Treatment of E0771 tumors in a syngeneic C57Bl/6 model with individual small molecules reduced tumor growth, while combination treatment with the two small molecules completely eliminated tumor growth. The treatment was halted after tumor disappearance. Three weeks later, the cured mice were re-challenged with E0771 cells, with complete, durable protection against tumor growth (Figure 1).

Conclusions

These results suggest that Ly6K inhibitors have anti-tumor properties and induce host-protective anti-tumor immunity. We are currently investigating the molecular basis of these findings in vivo. Our data implicate the novel biomarker Ly6K as a therapeutic target and immune modulator in multiple tumor types with high expression of Ly6K.

Figure 1. Treatment with Ly6K binding small molecule induce immunity

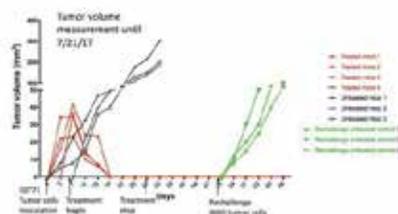


Figure 1: Ly6K binding small molecules induce immunity against tumor growth. Treatment of E0771 tumors in a syngeneic C57Bl/6 model with individual small molecules reduced tumor growth, while combination treatment with the two small molecules completely eliminated tumor growth. The treatment was halted after tumor disappearance. Three weeks later, the cured mice were re-challenged with E0771 cells, with complete, durable protection against tumor growth.

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P94

The RareCyte® platform for identifying rare antigen-specific circulating CD4 and CD8 T cells in Merkel cell carcinoma

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Background

Merkel cell carcinoma (MCC) is an often-lethal skin cancer associated with the Merkel cell polyomavirus (MCPyV) in 80% of cases. Although checkpoint immunotherapies have proven efficacious for a cohort of MCC patients, there is still a need to identify predictive biomarkers. Tumor antigen-specific T cells in peripheral circulation can be reflective of their phenotype within the tumor microenvironment making them attractive candidates as cellular biomarkers. However, the frequency of MCC antigen-specific CD4 and CD8 T cells can often be below the detection limit of flow cytometry making them difficult to find. We thus employed the highly sensitive RareCyte platform to detect and phenotype rare antigen-specific CD4 and CD8 T cells.

Methods

MCC patient peripheral blood mononuclear cells (PBMC) was stained and plated onto chamber well

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slides and imaged on the CyteFinder[®] 6-channel fluorescent scanning microscope. CD4 and CD8 T cells were identified by MCPyV-specific tetramer staining, and characterized with various exhaustion markers, including PD1, LAG3, TIM3, and CTLA4. Single MCPyV-specific CD4 T cells were picked and placed individually into tubes using an integrated computer-controlled retrieval system (CytePicker[®]). RNA was extracted and single cell sequencing was performed on the alpha and beta chains of the T cell receptor (TCR).

Results

We consistently identified MCPyV -specific CD4 and CD8 T cells below the detection limit of flow cytometry without the need for enrichment or large blood volumes. Furthermore we have high confidence in the cells identified and retrieved due to the morphologic information obtained with imaging that is not possible by flow cytometry. We were able to identify patients that had exhausted MCPyV-specific T cells in circulation and characterize the expression of exhaustion markers. We were also able to sequence the TCR from single rare MCPyV-specific CD4 T cells retrieved using the platform.

Conclusions

Using this approach to identify rare but functionally important MCPyV-specific T cells will enhance our understanding of the immune response against this oncogenic virus and may help guide patient selection for checkpoint therapies.

P95

Development and application of a 6-channel immunofluorescence assay for investigating PDL1 and IFR1 expression on circulating tumor cells

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Background

There is a need for non-invasive predictive biomarkers of response to anti-PD1/PDL1 therapies. Assessment of circulating tumor cells (CTCs) is a rational approach to non-invasive sampling of tumors to understand PDL1 phenotype. IFN-gamma signals through the JAK/STAT cascade to induce PDL1 via the Interferon Regulatory Factor-1 (IRF1) transcription factor and is a potent inducer of PDL1 expression in tumor cells. Previous studies comparing PDL1 and IRF1 tissue staining of melanoma showed that IRF1 had a higher predictive value than PDL1 of response to anti-PD1/PDL1 therapy [1]. Using CTC models and the RareCyte platform for CTC identification, we developed a multi-parameter assay that allows simultaneous PDL1 and IRF1 assessment after CTC identification. We applied this assay to clinical samples of breast and Merkel cell carcinoma (MCC).

Methods

Peripheral blood from normal donors or cancer patients was collected into RareCyte blood collection tubes. PDL1(+) and PDL1(-) CTC models were created by culturing A549 overnight with or without 10ng/ml INF-gamma. The A549 cells were then spiked into normal donor blood and buffy coats isolated from 7.5ml of blood by AccuCyte[®] separation and spread onto slides. Slides were stained with a 6-marker panel that included antibodies to pan-cytokeratin (CK), EpCAM, CD45, CD45, PDL1, IRF1, and a nuclear dye on the Leica Bond Rx auto-stainer. Slides were scanned with CyteFinder[®] and CTCs identified by CK and/or EPCAM positivity and negative CD45 staining. Confirmed CTCs were then assessed for expression of PDL1 and IRF1; cell compartment was also recorded for IRF1 staining.

Results

In unstimulated A549s, PDL1 staining was absent but cytoplasmic staining of IRF1 was often detectable. In contrast, IFN-gamma stimulated A549s demonstrated PDL1 staining and strong nuclear staining for IRF1. In patient samples, PDL1 was identified in a minority of CTCs in both breast

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cancer and MCC. PDL1(+) CTCs identified had concomitant strong nuclear IRF1 signal. IRF1 was seen in the majority of the PDL1(-) CTCs but staining could be either cytoplasmic and/or nuclear.

Conclusions

In this study we demonstrate the feasibility of combining PDL1 and IRF1 biomarkers with CTC identification for non-invasive assessment. The cellular distribution of IRF1 in combination with PDL1 expression may add predictive value to the assay.

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P96

Tumor mutation burden, microsatellite instability and chromosomal instability analysis using low pass whole genome sequencing of single circulating tumor cell

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Background

Tumor Mutation Burden (TMB), Microsatellite Instability (MSI) and Chromosomal Instability (CIN) represent the majority of Genomic instability (GI) in metastatic patients. Recent studies show that TMB and MSI are emerging immune checkpoint inhibitor drug sensitivity biomarkers, and CIN is a sensitivity marker for PARP inhibitors. Assessment of GI in bulk tumor samples is well explored, but it is limited by sample availability and tumor heterogeneity. Analysis of ctDNA is feasible for TMB and MSI but not CIN, and it also suffers in sensitivity and specificity in patients who harbor subclonal GI, limiting its clinical utility. Epic Sciences' Circulating Tumor Cell (CTC) platform employs a non-

enrichment based approach and could provide insight into subclonal heterogeneity. Here we present downstream single cell GI assay(s) for the detection of TMB, MSI and CIN from individual CTCs.

Methods

Contrived samples were prepared by spiking prostate cancer cell lines, LNCaP, PC3 and VCaP, into healthy donor blood. Red blood cells were lysed, nucleated cells deposited onto slides, slides immunofluorescently stained (DAPI, CK, CD45, and Androgen Receptor), and identified cancer cells individually isolated from the slides. Each recovered cell was lysed, whole genome amplified (WGA), shotgun library prepared, and low pass whole genome sequenced using Illumina NextSeq 500. Data were analyzed for TMB scores and large scale transitions. MSI was measured using Qiagen Type-It microsatellite PCR kit for four sites. Samples from metastatic castration resistant prostate cancer (mCRPC) patients were included to evaluate clinical feasibility.

Results

TMB scores for LNCaP (average 652) were significantly higher than PC3 (558), VCaP (548), and WBC from healthy donor (540) with $p < 0.01$. MSI assay confirmed that LNCaP is the only MSI-H cell line with insertions/deletions found in 3 of 4 microsatellite sites. No MMR deficiencies were found in PC3 and VCaP. LST analysis shows PC3 (average 33) and VCaP (33) have much higher LST scores than LNCaP (11). A wide range of TMB (375-861) and LST scores (0-70) are observed inter- and intra- mCRPC patient samples.

Conclusions

These data demonstrate the feasibility of detecting three types of genomic instabilities at the single cell level using the Epic Sciences CTC Platform. Inter- and intra-patient heterogeneity is observed in the small patient cohort. Additionally, the results confirm that MSI and HRD are likely mutually exclusive driver events, driving tumor selection in mCRPC. Further studies are on-going to investigate

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the correlation of GI markers with PARPi and I/O checkpoint inhibitor responses.

P97

Simultaneous characterization of rare immune cell subpopulations and PD-L1 expressing CTCs in peripheral blood of cancer patients

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Background

Expression of PD-L1 on tumor and immune markers in tumor tissue are associated with improved response to PD-1 and PD-L1 checkpoint inhibitors. However, each alone has limited predictive utility. Multimodal characterization of both the tumor and host immune system is an unmet medical need for the improved prediction of response to immunotherapy. Metastatic lesions are likely to be under-sampled and require a liquid biopsy, given tumor heterogeneity and evolution and temporal changes in the host immune system. We sought to examine expression of PD-L1 on circulating tumor cells (CTCs) as well as characterize rare immune cell populations with a non-invasive liquid biopsy. Examining dynamic biomarker changes in longitudinal samples could enable the development of novel diagnostic tools for response prediction and pharmacodynamics studies related to immunotherapy.

Methods

Blood samples from lung cancer patients were collected and shipped to Epic Sciences. Contrived samples were also developed by spiking cancer cell lines into healthy donor blood. Red blood cells were lysed, and nucleated cells plated onto glass slides. Slides were stained with DAPI as well as multiple immune cocktails and imaged. Targets included pan-CK, CD45, PD-L1, CD4, CD8, Ki-67, LAG-3, and TIM-3. Approximately 3 million nucleated cells per slide

were examined through advanced digital pathology pipelines to detect and quantify changes in T cell populations to infer immune activation, exhaustion, suppression, and circulating tumor burden.

Results

Epic Sciences' rare cell detection platform has an analytically validated limit of detection of 1 cell/mL of blood. Three immuno-panels were developed to profile leukocyte subpopulations and CTCs simultaneously. PD-L1+ leukocytes detected in 30 out of 33 lung cancer patients, and the incidence of PD-L1+ leukocytes ranged from 0% to 0.138% (average 0.0213%, median 0.0118%). PD-L1+ CTCs were observed in the presence of both high and low counts of PD-L1+ leukocyte populations.

Conclusions

The low limit of detection of the Epic Sciences CTC platform coupled with ability to archive patient blood samples allowed for retrospective precise quantification of leukocyte subpopulations and PD-L1 expression on CTCs retrospectively. Development of a liquid biopsy based platform that can simultaneously measure immune biomarkers in CTCs as well as on leukocytes will allow for real time assessment and monitoring of response to immune checkpoints inhibitors.

P98

Immunological profiling of baseline and resected biopsies from locally/regionally advanced/recurrent melanoma treated with neoadjuvant combination ipilimumab (3mg/kg or 10mg/kg) and high dose IFN- α 2B

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Background

Melanomas are the most responsive tumor type to immune checkpoint blockade, but clinical response rates remain suboptimal. Gene expression profiling of the pretreatment tumor biopsies is an attractive approach to developing predictive biomarkers of response because it is able to directly measure the activity of the immune system within the tumor. Furthermore, characterization of the transcriptional changes induced by treatment allows insights into the mechanism of action of the therapy. UPCI 11-063 is a clinical trial to assess the safety of two doses of ipilimumab combined with high dose IFN- α 2B in the neoadjuvant setting. Both treatment regimens induce clinical responses, with reduced toxicity observed in the low dose ipilimumab arm. This study is a follow on biomarker analysis to investigate gene expression in pretreatment and post treatment biopsies.

Methods

This was a Phase I trial of 30 patients with locally/regionally advanced/recurrent melanoma who received either high (10 mg/kg) or low (3 mg/kg) dose ipilimumab plus high dose IFN- α 2B. Tumor biopsies collected prior to treatment and at the time of surgery (6-8 weeks after starting neoadjuvant treatment) were profiled on the NanoString® platform.

Results

Transcriptional profiling of tumor biopsies collected prior to treatment or at the time of surgery was performed in order to find patterns of gene expression that correlated with pathological complete response (pCR), radiologic response (RR) or recurrence-free survival (RFS). In the baseline tumor biopsies, a number of genes were positively associated with pCR, including several members of the WNT signaling pathway. The T cell inflamed Gene Expression Profile (GEP), a signature of a peripherally suppressed immune response in the tumor, was associated with longer RFS. Differential gene expression of paired pre- and post-treatment biopsies identified a number of genes upregulated in response to ipilimumab/IFN treatment, including

signatures of multiple immune cell populations, including T cells and macrophages, which were associated with pCR.

Conclusions

The combination of ipilimumab/IFN as a neoadjuvant for treatment of melanoma has the potential to improve response rates by activating quiescent immune responses via multiple pathways. Expression profiling of pre and post treatment samples suggested a mobilization of immune cells to the tumor, consistent with activation of local immunity. The GEP, which predicts response to pembrolizumab in a variety of solid tumors, may also be useful in the setting of ipilimumab/IFN. Further work in this or similar sample sets may extend these observations.

Trial Registration

ClinicalTrials.gov Identifier NCT01608594

P99

Biomarker analysis from the OpACIN trial (Neo-/adjuvant ipilimumab + nivolumab (IPI+NIVO) in palpable stage 3 melanoma)

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Background

The combination of IPI+NIVO induces high response rates and improved overall survival in late stage

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melanoma. T cell checkpoint inhibition is of greatest value at the moment of TCR triggering and therefore dependent on the amount of antigen present, indicating that adjuvant immunotherapy will work most efficiently, when initiated prior to surgery.

Methods

Two-arm Phase 1b feasibility trial consisting of 20 high risk AJCC stage 3B/C melanoma patients with palpable nodal disease receiving the combination of IPI 3mg/kg and NIVO 1mg/kg, either adjuvant four courses after surgery, or split neo-adjuvant and adjuvant.

Results

In this update 20 patients are evaluable. Neo-adjuvant application of IPI+NIVO was feasible and no surgery-associated adverse events were attributed to (neo-) adjuvant therapy. 18/20 patients had to stop earlier due to grade 3/4 toxicities. Neo-adjuvant IPI+NIVO reduced tumor load in 8/10 patients (3 pCR, 4 near pCRs [minimal remaining micro metastases], 1 pPR [$<50\%$ vital tumor cells], 1 pSD and 1 pPD). So far, none of the responders in the neo-adjuvant arm has relapsed. Relapse was observed for the 2 non-responders within the neo-adjuvant arm and for 3 patients within the adjuvant arm.

We will present at the detailed biomarker analysis, comprising DNA and RNA sequencing, NanoString® RNA and protein profiling of PBMC, NanoString digital spatial profiling of tumors, multiplex immunohistochemistry of tumors and TCR sequencing. We found that mutational load did not correlate with response and that IFN signature, inflamed signature and BATF3 signature were mainly positive predictive. Using NanoString spatial profiling, we found that PD-L1, PD-1, $\beta 2M$ were associated with favorable outcome.

Conclusions

The combination of IPI+NIVO in the (neo-)adjuvant treatment setting for high risk stage 3 melanoma patients is promising and currently tested in an

international phase 2 randomized trial comparing different combination schemes (OpACIN-neo trial, NCT02977052) with the aim of preserving efficacy, but reducing toxicity. Biomarkers identifying patients responding upon neo-adjuvant IPI+NIVO and remaining relapse-free for a long time, will help to select the patients that need and don't need to be exposed to IPI+NIVO associated toxicity.

Trial Registration

ClinicalTrials.gov Identifier NCT02437279

P100

Pretreatment gene expression correlation with clinical response to pembrolizumab or nivolumab in metastatic melanoma

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Background

PD-1 checkpoint blockade with the therapeutic antibodies, pembrolizumab or nivolumab, improves survival in patients with metastatic melanoma. However, not all patients experience clinical benefit. The NanoString T cell Inflamed Gene Expression Profile (GEP) measures genes associated with CD8+ T cells, natural killer cells, IFN signaling, and antigen presentation that are reflective of a pre-existing, PD-1-suppressed adaptive immune response within the tumor, and associated with positive treatment outcomes to pembrolizumab in advanced-stage melanoma and other solid tumors. Here, we evaluate the GEP in pre-treatment tumor biospecimens obtained from patients receiving either nivolumab or pembrolizumab. Further, this study examines differential gene expression in PD-1-responsive vs. non-responsive melanomas to identify potential resistance mechanisms, which could be targets for therapeutic intervention.

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Methods

Formalin-fixed, paraffin-embedded pre-treatment tumor biopsies from patients receiving either nivolumab or pembrolizumab in the community setting were profiled with NanoString's nCounter gene expression codesets. A total of 110 patients were included from three independent cohorts. Clinical benefit to PD-1 therapy was defined as an immune-related complete or partial response. PD-L1 expression on tumor cells was evaluated by immunohistochemistry in 36 samples from the pembrolizumab-treated cohort using the 22C3 anti-PD-L1 antibody clone, and staining was scored by two independent pathologists.

Results

The GEP predicted clinical benefit from pembrolizumab (n=85), but was not predictive for nivolumab (n=25), although the small sample size may limit statistical power. We plan to expand the nivolumab cohort prior to presentation of these results. Additionally, the GEP was more predictive of response to pembrolizumab than PD-L1 IHC, as assessed by ROC curves. Furthermore, clinical response to either therapeutic antibody was associated with an enrichment of genes expressed by lymphoid cells, as determined by cell type-specific gene expression. Additional analyses of the total cohort are ongoing and will be presented.

Conclusions

The TIS predicted clinical response to pembrolizumab, but appears not to predict response to nivolumab. Validation and further studies will be required to determine if this observation holds true in larger cohorts, and if it relates to differences between the two agents.

P101

Multiplex biomarker detection in FFPE tumor samples

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Background

Immuno-oncology (IO) drug discovery research requires a multi-staged approach: pre-clinical *in vitro* assays, *in vivo* studies and histological analysis. We demonstrate the power of incorporating a specialist histological approach in formalin fixed paraffin embedded (FFPE) tissues early in the drug development process can provide high value data to complement functional *in vitro* assay readouts to inform on cancer immunotherapy clinical trial strategy.

Simultaneously imaging multiple IO markers on a section provides morphological, pathological and spatial information of the juxtaposition of different immune cell types (quantified by image analysis techniques).

Multispectral imaging approaches:

1. Single immunofluorescence (IF), immunohistochemistry (IHC) and RNAscope (mRNA)
2. Dual and triple IF, IHC and RNAscope
3. Multiplex IF, IHC and RNAscope
4. Mixed multiplex detection of protein, mRNA and/or protein-mRNA
5. Quantitative image analysis

Methods

4 µm FFPE sections were stained using validated protocols on automated staining platforms (Leica Bond III/RX). Imaging performed using colorimetric and fluorescent scanners.

Results

Automated single-plex IHC or IF demonstrates protein (Figure 1) and mRNA (Figure 2) expression. Multiple protein targets are visualised using colorimetric (Figure 3) and 4-plex protein IF staining (Figures 4-7). It is possible to stain 6 or 7 markers on one section (Figures 8 & 9), visualising the cellular location and spatial distribution of markers in relation to each other.

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Conclusions

Specialised histological staining approaches utilised during drug discovery research provide valuable insights into the spatial expression of IO biomarkers. A pragmatic approach to antibody selection and validation powerfully combines with RNAscope to facilitate demonstration of novel and challenging IO biomarkers *in situ*, and can complement pre-clinical *in vitro* assay data.

P102

Mining the periphery for tumor-relevant B and T cells

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Background

Identification of tumor-specific T and B cells is key to understanding the immune response to cancer, but tumor tissue is only available in rare circumstances. Here we identify biomarkers for more accurate identification of tumor-reactive B and T cells from peripheral blood.

Methods

Matched peripheral blood and tumor samples from four donors with either advanced head and neck cancer or melanoma were analyzed by flow cytometry using markers including CD3, CD4, CD8, CD19, CD20, CD28, CD38, HLA-DR, NKG2D and TIGIT. From the periphery, we index sorted CD3⁺CD8⁺PD1⁺ T cells and collected CD19⁺CD20⁻CD38⁺ plasmablasts (PBs), as these are phenotypes likely to harbor tumor-reactive cells, and generated paired α/β and heavy/ light chains from single cells.

For tumor samples, CD8⁺ T cells, CD19⁺CD20⁻CD38⁺ PBs and CD19⁺CD20⁺ B cells were sorted and RNA was extracted for deep sequencing of β and heavy chains. Tumor-derived cells were deep sequenced to identify the largest possible numbers of clones present in tumor. T cell lineages were defined as having identical β -CDR3 nucleotide sequences, while B cell lineages were defined as having identical V_H genes, and 80% amino acid similarity in the H-CDR3.

Results

We generated an average of 390 (314-468) paired α/β sequences from peripheral blood CD3⁺CD8⁺PD1⁺ T cells, and 4,692, 4,968, 7,346 and 108,032 clones from deep sequencing of tumor CD3⁺CD8⁺ T cells. 63% of peripheral CD8⁺PD1⁺ T cell clones overlapped with tumor-resident CD8⁺ clones. Among peripheral CD8⁺PD1⁺ T cells, CD28⁻ clones had significantly greater overlap with tumor-resident T cell clones than CD28⁺ clones ($p=0.005$, Fisher's exact test). There was also a trend toward greater overlap with tumor clones for the CD38⁺ subset of CD8⁺PD1⁺ peripheral T cells. Additional analyzes are underway to evaluate other T cell markers.

In analysis of B cell responses in two donors, 53.2% and 52.4% of peripheral plasmablast lineages overlapped with tumor B cell lineages. Additionally, the peripheral PBs that overlapped with the tumor population had a higher number of nucleotide mutations than the peripheral PBs that did not overlap (32.49 vs 30.08, $p=0.06$, two-sided t-test).

Conclusions

In this study, we demonstrated significant overlap between peripheral plasmablasts, peripheral PD1⁺CD8⁺ T cells and the tumor lymphocyte populations of advanced cancer patients. We further demonstrated that markers such as CD28 may be used to enrich for tumor-resident clones. Subsequent studies will expand upon these analyzes with additional donors and markers.

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P103

Tumor infiltrating lymphocyte (TIL) percentage as a prognostic biomarker for overall and relapse free survival in Hu14.18-IL2 treated resectable recurrent stage 3 or 4 melanoma patients

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Background

Recurrent stage 3 or 4 melanoma patients (pts) have a poor prognosis. The presence of tumor infiltrating lymphocytes (TILs) have shown prognostic value in pt survival in melanoma, particularly for pts receiving checkpoint blockade immunotherapy. We investigated the prognostic value of TILs identified on H&E sections of tumors from pts who have already undergone one cycle of

hu14.18-IL2 (an immunocytokine fusion protein of anti-disialoganglioside antibody and IL-2) immunotherapy compared to hu14.18-IL2 naïve tumors.

Methods

Between 2008-2012, twenty-three stage 3 or 4 recurrent, but resectable, melanoma pts were enrolled in CO-05601 (NCT00590824) and randomized to receive the first of 3 courses of hu14.18-IL2 either before or after surgical resection of all sites of disease. At the time of surgery, two pts within this trial were found to be not completely resectable and were taken off study. Following routine pathologic assessment, representative sections of each tumor were coded and given to our board certified hematopathologist (author EAR) for TIL assessment. Scatter plots were made for exploratory analyzes to interrogate the relationship between treated TILs and survival outcomes with stratification based on whether pts had hu14.18-IL2 treatment. Kaplan Meier survival curves interrogated the relationship between TILs and survival outcomes (relapse free survival [RFS] and overall survival [OS]) in relation to treatment status.

Results

There was a significant correlation between increased percentage of TILs and increased RFS ($p=0.0166$; $n=13$) and OS ($p=0.0223$, $n=13$) in pts whose tumors were obtained after hu14.18-IL2 treatment. These correlations were not seen in pts whose tumors were obtained prior to hu14.18-IL2 (not significant, $n=8$). In addition, when comparing pt tumors with high TILs (above the median of 5%, $n=11$) and low TILs (below 5%, $n=10$), there was an increase in RFS ($p=0.0446$, median survival: high TILs - not reached, low TILs - 4.65 months) and a trend towards increased OS ($p=0.0540$, median survival: high TILs - not reached, low TILs - 45 months, Figure 1a-b). Additionally, hu14.18-IL2 treated pt tumors with high TILs ($n=6$, median RFS and OS not reached) showed significantly increased RFS ($p=0.0159$) and OS ($p=0.0208$) compared to hu14.18-IL2 treated pt tumors with low TILs ($n=5$, median RFS - 4.2 months; OS - 41.3 months (Figure

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1C-D), whereas hu14.18-IL2 naïve tumors did not show this relationship.

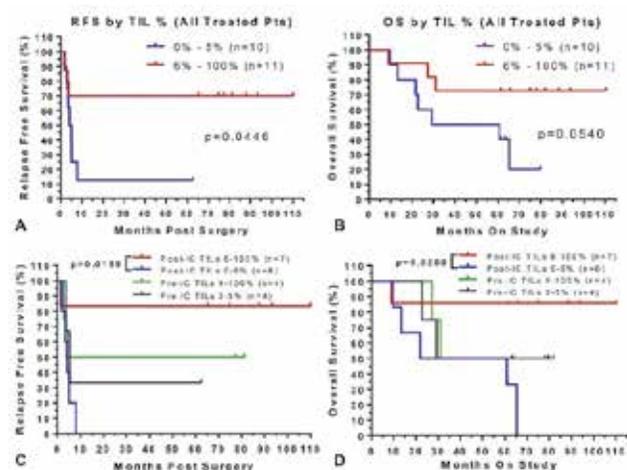
Conclusions

These results suggest that resection specimens and biopsies obtained post-hu14.18-IL2 immunotherapy should be further investigated to monitor for TILs, as they may be associated with a beneficial immunotherapeutic effect.

Trial Registration

ClinicalTrials.gov Identifier NCT00590824

Figure 1. TIL Percentage Prognosticates Increased OS and RFS in Hu14.18-IL2 Treated Melanoma Patients



P104

A study of PD-L1 diagnostic assay concordance in urothelial carcinoma

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Background

In urothelial carcinoma (UC), multiple pharmaceutical companies are investigating programmed cell death ligand-1 (PD-L1) expression using different antibody clones, staining protocols, and scoring algorithms. A stronger understanding of the comparative technical performance of these assays will allow appropriate interpretation of clinical outcomes for patients with UC treated with different anti-PD-1/PD-L1 therapies.

Methods

325 tumor biopsy samples from patients with UC were assessed using 4 commercially available PD-L1 diagnostic assays: Ventana SP263, Ventana SP142, Dako 28-8 and Dako 22C3. Assays were performed in an CLIA accredited laboratory, per the device protocol and scored for tumor cell (TC) and immune cell (IC) PD-L1 staining. Analytical concordance was calculated pairwise between assays using the Spearman (ρ) rank correlation coefficient. Classification concordance, including agreement between clinically relevant scoring algorithms, was investigated using overall/positive/negative percentage (OPA/PPA/NPA) agreement at multiple cut-offs.

Results

Data indicated good association, with a Spearman correlation coefficient of ≥ 0.8 for each pairwise comparison of the 4 assays for IC, and ≥ 0.7 for TC. When applying the SP263 clinical algorithm to 22C3 there is good agreement on positive cases with SP263 (OPA, PPA, NPA >80%) and vice versa.

Conclusions

Analytically, SP263, 22C3, and 28-8 assays showed similar performance for TC and IC PD-L1 expression. However, while SP142 showed similar analytical performance to the other 3 assays for IC staining, it appeared to be the least sensitive with the fewest samples showing PD-L1 staining on TC. This confirms previous observations from studies on assay performance in NSCLC and HNSCC. When comparing scoring algorithms, caution should be taken when applying any algorithms utilising TC

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measures of PD-L1 to SP142 due to differences in sensitivity of the assay. Concordance achieved with SP263 algorithm applied to the 22C3 assay and vice versa builds optimism that those 2 assays could be used interchangeably in informing patient treatment decisions for durvalumab.

P105

***Infino*: Bayesian inference to distinguish immune cell expression phenotypes, estimate immune infiltration into tumor microenvironment, and investigate exhausted T cell phenotype**

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Background

Robust quantification of immune cell infiltration into the tumor microenvironment may shed light on why only a small proportion of patients benefit from checkpoint therapy. Indeed, the immune contexture has recently been associated with prognosis and response to therapy [1], and the exhausted T cell phenotype is likely to be implicated in response to checkpoint blockade [2]. However, traditional measurement of immune cell content around a tumor using cell surface markers is low-throughput, requiring manual intervention by a pathologist. Computational alternatives instead estimate immune cell type abundances by deconvolving the gene expression mixture measured in the tumor microenvironment.

Methods

After constructing synthetic gene expression mixtures with known immune cell abundances, we evaluate the performance of four existing immune

cell mixture deconvolution methods [3]. Our analysis discovers that they have high confidence but substantial deconvolution error for certain mixtures. We then propose a new Bayesian method, *infino*, that learns the relationships between immune cell types to estimate their abundances in RNA-seq data. Modeling these relationships is crucial for our deconvolution ability, enabling investigation of phenotypes of interest.

Results

Using Bayesian generative modeling, *infino* estimates a probability distribution for the abundance of each immune cell type in an RNA-seq mixture. We demonstrate comparable performance to state-of-the-art infiltrate quantification methods, as well as more rigorous quantification through *infino*'s probabilistic estimates at all levels of the immune cell type hierarchy. Additionally, we extend *infino* to estimate stromal and tumor cell proportions for a complete accounting of the tumor microenvironment. We present results from a patient cohort, as well as *infino*'s estimation of the exhausted T cell phenotype, to investigate variation in response to checkpoint blockade.

Conclusions

By modeling the relationships between immune cell types, our novel Bayesian approach to immune infiltrate quantification produces robust deconvolution of gene expression mixtures like those found in the tumor microenvironment. Our results help probe the differential effectiveness of immunotherapy.

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P106

Bioinformatic analysis of the tumor immune landscape to prioritize combinations with antibody-drug conjugates (ADCs)

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Background

Immunotherapy has transformed the treatment of multiple tumors, yet significant unmet need remains. Antibody-drug conjugates (ADCs) have had limited success, with some evidence to support that they can reshape the tumor immune-environment to increase sensitivity to immunotherapy. Thus, combining immunotherapy and ADCs may enhance the benefit of both; however it is unclear how to prioritize such combinations. In this study, 52 ADC targets under clinical investigation were analyzed by bioinformatics approaches to examine the intersection of expression of these ADC targets and the tumor immune landscape to identify targets and pathways that may increase the likelihood of success of ADC through immunotherapy combinations.

Methods

Thirty-seven ADC targets were analyzed across 16 solid tumor types and 15 ADC targets were analyzed in 2 hematological cancers using transcriptome data in TCGA. Co-expression of ADC targets with inflamed or non-inflamed tumor microenvironment was defined based on a previously reported IFN γ mRNA expression signature that showed strong association with ORR and survival in patients treated with durvalumab. Top ranked IFN γ -associated ADC targets were further analyzed to

identify the common molecular signatures shared by these targets.

Results

Out of 592 combination counts of 37 ADC targets in solid tumors, 163 (28%) were associated with inflamed signature whereas 67 (11%) were associated with non-inflamed signature. Out of 30 combination counts of 15 ADC targets in hematological cancers (AML and DLBCL), 14 (47%) were associated with inflamed signature whereas 5 (17%) were associated with non-inflamed signature. Top ranked ADC targets were associated with inflamed signature in a majority of tumor types (>10 out of 16), including those known to be either highly responsive or less responsive to immune checkpoint inhibitors. In-depth profiling of the top ranked ADC targets identified epithelial-mesenchymal transition (EMT) and tumor invasion/metastasis to be the most commonly shared signatures among these ADC targets.

Conclusions

ADC target selection is usually based on differential expression between tumor and normal tissue but the tumor immune landscape is not considered. With the success of immunotherapy, our bioinformatics analysis suggests that selection of ADC targets co-expressed with immune inflamed, EMT and tumor invasion/metastasis signatures may be a strategy to further triage candidate ADC targets to increase the likelihood of success for effective combination of ADC and immunotherapies.

Cancer Vaccines

P107

CMV gB/pp65 eVLPs formulated with GM-CSF as a therapeutic vaccine against GBM

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Background

Failures of many past therapeutic cancer vaccines can be attributed to several common shortcomings including the inherently poor immunogenicity of the tumor antigens used, the failure to expand CD4⁺ T helper cell responses that could sustain potent CD8⁺ CTLs, and limited breadth of immunity directed against multiple antigens/epitopes that could avoid the rapid immunoselection of tumor escape variants.

Numerous independent laboratories using several techniques (ISH, immunohistochemistry, T cell recognition of autologous primary tumors) have demonstrated cytomegalovirus (CMV) antigens in over 90% of GBM tumors. Memory CD4⁺ and CD8⁺ T cells are most frequently directed against the gB and pp65 antigens at frequencies that can exceed 10%. Thus, CMV gB and pp65 represent attractive, highly immunogenic “foreign” antigen components of a vaccine against GBM that can exploit high precursor frequencies of CD4⁺ and CD8⁺ T cells.

Methods

Enveloped virus-like particles (eVLPs) are produced after transfection of HEK 293 cells with a plasmid encoding murine leukemia virus Gag plasmid fused in-frame with CMV pp65 antigen, which gives rise to particles. Co-transfected CMV gB plasmid enables particles budding from the cell surface to incorporate the gB protein into the lipid bilayer.

Results

In *ex vivo* studies using PBMCs from GBM patients we have demonstrated that gB/pp65 eVLPs restimulate both CD4⁺ and CD8⁺ T cells at frequencies comparable to those observed for healthy CMV⁺ subjects, and that formulation with GM-CSF augments IFN- γ and CCL3 secretion. Biodistribution studies have demonstrated that the gB/pp65 eVLPs remain at the intradermal injection site for at least two weeks, with eVLP particles appearing in draining lymph nodes within a few hours. Mechanistic studies demonstrate that

monocyte uptake of eVLP particles induces proinflammatory cytokines, which are amplified by gB expression on the surface of the gB/pp65 particles. Immunization of CMV⁺ rhesus macaques with a macaque-specific version of the vaccine candidate confirmed the safety of the vaccine and the ability to boost pre-existing IFN- γ -secreting T cell responses.

Conclusions

An IND for a phase I/IIa trial in recurrent GBM patients was filed with FDA in July, with subject enrollment expected in Q4 2017.

P108

Induction of folate receptor alpha-specific Th17 T cell immunity in ovarian cancer patients

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Background

The nature of the endogenous immune response to ovarian cancer (OC) is a strong predictor of clinical outcomes; regulatory T cells (Tregs) predict poor survival, whereas interleukin 17 (IL-17)-producing T cells (Th17s) are favorably prognostic. We have developed a method by which *ex vivo*-matured dendritic cells (DCs) can preferentially drive expansion of Th17s [1]. A vaccine utilizing OC-specific Th17-inducing DCs might diminish OC-related Tregs and reduce the rate of OC recurrence.

Methods

Patients with stage IIIC and IV OC were enrolled following completion of initial surgery and chemotherapy. DCs were generated from autologous peripheral blood mononuclear cells

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(PBMCs) that were cultured according to our Th17-inducing protocol [1] and pulsed with peptides from the OC antigen folate receptor alpha (FR α). Mature antigen-loaded DCs (FR α DCs) were delivered to patients via intradermal injection in a series of five immunizations and up to seven boosters. Patient PBMCs were collected before and after vaccine induction and were assessed via Enzyme-Linked ImmunoSpot (ELISpot) assay for FR α -specific T cells capable of secreting IL-17 or IFN γ . ELISpot responses were defined as at least a doubling of FR α peptide- or protein-specific cytokine-secreting cells.

Results

Nineteen patients were enrolled and immunized with vaccine, with one patient subsequently excluded due to ineligibility. No grade 3 or higher adverse effects were seen. Vaccination resulted in the development of both Th17 and Th1 immunity to vaccine constituent FR α peptides as well as whole FR α protein in the majority of patients. Antibody responses to FR α were also seen following vaccination in some patients.

Conclusions

Specific induction of Th17 immunity against self-antigens in cancer patients is feasible and safe. The results of this study pave the way to future trials aimed at determining if vaccine-induced Th17 immunity prevents disease progression and improves survival.

Trial Registration

ClinicalTrials.gov Identifier NCT02111941

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P109

Anti-NY-ESO-1 immune response and survival benefit after LV305 therapy in patients with advanced sarcoma and other solid tumors

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Background

LV305 is a dendritic cell targeting lentiviral vector encoding NY-ESO-1 designed to generate and expand anti-NY-ESO-1 T cells. This first-in-human study showed LV305 is safe, induced anti-NY-ESO-1 CD4/CD8 T cells, and provided durable disease control with a 1-yr overall survival (OS) of 81% in sarcoma patients (pts)(N. Somaiah, ASCO 2016). We now present immune response (IR) and long term OS data.

Methods

Previously treated, advanced NY-ESO-1+ solid tumor pts were eligible. Part 1 dose escalation, 4 cohorts received 3 or 4 intradermal injections every 3 weeks of 10⁸, 10⁹ or 10¹⁰ vector genomes (vg)/dose; 10¹⁰ vg/dose in part 2 expansion. Anti-NY-ESO-1 antibody (Ab) and T cell IR measured by ELISA, and ELISpot and T cell receptor sequencing, respectively.

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Results

As of March 31, 2017, 39 pts (13 synovial sarcoma, 6 myxoid round cell liposarcoma, 4 other soft tissue sarcoma (STS), 1 Ewing's sarcoma, 9 ovarian cancer, 5 melanoma, 1 NSCLC) were enrolled, median (m) duration of observation 17.2 months (mos). For the 24 sarcoma pts (mean age 48.1 yrs, 87.5% relapsed metastatic, 100% previously treated), mPFS 4.67 mos and mOS not reached (NR). Exploratory analysis of biomarker data showed: 1) Survival (mos (95% CI)) and presence of baseline anti-NY-ESO-1 Abs: For all pts combined, mOS was NR (17.9, NR) for NY-ESO-1 Ab+ (n=12) vs 27.8 (10.3, 27.8) for NY-ESO-1 Ab- (n=19), HR 0.28 p value 0.09. For STS patients (n=23), mOS was NR (NR, NR) (n=8) vs 18.8 (10.3, 27.8) (n=14), HR 0.0 p value 0.02, respectively. 2) Survival and LV305 induction of IR: Of 31 pts tested, 52% pts developed NY-ESO-1 specific T cells; 3% pts developed anti-NY-ESO-1 Abs after LV305 therapy. 3) Survival and IR: For all pts combined, mOS was 27.8 (18.8, 27.8) for IR+ (n=21) vs 10.3 (4.0, NR) for IR- (n=10), HR 0.32 p value 0.06. Pts who had anti-NY-ESO-1 IR both at baseline and induced had the best survival: mOS was 27.8 (17.9, 27.8) (n=14) vs 8.6 (6.0, NR) in pts who had no evidence of IR at baseline nor on therapy (n=4), HR 0.17 p value 0.018.

Conclusions

LV305 is a well-tolerated therapy which induces anti-NY-ESO-1 IR and may improve OS in sarcoma pts. Pts with baseline anti-NY-ESO-1 Abs or induced IR on LV305 therapy appear to have a better survival.

Trial Registration

ClinicalTrials.gov Identifier: NCT02122861P110

P110

MEK inhibition augments Talimogene laherparepvec (T-VEC)-mediated *in vitro* melanoma oncolysis and induces *in vivo* tumor regression

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Background

Herpes simplex virus, type 1 (HSV-1) encoding GM-CSF (Talimogene laherparepvec; T-VEC) is the first and only FDA approved oncolytic virus for the treatment of melanoma. We recently found that T-VEC induces apoptosis in melanoma cells *in vitro*, and this effect is enhanced with concurrent MEK inhibition. In this study, we sought to determine how MEK inhibition enhances tumor cell lysis and determine the therapeutic effects of combination treatment in a melanoma xenograft model

Methods

BRAF wild-type and mutated melanoma cell lines were plated in 96-well plates (10⁴ cells per well) and treated with T-VEC (MOI 0.001-1.0). Cell viability was assessed by standard MTS assay. After establishing baseline viability results, cells were also treated with MEK inhibitors (Trametinib or PD0325901 at 1-100 nM) and cell viability determined by MTS assay. Viral replication was measured by plaque assay and the infection metric was analyzed using Lumacyte single-cell optical density. Apoptosis was determined by Annexin V flow cytometry and cleaved PARP immunoblotting. For *in vivo* experiments, NSG mice were challenged with SKMEL-28 (5x10⁶) at day 0 and treated with intra-tumoral T-VEC (10⁶ PFU) biweekly and/or trametinib (0.1 mg/kg) by oral gavage for 2 weeks. Tumor growth was measured by calipers. Statistical comparisons between treatment groups were determined using the student's t test with P<0.05 being considered statistically significant.

Results

Combination T-VEC and trametinib significantly increased melanoma cell death (P<0.01). A similar effect was seen with a second MEK inhibitor (PD 0325901) (P<0.001). The combination caused a significant increase in viral replication and increased

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apoptosis. Combination treatment was associated with morphologic changes in tumor cells and an increased infection metric by Lumacyte. A PCA analysis distinguished virally-infected cells and those treated with both virus and MEK inhibitor from uninfected cells. A significant reduction in tumor volume was also observed in SKMEL-28 NSG xenografts during the combination treatment compared to monotherapy with T-VEC or MEK inhibition

Conclusions

Combination T-VEC and MEK inhibition enhances tumor cell killing and viral propagation *in vitro* and induces tumor regression *in vivo*. Combining MEK inhibitors with T-VEC represents an attractive therapeutic option and further studies are needed to understand the impact of this combination on induction of host anti-tumor immunity.

Figure 1. MEK Inhibition increases viral titers in T-VEC treated SKMEL28 Melanoma cell line

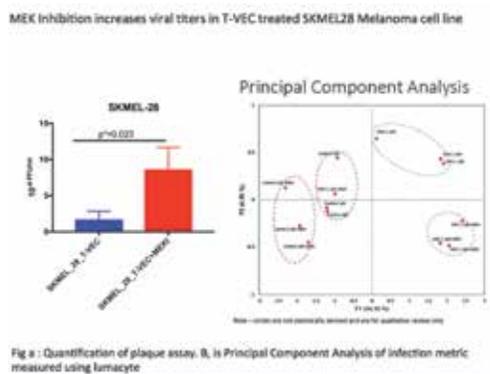
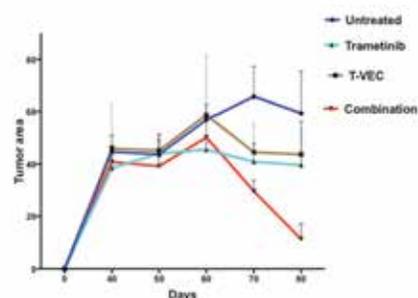


Figure 2. Trametinib augments T-VEC mediated tumor regression in Xenograft melanoma model

Trametinib augments T-VEC mediated tumor regression in Xenograft melanoma model



NSG mice with SKMEL28 xenograft treated with Trametinib and/or T-VEC

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P111

Initial safety and production efficiency of an autologous tumor lysate-loaded yeast cell wall particle vaccine

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Background

Yeast cell wall particles (YCWP) have been used to create an autologous tumor lysate, particle-loaded, dendritic cell (TLPLDC) vaccine with encouraging initial clinical results. Direct inoculation of an autologous tumor lysate loaded, particle only (TLPO) vaccine eliminates the need for *ex vivo* DC loading, greatly reducing production requirements compared to TLPLDC. Our preclinical work showed that YCWP can be loaded with autologous tumor lysate (TL) and capped with silicate (Si) to seal in the TL content and attract a monocytic infiltrate. We have shown that silicate capping enhances TL retention, DC phagocytosis and cytoplasmic delivery of the YCWP contents. Here, we present the initial production efficiency and safety data from an exploratory phase I basket trial of the autologous TLPO vaccine.

Methods

YCWP are created from *Saccharomyces cerevisiae* by NaOH/HCl digestion of all non-cell wall components and washed with isopropanol/acetone resulting in β -glycan shells. Autologous TL is created by freeze/thaw cycling of ≥ 1 mg of tumor and loaded into YCWP along with CpG oligonucleotides and a tetanus helper peptide by dissolution and freeze-drying. The TL-loaded YCWP are then capped with Si, aliquoted in single dose vials and cryopreserved. The TL protein content of production batches is determined by measuring the nitrogen content by combustion analysis, while Si cap thickness is calculated based on Si content by mass spectrometry. The vaccine is administered intradermally at 1.0×10^8 particles monthly x4 followed by boosters every 3 months until progression.

Results

Nine patients with stage I-IV malignancies (non-small cell lung, pseudomyxoma peritonei, prostate, ovarian, pancreatic, breast, desmoplastic small round cell tumor, esophageal, and renal cell carcinoma) aged 21-85 (median 52) have been enrolled and received between 1 to 3 inoculations of TLPO to date. Enrollment is ongoing. Vaccine

production has been successful in 100% of patients. In the production runs of the vaccine, the mean TL protein content was $16.56 \pm 0.56\%$ of each inoculation by weight. The Si cap thickness was 0.0615 ± 0.0009 microns (approximately 140 molecular layers of Si). In the 17 inoculations thus far, there has been no toxicity $>$ grade 2. Patients are due for their first set of imaging shortly.

Conclusions

TLPO vaccine is reliably and efficiently produced from autologous tumor tissue with sufficient loading of TL and sufficient Si capping to maintain TL within the YCWP. The vaccine is well tolerated with minimal toxicity thus far. The favorable production characteristics and safety profile combined with improved production efficiency of the TLPO compared to the TLPLDC vaccine warrant continued development of this vaccine platform.

P112

Telomerase peptide vaccine treatment of patients with locally advanced or metastatic non-small cell lung cancer: Report from a phase I/IIA trial

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Background

At the initiation of this study in 2013 second and later lines treatment of patients with stage III or IV NSCLC was chemotherapy or radiotherapy with limited median survival.

UV1 is a therapeutic cancer vaccine consisting of three long synthetic peptides of the enzyme telomerase (hTERT). The UV1 peptides comprise

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epitopes recognized by T cells from cancer patients experiencing long-term survival following vaccination with first-generation hTERT vaccines. The aim was to investigate the safety, induction of immune response (IR) towards the hTERT peptides and efficacy. Three different UV1-doses were investigated.

Methods

Patients with locally advanced or metastatic NSCLC received UV1 (100 µg, 300 µg or 700 µg) and 75 µg GM-CSF as adjuvant [EudraCT No. 2012-001852-20]. All patients had received prior lines of chemotherapy and/or radiation therapy, and had no evidence of disease progression at the time of inclusion. Safety was assessed according to CTCAE v. 4.0 and tumor responses according to RECIST v.1.1. Immune response (IR) against UV1 peptides was monitored by T cell proliferation assays and, if sufficient cell numbers, IFN-γ ELISPOT assays. Patients are to be followed up for five years after their first UV1 treatment. Results as of Q2-2017 are reported.

Results

18 patients (10 females), mean age 65.9 yrs. (48-76), with locally advanced or metastatic (stage III or IV) NSCLC were included. Ten patients had adenocarcinoma and eight had squamous cell carcinoma. Six patients were enrolled in each dose group. Patients received between 9 and 18 doses of UV1 each (mean 12.5 doses, total of 224). Treatment was generally well tolerated. Adverse events mainly included injection site reactions and fatigue. No treatment related serious adverse events were reported.

IR was induced against UV1 in 12 of 18 patients (67%). Fifteen patients were evaluated for tumor response after nine months, 12 patients had SD and three patients had PD. Median overall survival was 23.6 months. After two years, seven patients were alive, two and five in the 300 and 700 µg dose group, respectively.

Conclusions

UV1 is safe and well tolerated and induce an UV1 specific IR in the majority of the patients (67%). UV1 is currently under development in different solid tumors as combination therapy.

Trial Registration

EudraCT No. 2012-001852-20

P113

OncoPeptVAC: A robust TCR binding algorithm to prioritize neoepitope using tumor mutation (DNaseq) and gene expression (RNAseq) data

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Background

Neoepitopes are tumor-derived immunogenic peptides that arise from intracellular proteolytic processing of somatic mutation in protein coding genes. These peptides bind HLA Class I proteins and are presented on the surface by antigen presenting cells. Productive engagement of HLA Class I-bound peptide with T cell receptor (TCR) activates CD8⁺ T cells to generate cytotoxic T cells, which mediate lysis of the neoantigen-expressing tumor cells. Neoepitopes can be used as cancer vaccines to prime CD8⁺ T cells against tumor cells. As tumors accumulate hundreds of mutations during cancer development and only a small subset of these are immunogenic, identifying the neoepitopes requires accurate modeling of the steps involved in peptide production, presentation as well as TCR binding.

Methods

Most current pipelines prioritize neoepitopes based on the expression of mutant proteins and their HLA binding affinity. However, presentation of peptides on the surface is not sufficient to activate T cells. To improve the predictive power of our neoepitope prioritization pipeline and to circumvent selection

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biases that are inherent with using HLA binding as a proxy for neoepitope prediction, we developed a novel algorithm that prioritizes peptide interactions with the TCR. This algorithm uses features selected by analyzing crystal structures of TCR and HLA-peptide complex present in the Protein Data Bank. A neural network model was built to derive a composite score that includes, besides TCR binding, other features associated with the peptide, such as level of expression of the mutant allele, affinity of HLA binding, affinity of TAP binding and sensitivity to proteasomal processing.

Results

We validated our neural net prediction model on known immunogenic and non-immunogenic peptides and achieved superior accuracy, sensitivity and specificity of prediction compared to using the standard HLA binding affinity of ≤ 500 nM. By applying OncoPeptVAC neoepitope prioritization solution to 2.2 million unique somatic mutations, we identified ~700 immunogenic peptides derived from recurrent somatic mutations in all cancers. Several of these peptides were validated on a CD8⁺ T cell-activation assay.

Conclusions

Our *in silico* prioritization platform combined with the cell-based validation method is a powerful tool to identify therapeutic vaccines for personalized cancer immunotherapy applications.

P114

No toxicity and long-term survival up to 16-years from repeated oncolytic vaccine systemic immunotherapy in advanced melanoma: Teaching us lessons

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Background

We have previously reported very long-term complete responses with survival of ≥ 5 years to oncolytic melanoma vaccine therapy for patients with advanced melanoma. The Complete Response (CR) Rate was also higher than expected at 17% predominantly from therapy with vaccine alone [1]. Importantly, side-effects were negligible with no noticeable toxicity.

Methods

Extended analysis of patient survival was performed to investigate the presence of recurrence, late failure, morbidity, mortality and the longevity of responders.

Standard methods of outcome evaluation (CR, PR, SD, PD and overall survival) including Kaplan-Meier estimates, were performed.

Results

Clinical responses have continued to beyond 10-years with survival periods of now over 15-years in duration for some patients. These responders are being studied in more detail. CR's when obtained were durable in almost all cases.

Conclusions

Clinical responses and long-term survival using vaccine therapy alone is unusual, but continued to beyond 10-years with survival periods of now over 15-years is obtainable and this requires more investigation as a matter of priority. These responders are being studied in more detail. Some 84% of patients showed some clinically useful response, and no toxicity. These long-term survival [2] and high CR rates results were not from chance alone and were attributable to the vaccine administration & scheduling most likely creating successful in-vivo immunomodulation.

Trial Registration

Australian Clinical Trials Registry [ACTRN]
12605000425695

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P115

Inflammatory response after immunotherapy with a yeast-CEA therapeutic cancer vaccine in metastatic medullary thyroid cancer

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Background

Medullary thyroid cancer (MTC) accounts for approximately 4% of thyroid carcinomas. Although vandetanib and cabozantinib are approved for metastatic MTC, toxicity limits their use. There are ongoing trials at the NCI exploring the role of immunotherapy in MTC patients.

Methods

In a series of two clinical trials (including a phase II MTC study; NCT01856920), 30 MTC patients have been treated with a yeast-based vaccine targeting CEA (Bilusic M et al., *Immunol Immunother*, 2014). The vaccine was scheduled every 2 weeks for 3 months; then monthly for 9 months and quarterly in the second year for patients without radiographic disease progression.

Results

3/30 (10%) of patients developed tumor site-specific inflammatory responses which are not typically seen in MTC. A 39-year-old woman with

sporadic metastatic MTC and stable disease on restaging CT after 3 months of vaccine, developed dyspnea one week later. CT scan showed pleural and paricardial effusions at sites of known disease. After a work-up yielded no diagnosis, empiric steroids resolved symptoms in 48 hours and a follow-up CT-scan demonstrated resolution of the effusions. This patient from the phase I study was found to have robust antigen-specific T cell responses. Similarly, a 72-year-old woman with sporadic metastatic MTC presented with an enlarging neck mass at a known site of metastatic disease after 21 months of vaccine. Due to difficulty swallowing, she received high-dose steroids and empiric antibiotics at an outside hospital with resolution of her symptoms. A follow-up CT reported a decreased in size of the neck mass. Perhaps most interestingly, a 66-year-old woman with MEN2A and metastatic MTC returned to our clinic 17 months after completing the 2-year vaccine course with a rapidly enlarging lymph node at a known site of metastatic disease confirmed by CT. A biopsy of the lymph node was negative for tumor but demonstrated an abundance of CD3+ and CD20+ lymphocytes. The symptoms and lymph node size decreased within 1 week as did the patient's calcitonin in the following weeks.

Conclusions

We present 3 patients with metastatic MTC who presented with an inflammatory event after treatment with a yeast-CEA therapeutic cancer vaccine. These unexpected inflammatory events may demonstrate the potential for immunotherapy in MTC. A follow-up phase II is ongoing at the NCI treating patients (both with previous immunotherapy and immunotherapy-naïve) with pembrolizumab (NCT03072160) and analysis of the vaccine study is underway.

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P116

Heat-inactivated modified vaccinia virus Ankara (MVA) as vaccine adjuvant

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Background

Recent discoveries of cancer neoantigens and the potential of the combination of cancer vaccination with immune checkpoint blockade to enhance vaccination effects have generated excitement and renewed interest. Developing novel and effective vaccine adjuvants that can enhance antitumor immune responses is critical for the success of cancer vaccine. We have recently shown that intratumoral injection of Heat-inactivated MVA (Heat-iMVA; by heating MVA at 55°C for 1 h) generates systemic antitumor effects via a STING-dependent mechanism and requires Batf3-dependent CD103⁺/CD8 α ⁺ dendritic cells (DCs). Heat-iMVA infection of conventional DCs (cDCs) induces higher levels of type I IFN and proinflammatory cytokines and chemokines than live MVA. It also induces DC maturation in a STING-dependent manner. The role of Heat-iMVA as a vaccine adjuvant has not been explored previously.

Methods

To test whether Heat-iMVA is an effective vaccine adjuvant, we first tested whether co-administration of chicken ovalbumin (OVA) with Heat-iMVA improves anti-OVA T cell and antibody responses. We then tested whether co-administration of irradiated OVA-expressing B16 cells with Heat-iMVA delays tumor growth in a therapeutic vaccination model. We also tested whether systemic delivery of anti-PD-L1 antibody would further improve vaccination efficacy. Mice were intradermally implanted with B16-OVA, they were vaccinated with either irradiated B16-OVA, or B16- OVA + Heat-iMVA three times on the contralateral flank in the

presence of absence of anti-PD-L1 antibody. We measured tumor volumes and monitored survival of the mice.

Results

We found that addition of Heat-iMVA increased the percentage of OVA-specific CD8⁺ T cells and CD4⁺ T cells in the draining lymph nodes and spleens. The induction of OVA-specific CD8⁺ T cells was significantly reduced in Batf3^{-/-} mice. It also boosted the OVA-specific IgG2c and IgG1 in the serum. In the therapeutic vaccination model, we found that vaccination with irradiated B16-OVA + Heat-iMVA extended the median survival from 16 days (with irradiated B16-OVA vaccination) to 23 days (, P<0.01). With anti-PD-L1 antibody, vaccination with irradiated B16-OVA + Heat- iMVA extended the median survival from 20 days to 27 days (, P<0.01). In vitro studies demonstrated that Heat-iMVA enhanced antigen cross-presentation by DCs.

Conclusions

Our results demonstrate that Heat-iMVA is a safe and potent vaccine adjuvant for peptide and irradiated whole cell vaccinations. Future studies will evaluate the role of Heat-iMVA as a vaccine adjuvant for tumor neoantigens.

P117

DNA-based cancer vaccines designed by SynCon® technology break tolerance in genetically diverse pre-clinical models

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Background

Cancer vaccines targeting self-antigens have shown limited efficacy in the clinic due to self-tolerance

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mechanisms preventing or dampening an immune response against them. To overcome self-tolerance, we developed a novel, multi-phase DNA vaccine design strategy (SynCon[®] technology) that introduces subtle amino acid changes into native cancer antigens. These synthetic immunogens aim to generate cross-reactive T cells and/or promote epitope spreading. As a proof of concept, we first used SynCon[®] technology to design a novel DNA immunogen targeting the self-antigen FAP (SynCon[®] FAP) and investigated its ability to break tolerance in outbred CD-1 mice. Compared to commonly used inbred mouse models, CD-1 mice are more relevant for inferring immunogenicity in humans due to their genetic diversity. In addition, we designed a SynCon[®] immunogen targeting TERT (SynCon[®] TERT) and tested its ability to break tolerance in non-human primates (NHP), a highly relevant model for immunotherapeutic vaccine development.

Methods

CD-1 mice were immunized with either SynCon[®] FAP, a mouse native FAP vaccine, or vector control. Three immunizations were administered two weeks apart with electroporation following each immunization. For the NHP study, rhesus macaques were immunized with SynCon[®] TERT or native rhesus TERT vaccine as a control. Four immunizations were administered four weeks apart with electroporation following each immunization. Vaccine-induced immune responses were evaluated by IFN- γ ELISpot using species-specific native peptides.

Results

Compared to native mouse FAP, SynCon[®] FAP was more immunogenic in CD-1 mice (average 407 ELISpot-forming units (SFUs) per 10⁶ splenocytes for SynCon[®] vs. 160 SFU/10⁶ for native), suggesting that SynCon[®] FAP was capable of breaking tolerance. Importantly, 14/15 mice in the SynCon[®] FAP group generated an immune response above 100 SFU/10⁶, compared to only 9/15 mice in the native FAP group. In rhesus macaques, while the native rhesus TERT was not able to induce any detectable immune responses following the fourth immunization, SynCon[®] TERT elicited an average of 353 SFU/10⁶,

indicating that the SynCon[®] TERT immunogen was capable of breaking tolerance in NHPs.

Conclusions

Taken together, we demonstrate that SynCon[®] technology can be used to design DNA immunogens that are capable of breaking tolerance in genetically diverse preclinical models. SynCon[®] DNA immunogens have the potential to break tolerance, induce immune responses, and provide clinical benefit for patients with diverse HLA haplotypes. Further investigation of SynCon[®] DNA immunogens in clinical trials is warranted.

P118

Gp96-Ig/Costimulator combination vaccine improves T cell priming, enhances immunity, memory, and tumor elimination

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Background

The recent excitement in the field of immunoncology has been driven largely by the clinical success of checkpoint inhibitors. This success is tempered by the fact that monotherapy succeeds in only 10-40% of patients. It is widely believed that to improve patient outcomes, new approaches that combine treatments with more than one functionality will be required.

Methods

We have developed a next generation cellular vaccine platform – referred to as ComPACT (COMbination Pan-Antigen Cytotoxic Therapy) that incorporates a tumor antigen chaperone (gp96-Ig) with T cell costimulation (Fc-OX40L), into a single anti-tumor cell line that secretes them both (Cancer Immunol Res. 2016 Sep 2; 4(9):766-78).

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Results

CompACT primes both antigen-specific CD4+ and CD8+ T cells, and stimulates activation of CD127+KLRG-1- memory precursor cells. Systemic administration of OX40 agonist antibodies led to toxic proliferation of non-specific CD4+ T cells, Tregs and systemic inflammatory cytokine production. Importantly, CompACT led to high frequencies of IFN γ +, TNF α +, granzyme-b+ and IL-2+ antigen-specific CD8+ T cells at both priming and boosting, which enhanced rejection of established murine melanoma (B16.F10) and colon cancer (CT26) tumors and increased overall survival.

Conclusions

Here, we have assessed CompACT in combination with a checkpoint inhibitor and an additional systemic administered T cell costimulator (TL1A) and show that they synergize effectively with antagonist antibody therapies, amplifying antigen-specific T cells, programming a memory response, and eliminating tumors. CompACT/ α PD1 or α PD-L1/TL1a combinations may therefore translate into an efficacious approach to treat human cancers.

P119

Optimization of antigenic composition of a dendritic-cell targeting MIP3 α -antigen vaccine and the efficacy of additional IL-10 neutralization therapy in the B16F10 mouse melanoma model

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Background

The chemokine MIP3 α (CCL20) binds to CCR6 on immature dendritic cells. Vaccines fusing MIP3 α to melanoma antigen gp100 have been shown to be moderately effective in therapeutically alleviating melanoma in mouse models. To optimize the therapy, our laboratory is exploring two avenues. First, we added agents to neutralize interleukin 10

(IL-10) at the tumor site (i.t.) or vaccination site to modulate immune responses. Second, we examined the effects of substitution and addition of the highly immunogenic tyrosinase-related protein 2 (TRP2) antigen to the MIP3 α -gp100 vaccine construct.

Methods

The current studies utilize the B16F10 syngeneic, transplantable, mouse melanoma model system. The MIP3 α -antigen DNA vaccine is administered intramuscularly (i.m.) into the tibialis muscle, followed immediately by i.m. electroporation. Constructs utilized include MIP3 α fused to gp100, TRP2, or both. Vaccinations are given therapeutically, beginning at day 3 or 5 post challenge. Tumor sizes, growth, and survival were all assessed. Treatment responses were characterized by flow cytometric analysis of tumor infiltrate. The mechanism of i.t. α IL-10 efficacy was explored by RT-PCR and confirmed with a knockout mouse model.

Results

With this therapeutic protocol, we demonstrate for the first time that neutralizing IL-10 at either the vaccination or tumor site enhances the anti-tumor efficacy of a MIP3 α -gp100 vaccine, leading to significantly smaller tumors, slower growing tumors, and increases in mouse survival. We discovered that IFN α -4 transcripts in the tumor were significantly upregulated in mice given vaccine and i.t. α IL-10 compared to vaccine alone. A mouse model with *IFN α R1* knocked out eliminated the protection provided by i.t. α IL-10, demonstrating that the additional therapeutic value of i.t. α IL-10 is primarily mediated by type-I interferons. Also, we demonstrate that the MIP3 α -gp100-TRP2 dual antigen and the MIP3 α -TRP2 vaccine provided better protection compared to the MIP3 α -gp100 vaccine but were not significantly different from each other.

Conclusions

Efficient targeting of antigen to immature dendritic cells with a chemokine fusion vaccine offers a potential alternative approach to the *ex vivo*

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dendritic cell antigen loading protocols currently undergoing clinical investigation. Neutralizing IL-10 at both the vaccination site and tumor site have enhanced vaccine efficacy, the latter shown to be due to type-I interferons. Additionally, MIP3 α -TRP2 has proven to be a superior vaccine construct than MIP3 α -gp100 in our system, and the MIP3 α -gp100-TRP2 vaccine did not improve on the protection provided by MIP3 α -TRP2. Further potential therapy optimization currently undergoing investigation offers promise for this line of investigation to become a novel melanoma therapy.

P120

Streamlining approaches for the selection of T cell neo-epitopes: from mutanome to therapeutic personalized cancer vaccine

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Background

Next-generation sequencing has opened the door to precision cancer therapies targeting mutations expressed by tumor cells. However, most neo-epitopes selected by traditional T cell epitope prediction algorithms prove to be non-immunogenic. Poor predictive performance may partially be due to inclusion of mutated epitopes cross-conserved with self-epitopes recognized by the T cell receptor of regulatory (Treg), anergic or deleted T cells. Vaccination with self-epitopes can lead to weak effector responses, active immune suppression, and toxicity due to immune-mediated adverse effects.

Methods

We have developed Ancer, an advanced cancer T cell epitope identification and characterization tool that streamlines the selection of Class I and II neo-

epitopes. Ancer is leveraging EpiMatrix and JanusMatrix, state-of-the-art tools that have been extensively validated in prospective vaccine studies for infectious diseases [1, 2]. Use of Ancer in the oncology field allows for the prioritization of neo-epitopes exhibiting reduced potential for inducing Tregs, whose activation continues to curtail efficacy of current cancer therapies.

Results

We validated Ancer's predictive accuracy using datasets of HLA-bound peptides detected by mass spectrometry, which are independent of training sequence data used in model development. Analysis of sequences from Abelin et al. [3] shows a 96% agreement between Ancer predictions and peptides eluted from common Class I HLAs, while only 86% of these sequences are accurately recalled by NetMHC and NetMHCpan. Additional retrospective analyzes of a cancer immunogenicity study [4] demonstrate that Ancer selects immunogenic neo-epitopes with 72% accuracy, as compared to 21% accuracy when using public prediction tools.

Conclusions

These results demonstrate that Ancer may focus epitope candidate selection on higher value sequences than conventional algorithms. Neo-epitopes with low Treg activation potential may then be used to support the development of safer and more effective vaccines.

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P121

Phase 1 dose escalation trial of CV301

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Background

CV301 is a poxviral-based vaccine comprising a prime-boost strategy with Modified Vaccinia Ankara (MVA, prime) and fowlpox (FPV, boost), which contain transgenes encoding two tumor associated antigens, CEA and MUC-1, as well as 3 costimulatory molecules (B7.1, ICAM-1 and LFA-3, called TRICOM). Preclinical evidence suggests CV-301 activates CEA and MUC-1 specific T cell responses, potentially increasing the clinical benefit of immune checkpoint inhibition. An open label phase 1/1b/2 trial was initiated to evaluate safety of CV301 alone (phase 1) and in combination with PD-1/L1 inhibition (phase 1b) to be followed by a randomized phase 2 trial evaluating the combination compared with PD-1/L1 inhibition alone. Here, we report the phase 1 safety evaluation of CV301.

Methods

This open-label, 3 +3 design dose-escalation phase 1 trial evaluated 3 dose levels of the priming dose of CV301 (MVA): 1, 2 or 4 s.c. injections of 4×10^8 Inf.U/0.5 mL on days 1 and 29. FPV booster doses were scheduled for all subjects subcutaneously at a

1×10^9 Inf.U/0.5 mL dose on weeks 9, 11, 13, 15, 19, 23, 27, 31, 35, 39, 43, 47, 51, 65, 78, 91 and 104.

Results

There were no dose limiting toxicities in the phase 1 evaluation of CV301 alone. The minimum required patient number was enrolled in each dose level (DL1: 3, DL2: 3, DL3: 6, total: 12). The most frequent treatment-related AEs were temporary and self-limiting, grade 1/2, and included injection site reactions (erythema, pruritus, pain, induration and/or swelling) and general symptoms (fever/chills, flu-like symptoms, headache, fatigue/weakness, nausea/vomiting, myalgia and arthralgia). Seven of the 12 patients have microsatellite stable colon cancer. Of these seven, two had preliminary evidence of benefit. One patient with stable disease at first restaging has marked reduction (60% fall) in tumor markers at 10 weeks. Another patient had unconfirmed partial response at first restaging (6 weeks). When 3 month restaging scans indicated progression, a PD-L1 inhibitor was initiated. Subsequently, tumor markers fell by 60%.

Conclusions

CV301 priming doses (MVA) can be safely administered to at least 4 injection sites concurrently at 4×10^8 Inf.U/0.5 mL per site for a total of 1.6×10^9 Inf.U, which is the recommended phase 1b and phase 2 dose. Booster doses (FPV) can safely be administered s.c. at 1×10^9 Inf.U/0.5mL. Subsequent analyzes will include immune response data. The trial will continue to enroll phase 1b and, subsequently, the randomized phase 2.

Trial Registration

ClinicalTrials.gov Identifier NCT02840994

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P122

N-terminally extended proline residues influences the HLA class I antigen processing and decreases CTL responses

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Background

CD8+ T cells recognize peptide-MHC class I complexes presented on target cell surfaces; however, the precise mechanisms of antigen processing by which T cell epitopes are generated still remains unclear.

Methods

Here we extensively collected >800 naturally presented peptide sequences, and statistically analyzed an amino-acid profile of their N-terminal extensions. We combined natural HLA-A24 ligand sequences gained from HLA-ligandome analysis with their corresponding N-extension sequences gained from database search, and analyzed frequency of each amino acid at given positions of N-extensions (Position -15 to -1).

Results

Interestingly, we found that frequency of proline residue (Pro) was significantly reduced between P-3 and P-1, implying that peptide sequences following Pro might not be efficiently presented by HLA-A24. To further investigate this hypothesis, we prepared 293T cells expressing a panel of mini-gene constructs that carried N-extensions followed by a model epitope sequence, and ultimately found that presence of Pro at P-3 to P-1 significantly reduced IFN- γ production from the CTLs specific to the epitope.

Conclusions

Thus, we consider that presence of Pro could be a new signature in HLA class I antigen processing that

attenuates presentation and CTL recognition of the following epitope sequences.

P123

A novel oncolytic virus expressing multiple immune regulatory factors to change tumor immune microenvironment and to cause lasting complete tumor eradication in animal models

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Background

Oncolytic viruses (OVs) are among the most powerful approaches in cancer immunotherapy [1]. OVs not only cause cancer cell lysis but more importantly, their infection in tumors induces anti-tumor immune response from the host, resulting in lasting anti-tumor immunity. It has been recognized that anti-tumor immune response requires multiple immune regulatory factors that act synergistically and tumor microenvironment is critical for tumor to grow. Herpes simplex virus type-1 (HSV-1) has been approved by FDA as an oncolytic viral drug to treat melanoma [2]. One advantage of HSV-1 is its large genomic capacity for carrying multiple exogenous genes.

Methods

A HSV-1 oncolytic viral vector (VG161) was constructed to simultaneously express IL12, IL15 with its receptor alpha unit and a PDL-1 blocking peptide. Anti-tumor activity of VG161 was tested in both immune competent mice (CT26 and A20 tumor models) and nude mice for human tumor models (LNCaP and U87). Since CT26 and A20 are poorly permissive for HSV-1 replication, the mouse tumor models were able to demonstrate the anti-tumor

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immune response induced by VG161 while oncolytic activity of VG161 was demonstrated in LNCaP and U87 models since the immune system is compromised in those models.

Results

VG161 caused complete tumor eradication in all the models tested and the animals survived tumor-free for many months till sacrificed. VG161 virus induced tumor oncolysis caused complete tumor destruction in both LNCaP and U87 tumors. In the CT26 model, no tumor could be found after re-challenging with the same tumor cells. The anti-tumor immune response by VG161 was significantly stronger than similar viruses that did not express any immune stimulating gene or only express GM-CSF. Furthermore, in an A20 double tumor model, intratumoral injection into the tumor on one side caused tumor regression on both sides. Transcriptom analysis showed significant change in tumor microenvironment. Finally, memory T cells are evident in the treated animals demonstrated by multiple assays.

Conclusions

VG161 is a novel oncolytic virus that are both strong in stimulating anti-tumor immunity and oncolytic activity. Intratumoral expressing multiple immune regulatory factors by an oncolytic virus may significantly change the tumor immune microenvironment to enhance efficacy of the oncolytic virus.

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P124

***In vitro* mode of action of ilixadencel - a cell-based allogeneic immune primer for intratumoral administration**

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Background

Therapeutic cancer vaccines that target personal mutation-derived neoantigens in tumor cells have recently been shown to stimulate a potent immunological anti-tumor response in cancer patients. One obvious way to circumvent major technical difficulties associated with *ex vivo* production of neoantigen-based vaccines would be to use the patient's own tumor *in situ* as a direct antigen source by intratumoral administration of a potent immune primer. The immune-priming function would be to promote immunogenic cell death and activation of recruited DCs into T helper 1 polarizing DCs. For this purpose, a cell-based immune primer consisting of cryopreserved proinflammatory allogeneic DCs (international nonproprietary name ilixadencel; previously named INTUVAX), producing high amounts of T helper 1 associated chemokines and cytokines, has been developed and tested in a phase I/II clinical trial with promising immunological and clinical results [1]. In order to explore the potential mode of action, a batch of GMP-produced ilixadencel was investigated *in vitro*.

Methods

The effects of soluble factors released by ilixadencel, and cell-to-cell interactions between ilixadencel and co-cultured allogeneic immune cells, on NK cell recruitment and activation, and allogeneic bystander DC maturation and polarization were analyzed *in vitro*.

Results

Cell migration experiments revealed that allogeneic NK cells migrated towards chemotactic factors produced by ilixadencel. When cocultured with allogeneic NK cells, ilixadencel was found to be superior to IL-2 priming in terms of induced CD69-expression and IFN-gamma production by NK cells.

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The *in vitro* assay for cytotoxicity further revealed that the tumor-killing ability of NK cells was markedly enhanced after coculture with ilixadencel. Finally, supernatants from ilixadencel, and ilixadencel cocultured with allogeneic PBMCs, induced a phenotypic maturation of bystander DCs producing substantial amounts of IL-12p70 upon subsequent CD40-ligation.

Conclusions

Collectively, the presented *in vitro* results indicate that intratumorally injected ilixadencel will create an immune-priming environment leading to NK-cell mediated tumor cell death with release of tumor-derived antigens, including neoantigens, as well as maturation of bystander endogenous DCs into T helper 1 polarizing mature DCs.

References

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P125

Immunogenicity of human papillomavirus (HPV) specific DNA vaccine, INO-3112 (HPV16/HPV18 plasmids + IL-12) in HPV+ head and neck squamous cell carcinoma (HNSCCa)

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Background

Oropharyngeal HNSCCa is frequently associated with HPV. We hypothesized that immunotherapy with INO-3112 would generate immune responses in patients (pts) with HPV16+ or 18+ HNSCCa.

Methods

This Phase I/IIa trial included pts with p16+ locally advanced HNSCCa, ECOG PS 0-1. INO-3112 was delivered IM along with electroporation with the CELLECTRA[®] device, Q3 weeks x 4 doses. Cohort 1 (C1) pts received INO-3112 pre and post-surgery; Cohort 2 (C2) pts received INO-3112 post cisplatin-based definitive chemoradiation. 1[°] and 2[°] endpoints were safety and immune responses. HPV16/18 specific antibody levels were assessed by ELISA. IFN γ ELISpot and flow cytometry were used to quantify antigen specific T cell responses, at each dosing visit and q3 months (mos).

Results

Twenty two (22) pts were treated, completing accrual. C1: n=6, C2: n=16; 20 male, median age 57.5 years (32-76); base of tongue=10, tonsil=12; never smoker=10. All pts are alive, median follow up is 15.9 mos (1-26). INO-3112 was well-tolerated with no related Grade 3-5 AEs. Peak mean/median antibody responses to HPV16 E7 and HPV18 E7 antigens for 19 pts with evaluable samples were 1:1235/1:150 and 1:2853/1:450, respectively. As compared to baseline, 18 pts with evaluable samples showed elevated HPV16/HPV18 specific T cell activity (by IFN γ ELISpot). Flow cytometric analysis of PBMC revealed an increased frequency of INO-3112 specific (summed responses to HPV16 and HPV18) CD8+CD38+PD-1+ cells that were positive for Granzyme A or B and perforin from baseline (mean frequency of 1.19% and 1.097% for GrzA+Prf+ and GrzB+Prf+ within CD8+CD38+PD-1+, respectively) to post immunotherapy (mean frequency of 7.694% and 6.698% GrzA+Prf+ and GrzB+Prf+ within CD8+CD38+PD-1+, respectively) in 7 of 8 patients with evaluable samples. 3 pts have progressed; 1 pt received Nivolumab for progressive disease, and remains in CR.

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Conclusions

These data show that INO-3112 generated HPV-specific peripheral humoral and cellular immune responses in patients with HPV+ HNSCCa. The post therapy increase of INO-3112 specific (summed HPV16 and 18 responses) CD38+PD-1+ cells that express lytic granules suggests that INO-3112 promotes CD8+ T cell activation, and that these activated CD8+ T cells may be vulnerable to PD-L1 mediated suppression. Clinical trial information: NCT02163057

Trial Registration

ClinicalTrials.gov Identifier NCT02163057

P126

Cancer immunotherapy with novel vaccine nanodiscs for efficient elimination of mucosal tumors

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Background

Recently we have reported a novel vaccine nanodisc technology for elicitation of anti-tumor T cells that penetrate into subcutaneous flank tumors [1]. Here we aimed to apply the nanodisc technology for elimination of mucosal tumors, such as cervical carcinoma and lung tumors. Previous research has suggested that intranasal vaccination can recruit T cells to local mucosal tissues, and may control the growth of lung tumors. However, recruitment of T cells to distal mucosal sites, such as the reproductive tract, is very challenging. In contrast to mucosal vaccination, parenteral vaccination may elicit strong systemic T cell responses and allow for robust T cell infiltration into multiple, disseminated mucosal tissues, thus improving the overall therapeutic effect. To test this hypothesis, we established murine models of HPV-induced cancer in reproductive tract and lung metastasis and directly compared the anti-tumor efficacies of

subcutaneous vs. intranasal vaccination with nanodiscs.

Methods

The vaccine nanodiscs were prepared by conjugating the HPV antigen peptide and adjuvant CpG to pre-formed nanodiscs. The resulting vaccine nanodiscs were characterized by dynamic light scattering, gel permeation chromatography, and HPLC. The reproductive tract tumor model was established in C57BL/6 mice by intravaginal administration of luciferase-expressing TC-1 cells - a surrogate for HPV-induced human tumors, such as cervical carcinoma. The lung metastasis model was established by intravenous injection of luciferase-expressing TC-1 tumor cells. Tumor-bearing mice were vaccinated twice, and the growth of TC-1-luc intravaginal and lung tumors was monitored by IVIS. The antigen-specific T cell responses were measured by the tetramer staining assay.

Results

Subcutaneous administration of nanodiscs induced robust expansion of circulating antigen-specific CD8+ T cells with the peak frequency of ~35%, a 12-fold increase than that achieved by intranasal vaccination ($p < 0.0001$). Furthermore, subcutaneous administration of vaccine nanodiscs eliminated established TC-1 tumors both in the lungs and reproductive tract by day 20, whereas intranasal administration only eliminated tumors in the lung but failed to do so for intravaginal tumors.

Conclusions

Subcutaneous route of nanodisc vaccination is more efficient than intranasal route in terms of elicitation of anti-tumor T cell responses and elimination of mucosal tumors. These vaccine nanodiscs may offer a promising strategy for treatment of mucosal tumors, including cervical carcinoma and lung metastases.

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P127

Transduction of MAGE-A1, A3, A4, A10 and IL-12 by ZVex[®], a dendritic cell targeting platform induces robust multi-antigen T cell immune responses without antigenic interference or immunodominance

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Background

Expression of multiple antigens in tandem from viral vaccine vectors often results in immune dominance phenomena, which preclude generation of balanced immune responses to each antigen. To address this issue, we have developed a novel technology that allows for multiple antigens and/or immune-modulators to be expressed *in vivo* using the integration-deficient, dendritic-cell targeted lentiviral vector platform, ZVex. Utilizing a unique manufacturing process that generates reassortant vectors, we have designed a vector expressing the human cancer testis antigens MAGEA1, MAGEA3, MAGEA4, MAGEA10 and Interleukin 12, in order to generate robust T cell responses specific for all four antigens simultaneously.

Methods

In addition to the backbone plasmids encoding for essential vector components (such as capsid, envelope, Vpx and Rev proteins, polymerase, protease), five identical vector backbones each encoding a different full-length MAGEA or murine IL12 gene were co-transfected into a producer cell line for viral vector production. Because lentiviral vectors package two RNA molecules each, this approach results in the generation of potentially 15 different homozygous or heterozygous vector genotypes. Vector titers were determined by vector and gene-specific RT/PCR and by a cell-based

infectivity assay. Recombinant IL-12 was detected by ELISA. Female BALB/c mice were immunized with either reassortant vector products or a ZVex vector expressing single antigens. After two weeks, CD8+ T cell responses were evaluated for multi-functionality via intracellular cytokine staining.

Results

The product was shown to contain nearly equal amounts of MAGE-A1, A3, A4, A10 (ranging from 2.3-4.9x10¹⁰ genome copies/ml, 1.4-3.9x10⁹ infectious units/ml) and to contain IL12 at the expected proportion. A single subcutaneous injection generated specific T cell responses against all four of the encoded MAGEA antigens at similar levels (0.4 % antigen specific splenic CD8 T cells each). Furthermore, IL-12 was shown to consistently enhance immunogenicity with the response enhancement ranging from 2.9 to 3.8-fold for each of the four antigens.

Conclusions

Expressing multiple MAGE-A proteins and the immune enhancing cytokine IL-12 from a reassortant ZVex vector resulted in robust and balanced antigen specific CD4 and CD8 T cell responses in mice. No evidence of immunodominance or antigenic interference was observed in these experiments. Because MAGE-A1, 3, 4, and 10 antigens are expressed by a large number of solid tumors individually or in combination, this is a potentially broadly applicable, off-the-shelf cancer vaccine.

P128

PD-L1-specific T cells can be activated by interferon- γ and have an anti-tumor effect

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Background

Checkpoint inhibitory pathways, like the programmed death-1 receptor (PD-1) and its ligand (PD-L1), play key roles in inducing immune tolerance in the tumor microenvironment. PD-L1 is expressed in the tumor microenvironment by cancer cells as well as different immune regulatory cells, like dendritic cells. We have previously shown that the immune system has an anti-cancer mechanism that works via PD-L1-specific effector T cells *in vitro*. We described spontaneous CD8+ and CD4+ T cell reactivity against PD-L1 in peripheral blood of healthy donors and patients with various cancers. Those results suggested that PD-L1-specific T cells might modulate adaptive immune reactions by reacting to regulatory cells and cancer cells. Here we examined the function of PD-L1-specific T cells *in vivo*.

Methods

We hypothesized that PD-L1-specific T cells are present at steady state and are an important factor in regulating the immune system during inflammation. Healthy C57Bl/6 mice were injected with IFN γ intraperitoneal to simulate inflammation. After 6 days the mice were sacrificed and the spleen was removed for further analysis by Elispot with *ex vivo* stimulation of a murine PD-L1-derived epitope (mPD-L1long). Subcutaneously (s.c.) vaccination with mPD-L1long in Montanide was done in C57Bl/6 mice to further enhance the response. After a week, the mice were sacrificed and spleen and draining lymph node (dLN) removed and analyzed by Elispot. To test the anti-tumor effect of the mPD-L1long vaccination, C57Bl/6 mice were inoculated with B16F10 cells s.c. and vaccinated two times, and the tumor growth was monitored

Results

Ex vivo stimulation with mPD-L1long resulted in a PD-L1-specific T cell response in the spleen after just two IFN γ -injections, indicating a rapidly increase in PD-L1-specific T cells. It was possible to further increase the PD-L1-specific response by one vaccination with mPD-L1long. A strong PD-L1-specific T cell response was found in the spleen and

dLN of the mPD-L1long-vaccinated mice compared to the control mice. We also found that mPD-L1long-vaccination decreased the tumor growth.

Conclusions

We describe that PD-L1-specific T cells are expanded by IFN γ -injections, which suggest that PD-L1-specific T cells are already present and are activated due to a strong activation signal from their cognate targets (i.e. professional antigen-presenting cells) at inflammation sites. PD-L1-specific T cells are easily expanded by vaccination and they have an anti-tumoral effect. Thus, PD-L1-specific T cells are a particularly interesting example of the immune system's ability to influence adaptive immune responses by directly reacting against the immune-suppressive mechanisms employed by cancerous cells.

P129

Distinct patterns of clonotypic T cell responses in metastatic castration-resistant prostate cancer pts treated with standard sipuleucel-T (sip-T) compared with pts receiving a booster treatment

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Background

Sip-T is an FDA-approved autologous cellular immunotherapy for asymptomatic or minimally symptomatic mCRPC. Neoadjuvant sip-T induced activated T cell infiltration into prostate tissue [1] and broadened the TCR repertoire within prostate tumor tissue vs non-sip-T-treated patients [2]. To test if sip-T induces long-lasting memory T cell responses, we compared treatment-induced

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changes in TCR repertoire between patients who received standard sip-T treatment (STRIDE) vs patients retreated with sip-T as a boost (P10-1).

Methods

In P10-1 (N=8), patients were previously treated with sip-T in an androgen-dependent setting and retreated *after a median of 8.6 years* [3]. In STRIDE (N=52), patients received sip-T with concurrent or sequential enzalutamide [4]. Peripheral blood mononuclear cells (PBMCs) were collected at baseline and during/post-sip-T. Deep sequencing and sequence diversity of the TCR VDJ region were performed using the Immunoseq assay (Adaptive Biotechnologies) and the Shannon diversity index and clonality, respectively. TCR dynamics across time points was evaluated by Morisita's distance, intraclass correlation coefficient, and fold-change analysis [5].

Results

Baseline TCR diversity was similar between the two studies ($p=0.590$, Shannon frequency; $p=0.700$, clonality; Wilcoxon rank sum test). Significant increases in TCR diversity assessed by clonality were observed from baseline to week 4 ($p<0.001$) and week 6 ($p=0.030$) in STRIDE, but no significant changes were observed for post-treatment time points in P10-1. Morisita's distance in was significantly higher at week 2 ($p=0.040$) and 4 ($p=0.013$) in STRIDE vs P10-1, indicating a more consistent TCR repertoire in P10-1 across time points. Percent increases in clones at post-treatment time points were significantly higher in P10-1 vs STRIDE (week 6: $p=0.007$, week 26: $p=0.015$, week 52: $p=0.026$ (Figure 1). The extent of change within the top 100 most abundant TCR sequences (clonal shuffling) was greater and was initiated earlier in P10-1 vs STRIDE (Figure 2).

Conclusions

These data indicate that initial sip-T treatment of naïve mCRPC patients programs the TCR repertoire, which is maintained over time consistent with immunologic memory. Retreatment with sip-T expands the number of select T cell clones

persisting up to 1 year after retreatment, which is characteristic of immunological boosting following successful vaccination.

Trial Registration

NCT01338012 (P10-1), NCT01981122 (STRIDE)

Figure 1. Percent increase in clones in mCRPC patients retreated with sip-T (P10-1) compared with patients receiving a late booster treatment (STRIDE)

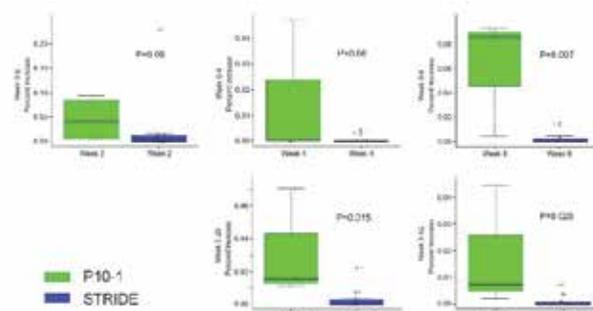
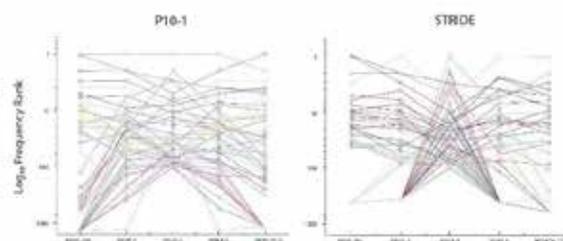


Figure 2. Clonal shuffling of top 100 clones at week 4 of sip-T treatment



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P130 Abstract Travel Award Recipient

***In situ* vaccination with Flt3L, radiation, and poly-ICLC induces a potent immune response in patients with follicular lymphoma**

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Background

Lymphomas are the 5th most common cancer in the US; roughly half of these are indolent non-Hodgkin's lymphomas (iNHLs), which outside of allogeneic stem cell transplant remain incurable. A previous trial of *in situ* vaccination (ISV) in iNHLs combined intratumoral (IT) CpG injection with localized radiotherapy (XRT) with some clinical success. One limitation in this previous trial may have been a paucity of IT dendritic cells (DCs), particularly Batf3⁺CD141⁺DCs known to cross-present antigen to T cells, and to express high levels of TLR3. Hence, a novel iteration of ISV was developed which incorporates Flt3-ligand (Flt3L), to increase IT DCs, and poly-ICLC.

Methods

In an ongoing clinical trial patients receive: 1) IT Flt3L for 9 days, 2) XRT two consecutive days, and 3)

IT Poly-ICLC on days 10, 14, 17, and weekly thereafter. Primary endpoints are safety/tolerability and overall response. Biopsy tissue and peripheral blood is taken before and during treatment to assess changes in tumor microenvironment by flow cytometry and CyTOF.

In the murine model of ISV in which sub-cutaneous A20 (B cell lymphoma) tumors is injected with Flt3L for 9 consecutive days, irradiated and then treated with poly-ICLC daily for 5 days, systemic anti-PD-1 was administered every 3 days.

Results

Biopsy tissue and peripheral blood from patients confirms that Flt3L induces an influx of CD1c and CD141 DCs into treated tumors, which attain a mature effector (PD-L1^{lo} CD80^{hi}) phenotype following initiation of poly-ICLC; similarly, intratumoral T cells attain a more mature—if not exhausted—phenotype. CyTOF performed on peripheral blood from 2 patients with clinical responses and 4 patients with progressive disease revealed larger populations of more terminally differentiated T cells with high expression of markers of exhaustion such as PD1, LAG3, and TIGIT (Figure 1).

Similar findings of an exhausted intratumoral T cell phenotype were seen in the murine ISV model. The addition of anti-PD1 to reverse this exhaustion resulted in significant improvement in survival and tumor regression, with over 75% of mice clearing tumors (Figure 2).

Conclusions

Though small numbers, data from this ISV trial suggest that non-responders have larger populations of exhausted T cells, also seen in the murine model of this ISV. The addition of PD-1 reverses this exhaustion in mice, and results in significantly improved tumor regression. These findings will inform a novel ISV which will incorporate checkpoint blockade in humans with lymphoma and peripherally accessible solid tumors.

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Figure 1. CD8 peripheral blood T cells in ISV responders and non-responders

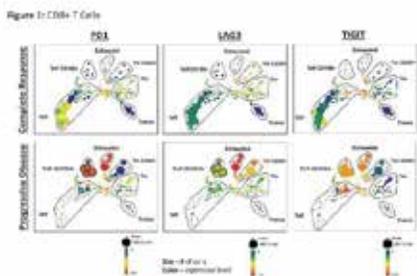
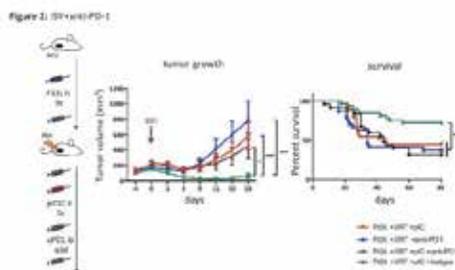


Figure 2. Murine ISV + anti-PD1 results.



Trial Registration

ClinicalTrials.gov Identifier NCT01976585

P131

Contrasting roles of cyclooxygenase-1 and cyclooxygenase-2 in cell-mediated immunity to *Listeria monocytogenes*

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Background

Listeria monocytogenes is an intracellular bacterium that is currently being developed as a cancer

immunotherapeutic platform due to its ability to elicit robust CD8⁺ T cell responses. Though the role of cytokines in *Listeria*-stimulated immunity has been extensively studied, the influence of lipid modulators of inflammation, known as eicosanoids, is less clear. Understanding how eicosanoids impact immunity is critical as current *L. monocytogenes*-based immunotherapy trials utilize nonsteroidal anti-inflammatory drugs (NSAIDs) as analgesics to alleviate patient discomfort. We hypothesized that eicosanoids, particularly those produced downstream of cyclooxygenases, influence the response to *L. monocytogenes*.

Methods

C57Bl/6, microsomal prostaglandin E₂ synthase-1 -/- (mPGES-1), or Cox-1 -/- mice treated with or without indomethacin (broad cyclooxygenase inhibitor), celecoxib (Cox-2 inhibitor), or acetaminophen were immunized with 10³ *DactA/DinIB L. monocytogenes* and challenged 30 days later with a lethal dose of wild type *L. monocytogenes*. Bacterial burdens in the spleens and livers were enumerated at 68-72 hours post infection to assess for protective immunity.

To assess acute T cell responses, mice were immunized with 10⁷ *DactA/DinIB L. monocytogenes* expressing B8R and OVA. B8R specific responses were examined at 7 days post immunization for CD8⁺ T cell populations producing IFN γ , TNF α , and IL-2.

Eicosanoid levels were assessed in the spleen from 4 to 48 hours post immunization with 10⁷ *DactA/DinIB L. monocytogenes*. Extracted eicosanoids were analyzed by high performance liquid chromatography and mass spectrometry.

Results

Treatment of mice with the non-specific cyclooxygenase inhibitor indomethacin led to decreased antigen specific T cells and protective immunity, suggesting a role of eicosanoids in the context of *L. monocytogenes* stimulated immunity. Immunization of Cox-1^{-/-} deficient mice suggested

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that cyclooxygenase-1 activity was detrimental to immunity. In contrast, treatment with celecoxib demonstrated that cyclooxygenase-2 activity was required for immunity. Analysis of eicosanoid levels in the spleen through 48 hours following immunization revealed an acute upregulation of the eicosanoid PGE₂ in C57Bl/6 mice twelve hours post immunization. Importantly, we found that PGE₂ induction is critical for immunity as mice deficient in mPGES-1 showed an impaired cell-mediated immune response. Finally, we demonstrated that the non-NSAID analgesic acetaminophen did not influence the development of cell mediated immunity during *L. monocytogenes* immunization.

Conclusions

These results indicate that eicosanoids impact the immune response to *L. monocytogenes*. Production of eicosanoids downstream of cyclooxygenase-1 is detrimental to cell-mediated immunity where cyclooxygenase-2 is critical, particularly through PGE₂ production. Understanding how eicosanoids modulate immunity could present novel mechanisms to improve the use of *L. monocytogenes* as an immunotherapeutic.

P132

Overexpression of androgen receptor (AR) in prostate cancer cells following androgen deprivation increases recognition by AR-specific CD8+ T cells

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Background

Androgen deprivation therapy (ADT) is the primary treatment for recurrent and metastatic prostate cancer. There has been a lot of interest in

combining ADT with targeted immunotherapies, because ADT has demonstrated immunostimulatory properties. In this study, we hypothesized that overexpression of AR as a mechanism of resistance to ADT may result in enhanced AR-specific T cell recognition and cytotoxicity, suggesting that AR-targeted immunotherapies might specifically best be combined with androgen deprivation.

Methods

We examined the expression of AR in 22RV1 cells cultured in serum-replete or androgen-depleted media and determined whether increased expression of AR would result in increased AR-specific T cell immune recognition. We then examined whether ADT could be combined with a DNA vaccine encoding the AR to increase the anti-tumor response using two mouse models of prostate cancer (prostate-specific PTEN-deficient mice and MycCaP).

Results

Culturing 22RV1 cells in androgen-depleted medium resulted in increased AR expression compared to 22RV1 cells cultured in complete medium. The overexpression of AR resulted in increased recognition and cytotoxic activity of AR-specific CD8+ T cells. When ADT was combined with a DNA vaccine encoding the AR, there was improved anti-tumor responses, as demonstrated by smaller tumor volumes and delays in the emergence of castration-resistant tumors using two models of prostate cancer. Furthermore, depletion of CD8+ T cells abrogated this delayed growth of castration resistant tumor growth, demonstrating that the anti-tumor response of the DNA vaccine and ADT is mediated by CD8+ T cells.

Conclusions

Together these data suggest that combining ADT with an AR-targeted vaccine could be investigated for the treatment of prostate cancer to delay or prevent the development of castration resistance due to overexpression of AR.

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P133

A heterologous prime-boost vaccination strategy combining a *Listeria* and DNA-based vaccine encoding prostatic acid phosphatase (PAP) elicits a strong antigen-specific, anti-tumor response

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Background

Immunotherapies have demonstrated clinical benefit for many types of cancers. Sipuleucel T, an autologous cell-based vaccine targeting the prostate tumor antigen prostatic acid phosphatase (PAP), received FDA approval in 2010 for the treatment metastatic, castration-resistant prostate cancer. Given the cost and logistics of this autologous cell product, off-the-shelf vaccines are highly desirable. We have investigated a DNA vaccine encoding PAP (pTVG-HP) in two clinical trials in patients with biochemically recurrent prostate cancer. In both trials, persistent PAP-specific, Th1-type immunity could be elicited. In the current study, we sought to evaluate whether the immunogenicity and anti-tumor efficacy of this vaccine could be augmented using a prime-boost vaccination strategy with another genetic vaccine approach. Specifically, we investigated a live attenuated double-deleted strain (LADD) of *Listeria monocytogenes* encoding PAP, given alone or in sequence with the pTVG-HP DNA vaccine, and using a humanized murine tumor model.

Methods

Transgenic mice expressing HLA-A2.01 and HLA-DRB10101, but not murine MHC class I or class II (A2/DR), were immunized alone or with heterologous prime-boost strategies using a DNA (pTVG-HP) or listeria-based vaccine (Lm-PAP), each targeting PAP. Splenocytes from immunized mice were evaluated for immune responses to MHC class

I and class II-restricted, PAP-specific epitopes using IFN γ ELISPOT and intracellular cytokine staining (ICCS). Anti-tumor response to PAP-expressing syngeneic tumors were also assessed.

Results

A2/DR mice immunized with LM PAP or pTVG-HP alone developed CD4+ and CD8+ PAP-specific immune responses and anti-tumor responses. However, A2/DR mice immunized with Lm-PAP generated primarily an immune response to a PAP-specific MHC class I epitope while mice immunized with the DNA vaccine developed an immune response to PAP-specific, MHC class II epitopes. A2/DR mice primed with pTVG-HP and then boosted with Lm-PAP developed more robust immune responses and anti-tumor responses. The greater anti-tumor efficacy with this specific sequence was dependent on the development of a CD4+ T cell response and was not due to an adjuvant effect from *Listeria*. Differences in T cell immunity elicited were also not due to differences in B cell antigen presentation following DNA immunization.

Conclusions

Prime-boost vaccination with recombinant DNA and *Listeria* vaccines elicited CD4+ and CD8+ T cell immunity and anti-tumor responses in a relevant murine model. The sequence of immunization, with DNA priming prior to a heterologous immunization approach, was superior, consistent with studies in other systems, and is likely due to the early generation of Th1-biased CD4+ T cell immunity.

P134

Preclinical *in vivo* model validation for the development of a novel vaccine based strategy targeting IDO1

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Background

IDO1 (indoleamine 2, 3-dioxygenase 1) is a tryptophan catabolizing enzyme that fosters a tumor-promoting inflammatory microenvironment. IDO1 acts to subvert T cell immunity at multiple levels, including suppressing effector T cells and inducing/activating Tregs. The therapeutic potential of IDO1 inhibitors, particularly in combination with current 'immune checkpoint' agents, is now being actively explored in multiple clinical trials, including Phase 3 studies. In this context, a particularly intriguing finding has been the observation that humans exhibit MHC class I restricted, cytotoxic T cell reactivity against IDO1-expressing cells. IDO1 reactive T cells are found not only in cancer patients, where pathophysiological elevation of IDO1 has been frequently noted, but even in healthy individuals. This finding implies the existence of a T cell-mediated, counter-regulatory mechanism directed against IDO1. The results from initial clinical studies employing a peptide-based vaccine approach to harness this anti-IDO1 response for the benefit of cancer patients have thus far been encouraging. However, there is a pressing need to establish preclinical models for investigating the underlying basis of the anti-IDO1 response in order to inform treatment optimization.

Methods

See Figure Legends.

Results

To develop a surrogate mouse model to mimic the observed MHC I-restricted response to human IDO1 peptides, computer algorithms were used to rank peptides. H2^d restricted peptides were specifically queried to be compatible with the Balb/c strain based, CT26 colon carcinoma model, selected based on high levels of IDO1 expression and responsiveness to IDO1 inhibition reported for these tumors. A subset of these peptides were

found to be capable of stimulating IFN γ production in a recall response assay. Prophylactic vaccination of mice with each of the peptides produced varying degrees of growth suppression against CT26 tumors, which unexpectedly, did not directly correspond with their effectiveness in the recall response assay. Therapeutic treatment of established CT26 tumors with a combination of peptide vaccine and anti-PD-1 antibody treatment produced a combinatorial anti-tumor response beyond what was achieved with either agent alone. Studies into the underlying nature of the IDO1-directed, anti-tumor response are currently ongoing.

Conclusions

As noted initially in humans, our results demonstrate that mice are capable of mounting an effector T cell response against IDO1, confirming that this endogenous protein is somehow excluded from normal tolerance mechanisms. Initial findings that IDO1 peptide derived vaccines can elicit effective anti-tumor responses confirms the utility of mouse tumor models for further exploration and refinement of this novel approach to IDO1 directed cancer therapy.

P135

PEGylated tumor membrane nano-vesicles for eliciting adaptive immune responses against melanoma

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Background

Cancer immunotherapy has progressed greatly over the past decade with the approval of checkpoint blockade therapies and numerous cancer vaccine clinical trials. However, lack of consistent response rates and presence of immune evasion require further improvements. This project utilizes the wide antigen repertoire within tumor cell lysate to elicit cytotoxic T cell lymphocyte (CTL) and humoral

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responses against tumors in mice. We have prepared membrane vesicles from a model melanoma cell line, B16F10-OVA, and stabilized them by inserting poly (ethylene glycol) (PEG) layer on the surfaces of membrane vesicles. The resulting nano-vesicles effectively drained to secondary lymphoid organs and co-delivered antigens and adjuvants to antigen presenting cells, initiating T cell and IgG responses against model tumor antigens. Additionally, combination therapy with checkpoint blockade inhibitor, anti-PD-1 antibody, was utilized to enhance the efficacy of the vaccine.

Methods

Tissue culture-grown B16F10-OVA cells were harvested and prepared into cell lysate through freeze-thaw cycles followed by probe-tip sonication. After washing, the remaining fraction was incubated with calcium chloride to allow for fusion of membrane vesicles. The resulting vesicles were washed to remove unincorporated proteins and materials, followed by PEGylation and incorporation of CpG (a potent TLR9 agonist) by the post-synthesis insertion method. Stabilized nano-vesicles were administered subcutaneously at the tail-base utilizing a prime-boost schedule either in naïve or tumor-bearing C57BL/6 mice.

Results

PEGylated nano-vesicles maintained average hydrodynamic size of approximately 160 nm with good polydispersity index (<0.200) for over three weeks at 4°C, whereas cell lysate without PEGylation aggregated within a few days. After subcutaneous administration in C57BL/6 mice, PEGylated membrane vesicles accumulated 2.5-fold more effectively in the draining lymph nodes, compared with non-PEGylated lysate. Naïve C57BL/6 mice vaccinated with B16F10-OVA vesicles containing CpG generated modest CTL responses against OVA, while also eliciting IgG antibodies against whole cell lysate. Interestingly, in tumor-bearing mice, vaccination with membrane vesicles with CpG resulted in strong anti-OVA CTL immunity (reaching 5% of all CD8+ peripheral blood mononuclear cells), which was also enhanced by

combination therapy with anti-PD-1 antibody. Vaccinated animals exhibited a prolonged median survival time, compared to untreated animals.

Conclusions

Tumor cell membrane vesicles stabilized by surface PEGylation can be stored and administered as a potent vaccine vehicle. Effective co-delivery of antigens and adjuvants to the draining lymph nodes of tumor-bearing animals allowed for enhanced immune responses and prolonged survival in a therapeutic setting.

P136

Dendritic cell vaccination combined with chemotherapy for advanced or relapsed head and neck cancer patients induces immunological and clinical responses

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Background

The prognosis of patients with advanced squamous-cell carcinoma of head and neck is poor with a 5-year overall survival rate of 20-40%. In the current study, we have evaluated the clinical and immunological responses in patients with advanced or relapsed head and neck cancer who received dendritic cell (DC) vaccination in combination with platinum-based chemotherapy.

Methods

Ten patients (8 males, 2 females; aged 49-85 years) were enrolled in the present study. Autologous DCs were generated by culturing adherent mononuclear cells with interleukin-4 and granulocyte-macrophage colony stimulating factor. DCs were then loaded with synthetic peptides derived from Wilms' tumor 1 (WT1) and/or MUC1 mucin following maturation by prostaglandin E₂ and a toll like receptor 4 agonist, OK432. Peptide-loaded mature DCs and OK432 were administered intradermally every 2 weeks for 7 times and

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repeated biweekly until tumor progression. Induction of vaccine-induced immune responses was evaluated using an ELISpot assay, a HLA-tetramer assay and a flow cytometry analysis.

Results

The treatment was well tolerated and none of the patients experienced serious adverse events during the treatment period. Of 10 patients, 5 had stable disease (SD) and 5 had disease progression (PD) after one course of vaccination. Median overall survival was 14.5 months. Survival of patients achieving SD after DC vaccination (responder) was longer than those who did not respond to the treatment (non-responder) (median duration of survival; 17 vs 6 months). ELISpot assay showed the increase in spot-positive cells in both responders and non-responders after one course of vaccination. However, increment in positivity was marked in responders in comparison with non-responders; 24.3 and 3.5 fold in responders and non-responders, respectively. Similarly, HLA-tetramer assay showed the increase in positivity of WT1-specific CD8⁺ T cells in responders. Percentage of NK and NKT cells were almost same before and after vaccination. On the other hand, percentage of both CD14⁺HLA-DR⁻ and CD15⁺CD11b⁺CD33⁺ myeloid-derived suppressor cells decreased by 65.1% and 18.2%, respectively, indicating that DC vaccination may contribute to the reversal of immunosuppression by these cells.

Conclusions

DC vaccine-based immunotherapy combined with conventional chemotherapy was demonstrated to be safe and elicit acquired cellular immune responses correlated with clinical effects. These results suggest that add-on DC vaccination might be a promising novel strategy for the treatment of patients with advanced or relapsed head and neck cancer.

Trial Registration

University hospital Medical Information Network, Japan ID: UMIN000027279

P137

Immune mechanisms of the response to TG4010, a viral-based vaccine, in patients with advanced non-small cell lung carcinoma

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Background

TG4010 (MVA-MUC1-IL2) is a viral-based vaccine encoding mucin-1 and interleukin-2 which has been developed as an immunotherapy for epithelial cancers displaying abnormal MUC1. TG4010 has shown clinical benefit in patients with advanced NSCLC in combination with chemotherapy in phase II trials, and is currently assessed in combination with nivolumab at UC Davis (NCT 02823990). In this perspective, it has been essential to demonstrate the synergy between TG4010 vaccination and immune checkpoint inhibition; more specifically that improved overall survival (OS) correlates with the onset of a specific and diverse CD8⁺ repertoire, and to identify patient subpopulations most likely to benefit from TG4010 vaccination.

Methods

Samples were collected from patients of the TIME study. PBMCs were analyzed by using combinatorial MHC multimer staining to detect CD8⁺ T-cells reactive to MUC1 epitopes and other tumor-associated antigens (TAAs). Monitoring of immune background also included the phenotyping of peripheral blood cells and of circulating cytokines to identify predictive and prognostic biomarkers.

Results

We report that improved OS under TG4010 treatment correlated with development of specific CD8⁺ T-cell responses against MUC1 epitopes. Interestingly, MUC1 specific response also correlated with the onset of T cell clones against several lung TAAs (11 previously described epitopes for lung cancer were assessed + 4 predicted neo-

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epitopes). These results support the causality of specific CD8⁺ T cell responses in clinical response to TG4010, and constitute the first clinical report of epitope spreading after vaccination with a viral vector. We could also observe a benefit in subpopulations with unfavorable immune background; OS increased from 9.5 to 14.6 months (HR 0.56 [0.37-0.86]) upon vaccination with TG4010 in patients with high levels of Tregs at baseline, and from 10.3 to 15.2 (HR 0.59 [0.39-0.89]) in patients with low level of innate immunity at baseline (low NK cells).

Conclusions

Immune monitoring of TG4010-treated patients has confirmed the involvement of MUC1 specific CD8⁺ T-cell responses, and has demonstrated an original mechanism of epitope spreading. Clinical outcome correlated positively with the diversity of T-cell repertoire, suggesting the major driver function of viral-based vector.

P138

Tumor-specific CD8 T cell checkpoint expressions in melanoma patients that received adenovirally-engineered DC vaccine +/- IFN- α

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Background

Despite durable clinical responses, only 30-40% of melanoma patients respond to treatment with immune checkpoint blockade. It is critical to identify rational combination approaches that will enhance responses. Combinations of immune checkpoint blockade with cancer vaccines can promote and/or enhance anti-tumor T cell responses. We developed an adenovirus-based DC vaccine encoding three full length melanoma antigens, Tyrosinase, MART1 and

MAGE-A6 (AdVTMM2) designed to promote broad, polyclonal CD8⁺ and CD4⁺ T cell immunity, and tested it in a Phase I clinical trial to treat late-stage melanoma patients

Methods

PBMC from enrolled HLA-A2⁺ patients were banked at baseline, post-vaccination (day 43) and at day 101 after either observation or IFN- α administration. Dextramers were used to assess the frequencies of CD8⁺ T cells specific for peptides encoded by the vaccine (Tyros₃₆₉₋₃₇₇, MART-1₂₇₋₃₅, and MAGE-A3₂₇₁₋₂₇₉) before and after vaccination. IFN- γ -producing CD8 T cells specific for tumor antigens were measured by ELISPOT. The expression of CTLA-4, TIM3 and PD-1 by CD8⁺ T cells were analyzed by multicolor flow cytometry.

Results

There were 17 HLA-A2⁺ out of 35 enrolled patients that received all 3 AdVTMM2 DC vaccines. There was a 2.85-fold increase in MAGE-A3₂₇₁₋₂₇₉, and a 1.37-fold increase in MART-1₂₇₋₃₅-specific T cells post-vaccination compared to baseline, and the increased frequency of both tumor-specific CD8 T cells was still evident by day 101. The ELISPOT assay showed a 3.5-fold increase in MART-1 specific CD8 T cells after vaccination. Patients previously treated with α -CTLA-4 with/without α -PD-1 therapy had more tumor-specific IFN- γ -producing T cells before and after vaccination compared to patients that received no prior checkpoint blockade. These patients also had lower frequencies of CTLA-4⁺TIM3⁺ tumor-specific CD8⁺ T cells. While most patients examined expressed PD-1 in ~25% of tumor-specific CD8 T cells, 2 patients with no prior α -PD-1 treatment had lower levels of PD-1 on tumor-specific CD8⁺ T cells that correlated with good clinical outcome. IFN- α treatment did not enhance anti-tumor specific CD8 immunity after DC vaccination.

Conclusions

Results show that vaccination with AdVTMM2 DC increased the frequency of circulating tumor-specific CD8 T cells. Patients that received

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checkpoint blockade prior to DC vaccination had a higher number of anti-tumor specific CD8 T cells compared to patients that received DC vaccination alone, and IFN- α did not increase frequencies, suggesting that combining DC vaccination with checkpoint blockade is a rational approach to enhance anti-tumor immunity.

Trial Registration

NCT01622933: Multiple Antigen-Engineered DC Vaccine for Melanoma

References

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P139

A viral vectored vaccine based on shared tumor neoantigens for prevention and treatment of microsatellite instable (MSI) cancers

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Background

Tumor neoantigens are very appealing targets for cancer vaccines for the low risk of self-tolerance and autoimmunity. Most neoantigens are specific to an individual tumor and therefore can only be utilized

for personalized vaccination approaches. There is, however, a group of cancers whose neoantigens are shared among patients: microsatellite instable (MSI) tumors, caused by a defective DNA mismatch repair system. In these tumors, mutations accumulate mainly in microsatellite regions of mononucleotide repeats (MNR) as deletions of 1 nucleotide, and affect a limited number of genes. Here, we describe a method to select shared MSI-associated neoantigens for designing a universal vaccine for prevention and treatment of MSI tumors.

Methods

Exome and RNA sequencing data were analyzed for 69 colorectal, 85 gastric and 166 endometrial MSI cancers and matched normal samples in the TCGA database. We selected deletions of 1 nucleotide at MNR of coding genes that result in the formation of tumor-specific neoantigens, called frame shift peptides (FSPs). Three key parameters were taken into account by our pipeline to prioritize FSPs for an "off-the-shelf" MSI vaccine: (i) expression of the mutated gene; (ii) sharedness of the mutation among MSI patients; (iii) length of the FSP. An algorithm was then designed to choose the minimal subset of FSPs that ensured optimal coverage of MSI tumors.

Results

Nouscom proprietary pipeline selected 209 FSPs (for a total length of 6021aa) for inclusion in the MSI vaccine. These FSPs were found at expected frequencies in tumor Flash Frozen (FF) biopsies from 6 MSI patients. The 209 FSPs were assembled into 4 artificial genes and cloned into Great Apes adenoviral vectors (GAd) and Modified Vaccine Ankara (MVA). Potent immunogenicity of the vaccine in heterologous prime/boost regimen was demonstrated in mice.

Conclusions

In targeting a large set of shared neoantigens, the MSI vaccine has a high probability to reach the necessary breadth of immune responses to prevent tumor occurrence in Lynch syndrome carriers affected by a genetic defect of DNA mismatch repair

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system. Moreover, the vaccine can be used therapeutically in established MSI tumors to potentiate the activity of checkpoint inhibitors.

P140

NKTR-214 enhances anti-tumor T cell immune responses induced by checkpoint blockade or vaccination

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Background

High dose IL-2 has been used in treatment of metastatic melanoma and renal cell carcinoma. However, expansion of suppressive Tregs and physiologic toxicities associated with IL-2 treatment, limited its use in anti-cancer therapies. NKTR-214 is an engineered IL-2 cytokine, designed to provide a non-toxic, stable and more efficient alternative to IL-2. NKTR-214 provides sustained activation of the IL-2 pathway through controlled release of active CD122-biased (IL-2R $\beta\gamma$) cytokine. In the present study, we assessed the synergy of NKTR-214 with anti-cancer therapies in treatment of solid murine tumors. We investigated whether NKTR-214 can promote expansion and function of tumor specific CD8+ effector T cells, induced by T cell checkpoint blockade therapy or peptide-based vaccination. We also studied how NKTR-214 impacts the proliferation and apoptosis of effector CD8+ T cells vs. immunosuppressive Tregs.

Methods

To assess synergy between NKTR-214 and checkpoint blockade, CT26 colon carcinoma tumor bearing mice were treated with either NKTR-214 or checkpoint blockade or combination of both therapies together. Tumor-specific effector T cell response was also analyzed in blood. To understand

the effect of NKTR-214 on antigen-specific CD8+ T cells, we adoptively transferred naive gp100-specific TCR transgenic PMEL-1 CD8+ T cells into mice bearing established subcutaneous B16 tumors, followed by vaccination (gp100 peptide + anti-CD40 mAb + TLR-7 agonist) alone or in combination with NKTR-214 or IL-2. Mice then received NKTR-214 or IL-2 every 8 days. Tumor growth, survival and T cell response in blood was monitored. To identify effect of NKTR-214 on proliferation/apoptosis of effector CD8+ T cells and Tregs, expression of proliferation marker Ki67 and apoptosis marker Annexin V was also analyzed.

Results

We observed that NKTR-214 demonstrated efficient therapeutic synergy with checkpoint blockade. NKTR-214 also enhanced anti-tumor effect of vaccination and improved survival of mice by potently suppressing tumor growth as compared to vaccination with IL-2. As compared to IL-2, NKTR-214 significantly promoted proliferation of PMEL-1 CD8+ T cells while reduced the proliferation of Tregs in tumor as well as in spleen. Furthermore, Annexin V staining showed that vaccine combined with NKTR-214 supported the survival of PMEL-1 CD8+ T cells while promoting apoptosis of Tregs in tumor.

Conclusions

NKTR-214 synergizes with checkpoint blockade and with vaccination and improved the survival, proliferation and tumor infiltration of effector CD8+ T cells without promoting the intratumoral accumulation of Tregs. These preclinical results suggested that NKTR-214 can exclusively enhance intratumoral CD8+ effector T cell/Treg ratios to establish anti-tumor immunity.

P141

A multi-center study of hTERT immunotherapy in adults with solid tumors at high risk of relapse post-standard therapy

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Background

Human telomerase (hTERT) consists of a catalytic reverse transcriptase subunit and an internal RNA template that recognize and elongate telomeric DNA ends. hTERT is expressed in 85-90% human cancer, and can be recognized by cytotoxic CD8⁺ T cells. The administration of optimized full length DNA sequences followed by in vivo electroporation (EP) has generated potent CD4⁺ and CD8⁺ T cell responses against a variety of antigens in preclinical and clinical studies. In this phase 1 dose-escalation study, synthetic optimized DNA plasmids that target hTERT (INO-1400, or mutant hTERT; and INO-1401, or SynCon[®] TERT) are delivered followed by EP with the CELLECTRA device, to assess the safety, tolerability, and immune effects of immunotherapy with INO-1400 or INO-1401, alone or co-administered with a plasmid encoding for hIL-12 (INO-9012), in adult patients with cancer.

Methods

Following neoadjuvant or adjuvant chemoradiotherapy and/or surgery, and screening and evaluation, patients with one of 9 solid tumors at high risk of relapse but without evidence of residual disease are enrolled to one of 10 arms and receive four doses, four weeks apart, of either INO-1400 or INO-1401 alone or in combination with INO-9012 by intramuscular (IM) injection, followed by EP. Patients are followed for tolerability, immunogenicity and clinical response.

Results

As of July 26, 2017, 70 patients have received either INO-1400 or INO-1401±INO-9012. Doses of 2 or 8 mg of INO-1400/01, and of 0.5 or 2 mg INO-9012 have been well-tolerated, with the majority of reported adverse events (AE) being low-grade and related to IM+EP administration. Two related SAEs have been reported: one patient with breast cancer post-breast revision with cellulitis at the prior surgical site; and a second patient with pancreatic cancer with abdominal pain and elevated lipase. One dose-limiting toxicity, rash, has been reported, with no other dose-limiting events to date. Preliminary immunogenicity by ELISpot for antigen-specific interferon-gamma (IFN-γ) secreting cells suggests that patients can generate an hTERT-specific CD8⁺ T cell population. The majority of patients continue on study in long-term follow up, with several patients on study for >1 year. To date there is no evidence of a dose effect with respect to AEs or immunogenicity, but data assessment is ongoing.

Conclusions

INO-1400/01±INO-9012 given IM with EP is well-tolerated in adults with solid tumors. Following administration of INO-1400/01, patients can generate hTERT-specific IFN-γ secreting cells, suggestive of an ability to break tolerance. Dosing in this study is continuing, and follow-up is ongoing.

Trial Registration

NCT02960594

P142

***In situ* immunocytokine vaccination, radiation and checkpoint blockade therapy prevents engraftment of brain metastases in a murine melanoma model**

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Background

BACKGROUND: Brain metastases develop in >60% of advanced melanoma patients, and are now treated with surgery and/or radiation. Recent clinical checkpoint blockade studies show promising results for melanoma brain metastases. We previously showed that combining radiation and anti-CTLA-4 checkpoint blockade with *in situ* vaccination via intratumoral (IT) injection of hu14.18-IL2 immunocytokine tumor-specific anti-GD2 antibody fused to IL2 improved survival and reduced tumor burden in a syngeneic metastatic lung cancer model. Here we test whether this *in situ* vaccination strategy of combining local radiation, checkpoint blockade and *in situ* immunocytokine strategy prevents engraftment of melanoma brain metastases.

Methods

METHODS: GD2⁻ B16 or GD2⁺ B78 (derived from B16 cell line) melanoma xenografts were implanted into flank or stereotactically implanted into brain of C57BL/6 mice. Melanoma flank xenograft were irradiated (12Gy), injected with IT-IC, and/or intraperitoneal anti-CTLA-4 given to tumor-bearing mice. Overall survival, immune memory response, and histopathology performed for analysis.

Results

RESULTS: In mice bearing a single B78 flank tumor, 73% were rendered disease-free by *in situ* vaccine (12 Gy + IT-IC) + systemic anti-CTLA-4 treatment. Testing of these pre-treated mice confirmed immune memory response after greater than 90 days, with all mice rejecting B78 cellular engraftment in contralateral flank, and 60% rejecting sequential implanted B78 cellular engraftment in brain over 20 days later. Increased CD8⁺ and CD4⁺ T cells were observed at implanted

brain sites compared to control contralateral striatum. In contrast, all naïve control mice implanted with B78 developed flank or brain melanomas with sparse immune infiltrates. Compared to naïve mice, pre-treated mice showed improved survival after implanting B78 cells into brains (median not reached vs 28 days, $p=0.001$). In mice with 5 weeks growth of a B78 flank melanoma xenograft, and a 1 day implanted B16 brain melanoma xenograft, combining *in situ* IT-IC vaccine + systemic anti-CTLA-4 improved survival compared to anti-CTLA-4 alone (Mean 33 days vs 20 days, $p=0.017$). Immunohistopathological assessment of brains from the above treatment cohorts showed a trend toward increased CD4⁺, CD8⁺, and CD16⁺ cells in brain metastases in the *in situ* vaccinated mice.

Conclusions

CONCLUSIONS: In this preclinical model, *in situ* vaccination of melanoma flank xenografts with hu14.18-IL2 combined with radiation and anti-CTLA-4 checkpoint blockade enhances the anti-tumor immune response and prevents engraftment of brain melanoma metastases.

Cellular Metabolism and Antitumor Immunity

P143

T cell metabolic insufficiency explains the dysfunctional immune response in renal cell carcinoma

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Background

Renal cell carcinoma (RCC) is considered an immunogenic tumor with a prominent dysfunctional immune cell infiltrate, unable to control tumor growth. Although tyrosine kinase inhibitors have improved the outlook for some patients, many patients incur disease progression, symptoms and reduced life expectancy. Following the limited success of cytokine therapy, nivolumab checkpoint-inhibitor therapy for second line treatment has reiterated the potential for immune manipulation to significantly impact on this disease. However, the response to nivolumab is limited to 25% of patients with a median duration of only 12 months. There is an urgent need to improve the potential efficacy of these new targeted immunotherapies. The immune environment created by the tumor recapitulates that present in chronic viral infections in which inappropriate and excessive antigen stimulation leads to intra-tumoral T cell exhaustion. These exhausted T cells exhibit defective proliferative capacities and cytokine production and display an overall phenotype of metabolic insufficiency, characterized by extensive mitochondrial alterations. We set out to comprehensively identify mitochondrial defects in exhausted RCC TILs and correlate these findings with the clinicopathological characteristics of the tumor.

Methods

Tumor cell suspensions were prepared from freshly resected RCC tumors using a combination of mechanical (GentleMACS Dissociator) and enzymatic dissociation. Exhausted T cells were analysed by multicolour FACS on paired PBMC and tumor-cell suspensions using fluorescently conjugated antibodies. The functional status of PBMC and tumor infiltrating lymphocytes (TILs) from RCC patients was evaluated for cytokine production and glucose uptake in response to CD3 cross-linking. The metabolic fitness of sorted stimulated CD3 T cells from dissociated tumors was assessed using a Seahorse XF extracellular flux analyzer and a range of mitochondria-targeted dyes.

Results

Flow cytometric analysis of TILs from late stage RCC specimens expressed a high level of PD-1 which correlated with a depolarized mitochondrial phenotype and abnormal mitochondrial function (defect in oxygen consumption rate with loss of spare respiratory capacity). These exhausted CD8+ TILs displayed a depressed glucose competency and had the lowest cytokine production. This dysfunctional phenotype was specific to TILs from advanced stage cases and not observed in the corresponding blood of RCC patients.

Conclusions

The clinical relevance of mitochondrial alterations within the exhausted RCC TILs will be determined using mitochondria-targeted antioxidant compounds to try to achieve significant functional restoration of the T cells. The ultimate aim is to combine modifying the bioenergetics of the T cells with remodeling of the dysregulated metabolism of the RCC tumor microenvironment to create a more permissive environment for T cell anti-tumor activity.

P144

Investigating the kynurenine pathway as a central effector mechanism of tumor immune-suppression and its potential as therapeutic target

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Background

Although immunotherapies have had notable impact on cancer treatment, multiple mechanisms of immune resistance prevent optimal response. The catabolism of Tryptophan into metabolites

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known as Kynurenines (Kyn) by enzymes like IDO or TDO has been described as a major immunosuppressive player in different tumor types, however, the mechanisms are still poorly understood. Recently it has been shown that Kyn is an agonist of the Aryl hydrocarbon Receptor (AhR), promoting tolerogenic DCs and skewing CD4⁺T cells towards a regulatory T cell phenotype. Here we sought to characterize the mechanisms of immunosuppression associated with activation of the AhR pathway by Kyn in pre-clinical models of melanoma and to evaluate AhR's potential as therapy target.

Methods

B16 wild-type (B16-WT) and IDO-overexpressing B16 (B16-IDO) melanoma cells were implanted orthotopically in c57bl6 mice. Gene-expression was performed using Fluidigm qRT-PCR system. Macrophages were depleted using clodronate-liposomes (CL) twice a week starting on day 3 after tumor implantation (T.I). Tumor-infiltrating leukocytes were analyzed by Flow Cytometry on days 10, 16 and 23 after T.I. Tumor-bearing mice are under treatment with a specific AhR antagonist (CH-223191) daily at 50 mg/kg starting at day 7 after T.I.

Results

We found that B16-IDO tumors grow at a faster rate than B16-WT *in vivo* and present reduced expression levels of many Type 1 inflammatory genes, including IFN γ , IL1B, TNF α , Granzyme B and CD40. In addition, B16-IDO tumors present higher infiltration of macrophages (CD11b⁺F4/80⁺Ly6G⁻), which peaks 16 days after T.I. and persists as tumors progress. B16-IDO TAMs have up-regulation of classic AhR-regulated genes (Cyp1a1 and Cyp1b1) and are differentially skewed towards an immunosuppressive M2 phenotype, characterized by higher expression of CD206, STAB1, Fizz1 and lower expression of several M1 markers (IL1, IL6, IL12, NOS2). Also, B16-IDO TAMs have the ability to suppress activation of CD8⁺T cells *in vitro* and depletion of TAMs using CL abrogated B16-IDO tumor growth phenotype. Importantly, inoculation of B16-IDOs in Rag^{-/-} and FoxP3-depleted mice

abrogated the accumulation of TAMs. Studies using a specific AhR antagonist (CH-223191) are in progress to evaluate tumor growth and survival in this model.

Conclusions

Here we identified that the activation of the AhR pathway by Kyn in IDO-overexpressing melanomas promotes tumor growth in a macrophage-dependent manner. Importantly, we found that AhR-activated intratumor FoxP3⁺T cells are associated with the suppressive myeloid phenotype in IDO⁺ tumors. All together, our findings demonstrate that targeting the Kyn pathway through AhR-inhibition represents a promising approach in cancer patients who are resistant to immune-checkpoint blockade.

P145

CD38-NAD⁺axis regulates immunotherapeutic anti-tumor T cell response

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Background

Adoptive T cells therapy (ACT) has shown great prominence in immunotherapy of cancer. However, ACT is only able to cure a small proportion of the patients because of the rapid functional exhausting and short-term persistence in tumor bearing host. Therefore, identifying factors governing the maintenance of functional phenotype and survival of anti-tumor T cells is of utmost interest.

Methods

Quantitative PCR (q-PCR), flow cytometry, and metabolomic analysis were used to evaluate the expression of various metabolism and stemness-associated genes as well as protein expression in the T cells. To compare the metabolic commitment in T cells real time metabolic flux analyzer (Seahorse Biosciences, USA) and radioactive tracer studies

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were used. Melanoma epitope gp100 or tyrosinase reactive T cells from either PMEL or TRP-1 mice respectively were used for adoptive T cell transfer (1×10^6 /mouse) in B6 mice bearing 10 days subcutaneously established B16 melanoma.

Results

Recently, we found that Th1/17 cells which can be differentiated by combining the culture conditions of Th1 and Th17 cells, exhibited durable and superior anti-tumor activity *in vivo* as compared to Th1 and Th17 alone. Since metabolic fitness affects the persistence and functionality of the adoptively transferred T cells, we comprehensively characterized the metabolomic profile of Th1, Th17 and Th1/17 cells and identified that increased level of intracellular NAD⁺ is a key in regulating the anti-tumor potential of the Th1/17 cells. NAD mediated this effect mainly through providing substrate for the SIRT1 deacetylation activity since pharmacological blockade or genetic ablation of SIRT1 in Th1/17 cells completely abolished its anti-tumor activity. We further provided data in support of this observation by using CD38 deficient T cells which has very high level of NAD due to absence of NAD lyase CD38. We found that CD38 deficient T cells without any *in vitro* differentiation could mount potent anti-tumor response in mice bearing melanoma.

Conclusions

These data together suggest that strategies to maintain high level of intracellular NAD in anti-tumor T cells by blocking CD38 will have a potential therapeutic application in improving ACT.

P146

Co-expression of CD39 and CD103 identifies tumor-reactive CD8 TIL in human solid tumors

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Background

Identifying tumor antigen specific T cells from cancer patients has been a goal of tumor immunologists for several decades.

Methods

none

Results

Here we identified a subset of tumor-infiltrating CD8 T cells (CD8 TIL) characterized by co-expression of CD103 and CD39 in human solid tumors. This cell population was only found in TIL from primary and metastatic tumors, exhibited features of chronic stimulation and displayed characteristics of tissue-resident memory T cells. Double positive CD8 TIL had a distinct TCR repertoire compared to other CD8 TIL subsets, were highly enriched for tumor antigen recognition and efficiently killed autologous tumor cells. Finally, patients with head and neck cancer whose CD8 TIL contained a higher frequency of CD39⁺CD103⁺ cells experienced a greater overall survival.

Conclusions

This work describes a simple method for detecting tumor-reactive TIL, which should help define mechanisms of current immunotherapies and may lead to the development of future immunotherapies.

P147

Reduced tumor-associated glycosphingolipids in cell membrane lipid rafts and a diminished proliferation rate in mammary breast carcinoma cultures after incubation with glyphosate-based formulations

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Background

The glycosphingolipid-containing cell membrane lipid rafts are important structural and functional cellular components and as so have a primary role in cell integrity and signal transduction. Our question was how glyphosate-based herbicides (intensively used worldwide, leading to environmental and food contamination), their active ingredient glyphosate (revealed as a chemical chelator) and formulants influence the growing potential of mammary carcinomas and other sensitive cell types. Our specific aims were to define the effects of glyphosate and its formulating surfactant on various, intensively growing cell types at cellular and molecular levels.

Methods

Glyphosate-based herbicide formulation Roundup Classic and its polyethoxylated tallowamine detergent POEA were tested in 2% 50X diluted and 0.32% 300X diluted forms, respectively, while glyphosate (as isopropylamine salt) (62%) was 46.3X diluted; and 5-step series of dilutions of each stock solution were added into cultures of MCF7, MDA-MD-231 and other sensitive and control (HEK293) cells. Cell proliferation in the cultures was measured with the MTT assay, and microcultures were scanned regularly by inverted light microscopy. Harvested control, and treated cell suspensions were analyzed by immunofluorescence microscopy and high quality RNA was prepared for DNA microarray gene expression analysis.

Results

Reduced proliferation was experienced in the MDA-MD-231 and MCF7 cell lines. The effect was

different in ER-positive and -negative cells, and a lower tumor-associated glycosphingolipid expression could be detected by indirect immunofluorescence assay and fluorescence microscopy. The expression change of GD3 gangliosides, highly tumor-associated antigens was especially remarkable. First-line gene expression analysis (Agilent GeneSpring GX) and the Ingenuity Pathway Analysis Qiagen revealed different gene expression patterns upon exposure to either Roundup or POEA, or to glyphosate. Differences were also shown in gene expression regulation in the two breast cancer cell lines, indicating the involvement of GD3 synthase gene machinery.

Conclusions

Our data show a new aspect of the glyphosate and Roundup treatments of various cells, emphasizing the cell membrane integrity damaging potential of these xenobiotics *via* glycosphingolipid-containing lipid rafts. The characteristic transcriptional changes found by gene expression analysis support the diminished GD3 disialylated glycosphingolipid expression found by immunofluorescence microscopy. The study provides important information on the proliferation inhibition and apoptosis promoting effects of adjuvants in glyphosate formulations, attracts attention on epidemiological questions, and provides data to be harnessed in developing novel anti-cancer strategies.

P148

KHK2455, a long-acting selective IDO-1 inhibitor, in combination with mogamulizumab, an anti-CCR4 monoclonal antibody, in patients with advanced solid tumors: preliminary safety report and pharmacodynamic activity from a first-in-human study

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Background

KHK2455 is an oral, long-acting, once-daily, selective IDO-1 inhibitor. Mogamulizumab is a monoclonal antibody with enhanced antibody dependent cellular cytotoxicity activity against CCR4⁺ T cells expressed by Th2 regulatory lymphocytes. Both KHK2455 and mogamulizumab have demonstrated immunologic and antitumor activities during *in vitro* and *in vivo* preclinical studies. Clinically, IDO-1 inhibitors have demonstrated synergy in combination with immunotherapy in several tumor types.

Methods

In this first-in-human study, patients with advanced solid tumors will receive escalating doses of oral KHK2455 (0.3, 1, 3, 10, 30, and 100 mg once daily) as monotherapy for 4 weeks (Cycle 0) followed by combination with intravenous mogamulizumab 1 mg weekly for 4 weeks (Cycle 1) and then biweekly (Cycle 2 and beyond) using a standard 3+3 design. Cohort expansion will occur at the maximum tolerated dose or the highest tested dose. The primary objective is to determine the safety, tolerability, and dose-limiting toxicities (DLTs) of KHK2455 in combination with mogamulizumab. Secondary objectives include pharmacokinetic, pharmacodynamic, biomarker and immunologic monitoring, and determination of best overall responses.

Results

By May 2017, twelve patients were enrolled in cohorts that received KHK2455 at 0.3 and 1 mg dose level, including patients with head and neck cancer

(n=4), ovarian cancer (n=3), osteosarcoma, pancreatic, gastric, gallbladder, and adrenal cancers (each n=1). Eight patients continue treatment. There were no DLTs. The most frequent adverse events included maculopapular rash, thrush, dysphagia, thrombotic event, and tachycardia, none of which were considered related to KHK2455 although one case of rash (grade 3) was considered related to mogamulizumab.

The effective half-life for KHK2455 exceeded 1.5 and 2.5 days at 0.3 and 1 mg once daily, respectively. Potent dose-dependent inhibition of kynurenine production was demonstrated in plasma samples and *ex vivo* stimulation assays. Mean change from baseline of kynurenine/tryptophan ratio showed a dose-dependent decrease of 25% at the 1 mg dose level and no PD effect in serum samples from the 0.3 mg subjects.

Conclusions

Preliminary data from two cohorts (0.3 and 1 mg once daily) suggest that KHK2455 in combination with mogamulizumab has a good safety profile, demonstrated a prolonged effective half-life up to 2.5 days, and an ability to suppress kynurenine production in a dose-dependent manner. These data support the continuation of KHK2455 dose escalation.

Trial Registration

NCT02867007

P149

In vitro and in vivo characterization of KHK2455, a highly potent and selective indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor with a novel mechanism of action

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Background

IDO1 catalyzes the degradation of tryptophan to kynurenine (kyn) metabolites that can inhibit T cell function and may be one mechanism by which tumors avoid anti-tumor activity. In the clinic, combination of IDO1 inhibitors (IDO1i) with checkpoint inhibitors has been shown to improve response rates in several cancer indications [1][2]. Our IDO1i program identified KHK2455, a highly potent and specific IDO1i with a novel mechanism of action that is currently in a phase I clinical trial.

Methods

Here we describe the pre-clinical characteristics of KHK2455 IDO1 inhibition. In vitro dose-dependent potency and selectivity were analyzed in IDO/TDO expressing 293T cells. In vivo pre-clinical pharmacokinetic (PK) properties, pharmacodynamic (PD) activity and toxicology were examined in mice and in cynomolgus monkeys. Anti-tumor activity of KHK2455 in combination with anti-CTLA-4 antibody (9D9) was demonstrated in B16-F10 derived tumor bearing mice.

Results

KHK2455 had potent inhibition of kyn production in IDO1-expressing cells ($IC_{50} = 14$ nM) but not in IDO2 or TDO2-expressing cells ($IC_{50} >10,000$ or 1,000 nM). Unlike other IDO1i molecules, KHK2455 primarily bound to apo form of IDO1 enzyme and inhibited the enzyme activity in vitro, indicating a unique and possibly more potent mechanism of action.

Mice and cynomolgus monkeys administered KHK2455 demonstrated plasma KHK2455 concentration was increased with increasing dose from 0.1 mg/kg to 10 mg/kg (mouse) and from 0.03 mg/kg to 3 mg/kg (monkey). Corresponding Kyn/Trp ratios reached a minimum 24 hours after a single oral administration of KHK2455 and the ratio was reduced with dose increment, supporting once daily dosing for clinical study.

KHK2455 demonstrated synergistic tumor growth inhibition with anti-CTLA-4 antibody (9D9) in B16-F10 derived tumor bearing mouse models.

Conclusions

KHK2455 is an IDO1 inhibitor with a novel MOA that potently and selectively inhibits kyn production in preclinical models, and demonstrates synergistic inhibition of tumor growth in a mouse tumor model with anti-CTLA-4 antibody. Together, these data support the clinical evaluation of KHK2455 in a phase I study.

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P150

Immunomodulatory effects of the senescence-associated secretory phenotype on NK cells in renal cell carcinoma

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Background

Metastatic renal cell carcinoma (mRCC) is highly resistant to chemo- and radiotherapy and the 5-year survival rate for mRCC remains low. RCC has long been considered as an immunogenic tumor and infiltration of NK cells rather than T cells is beneficial for patient survival. Thus, augmenting the activity of NK cells in patients with mRCC could lead to prolonged survival. Senescence is a cellular state

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of cell cycle arrest, but greater metabolic activity. NK cells play an important role in the clearance of senescent tumor cells. Furthermore, senescent tumor cells secrete a variety of factors, i.e. senescence-associated secretory phenotype (SASP). However, it is unknown if the SASP will alter the activity of NK cells. In this study, we sought to investigate if drug-induced senescence in RCC influence NK cell activity and to further identify rate-limiting factors within the SASP that cause alterations in NK cell activity. With this knowledge, strategies to better augment the activity of NK cells can be developed for patients with mRCC.

Methods

Senescence was induced by treatment with the mdm2-inhibitor Nutlin-3a and detected by senescence-associated β -gal staining and by ELISA for IL-6 and IL-8. NK cell phenotype, proliferation, IFN-gamma production, and lysis were analyzed by flow cytometry, thymidine incorporation, ELISA, and ^{51}Cr -release assay respectively.

Results

RCC cell lines expressing wt p53 are sensitive to mdm2 inhibition and become senescent indicated by higher levels of SA- β -gal and increased levels of IL-6 and IL-8 in the supernatants treated compared with untreated RCC cells. Furthermore, changes in the surface expression of MIC A/B and MHC class I was observed in Nutlin-3a compared with untreated cells. Unlike previous studies, no change in the sensitivity between senescent RCC cells to NK cell killing was observed. However, upon exposure of supernatants of senescent RCC, NK cells showed an increased proliferation. In contrast, no changes in NK cell cytotoxicity or degranulation were observed upon co-culture with supernatants of senescent RCC and untreated RCC. Our current focus is to screen the secretome of senescent RCC using to identify components in the SASP that enhance NK cell proliferation.

Conclusions

Senescent RCC produce factors that enhance the proliferation of NK cells. Secretome analysis to

identify what factors within the SASP that are responsible to augment the proliferation of NK cells are currently ongoing. The identification of such factors will lead to increased knowledge to sustain NK cell proliferation and better tailored NK cell-based immunotherapies for patients with RCC.

P151

Profiling the immunogenic cell death (ICD) mechanisms induced by Nano-Pulse Stimulation (NPS) treatment in mouse B16-F10 melanoma tumors using NanoString technology

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Background

Nano-Pulse Stimulation (NPS) is a non-thermal tumor therapy that delivers ultrashort electrical pulses to tumor cells. NPS opens nanopores in the membrane of the ER, allowing the efflux of Ca^{2+} thus causing ER stress, ROS production and ICD [1]. To date, the primary mechanism of action of most known ICD inducers is ER stress and ROS production leading to intrinsic mitochondrial apoptosis, and the release and translocation of DAMPs [2]. Here we sought to profile the pathways involved in ER stress, apoptotic cell death and the immune response after NPS treatment, using the NanoString PanCancer Immune Panel with an additional 30 spike-in genes designed to investigate cell death pathways.

Methods

C57/B6 albino mice were inoculated intradermally with 1-million B16-F10 melanoma cells into the left flank. When tumors reached $\sim 5\text{mm}$ in diameter they were treated with NPS (500 pulses, 200 ns in duration applied at 25 kV/cm at 5 pps). Tumors

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were harvested 2-hrs, 4-hrs and 24-hrs after treatment and placed into formalin for fixation followed by embedding in paraffin. mRNA was extracted and hybridized to bar-coded probes that correspond to 800 gene transcripts (770 PanCancer Immune Panel; 30 spike-in). Transcripts were read using the NanoString nCounter[®] and analyzed with nSolver software.

Results

NanoString analysis showed that genes involved in ER-stress were upregulated 24-hrs after NPS treatment, including several in key pro-apoptotic signaling pathways. Studies have demonstrated that calcium released from the ER is taken up by the mitochondria, causing release of cytochrome C and initiating intrinsic mitochondrial apoptosis [2]. Genes in these pathways were upregulated by 24-hrs, as were those coding for DAMPs, PRRs and the cascade of inflammatory mediators that initiate the maturation of DCs, promoting T cell recognition and priming of the adaptive arm of the immune response.

Conclusions

NanoString profiling revealed that transcripts for specific ER stress-related factors, intrinsic apoptotic pathways and immune mediators, previously identified as important components of ICD, were upregulated in tumor tissues 24-hrs after NPS treatment. We plan to continue to utilize the NanoString platform in future studies to help us to further understand the cell death and immune mechanisms involved in NPS-treatment.

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P152

41BB costimulation enables PD-1 blockade therapy by inducing T cell mitochondrial function and biogenesis

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Background

Tumors create a suppressive microenvironment that prevents antitumor immunity through a number of mechanisms like recruitment of regulatory T cells or ligation of co-inhibitory molecules. Targeting these molecules has become a major success in cancer treatment; however, some patients fail to respond to these therapies. It has become clear in recent years that the lack of nutrients in the tumor microenvironment may also play an immunosuppressive role and cause resistance to immunotherapy. We have previously shown that T cells infiltrating the tumor have repressed mitochondrial activity and biogenesis that leads to loss of metabolic sufficiency, a state that cannot be rescued by PD-1 blockade therapy alone. The co-stimulatory molecule 41BB is present on activated T cells can increase T cell survival and is a major clinical therapeutic target. We hypothesized that 41BB signaling might provide metabolic support to tumor-infiltrating T cells.

Methods

Non-tumor bearing mice or mice injected with B16 were treated with 200 µg anti-PD1 (clone J43, Bio X-Cell) or 50 µg anti-41BB (clone 3H3, Bio X-Cell), or respective hamster or rat isotype controls, intraperitoneally every other day for 3 days (in non-tumor bearing experiments) or every other day for the duration of the experiment (in tumor experiments). CD8 T cells were isolated from lymph nodes and tumors and tested for cytokine

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production by flow cytometry, metabolic function with Seahorse XFe96 Bioanalyzer, and signaling by western blot.

Results

We show that 41BB costimulation of previously activated, murine T cells results in enhanced mitochondrial activity through PGC1a-mediated mitochondrial biogenesis. In naïve T cells, 41BB can substitute for CD28-mediated costimulation, leading to activated T cells with high respiratory capacity and increased mitochondrial activity. Mice treated with agonistic anti-41BB *in vivo* produced T cells with higher respiratory capacity and oxidative function. In melanoma-bearing mice, 41BB stimulation alone does not induce robust anti-tumor immunity, but leads to increased mitochondrial mass in tumor-infiltrating T cells. Pairing 41BB agonism with PD-1 blockade therapy results in robust anti-tumor immunity and intratumoral T cell function.

Conclusions

Our results suggest 41BB functions to bolster T cell metabolism, highlighting the importance of combining 41BB stimulation with other immunotherapies to support T cells such that they can overcome the metabolic barriers in the tumor microenvironment.

P153

Nanoparticles to target tumor-draining lymph nodes conjugated with an antagonist for vasoactive intestinal peptide to promote an anti-tumor immune response

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Background

Vasoactive intestinal peptide (VIP) is a neuropeptide that regulates co-inhibitory pathways in the immune system. In previous studies, mouse models of bone marrow transplant show the potent immunosuppressive activity of VIP¹. Further, VIPhyb, an antagonist of VIP, increases the number of effector/memory CD8⁺ T cells and mature natural killer cells. Additionally, in a mouse leukemia model, VIPhyb downregulates the expression of PD-1 and PD-L1 in activated T cells and dendritic cells¹. In this study, we hypothesize that in solid tumor immunotherapy, the immunomodulatory effect of VIPhyb can be further augmented by conjugation with nanoparticles (NP) specifically designed to target tumor draining lymph nodes (TDLN)². Since TDLNs are a major tissue involved in draining tumor associated antigens, targeted delivery of VIPhyb to TDLNs could improve the anti-tumor immune response and hamper overall growth of solid tumors susceptible to immunotherapy.

Methods

Splenocytes were extracted from WT and VIP-KO mice treated with B16-F0 and B16-F10 melanoma, and analyzed with flow cytometry for Granzyme B (GrZ B) and CD8 expression. VIPhyb was conjugated to a biocompatible, non-toxic 30 nm NP through emulsion polymerization and tested for purity and stability. A mixed lymphocyte reaction (MLR) using luciferase-expressing splenic T cells from B6 Luciferase mice were co-cultured with irradiated splenic T cells from FVB mice. VIP, VIPhyb, and VIPhyb-NP at concentrations of 0 ug/mL to 975 ug/mL were added to the MLR on 3 consecutive days and their effect on T cell proliferation assessed with bioluminescent imaging.

Results

The addition of VIPhyb and VIPhyb-NP counteracted the inhibitory effects of VIP by increasing T cell proliferation *in vitro*. VIPhyb had a peak efficacy at a concentration of 300 ug/mL (Figure 1) while the effect of VIPhyb-NP was dose-dependent (Figure 2). Melanoma growth in VIP-knockout (KO) mice results

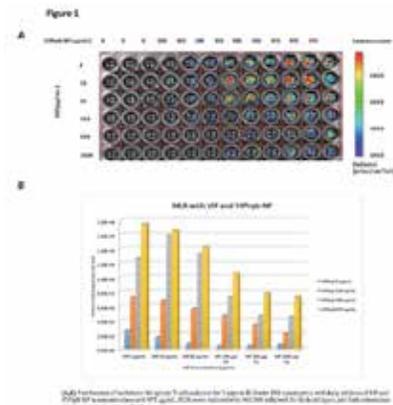
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in increased amounts of GrZ B and cytotoxic CD8⁺ splenocytes (Figure 3).

Conclusions

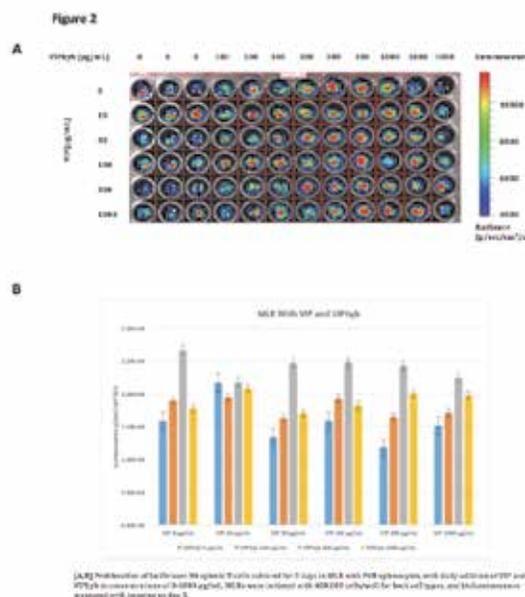
Blocking VIP-signaling with VIPhyb, especially when coupled with a TDLN targeting NP, thus represents a potential novel immunotherapeutic approach to bolster an adaptive T cell response and improve pharmacokinetics for advanced solid tumors in the clinic.

Figure 1: MLR with VIP and VIPhyb-NP



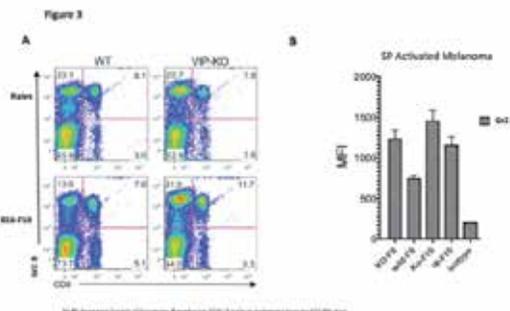
(A,B) Proliferation of luciferase+ B6 splenic T cells cultured for 3 days in MLR with FVB splenocytes, with daily addition of VIP and VIPhyb-NP in concentrations of 0-975 µg/mL. MLRs were initiated with 400,000 cells/well for both cell types, and bioluminescence measured with imaging on day 3.

Figure 2: MLR with VIP and Free VIPhyb



(A,B) Proliferation of luciferase+ B6 splenic T cells cultured for 3 days in MLR with FVB splenocytes, with daily addition of VIP and VIPhyb in concentrations of 0-1000 µg/mL. MLRs were initiated with 400,000 cells/well for both cell types, and bioluminescence measured with imaging on day 3.

Figure 3: Flow Cytometry Analysis of VIP-KO Mice



(A,B) Increased levels of Granzyme B producing CD8⁺ T cells in melanoma bearing VIP-KO mice

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P154

Tumor-derived PGD2 and NKp30-B7H6 engagement drives an immunosuppressive ILC2-MDSC axis in acute promyelocytic leukemia

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Background

Innate Lymphoid Cells (ILCs) were recently recognized as a distinct family of cells able to play a crucial role in immune processes. They are subdivided into 3 subsets (ILC1, ILC2 and ILC3) that functionally resemble CD4 T helper cells. ILCs are dysregulated in acute myeloid leukemia and are associated with a reduced susceptibility to graft-versus-host disease. However, their role in tumor immunosurveillance is still far to be well established. We aim to identify their role in acute promyelocytic leukemia (APL).

Methods

We characterized by multiparametric flow cytometry the ILC compartment present in both the peripheral blood and the bone marrow of 22 APL patients at diagnosis and in remission after all-trans retinoic acid (ATRA) therapy. In parallel, we monitored the presence of myeloid derived suppressor cells (MDSC) and of the different T helper cell subsets. In addition, the serum levels of various cytokines and soluble mediators involved in regulating ILC functions were determined by ELISA or Luminex. We also used 3 APL mouse models, namely FVB/NJ, C57BL/6 and humanized NSG mice injected with APL lines, to address the role of ILC during leukemogenesis and to target *in vivo* the key players of the newly identified immunosuppressive axis.

Results

We find that, in comparison to the other subtypes of acute myeloid leukemia, APL is specifically characterized by increased ILC2. ILC2 are hyper-activated to produce high amount of IL-13 via the CRTH2-PGD2 and NKp30-B7H6 bindings. Through IL-13, ILC2 recruit and promote functional mMDSC both *in vitro* and *in vivo*. In line with these findings, APL patients show increased circulating MDSC and

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elevated concentrations of PGD2 and IL-13. Of note, APL patients that achieve remission show normal frequencies of ILC2 and MDSC as well as normal concentrations of PGD2 and IL-13. The APL mouse models confirm that the enhancement of ILC2 is a consequence of the disease, and directly drives MDSC expansion. By treating the mice with blocking antibodies against NKp30, PGD2 and IL-13, ILC2 and MDSC levels are partially restored, APL growth is delayed and the survival of the mice enhanced.

Conclusions

Our data suggest that APL cells are able to recruit and activate ILC2 through PGD2 secretion and B7H6 expression. ILC2 are in turn able to recruit and activate MDSC, favouring a pro-leukemic environment. The ILC2-MDSC tandem might represent a novel target in tumors with enhanced and hyper-activated ILC2.

P155

Targeting CSK kinase activity to enhance anti-tumor immunity

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Background

To mount an effective antitumor immune response, a T cell receptor (TCR) must first recognize a cognate antigen presented by malignant cells. Successful effector T cell activation initiates an intracellular signaling cascade that results in the release of cytotoxins and pro-inflammatory cytokines that ultimately result in the elimination of diseased antigen-presenting cells. A key regulator of this signaling cascade is the C-terminal Src (CSK) kinase. In lymphocytes, CSK inhibits T cell activation by phosphorylating the SRC kinase family member LCK at tyrosine 505 and thereby terminates the signaling cascade. It is possible that relieving CSK-

mediated regulation of TCR signaling could lead to a more robust T cell response, improving antitumor immunity.

Methods

Both genetic and pharmacologic approaches to inhibit CSK activity were developed. A tamoxifen-inducible transgenic CSK knock-out (KO) mouse was engineered to bypass the embryonic lethality observed with constitutive systemic CSK depletion. Small molecule inhibitors of CSK were identified in a high-throughput screen and assessed in vitro for inhibitory activity or in vivo for antitumor efficacy alone or in combination with anti-PD-1.

Results

As expected, loss of CSK enhanced cytokine production and increased the antitumor immune response in the MC38 syngeneic tumor mouse model. Potent and selective CSK small molecule inhibitors, in the presence of TCR stimulation, similarly activated the proximal TCR signaling pathway and increased cytokine production and proliferation. In an in vivo efficacy study, however, an optimized CSK small molecule inhibitor dosed as monotherapy or in combination with anti-PD-1 did not improve the antitumor immune response at the dose levels tested.

Conclusions

These seemingly discrepant findings in tumor response observed in the tamoxifen-inducible CSK KO mice compared to mice treated with the CSK inhibitor may reflect the need for a more optimized dosing regimen and improved target coverage. In this poster, we will present our findings on the role of CSK inhibition in tumor immunity.

P156

Immunometabolic requirements for T cell exhaustion

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Background

CD8+ tumor-infiltrating T lymphocytes (TIL) in the tumor microenvironment (TME) are unable to effectively kill targets due to tumor cell and regulatory immune cell-mediated suppression. Poor metabolite availability has also been found to negatively impact TIL, as T cells have high energy demands that are not met in the TME due to competition with surrounding tumor cells. Consequently, tumor growth is allowed to progress as TIL becomes exhausted and dysfunctional. Recently, we found that exhausted TIL also exhibit a profound loss of functional mitochondria, which is due in part to the repression of TIL mitochondrial biogenesis. Enforcing mitochondrial biogenesis in T cells not only lead to increased mitochondrial function, but improved TIL functionality, decreased tumor burden, and increased survival in mouse melanoma. Additional studies suggest that simply causing progressive functional mitochondrial loss is enough to induce T cell dysfunction. Thus, we hypothesize that TIL dysfunction is driven by metabolic insufficiency, and altering the T cells' ability to compete for nutrients will increase TIL functionality.

Methods

T cell metabolic output was measured by Seahorse extracellular flux analysis. The TME was modeled *in vitro* by co-culturing mouse melanoma tumor cells or T cell activation beads with transgenic T cells either in normoxia or hypoxia (1.5% O₂).

Results

To determine the specific drivers of T cell dysfunction, we developed a model system to identify how metabolic insufficiency and chronic activation can induce T cell dysfunction *in vitro*. Chronically activated T cells and those cultured in hypoxic conditions were still able to carry out effector function, but chronic activation and hypoxia together induced profound T cell dysfunction, characterized by a PD-1^{hi}, Tim-3⁺ phenotype and drastically reduced cytokine

production. To specifically understand the contribution of mitochondria to the avoidance of exhaustion, we generated a model in which T cells progressively lose functional mitochondria *in vitro*. We found that T cells lacking functional mitochondria could not utilize oxidative phosphorylation, causing decreased cytokine and cytotoxic functionality and increased co-inhibitory marker expression, all hallmarks of TIL. Preliminary data suggests that this dysfunction is not due to energetic insufficiency, but rather to alterations in the epigenetic signature of activated T cells.

Conclusions

Our data support a model in which chronic activation and metabolic insufficiency show synergistic effects in the promotion of long-term T cell dysfunction. Reversing metabolic insufficiency may be an attractive strategy to improve the efficacy of cancer immunotherapy.

P157  Abstract Travel Award Recipient

Mitochondrial Reactive Oxygen Species are a Biomarker of Metabolic Fitness of T Cells in Tumors

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Background

Tumor microenvironments drive T cell metabolic dysfunction resultant in inhibition of anti-tumor capability. Biomarkers that indicate T cell metabolic distress may prove useful to therapeutically target metabolic dysfunction. We hypothesized that accrual of mitochondrial reactive oxygen species (mtROS) may indicate poor metabolic fitness of T cells.

Methods

We used FACS analysis to measure accumulation of mtROS in mouse and human T cells. Further, we

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FACS sorted mtROS⁻ and mtROS⁺ T cell subsets to measure spare respiratory capacity, mitochondrial permeability transition pore (MPTP) opening, and dependence on glucose consumption between subsets. *In vitro* and *in vivo* mouse and human T cells were treated with N-acetylcysteine (NAC) as a method to reduce mtROS accumulation and T cell anti-tumor immunity was measured.

Results

We found that accumulation of mtROS in mouse and human T cells was consistent with T cell aging and cell death. T cells that amassed mtROS had reduced spare respiratory capacity, increased mitochondrial permeability transition pore (MPTP) opening, and shifted toward a dependence on glucose consumption for biofuel. *In vitro* anti-oxidant treatment with NAC was able to reduce mtROS accumulation, restore SRC, and inhibit T cell apoptosis. In both mouse models and human tumors we found that T cells accumulate mtROS and that PD-1⁺ T cell subsets had a particularly robust expression of mtROS. In multiple tumor models treatment with NAC *in vivo* diminished mtROS accumulation, reduced T cell death, and remodeled PD1⁺ T cell populations.

Conclusions

A combination of NAC and anti-PD-1 antibody may greatly augment the effect of either therapy alone. Together our data suggest that mtROS is a biomarker of poor metabolic fitness of T cells in tumors and that anti-oxidant therapies may promote tumor control when combined with checkpoint blockade therapies in the clinical setting.

P158

PBMC-humanized preclinical tumor models serve as a valuable platform to evaluate *in vivo* activities of novel immune-oncology drug candidates

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Background

Treating cancer by taking advantage of a patient's own immune system has demonstrated considerable success in oncology research. The lack of experimental immunotherapy models is a major obstacle towards better evaluating new immunotherapeutics and testing combination strategies. More and more relevant and sophisticated preclinical tumor models are required in the I/O drug discovery industry.

Methods

We have established and validated a series of new preclinical *in vivo* models, PBMC-humanized tumor models, named MiXeno. Human xenograft tumors were developed and the human immune system was partially established by infusing human PBMCs in NOG/NSG (or similar background) immunocompromised mice.

Results

MiXeno models were established and the inoculation conditions were optimized or sub-optimized. The xenograft tumor establishment and human immune component reconstitution can be achieved either simultaneously or sequentially. These MiXeno models were characterized with regards to reconstitution of T cells, tumor response to anti-PD-1 and anti-CTLA-4 antibodies, and onset of possible graft versus host disease (GVHD) or graft versus tumor response (GVT).

Conclusions

A series of MiXeno tumor models have been established and validated where human immune cells were reconstituted in the mouse system for evaluation of human-origin bispecific antibodies, immune checkpoint inhibitors or immune-modulators. Further studies are needed to expand

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model collections and to extend their applications in I/O space.

P159

Clinical features and outcomes with talimogene laherparepvec in melanoma patients with comorbidities: a single-center experience

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Background

Patients with unresectable in-transit or limited cutaneous metastatic melanoma (MM) may benefit from intralesional injections with talimogene laherparepvec (TVEC), a modified oncolytic herpesvirus [1]. However, predictors of response and outcomes in patients with adverse prognostic features (e.g. poor performance status (PS), prior therapy and co-morbidities are not well studied [2].

Methods

We performed a single-center retrospective analysis of patients with MM treated with TVEC. Age, comorbidities, PS, and prior therapies, as well as treatment details, outcomes, and adverse events were obtained. Durable response rate (DRR) was defined as > 6 month of progression free survival (PFS).

Results

We identified 27 patients treated with TVEC. Most patients were elderly (median age 74, 8>80), 26% had poor PS (ECOG >1), and 70% with >2 co-morbidities. Most patients had stage IIIC disease (52%) and received prior therapies (56%), including 48% with immune checkpoint inhibitors (ICI). Overall, there was a DRR of 44%; median PFS (mPFS) was 5.9 months (m) and median overall survival (mOS) was not reached (median follow-up of 10.4m). Smaller disease burden (largest lesion

diameter of <2cm) was associated with substantially improved DRR (63% vs. 11%), mPFS (not reached vs. 2.1m) and mOS (not reached vs. 10.8m) compared with bulkier tumors. Poor PS was associated with significantly worse mPFS (1.6m vs not reached, p=.007) and mOS (4m vs not reached, p<.0001) compared to patients with ECOG 0-1, although occasional durable responses were noted (14% DRR). There was no association with clinical outcomes for prior ICI (DRR 42% vs. 46%) or age >75 years (DRR 43% vs. 46%).

Conclusions

Overall, TVEC produced responses in patients regardless of age or prior ICI therapy. Poorer survival was associated with poor PS and higher initial disease burden. These findings will need to be validated in larger prospective studies.

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Cellular Therapy Approaches

P160  Abstract Travel Award Recipient

Chimeric antigen receptors interact with multiple endogenous T cell proteins and induce tonic signaling through phosphorylation of endogenous CD3ζ

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Background

Immunotherapies based on chimeric antigen receptor (CAR)-T cells are expected to become the first line of treatment for multiple malignancies [1]. However, their mechanism of action is not fully understood. In order to gain insight into the molecular events that govern CAR-T cell activity, we conducted a systematic analysis of the CAR “protein interactome” and “signalosome” in human primary CAR-T cells.

Methods

Interactome analysis was based on immunoprecipitation of CARs followed by protein identification using mass spectrometry (LC-MS/MS). Technical validation of the results was performed using Western blot and flow cytometry. A variation of the SILAC [2] method was used for signalosome analyses, allowing for *in-vitro* stimulation of human primary CAR-T cells with pancreatic cancer cells. Metabolic labeling was used for *post-hoc* analysis of the phosphoproteome in the CAR-T cell compartment.

Results

We found that second-generation CARs interacted with, and induced spontaneous phosphorylation of, the endogenous CD3 ζ . This phenomenon was not exclusive of any specific scFv, or any co-stimulatory, hinge, or transmembrane modules. However, the intensity and site of phosphorylation were affected by the choice of transmembrane and co-stimulatory domains, respectively. CAR signalosome analysis revealed activation of multiple signaling networks including TCR signaling, Actin Cytoskeleton Signaling, Glycolysis, among others, in concordance with the changes in gene expression patterns observed by microarray analyses. By comparing second- versus third-generation CARs sharing the

same antigen-binding domain (anti-PSCA), we observed that the ability of a CAR to engage the endogenous CD3 ζ was associated with stronger phosphorylation of downstream secondary messengers, and superior antitumor efficacy *in vivo*.

Conclusions

We report a detailed analysis of the CAR interactome and signalosome, and we describe the establishment of tonic signaling mediated by CAR-induced phosphorylation of endogenous CD3 molecules. In addition, we present new immunoproteomic tools for the study of the relationship between CAR structure and T cell function. We plan to integrate the knowledge gained in these studies to optimize combination therapies, and to design the next generation of CAR-T cells, integrating customized sensor/effector circuits for superior therapeutic efficacy [3].

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P161

Engineering adoptive T cell therapy for efficacy in ovarian cancer

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Background

Over 20,000 women are diagnosed with ovarian cancer annually, and >50% will die within 5 years. This rate has changed little in the last 20 years, highlighting the need for innovative therapies. A promising new strategy has the potential to control tumor growth without toxicity to healthy tissues, by employing immune T cells engineered to target proteins uniquely overexpressed in tumors. Recent technological advances have helped identify and validate Wilms' Tumor Antigen 1 (WT1) and mesothelin (MSLN) as valid immunotherapy targets in ovarian cancer, as these proteins contribute to malignant and invasive phenotypes and have limited expression in healthy cells.

Methods

T cells were engineered to express high-affinity T cell receptors (TCRs), using lenti- or retro-viral transduction. The peritoneally disseminated ID8_{VEGF} mouse ovarian cancer model in immunocompetent mice was used for preclinical studies. Transcriptome profiling (ThermoFisher), flow cytometry, intracellular cytokine stimulation assays, mass spectrometry, the Seahorse platform, and immunohistochemical analyses were performed to characterize the human and murine tumor microenvironment (TME), and to measure intra-tumor T cell phenotypes and function.

Results

Using either patient-derived cell lines or mouse ID8_{VEGF} ovarian tumor cells, we found that T cells engineered to express either a human or mouse WT1- or MSLN- specific high-affinity TCR can kill human and murine ovarian tumor cells respectively *in vitro*. Moreover, in the ID8_{VEGF} *in vivo* murine model, adoptively transferred TCR-engineered T cells preferentially accumulated within established tumors, diminished ovarian tumor growth and prolonged mouse survival. However, our data also revealed that the TME can limit engineered T cell persistence and killing capacity. Immunosuppressive cells, inhibitory ligands that reduce T cell function, and cell death-inducing ligands are abundant within ID8_{VEGF} tumors. Cellular and molecular analyses of

human ovarian cancer specimens showed similar TME-mediated obstacles exist for human T cell therapy. The ovarian cancer TME is a nutrient- and oxygen-deprived milieu, which has protean effects on T cell function. Ongoing studies exploring strategies to disable specific TME elements that facilitate immune evasion by ovarian cancer and are common to human and murine tumors will be discussed. Both direct modulation of cellular and molecular components of the TME and T cell engineering are being pursued to overcome critical obstacles and thereby promote T cell survival and function.

Conclusions

Strategies that modulate inhibitory pathways have the potential to enhance T cell function and improve anti-tumor efficacy by reducing limitations posed by the TME. Our studies suggest such efforts will enhance therapy of ovarian cancer.

P162

Preclinical evaluation of BCMA-specific TAC receptor-engineered T cells for multiple myeloma

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Background

Multiple myeloma is an incurable hematologic malignancy, with poor prognosis and limited therapeutic options for patients who relapse post-transplant. Recent clinical trials with chimeric antigen receptor (CAR) T cells redirected against B-cell maturation antigen (BCMA) confirmed that myeloma is susceptible to treatment with engineered T cells [1,2]. We constructed a novel chimeric receptor, called the T cell antigen coupler (TAC), which was designed to co-opt signalling through the native T cell receptor to provide engineered T cells with a natural way of auto-

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regulating their activity. Herein, we discuss our results with several BCMA-TAC receptors.

Methods

We generated a series of BCMA-TAC receptors using previously-described and novel BCMA-specific single chain antibody fragments (scFvs) in the antigen-binding domain. Functionality of primary human T cells engineered with BCMA-TACs was evaluated *in vitro* and *in vivo*. Anti-tumor efficacy was measured using a xenograft myeloma model based on a luciferase-expressing KMS-11 cell line.

Results

Upon activation with BCMA⁺ myeloma cells *in vitro*, BCMA-TAC T cells proliferated, secreted cytokines, and killed target cells in an antigen-specific manner. *In vivo*, treatment of mice bearing disseminated myeloma with 4 x 10⁶ BCMA-TAC⁺ T cells consistently resulted in rapid disease regression, with many mice clearing their myeloma completely, and showing no signs of relapse 6 months after T cell infusion. These results were reproducible across multiple experiments, with T cells derived from different donors performing equally well in male and female myeloma-bearing mice. It was interesting to note that relapses were typically extramedullary, occurring at sites distinct from the primary site of disease, indicating that relapse was not a consequence of failure of the engineered T cells to clear the primary tumor site.

Conclusions

Our findings support further evaluation of BCMA-TAC T cells in human trials. Understanding the mechanisms of extramedullary relapse should provide strategies to enhance therapeutic efficacy.

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P163

Enhanced functional profile of CAR-T cells generated in the presence of mTOR kinase inhibitor

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Background

Manufacture of CAR-T cells requires activation and expansion of T cells for clinical dosing. This process results in a portion of the T cell drug product that may, in some cases, be driven to a state of terminal “exhaustion”, in which multipotency and T cell replicative potential are diminished. Drivers of exhaustion can include signaling through PI3 kinase (PI3K)-mTOR, which drives rapid growth and active effector response. Inhibition of PI3K-mTOR was shown to promote memory T cell differentiation and improved efficacy in adoptive immunotherapy models [1, 2, 3]. We explored whether inhibition of mTOR kinase (TORK) during CAR-T cell production would promote improved expansion, survival and function in response to cognate antigen.

Methods

We used a CD19-directed CAR-T cell which contains a 41BB-CD3z endodomain. CAR-T cells were generated by lentiviral transduction and subsequent expansion *in vitro* prior to cryopreservation. During expansion, cells were cultured with or without inhibitors (TORKi and pan-PI3K/mTOR inhibitor). Cryopreserved samples were thawed and assessed for function (target killing, cytokine secretion, and intracellular cytokine expression) and expansion or survival after stimulation through the CAR. Stimulation of CAR-T cells was by co-culture with CD19-expressing cells or anti-idiotypic antibody binding.

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Results

When cultured under sustained antigen conditions, inhibitor-modified CAR-T cells displayed increased expansion with enhanced survival upon antigen withdrawal. Inhibitor-modified CAR-T cells killed antigen-bearing target cells at levels similar to controls, but secreted higher cytokine levels. The enhanced cytokine functionality of inhibitor-modified CAR-T cells was retained throughout sustained antigen exposure.

Conclusions

The observed enhanced expansion and survival of inhibitor-modified CAR-T cells may lead to a potential for improved in vivo persistence. Additionally, the ability of inhibitor-modified CAR-T cells to retain improved cytokine capacity through sustained antigen exposure indicates that these cells may be resistant to functional exhaustion. Taken together, these data suggest that improved clinical durability of response could be achieved through use of TORK-inhibitors during CAR-T cell manufacturing.

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P164

A temporal and spatial analytical system to measure CAR-T cell function

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Background

The cell functional assays that currently exist to test the in vitro functional performance of CAR-T cells are indirect read outs. The field could benefit from a continuous live-cell imaging and analysis of the spatial and temporal kinetics of the effector-target interactions in simulated effects of the tumor environment. Ideally, these assays, could be implemented in high throughput to evaluate and isolate perturbations that illuminate the basic biological principles and allow for testing of specific hypotheses.

Methods

We generated and tested primary human CAR T cells as effector cells in co-culture assays with antigen-matched target cell lines. Cells were co-cultured for 48h on 96 well plates, and evaluated using the Essen BioScience InCuCyte Live-Cell Imaging System, an automated microscope that resides inside the culture incubator (Figure 1). We analyzed spatial and kinetic imaging in both phase contrast and fluorescence. Scans were scheduled and images collected and developed to define and test a Processing Definition analysis system. We established the parameters to be measured in all images and defined the metrics of object counts, average area, and mean intensity to be evaluated over time.

Results

The dynamic antigen-specific lysis of target cells could be visualized and quantified during the entire analysis period. The kinetics of the morphologic, proliferative, and surface area occupancy of both T cells and tumor cells were quantified. Our initial significant findings include: rapid clustering of CAR-T cells surrounding cancer target cells, and migration and proliferation of CAR-T cells stimulated by the presence of antigen. In addition, we simulated an inhibitory tumor microenvironment by the addition of human T regulatory cells to the culture, and

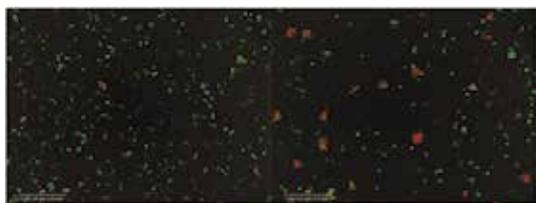
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noted changes in the spatial and temporal function of primary human CAR-T cells. Each one of these aspects was quantified.

Conclusions

This processing definition analysis system allowed us to measure and quantify biological parameters that allow us to understand the dynamic nature of CAR T cells over time in simulated tumor microenvironments. This system has potential to be tested as a potency assay, which is currently an unmet need in the field of cell-based immunotherapy.

Figure 1. U87-GFP cell line and anti-EGFR-CAR T cells vs unspecific CAR-T



Glioblastoma cells (U87, modified to express Green Fluorescent Protein), incubated with CAR T cells (also expressing red mCherry). Left, photomicrograph immediately after mixing; right, photomicrograph of the same area 24 hours later.

P165

High throughput label-free impedance-based technology for kinetic *in vitro* functional potency assessment of immune cell-mediated cytotoxicity and immune checkpoint modulation

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Background

In vitro characterization of reagents efficacy for cancer immunotherapy protocols is necessary

before moving to expensive animal models and clinical studies. However, current standard assays like Chromium-51 release, ATP-based luminescence cell monitoring or flow cytometry are difficult to implement in a high throughput environment and are based on end point methodologies that are unable to capture the full dynamic of the immune response. Here we present an impedance-based platform for monitoring cytotoxic activity of immune cells in the context of cancer immunotherapy assays.

Methods

The technology detects cell death and proliferation of adherent cells by measuring changes in conductance of microelectrodes embedded in 96 and 384-wells cell culture plates, without the use of labeling or cell modification. Because immune cells are unable to bind the microelectrodes, the technology monitors directly adherent target cells proliferation/survival without signal interference by effector cells. Furthermore, the approach can be adapted to suspension target cells, like B cell leukemia, through adhesion mediated by coating antibodies.

Results

We provide a large set of validation including most of the tolls that are currently utilized in the immunotherapy space: CD19 Car T models, EpCAM/CD3 BiTE antibodies and immune checkpoint inhibitor combination therapies. Based on our observation, PD-1 specific antibody can dose-dependently modulate the potency of human-derived PBMC against PC3 prostate cancer cell lines. Using PD-1 checkpoint antibody in combination with antibodies directed against other checkpoint proteins including Tim-3, Lag-3 and in combination with PDL-1 antibody, we can demonstrate either additive effects or no net increase in potency. Data comparison with Annexin V staining/Flow Cytometry shows perfect correlation between the drop in impedance signal and % of apoptotic cells, as well as correlation between immune checkpoint protein expression in PBMC populations and immune blockade.

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Conclusions

In summary, we have developed a quantitative and reproducible functional assay that can be used to characterize immune cytotoxic response across the temporal scale, an aspect that is otherwise very difficult to assess with more canonical end point assays. Thanks to the availability of 384-wells format and minimal sample handling, the technology is also ideally suited for applications in large drug screening campaigns or therapeutic protocol validation directly on patient samples.

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H3.3K27M mutation-derived novel neoantigen – characterization of the HLA-A2-binding epitope and a specific T cell receptor for development of T cell-based immunotherapy

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Background

The median overall survival for children with diffuse intrinsic pontine glioma (DIPG) is less than a year. The majority of diffuse midline gliomas (DMG), including over 70% of DIPG, harbor an amino-acid

substitution from lysine (K) to methionine (M) at the position 27 of histone 3 variant H3.3 (the H3.3K27M mutation hereafter) [1]. H3.3K27M mutation is associated with shorter survival compared to patients with non-mutated H3.3.

Methods

In vitro stimulation of human CD8⁺ T cells with a 10-mer peptide encompassing the K27M mutation (H3.3K27M peptide, hereafter) led to generation of specific CTL clones and cDNA for T cell receptor (TCR) α - and β -chains was isolated. Mass spectrometry analysis was conducted to determine whether the H3.3K27M-derived epitope is presented on human leukocyte antigen (HLA)-class I of HLA-A2⁺ H3.3K27M⁺ glioma cells. T cells transduced with the TCR were evaluated for cytotoxicity against HLA-A2⁺ H3.3K27M⁺ glioma cells *in vitro* and in mice bearing glioma xenografts.

Results

As predicted by the NetMHC3.4 algorithm, a competitive binding inhibition assay demonstrated that the H3.3K27M peptide, but not the corresponding non-mutant peptide, has an excellent binding affinity to HLA-A2. Importantly, mass spectrometric analysis of H3.3K27M⁺ glioma cells demonstrated that the H3.3K27M peptide is naturally processed and presented by HLA class I. H3.3K27M-specific CD8⁺ T cell responses were detected in patients' peripheral blood mononuclear cells by IFN- γ ELISPOT assay. Repeated stimulation of HLA-A2⁺ CD8⁺ T cells with the H3.3K27M peptide led to establishment of H3.3K27M-reactive CD8⁺ T cell clones. From one such clone, cDNA for TCR α - and β -chains were cloned into a retroviral vector. Human HLA-A2⁺ T cells transduced with the TCR efficiently killed HLA-A2⁺ H3.3K27M⁺ glioma cells in an antigen- and HLA-specific manner. Adoptive transfer of TCR-transduced T cells significantly suppressed the progression of orthotopic xenografts in mice. Furthermore, alanine-scanning assays demonstrated that there are no known human proteins that share the set of key amino acid residues required for recognition by the TCR, strongly suggesting that the TCR could be safely

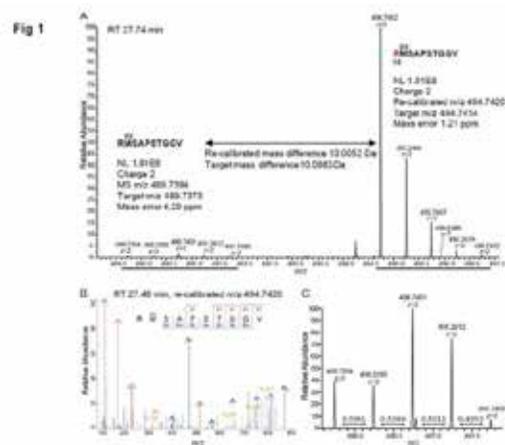
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used in patients without causing off-target toxicity (Figure 1, 2, 3, 4, 5).

Conclusions

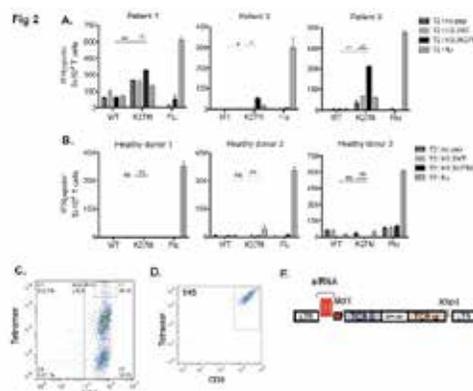
These data provide us with a strong basis for developing peptide-based vaccines as well as adoptive transfer therapy using autologous T cells transduced with the TCR.

Figure 1. The H3.3K27M peptide is detectable by LC-MS/MS in the HLA-class I immunopeptidome of glioma cells bearing the H3.3K27M mutation



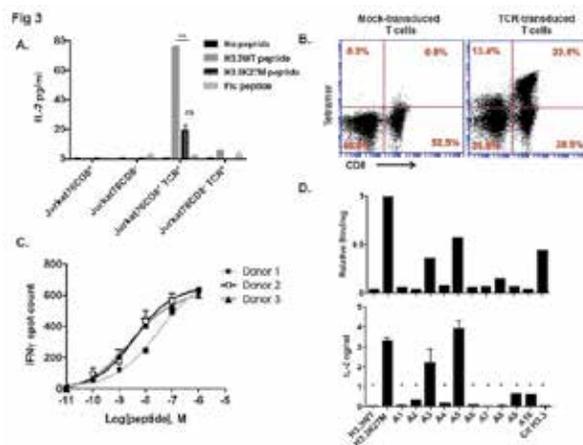
HLA-class I peptides were biochemically purified from U87H3.3K27M glioma cells and analyzed by LC-MS/MS with a synthetic heavy version of the H3.3K27M peptide as the reference. A. U87H3.3K27M HLA-class I immunopeptidome shows two co-eluting isotope patterns corresponding to the target m/z and mass difference of the oxidized forms of the heavy and the endogenous H3.3K27M peptides. B. Fragmentation spectrum of the heavy peak, showing identification of the oxidized heavy H3.3K27M peptide. C. Zoom-in of the light isotope pattern shows m/z values and distances between peaks as expected from the endogenous H3.3K27M peptide.

Figure 2. H3.3K27M peptide induces specific CTL clones from HLA-A2+ donor PBMC



A, B. Patient-derived PBMCs from HLA-A2+H3.3K27M+ tumor patient and healthy donor PBMCs were stimulated with H3.3WT, H3.3K27M peptide, flu peptide, or without peptide (indicated in the X-axis), and co-cultured with T2 cells pulsed with H3.3WT, H3.3K27M, flu peptide, or without peptide. Figure represents numbers of IFN- γ spots generated in each stimulation condition. Counts shown are normalized to no peptide stimulation control. n=3 in each group. p

Figure 3. Characterization of the functional avidity of TCR in recognition of HLA-A2-peptide complex and alanine scanning for safety of TCR use.

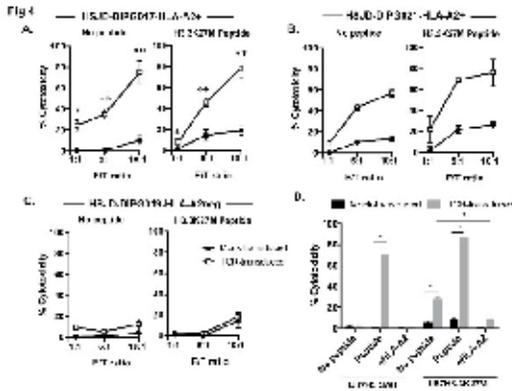


A. T2 cells loaded with or without H3.3K27M peptide, H3.3WT peptide or irrelevant HLA-A2

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binding flu peptide (10 µg/ml) were co-cultured with either control or TCR-transduced Jurkat76CD8+ cells and Jurkat76CD8- cells in 1:1 ratio and assessed for IL-2 production by ELISA. Data represent three independent experiments with similar results. p

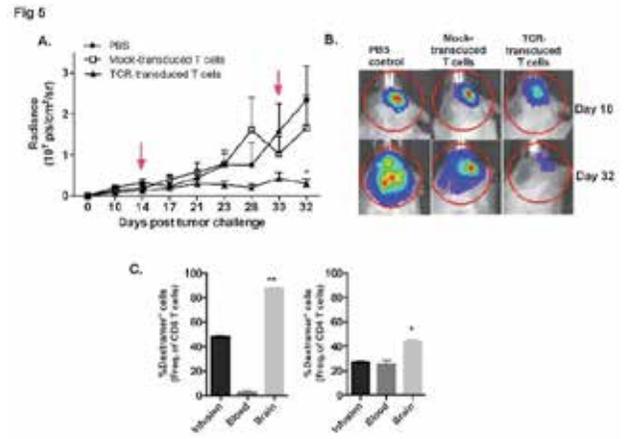
Figure 4. TCR-transduced T cells lyse H3.3K27M+HLA-A2+ glioma cells in an HLA-A0201- and H3.3K27M-dependent manner.



Cytotoxicity of TCR-transduced T cells was evaluated by lactate dehydrogenase (LDH) cytotoxicity assay. A,B, C. TCR-transduced or mock-transduced T cells were co-cultured with H3.3K27M+HLA-A0201+ HSJD-DIPG-017 cells, HSJD-DIPG-021 cells or control H3.3K27M+HLA-A0201- HSJD-DIPG-019 cells at E/T ratio of 1, 5, and 10 for 24 hrs. D. TCR-transduced or control T cells were co-cultured with HLA-A0201+ U87H3.3K27M cells or U87H3.3WT cells at E/T ratio of 5. Each group was assessed in triplicate. Data represent two independent experiments with similar results.

Figure 5. Adoptive transfer of TCR-transduced T cells but not mock-transduced T cells results in

inhibition of intracranial H3.3K27M+ glioma in NSG mice.



NSG mice bearing intracranial U87H3.3K27M luciferase+ gliomas received intravenous infusion with PBS, mock-transduced T cells or TCR-transduced T cells. A. Tumor growth is presented as radiance (10⁷ p/s/cm²/r) using BLI (n=8 per group). Arrows indicate days on which mice received treatment. B. Representative BLI images of mice on Day10 and on Day 32 post tumor inoculation. The background BLI signals were defined based on the levels seen in non-tumor bearing mice. C. Preferential accumulation of TCR+ T cells in the tumor site. At the time of intravenous infusion, approximately 50% and 30% of the infused CD8+ and CD4+ T cells, respectively, were TCR-Dextramer+. On Day 2 following second intravenous infusion, the percentage of Dextramer+ cells among CD8+ T cells and CD4+ T cells were evaluated in the peripheral blood and the brain of mice that received TCR-transduced T cells. Data indicate % Dextramer+ cells among total live CD8+ or CD4+ T cells (n=5 per group).

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Translation of highly functional CMV-induced “adaptive” NK cells to treat cancer

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Background

Cytomegalovirus (CMV)-induced “adaptive” natural killer (NK) cells express more adaptive marker NKG2C and maturation marker CD57, are epigenetically reprogrammed, and have unique functional properties including increased cytokine production after target exposure, increased antibody-dependent cellular cytotoxicity (ADCC), enhanced persistence, and inherent resistance to myeloid derived suppressor cells and regulatory T cells. We have shown that higher frequencies of adaptive NK cells in patients who reactivate CMV after reduced intensity hematopoietic cell transplantation have lower rates of relapse. Thus, in partnership with Fate Therapeutics, we have developed FATE-NK100, a first-in-class allogeneic NK cell therapy product enriched for cells with “adaptive” phenotype and function.

Methods

CD3 and CD19 depleted peripheral blood NK cells from CMV seropositive donors are cultured with IL-15 and a small molecule inhibitor of glycogen synthase kinase (GSK) 3.

Results

Inhibition of GSK3 during ex vivo expansion led to marked increases in the expression of T-BET ($p = 0.0005$), ZEB2 ($p = 0.0001$) and BLIMP-1 (0.0002), which are transcription factors that drive late-stage NK cell maturation. Further, the cultured cells produce significantly more TNF and IFN- γ and

mediate better ADCC as compared to conventional NK cells. Relative to control cultures with IL-15 alone, FATE-NK100 cells expressed significantly more KIR ($p = 0.014$), LFA-1 ($p = 0.011$) and 2B4 ($p = 0.009$) and markedly lower levels of NKG2A ($p = 0.002$). A Phase I clinical trial testing FATE-NK100 given after lymphodepleting chemotherapy (Fludarabine 25 mg/m² x 5 days and Cyclophosphamide 60 mg/kg x 2 days) followed by subcutaneous IL-2 (6 doses of 6 million units every other day) to treat refractory AML has opened to patient accrual. The first patient treated at the lowest dose cohort (1×10^7 TNC/kg) showed enhanced persistence of FATE-NK100 at Day +14 (45% donor, with an absolute FATE-NK100 cell count of 27 cells/uL of blood). A second Phase 1 clinical trial treating patients with recurrent ovarian cancer via intraperitoneal administration of FATE-NK100 will open in Q3 of 2017.

Conclusions

FATE-NK100 is a first-in-class, adaptive NK cell therapy product for adoptive cancer immunotherapy. The data demonstrate FATE-NK100 has highly-differentiated properties and superior antitumor activity. Clinical trials of FATE-NK100 in refractory AML, ovarian cancer and advanced solid tumors in combination with monoclonal antibody therapy are being conducted.

Trial Registration

NCT03081780 and NCT03213964

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CBLB gene editing potentiates the effector function of tumor-targeted engineered-TCR human T cells

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Background

Providing optimal outcomes with engineered T cell therapies in solid tumors will likely require strategies to overcome suppressive and immune-evasive mechanisms often present in solid tumor environments. The E3 ligase, CBLB, functions as a negative regulator of T cell activation and acts as an intracellular immune checkpoint regulator. In mice, *Cblb* deletion has been reported to induce autoimmunity and enhanced rejection of spontaneous and implanted tumors [1] due to loss of CD28 dependent T cell activation. We investigated the functional consequences of CRISPR/Cas9 mediated *CBLB* deletion in human engineered T cells in response to antigen in the presence or absence of co-stimulation.

Methods

Human CD4+ and CD8+ T cells were isolated from healthy donor apheresis, activated and, in some cases, transduced to express an engineered TCR (eTCR). Non-transduced or eTCR transduced T cells were transfected with *CBLB* targeted guide RNA (gRNA) in complex with Cas9 and expanded in the presence of added cytokines prior to analysis. *CBLB* deletion was confirmed by sequencing and western blot analysis. Responses to TCR stimulation with or without co-stimulation were analyzed following culture of *CBLB* edited non-transduced or eTCR transduced T cells, with anti-CD3 (with or without anti-CD28) or HLA-matched T2 cells pulsed with a peptide recognized by the eTCR (with or without CTLA-4-Ig), respectively. Cytokine production, target cell killing and proliferation were assessed at 24-72 hours post initiation of culture.

Results

CBLB editing in T cells resulted in a >90% reduction in CBLB expression as compared to transfection controls. Edited T cells displayed enhanced production of IFN γ , IL-2 and TNF α in response to sub-optimal TCR stimulation (in the absence of anti-CD28 or in the presence CTLA-4-Ig mediated inhibition of co-stimulation). Edited T cells also

demonstrated greater proliferative capacity and survival rates in response to antigenic stimulation in the absence of supplemental cytokines. Furthermore, *CBLB*-edited eTCR transduced T cells were more efficient killers of antigen presenting target tumor cells with an EC50 approximately 10-fold lower than that observed for unedited controls.

Conclusions

Manipulation of co-stimulatory molecules by tumor cells can, in some contexts, limit the effectiveness of adoptive T cell therapies. Our data show that *CBLB* editing of eTCR transduced T cells can enhance antigen sensitivity, cytokine production, proliferation and cytotoxicity in the absence of CD28 co-stimulation. These data suggest that *CBLB* editing may boost T cell function in a suppressive tumor environment.

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Mass-spectrometry-guided target selection and TCR profiling: towards the development of safer TCR-engineered T cell therapies

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Background

A major constraint for the broad and safe applicability of Adoptive Cellular Therapy (ACT) is the limited number of known tumor-specific targets, especially for solid tumors. For T cell receptor (TCR)-based ACT approaches, presentation of the targeted

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HLA-peptide complex on normal tissues can lead to on-target / off-tumor toxicity. Independently, TCRs with promiscuous peptide-binding motif are known to cause dramatic off-target adverse events.

Methods

Here, we present the capabilities of our XPRESIDENT® platform to not only identify novel tumor targets, but also screen TCR candidates against off-target toxicities in absence of relevant *in vivo* models. The XPRESIDENT platform allows for direct identification of HLA-restricted peptides via ultra-high sensitive mass spectrometry. Peptide sequencing of a wide-ranging collection of healthy tissues and tumor biopsies enabled the discovery of a pipeline of >80 targets over-represented in human tumors. Immatics' high-throughput TCR platform can generate large panels of unique, target-specific TCR sequences from the natural human repertoire. Positional scanning helps identify the peptide-binding motif of lead TCR candidates, and TCRs showing cross-recognition to analogous peptides expressed in healthy tissues (XPRESIDENT) can therefore be negatively-selected at an early preclinical stage.

Results

In addition to expression data, we will present a comprehensive preclinical data package of some of our lead TCR candidates. GMP-compliant manufacture of ACTengine T cells shows robust TCR expression and T cell expansion. ACTengine T cells preserve a stem-cell-like memory phenotype, which is known to correlate with longer persistence *in vivo*. Finally, ACTengine T cells selectively recognize and kill tumor cell lines presenting the target peptide, while sparing target-devoid primary healthy cells.

Conclusions

The XPRESIDENT-guided targets currently considered for ACTengine are presented in various solid and liquid tumor types, while not detected on healthy tissues. Immatics' TCR-based ACTengine® programs, in collaboration with MD Anderson

Cancer Center, aim to deliver safer, best-in-class immunotherapies.

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Exploiting CD4 T cells for adoptive cell therapy in cancer

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Background

T cell based immunotherapy represents an attractive strategy for the treatment of cancer. Whereas cellular anti-tumor immune responses have typically been attributed to CD8 T cells, CD4 T cells play a critical role in tumor elimination and the priming and maintenance of CD8 T cell responses. Combining HLA class I- and class II-restricted TCRs for T cell redirection may provide a more potent therapeutic effect in adoptive T cell therapy. Furthermore, HLA class II-restricted TCRs may be of therapeutic value both in haematopoietic malignancies and in melanoma where tumor cells frequently express HLA class II.

Methods

We have isolated CD4+ T cells reactive against tumor antigens from patients who experienced clinical benefit from treatment with cancer vaccines targeting universal tumor antigens and frequent neoantigens.

Results

Strong T cell responses against the vaccines or unrelated cancer antigens suggesting epitope spreading correlated with enhanced survival and tumor regression in late stage cancer patients. These HLA class II restricted T cell clones recognised target cells loaded with long peptides or protein and for some CD4+ T cell clones we could also show direct tumor recognition. TCRs were expressed in

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expanded donor T cells by mRNA electroporation or retroviral transduction and found functional in both CD8+ and CD4+ T cells producing TNF- α , IFN- γ with the capacity of target cell killing. We also show preliminary *in vivo* data for one of our broadly applicable TCRs recognizing a universal antigen, hTERT, presented on one of the most frequent HLA alleles, HLA-DP4.

Conclusions

Our findings open a way to more broadly applicable cancer treatment that will soon be tested in patients.

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Preclinical evaluation of mesothelin-specific T cell receptor (TCR) fusion constructs (TRuC™s) utilizing the signaling power of the complete TCR complex: a new opportunity for solid tumor therapy

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Background

Anti-CD19 chimeric antigen receptor (CAR) T cell therapies for B cell malignancies have demonstrated impressive clinical results. However, clinical trials in solid tumors have been much less promising. We reasoned that T cell activation triggered by CARs is not adequate to overcome the immunosuppressive microenvironment of solid tumors. We therefore developed the T Cell Receptor Fusion Construct (TRuC™) platform. It is based on the recombinant fusion of a target binding domain to subunits of the T cell receptor complex other than CD3 ζ . Different from CARs, which only use the CD3 ζ of the six available TCR subunits for signaling, TRuC™ variants are integrated into the complete TCR and can thereby trigger a more diverse signaling cascade through all TCR components. In this study, we

tested the potency of the TRuC™-T cells targeting the solid tumor antigen mesothelin (MSLN) in preclinical studies.

Methods

We generated a panel of TRuC™ variants by recombinantly fusing MSLN-specific single-domain antibodies (sdAbs) of high or low target affinity to TCR β , CD3 γ , or CD3 ϵ subunits, respectively. The constructs were introduced into primary human T cells via lentiviral transduction. After standard stimulation and expansion, TRuC™ surface expression and T cell activation were analyzed by flow cytometry. Cytokine release and tumor cell lysis were investigated *in vitro* prior to testing the anti-tumor efficacy and persistence of TRuC™-T cells in a mesothelioma mouse model.

Results

Of the various TRuC™s made, the ϵ -TRuC™ variant showed the best surface expression irrespective of the MSLN-specific binder used. Upon target cell engagement, phosphorylation of CD3 ϵ was observed with the MSLN- ϵ TRuC™ version but not with MSLN-specific CAR constructs, indicating that the TRuC™ was integrated into the TCR complex. TRuC™-T cells showed target-dependent upregulation of activation markers CD69 and CD25. Further, TRuC™ T cells demonstrated potent killing of MSLN-positive tumor cells and showed increased levels of degranulation marker CD107a. Of note, despite comparable cell lysis potential *in vitro*, TRuC™-T cells produced less cytokines than CAR-T cells. When tested for anti-tumor activity in a mesothelioma xenograft model, MSLN-specific TRuC™-T cells were more potent than CAR-T cells. TRuC™-T cells eradicated primary solid tumors and protected mice from a later re-challenge.

Conclusions

Our results demonstrate that T cells expressing a TRuC™ with a low-affinity MSLN-specific sdAb can potentially kill tumor cells *in vitro* and appear to be superior in potency over CAR-T cells. In our preclinical assessment, MSLN-specific ϵ -TRuC™ T

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cells showed the highest potential for the treatment of MSLN-positive solid tumors.

P172

iRGD enhances T cells infiltration and augments response to PD-1 gene knockout immunotherapy in gastric cancer

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Background

Poor infiltration of activated lymphocytes into tumors can be a fundamental factor limiting their efficacy and impeding the therapeutic effect of the checkpoint blockade immunotherapy [1,2]. A tumor-penetrating peptide, iRGD, has a well-defined role in delivering drugs into extravascular tumor tissues in both the combination regimen and conjugated pattern [3,4]. Here, we explored for the first time whether this cycled peptide could facilitate the infiltration of lymphocytes into tumor and furtherly overcome resistance to PD1 gene knockout immunotherapy.

Methods

We used polyethylene glycol-conjugated phospholipid (PEG-lipid) derivatives, a time-efficient and versatile platform, to immobilize iRGD on T cell membrane. The ability of iRGD modified or co-applied lymphocytes infiltration was detected in both the 3D tumor spheroids in vitro and subcutaneous tumor model and peritoneal tumor model of gastric cancer in vivo. Furthermore, the synergistic effect of iRGD modification and PD-1 gene knockout in adoptive T cell transfer immunotherapy was examined in a xenograft model of EBV-associated gastric cancer.

Results

In this study, we showed that T cells could be modified by the synthetic iRGD-PEG-lipid without compromising their vitality, expansion, phenotype and effector function. In vitro, co-administration of iRGD could promote the infiltration of T cells while iRGD modification made T cells spread more extensively throughout the multicellular spheroids. Near infrared results showed that iRGD modification made a tenfold improvement infiltration of T cells into tumors without a parallel increase in normal tissues. Most importantly, we demonstrated that iRGD modified T cells had superior antitumor efficiency owing to sufficiently increased T cells infiltration, and exhibited robust synergistic effect with PD-1 gene knockout immunotherapy.

Conclusions

Our study indicates that modification of T cell membrane with iRGD might be a potent strategy to increase T cells infiltration, thereby overcome the bottleneck of solid tumor immunotherapy.

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Mass cytometry based immunophenotyping of tumor-reactive T cells in human cancer reveals subpopulations with coordinate inhibitory molecule expression

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Background

The presence of tumor infiltrating lymphocytes (TILs) is a positive prognostic factor in various cancers. TIL function can be suppressed by the expression of immune checkpoint molecules (ICMs) and antibody-based blockade of these molecules can re-invigorate suppressed TIL function to evoke tumor regression. However, multiple checkpoint blocking antibodies exist, and approaches to rationally designing complex combination immunotherapies remain elusive. Identifying tumor-reactive TILs in cancer and understanding the complex immunobiology of these cells may drive the rational development of effective combination immunotherapies. CD137 (TNFRSF9, 4-1BB) is a biomarker for tumor-reactive TILs in human cancer [1]. CD137+ TILs recognize autologous tumor antigens, produce proinflammatory cytokines, and suppress tumor outgrowth in xenograft models; CD137- TILs do not. CD137+ TILs express ICMs, however, which and how many specific ICMs are coordinately expressed by tumor-reactive TILs is unknown. Accordingly, such knowledge may reveal new opportunities for rationally designed combination therapies.

Methods

Cytometry by time-of-flight (CyTOF) was performed on enzymatically-digested human ovarian cancers to immunophenotype TILs using a 34 analyte panel. CyTOF data were analyzed using visNE and PhenoGraph algorithms to identify TIL

subpopulations. Tumors were treated with anti-PD-1 and/or CD137 agonist antibodies, and TIL cytotoxic molecule expression was analyzed by flow cytometry.

Results

CD137+ TILs preferentially co-express multiple ICMs that may inhibit their anti-tumor abilities (e.g. PD-1, CTLA-4, Tim-3, 2B4, etc.) compared to CD137- TIL. Tumor-reactive TILs selectively sub-cluster into phenotypic subsets revealing coordinate ICM expression with some tumor-reactive TIL subsets expressing more ICMs than others. Agonizing CD137 on TILs significantly increased their cytotoxic phenotype, with elevated IL-2, TNF α , and IFN γ , while blocking PD-1 on TILs significantly increased the expression of cytotoxic molecules in the CD137+ TIL subsets, but not CD137- TIL. Preliminary results also showed increased patient tumor lysis in response to CD137 agonism.

Conclusions

Tumor-reactive TILs co-express multiple ICMs, sub-cluster into defined subsets revealing ICM patterning, and exhibit an enhanced cytotoxic profile in response to PD-1 blockade or CD137 agonizing. The biology underlying this sub-clustering phenomenon remains under investigation and other immunomodulatory antibodies are under assessment using this bio-assay system. Overall, our system provides the potential to predict immunomodulatory combinations that may enhance tumor-reactive T cells' cytotoxic activity and may serve as a guidepost for the rationale design of combination immune checkpoint inhibition trials.

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Removal of double stranded RNA using a novel method with RNaseIII produces robust mRNA chimeric antigen receptor T cell therapy

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Background

Chimeric Antigen Receptor (CAR) T cells made with mRNA offer a transient and safe alternative to viral CARs, mitigating the concern for persistent unwanted side effects from constitutively active T cells. Previous studies have shown that mRNA CARs are transiently effective, but lack CAR persistence and have struggled to show success across tumor types. We hypothesized the efficacy of mRNA CARs could be improved by utilizing recent advancements in RNA technology including the use of modified uracil and a novel purification method with RNaseIII to prevent double stranded RNA (dsRNA) that induces toxicity.

Methods

Using the established CD19 CAR model in B cell acute lymphoblastic leukemia, we created CD19-directed CAR T cells using unmodified, unpurified mRNA and compared them to CAR T cells created using modified uracil and/or purification to remove any aberrant dsRNA. A simple, novel procedure using RNaseIII enzyme was used for purification: in vitro transcribed mRNA was incubated with RNaseIII for thirty minutes then isolated via phenol-chloroform extraction. CAR T cells were evaluated for cytotoxicity, CAR expression, negative checkpoint regulator expression, and genomic profiling using Nanostring RNA sequencing.

Results

Both modified and purified mRNA CAR T cells showed a two-fold increase in expression of the CAR on their surface initially ($p < 0.0001$), as well as a two-fold improvement in cytotoxic killing of leukemia cells in vitro that persisted for up to five days ($p < 0.0001$). Both the modified and purified mRNA CAR T cells also showed reduced expression of negative checkpoint regulators PD1 and LAG3 compared to original RNA CAR T cells ($p < 0.0001$). However, in vivo studies using a patient-derived xenograft model with a single dose of CAR T cells revealed purified RNA CAR T cells offered the most robust 2-log enhanced suppression of leukemic burden ($p < 0.02$). In addition, genomic profiling revealed a differential pattern of activation among the mRNA CAR T cell constructs, indicating that using different mRNA products can alter the complex and often detrimental activation that occurs with mRNA electroporation.

Conclusions

RNaseIII is a novel purification technique that has not yet been reported in the literature for RNA manufacturing. DsRNA is intensely immunogenic; removal allows for increased protein translation, and here provides improved CAR T cell cytotoxicity, in particular in vivo. Our results provide a time efficient purification method that can be easily incorporated into RNA production for use in clinical trials, and poise RNA CARs for increased efficacy as new CAR targets emerge and are being tested.

P175

A rapid and streamlined method for the culture of Tumor Infiltrating Lymphocytes (TIL) from melanoma tumor fragments

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Background

The Moffitt Cancer Center TIL program has manufactured 45 TIL products for the treatment of melanoma patients. Currently, the pre-rapid expansion (pre-REP) manufacturing process takes 24-38 days to generate a sufficient cell dose for subsequent clinical dose production, during which time the patient is at risk of disease progression and treatment ineligibility. Here we present a simplified, rapid method for the culture of TIL from melanoma tumor fragments using gas-permeable 24-well culture plates.

Methods

Melanoma tumor fragments (1-3mm³) were cultured in polystyrene or gas-permeable (G-REX, Wilson-Wolf) 24-well culture plates. Each fragment was cultured in a separate well in complete media supplemented with IL-2 (6000 IU/ml) and agonistic anti-41BB antibody (10 µg/ml). TILs cultured in polystyrene plates were re-fed and split upon confluence into secondary 24-well polystyrene plates according to standard protocol and harvested on day (D) 24/25 of culture. The optimal media replacement schedule for TIL culture in G-REX wells was investigated and determined to be 3X/week. TILs cultured in G-REX plates were fed 3X/week, kept in their original wells throughout the culture period and harvested on D17/18 or D24/25. Cell count, viability, immunophenotype, and tumor reactivity were assessed.

Results

Sufficient TIL yield for rapid expansion was achieved using a single G-REX well per fragment (4.4e7±4.3e7, D17/18) a full seven days prior to a comparable yield from multiple polystyrene wells (5.1e7±5.3e7, D24/25) (p=0.32). Prolonged culture in the G-REX well did not significantly increase the number of TIL per fragment (4.6e7±4.4e7, D24/25) (p= 0.45). TIL grown in G-REX wells showed higher viability (91±3%) on D17/18 compared to polystyrene on D24/25 (79±5%) (p<0.000001). The viability of TIL in G-REX wells decreased on D24/25 (78±8%) suggesting peak expansion occurred at the earlier time point. TIL in G-REX wells showed higher

percentages of CD8+ T cells (88±10%, D17/18; 94±8%, D24/25) than TILs from polystyrene (76±14%, D24/25 (p<0.001) and lower percentages of NK cells (G-REX: D17/18, 2.4±3%; D24/25, 3±4%; polystyrene: 12±9% (p<0.001). Tumor-specific activity was similar, as measured by IFN-γ secretion between the two culture conditions.

Conclusions

G-REX culture plates allowed us to obtain sufficient pre-REP TIL (60e6 viable cells) for clinical dose manufacture in one week less than conventional polystyrene culture plates. Reducing the manufacturing period by one week potentially decreases patient ineligibility due to disease progression during TIL manufacturing. TIL culture in G-REX 24-well plates from tumor fragments is a simple and rapid method that may facilitate adoption of TIL therapy to other clinical sites.

P176

AdoptCell[®]-NK: *Ex vivo* generation of highly purified and activated natural killer cells from human peripheral blood in accordance with GMP/GCTP for clinical studies

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Background

Cancer immunotherapy has been established as a new therapeutic category since the recent success of immune checkpoint inhibitors and a type of adoptive immunotherapy, namely chimeric antigen receptor-modified T cells (CAR-T). Although CAR-T demonstrated impressive clinical results, serious adverse effects (cytokine storm and on-target off-tumor toxicity) and undefined efficacy on solid tumors are important issues to be solved.

As an alternative adoptive immunotherapy approach, we recently developed a new, simple method to generate highly active and expanded human NK cells from PBMCs. NK cells play a crucial

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role during the innate immune responses against various diseases, including infectious diseases and malignancies. Therefore, adoptive immunotherapy using NK cell is emerging as promising treatments for intractable malignancies; however, there has been still developing because of difficulties in culture, shortage of overall effector numbers, contamination of considerable numbers of T cells, and their limited anticancer potencies. We here established the simple feeder-free method to generate purified (>90%) and highly activated NK cells from PBMCs in accordance with GMP/GCTP for clinical studies.

Methods

Under approval of the institutional ethical committee, PBMCs were collected from healthy volunteers by using CliniMACS Prodigy[®] (automatic/closed system). CD3⁺ and CD34⁺ cells were depleted by CliniMACS beads, and the cells were cultured at a concentration of 5 x 10⁵ cells/ml with high concentration of hIL-2 and 5% human AB serum for 14 days. Then, we confirmed the expression of surface markers, CD107a mobilization and cell-mediated cytotoxicity against various tumor cells and normal cells with or without monoclonal antibody drugs *in vitro* and antitumor effects against K562 *in vivo*.

Results

Among the several parameters, we found that simply 1) only CD3/CD34-depletion, 2) high dose IL-2, and 3) use of specific culture medium were sufficient to obtain the highly purified, expanded (~200-fold) and activated CD3⁺/CD56⁺ NK cells from PBMCs. Almost all activated NK cells expressed lymphocyte-activated marker CD69, and showed dramatically high expression of NK activation receptors (i.e. NKG2D, NKp30, NKp46, etc.), interferon- γ , perforin and granzyme B. Importantly, only 2 hours' reaction at effector/target ratio=1:1 was sufficient to kill almost all K562 and Raji cells, and antitumor activity was also representative on tumor bearing mice *in vivo*. Cytolysis was specific for various tumor cells, but not for normal cells, irrespective of MHC class I expression.

Conclusions

We now started GMP/GCTP production of this new NK cells and first-in-man clinical trials in use of "haploidentical AdoptCell[®]-NK" will be initiated on 2Q 2018.

P177

Engineering artificial lymph nodes for immunotherapy

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Background

One of the challenges of adoptive immunotherapy is generating enough highly functional CD8⁺ T cells. Our lab has developed artificial antigen-presenting cells (aAPCs) to bypass immunosuppressed antigen-presenting cells to generate more functional CD8⁺ T cells. We hypothesized that an additional important variable to control T cell stimulation is the microenvironment—traditionally stimulation occurs just in a plastic culture dish. Inspired by the composition and structure of the lymph node, we engineer stimulatory microenvironments to improve numbers of highly functional antigen-specific T cells.

Methods

aAPCs were prepared by attaching dimeric antigen-loaded pMHC and anti-CD28 to nanoparticles. These were used to activate B6 CD8⁺ T cells on either tissue culture plates (TCPs) or on prepared hyaluronic acid hydrogels (Hyd). To remove the need for aAPCs we also conjugated the stimulatory signals directly to the hyaluronic acid hydrogels, which we term an artificial lymph node (aLN). To further mimic the reticular fiber network of the lymph node, polymeric (polycaprolactone) electrospun nanofibers were added to aLNs to form a composite material. On day 7 cells were counted and stained with cognate and non-cognate dimer pMHC. Functionality was measured by staining

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cytokines produced after 7 days of culture through intracellular staining of INF-g, TNF α , IL-2, and CD107a+ T cells.

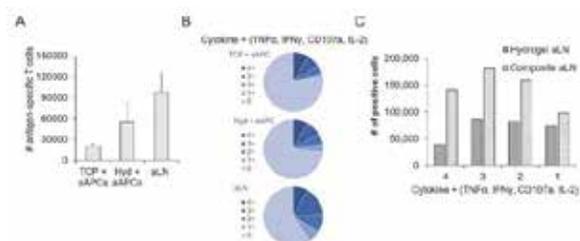
Results

aLNs generated more than triple the total antigen-specific CD8+ T cells than those stimulated by aAPCs on TCP (Figure 1A). Even stimulating with the same aAPCs but just on a hydrogel surface demonstrated nearly double the antigen-specific T cell number. These conditions were also more functional than traditional stimulation methods (Figure 1B). Using composite aLNs with polymeric nanofibers induced greater numbers of high functional T cells compared to hydrogel aLNs (Figure 1C).

Conclusions

We engineered environments which produce high number of functional antigen-specific T cells. Interestingly, having an extracellular matrix hydrogel was a critical factor. We used this data to further design our material to mimic the lymph node by incorporating polymeric nanofibers for similar structure, stiffness, and porosity. Beyond demonstrating the importance of the microenvironment, these data have implications for use of these cells and materials for *in vivo* immunotherapies.

Figure 1. Activation of antigen-specific T cells is influenced by material microenvironment



(A) Endogenous antigen-specific CD8+ T cells is enhanced by hydrogel and aLN environments. (B) Functionality is also increased as measured by cytokine production. (C) Enhanced numbers of polyfunctional cells from composite stimulation.

P178

Combined killing of cancer cells and cross presentation of tumor antigen by V γ 9V δ 2 T cells

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Background

The human V γ 9V δ 2 T cells are a unique T cell type, and recent studies of the biology of V γ 9V δ 2 T cells emphasize the potential exploitation of these cells in immunotherapy of cancer. V γ 9V δ 2 T cells exhibit dual functionality in that they are both antigen presenting cells (APC) and cytotoxic towards cancer cells.

Methods

In vitro assays that were included in this study were; ELISPOT, chromium cytotox assay, xCELLigence, flow cytometry and zoledronic acid expansion protocol for V γ 9V δ 2 T cells.

Results

We show that V γ 9V δ 2 T cells can kill cancer cell lines from various cancer types such as leukemia, melanoma, prostate-, and breast cancer, with a significantly increased killing upon treatment of the cancer cells with Zoledronic acid. In addition, we show that V γ 9V δ 2 T cells take up tumor antigens gp100 and MART-1 (long peptide and recombinant protein, respectively), and process these antigens for presentation of class I restricted peptides in the context of the HLA-A02.01 molecule, to be recognized by peptide specific cytotoxic CD8 T cells. Moreover, we show that specific inhibition of the proteasome by lactacystin impair recognition by

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peptide specific CD8 T cells, strongly suggesting proteasome involvement in presentation of the relevant class I restricted peptides.

Conclusions

The dual functions; killing and antigen presentation combined with the ease of expanding V γ 9V δ 2 T cells in vitro from peripheral blood lymphocytes to billions of cells, makes V γ 9V δ 2 T cells attractive vehicles for adoptive cell therapy (ACT) in cancer therapy. Thus, V γ 9V δ 2 T cells are broadly tumor specific killers that concurrently could induce or support tumor specific $\alpha\beta$ -T cell responses.

P179

NKG2D ligand–inducing immune therapy plus NKG2D+CD8+ T cell therapy overcomes chemoresistance in tumors

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Background

Though chemotherapy is still the first-line therapy for most cancers, tumors ultimately develop resistance to chemotherapy, allowing tumor relapse and metastasis, the primary cause of cancer patient death. Immune therapy has become a viable alternative for treating chemo-resistant tumors. Notably, CD20/19-CAR-T cell therapy has achieved great success in eliminating leukemia, and immune checkpoint blockers have extended overall survival in melanoma. For most solid tumors, however, neither approach has been effective; the poor efficacy is attributed to tumor heterogeneity, an inert tumor microenvironment, and the difficulty in penetration.

Methods

During the past years, our group has identified a simple combination of an immune stimulatory

signal (interleukin-12) and chemotherapy (doxorubicin) that persistently induces NKG2D ligand on tumor cells in vivo across tumor types [1] (Figure 1) and developed a novel CD28 stimulation based approach for fast induction of NKG2D receptor on CD8+ T cells [2] (Figure 2).

Results

We show here that adoptive transfer of NKG2D+CD8+ T cells to NKG2D ligand–induced tumors overcame tumor resistance to chemotherapy in both murine and humanized tumor models (Figure 3).

Conclusions

The mechanism underlying this tumor-specific and persistent NKG2D ligand–induction therapy has several components: (1) it boosts NKG2D+CD8+ T cell accumulation in tumors by inducing T cell attracting chemokines; and (2) it transforms the tumor microenvironment to enable the accumulated T cells to effectively kill tumor cells by engaging with the induced NKG2D ligand. Furthermore, it is independent of the known transcription mechanism but is dependent on epigenetic modifiers Kat2a/2b. Notably, the stress-induced NKG2D transcription lasts only for 1-2 days, but this Kat2a/2b-dependent induction lasts for at least 8 days and is CD8+ T cell–dependent. Depletion of CD8+ T cells impairs this NKG2D ligand induction in tumors (Figure 4), impeding the associated tumor eradication (Figure 5). Details of the molecular and immune mechanism will be discussed.

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Figure 1. Rae-1 is induced by IL12 plus doxorubicin in four tumor models.

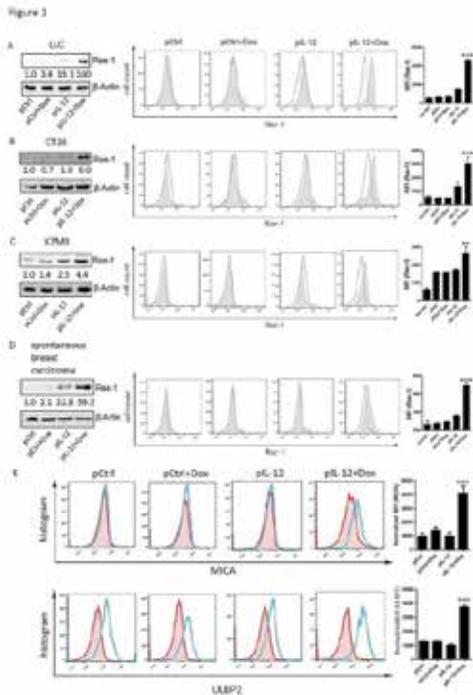


Figure 2. CD80 binding-mediated CD28 activation induces sustained expression of the NKG2D receptor on CD8+ T cells.

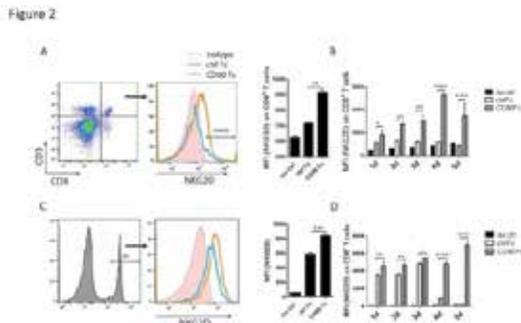


Figure 3

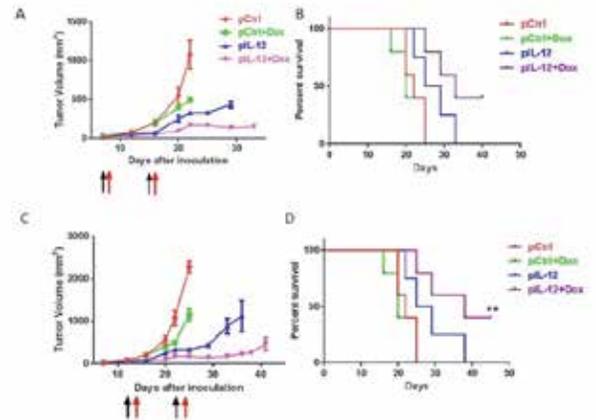
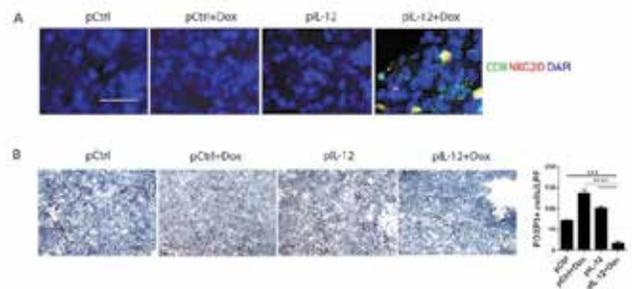


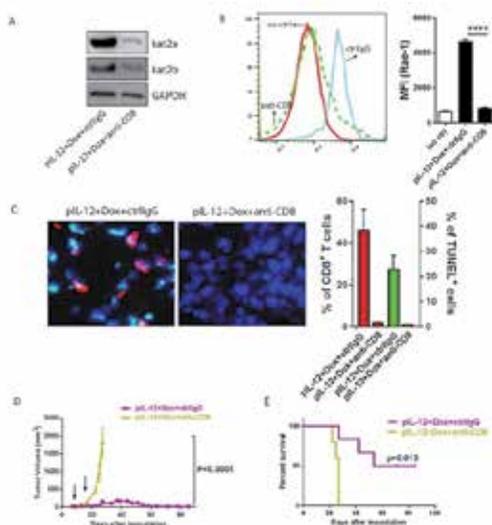
Figure 4. IL-12 plus doxorubicin enhanced the accumulation of NKG2D+CD8+ T cells, and reduced the infiltration of Treg cells in tumors.

Figure 4



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Figure 5



References

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P180

Chemokine receptor engineering of T cells with CXCR2 improves homing towards subcutaneous human melanomas in xenograft mouse model.

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Background

Adoptive cell therapy (ACT) using in vitro expanded tumor infiltrating T lymphocytes (TILs) from biopsy material represents a highly promising treatment of disseminated cancer. A crucial prerequisite for successful ACT is sufficient recruitment of transferred lymphocytes to the tumor site; however, despite transfer of billions of lymphocytes, T cell infiltration into the tumor post ACT is limited.

Methods

To identify potential chemokine/chemokine receptor axes in melanoma, chemokine expression and secretion of 12 selected chemokines were analysed by PCR and multiplex chemokine assays performed on cDNA and supernatants of 20 human melanoma cell lines. Expression of cognate chemokine receptors, CCR2, CCR4, CCR5, CXCR2, CXCR3, CXCR4 and CXCR6, was assessed by flow cytometry of tumor infiltrating lymphocytes from 10 patients with metastatic melanoma.

For expression of selected chemokine receptors, human primary T cells (from PBMC of healthy donors) were genetically engineered using lentiviral transduction. In vitro signalling and chemotaxis via the receptors were assessed by Ca²⁺ influx assay, and transwell migration assay towards both ligand and melanoma cell line supernatant. In vivo homing of T cells genetically engineered with chemokine receptor, CXCR2, was evaluated in a xenograft NOG mouse model. Mice with subcutaneous human melanomas were treated by ACT of MAGE-A3 specific T cells transduced with either CXCR2 or MOCK. Tumor infiltration of T cells was quantified by flow cytometry and immunohistochemistry.

Results

We found that a majority of malignant melanoma (MM) cell lines expressed chemokines CXCL8/IL-8, CXCL12/SDF-1 and CCL2. Successful engineering of TILs and peripheral blood T cells significantly increased receptor expression of the corresponding

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chemokine receptors CXCR2, CXCR4 and CCR2. All three chemokine receptors are functional *in vitro* and show ligand specific transwell migration of engineered T cells as well as increased migration towards MM conditioned medium. *In vivo* homing was assessed in a xenograft NOG mouse model. Mice with subcutaneous human melanoma were treated with ACT of MAGE-A3 specific T cells transduced with either CXCR2 or GFP. Transducing T cells with CXCR2 increased tumor infiltration. In comparison mock transfected T cells appeared to be allocated to other organ-compartments.

Conclusions

CXCR2, CXCR4 and CCR2 engineered T cells are functional *in vitro*, and transduction with CXCR2 improve *in vivo* homing of T cells to tumor site, setting the stage for mixing and matching chemokine-receptor expression to tumor microenvironments. Longitudinal studies assessing cell trafficking and tumor control using *in vivo* imaging are currently ongoing, and results will be presented at the meeting.

P181

Production of site-specific allogeneic CD19 CAR-T cells by CRISPR-Cas9 for B-cell malignancies.

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Background

We have applied CRISPR/Cas9 technologies to develop anti CD19 Allogeneic chimeric antigen receptor T cells (CAR-T) with reduced GVHD potential and reduced rejection potential for the treatment of CD19 positive malignancies. The efficiency of the CRISPR/Cas9 system enables rapid production of homogeneous CAR-T product from

prescreened healthy donors and thus can potentially be developed as an “off-the-shelf” therapy for efficient delivery to patients. Autologous CAR-T therapeutics targeting CD19 have shown impressive responses in B-cell malignancies but currently require significant individualized manufacturing efforts and can suffer from manufacturing failures. In addition, these autologous CAR-Ts are produced using retrovirus or lentivirus, for which the variable nature of integration can lead to a heterogeneous product. Allogeneic or “off-the-shelf” CAR-T products with site-specific CAR integration generated with gene editing technologies may address some of these significant challenges seen for Autologous products.

Methods

We have utilized the CRISPR-Cas9 technology in primary human T cells to produce allogeneic CAR-T cells by multiplexed genome editing. We have developed a robust system for site-specific integration of CAR and concurrent multiplexed gene editing in single T cells by utilizing homology-directed repair (HDR) with Cas9 ribonucleoprotein (RNP) and an AAV6-delivered donor template.

Results

With CRISPR/Cas9 editing technology we have achieved high frequency knockout of the constant region of the TCR α gene (TRAC) with ~98% reduction of TCR surface expression in human primary T cells from healthy donors, which aims to significantly impair graft-versus-host disease (GVHD). High frequency knockout of the β -2-microglobulin (B2M) gene could also be obtained, which aims to increase persistence in patients, potentially leading to increased potency overall. TRAC/B2M double knockout frequencies have been obtained in ~80% of T cells without any subsequent antibody-based purification or enrichment. Human T cells expressing a CD19-specific CAR from within a disrupted TRAC locus, produced by homology-directed repair using an AAV6-delivered donor template, along with knockout of the B2M gene have been consistently produced at a high efficiency. This site-specific integration of the CAR

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protects against the potential outgrowth of CD3⁺CAR⁺ cells, further reducing the risk of GVHD, while also reducing the risk of insertional mutagenesis associated with retroviral or lentiviral delivery mechanisms. These engineered Allogeneic CAR-T cells show CD19-dependent T cell cytokine secretion and potent CD19-specific cancer cell lysis.

Conclusions

We are able to use genome editing with the CRISPR-Cas9 system to efficiently create an Allogeneic or “off-the-shelf” CAR-T cell product that demonstrates potent and specific anticancer effects for patients with CD19-expressing human cancers.

P182

Development of chemically defined medium for *ex vivo* expansion of natural killer cells

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Background

NK cells belong to the innate immune system and are a type of cytotoxic lymphocyte. They comprise between 5-20% of the human peripheral blood lymphocytes and are characterized by expression of CD56 and absence of CD3 (CD56⁺CD3⁻). NK cells recognize and attack abnormal cells, such as cancer or viral infected cells without pre-activation. This ability to target and destroy cancer cells makes NK cells valuable for immunotherapy applications. However, this requires large doses of NK cells to be infused into the patient. Furthermore, consistent *ex vivo* expansion of NK cells retaining their functional properties remains a challenge despite extensive efforts in the field. In this study, we present a chemically-defined medium capable of supporting the *ex vivo* expansion of NK cells that retain their cytotoxic functionality.

Methods

Peripheral Blood Mononuclear Cells (PBMCs) or NK92 cells (immortalized NK cell line) were

stimulated with 100U/L IL-2 in the presence or absence of mitomycin C treated K562 feeder cells. Cells were expanded for 14 days, with media changes at days 3, 7, and 10. During the course of media development, we applied our Rational Media Design™ approach to eliminate serum and identify non-animal derived components that could improve NK cell expansion and performance. The performance of media was evaluated by the total nucleated cell count, CD56⁺CD3⁻ percentage, and cytotoxicity against K562 cells.

Results

By using a Rational Media Design™ approach we were able to formulate a chemically-defined medium that was able to support expansion of NK cells derived from PBMCs and NK92 cells, in the presence or absence of feeder cells. When NK92 cells and PBMCs from multiple donors were compared, our chemically-defined medium delivered a similar or higher specific fold increase of CD56⁺CD3⁻ cells and comparable cytotoxicity profiles over two other commercially available serum-free media.

Conclusions

The medium we developed is a product that combines translational quality, boasts high performance, and production under cGMP conditions. These features make it ideal for use in clinical studies for applications of both PBMC derived NK or NK92 cells.

P183

Stem cell differentiation towards tumor-recognizing CD4 T cells provides robust anti-tumor effects in combination with CD8-TCR-T cell therapy

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Background

T cell receptor (TCR) engineering of patient's mature T cells has shown to be a promising approach, but unfortunately, the anti-tumor effect is largely short-lived. We have recently discovered a novel and distinct subset of human CD4⁺ Th1 cells and cloned their TCRs which directly recognize NY-ESO-1 tumor antigen naturally presented by MHC II on cancer cells. Our central hypothesis is that CD4TCR-engineered human hematopoietic stem/progenitor cells (hHSC) will lead to durable in vivo supply of fully active Tumor Recognizing (TR)CD4 cells with anti-tumor activity, and provide sustained help to co-injected CD8TCR-transduced effector T cells (which will serve to immediately debulk the tumor), leading to long-lasting tumor rejection.

Methods

We generated lentiviral vectors for cell transduction with two unique NY-ESO-1 (TR)CD4TCRs (MHC-II restricted HLA-DR1 and DP4), and a novel CD8 (MHC-I HLA-A2.1)TCR for NY-ESO-1. We created new transgenic mouse models based on a highly immunodeficient NSG background with expression of human MHC-II-DP4 and DR1 in order to study hHSC differentiation/function in vivo and to test anti-human cancer activity with our adoptive cell transfer (ACT) platform.

Results

Transduction efficiency of hHSC was high with transgene expression levels of 40-51% (0.5-1 vector copies/cell); and 65-92% tetramer+ for mature T cells. We confirmed specific functional activity of all TCRs by co-incubating transduced T cells with various tumor targets (SKMEL-37, MZ19, or aAPC:K562/DR1/DP4/A2.1 +/- cognate peptides) by ELISA and intracellular staining for IFN γ . We injected mice with hHSC transduced with (TR)CD4-TCR and after 2-3 months we confirmed generation of engineered TCR-expressing human T cells by FACS through blood sampling. We then tested anti-tumor efficacy in vivo with s.q. injection of human melanoma (MZ19) or human ovarian carcinoma (A2780/A2/NY) cells in mice injected with (TR)CD4-TCR-transduced or non-transduced-control hHSC.

We injected a low dose (5×10^5 cells) of NY-ESO-1 HLA-A2.1 CD8TCR-transduced hPBMC and followed tumor growth. Remarkable tumor control was obtained ($p < 0.05$) in the mouse group that received (TR)CD4-TCR-transduced hHSC (tumor size = 28 mm^2 ; SE +/- 11) versus mice that received untransduced hHSC (152.7 mm^2 ; SE +/- 24) or control untreated (no hHSC, no CD8 T cell ACT) (196 mm^2 ; SE +/- 40) assessed at day 32 after tumor (A2780/A2/NY) injection.

Conclusions

Here we demonstrate for the first time that a combined ACT approach of (TR)CD4-TCR-transduced hHSC with CD8-TCR-transduced PBMC leads to a synergistic and efficient in vivo control of tumor burden. These results provide basis to pursue a Phase I/IIa clinical trial based on our novel ACT platform to benefit patients with advanced solid tumors.

P184

Genetic engineering of human NK cells to express CXCR2 improves migration to renal cell carcinoma

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Background

Adoptive natural killer (NK) cell transfer is being increasingly recognized as a therapeutic approach to treat cancer patients. However, clinical responses have so far been limited to patients with hematological malignancies. A potential rate-limiting factor in patients with solid tumors is

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defective homing of the infused NK cells to the tumor site. Chemokines regulate the migration of leukocytes via corresponding chemokine receptors. Various solid tumors, including renal cell carcinoma (RCC), secrete ligands for the chemokine receptor CXCR2. We hypothesize that infusion of NK cells expressing high levels of CXCR2 will result in increased influx of the transferred NK cells into tumors, and improved clinical outcome in patients with cancer.

Methods

Blood and tumor biopsies from primary RCC patients (n=14) were assessed by flow cytometry and chemokine analysis. Primary NK cells were retrovirally transduced with human CXCR2; transgene expression ranged from 23 to 93%. CXCR2 receptor functionality was determined by Calcium flux and NK cell migration was evaluated in transwell assays.

Results

We detected 10- to 186-fold higher concentrations of CXCR2 ligands in tumors compared with plasma of RCC patients. In addition, CXCL5 levels correlated with the intratumoral infiltration of CXCR2-positive NK cells (n=9, p=0.039). However, frequencies of CXCR2-positive NK cells were lower in the tumors compared with peripheral blood (p=0.0003). Moreover, healthy donor NK cells rapidly lost their CXCR2 expression upon *in vitro* culture and expansion. Genetic modification of human primary NK cells to re-express CXCR2 improved their ability to specifically migrate along a chemokine gradient of recombinant CXCR2 ligands 3.4-fold (p=0.0083) or RCC tumor supernatants 2- to 2.5-fold (p<0.05) compared with control cells. The enhanced trafficking resulted in increased killing of target cells (p=0.0039). In addition, while their functionality, including cytotoxicity, degranulation and cytokine production, remained unchanged compared with control cells, CXCR2-transduced NK cells obtained increased adhesion properties and formed 53±21% more conjugates with target cells (p=0.0128).

Conclusions

To increase the success of NK cell-based therapies of solid tumors, it is of great importance to promote their homing to the tumor site. In this study, we show that stable engineering of human primary NK cells to express a chemokine receptor thereby enhancing their migration is a promising strategy to improve the efficacy of adoptive cellular immunotherapies.

P185

A novel chimeric T cell receptor that operates through the endogenous TCR diverges in biology from conventional CAR T cells

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Background

T lymphocytes engineered with chimeric antigen receptors (CARs) have generated significant enthusiasm based on remarkable clinical successes in the treatment of B cell leukemias. Conventional CARs seek to recapitulate TCR and costimulatory signals through integration of signaling elements into a single receptor. The robust anti-tumor activity of CARs is associated with over exuberant T cell activation and cytokine production, often resulting in debilitating toxicities. Our lab has generated a novel chimeric receptor termed T cell antigen coupler (TAC), which is designed to co-opt the native T cell receptor for cellular activation and retain the MHC-independence of CARs. Comparatively, TAC T cells display enhanced anti-tumor activity and diminished cytokine production *in vivo* relative to CAR T cells.

Methods

Activated human PBMCs were transduced with lentivirus encoding the chimeric receptors. For RNAseq, T cells were flow sorted based on

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expression of the chimeric receptor and either CD4 or CD8. Total RNA from three biological replicates was sequenced via Illumina HiSeq platform. Flow cytometry was used to analyze expression of chimeric receptors, memory markers, checkpoint receptors, cytokine production, and T cell proliferation. For proliferation assays, T cells were labeled with CFSE and stimulated with either antigen-coated beads or antigen-expressing cell lines.

Results

Unsupervised hierarchical clustering from RNAseq determined that, regardless of donor, non-stimulated TAC and control T cells had very similar transcriptomes, whereas CAR T cells had a distinct transcriptional profile. Transcripts associated with effector T cells were differentially expressed in CAR T cells relative to TAC and control T cells, suggestive of elevated basal signaling in the CAR T cells. CARs produced cytokines and displayed elevated expression of checkpoint receptors in the absence of antigen exposure, indicative of auto-activation. Consequently, TAC T cells retained a less differentiated phenotype than CAR T cells when analyzed for memory-associated markers such as CD45RA and CCR7. To better understand antigenic requirements for activation, T cells were stimulated with antigen on beads or antigen on cells. While CAR T cells reacted to antigen regardless of the context, TAC T cells responded weakly to protein on beads, demonstrating that TAC T cells have enhanced ability to distinguish antigen context.

Conclusions

These studies confirm that TAC receptors and CARs operate through distinct biology. Understanding the differences between the two chimeric receptors will aid in the selection of diseases that is most suitable for treatment with the individual receptors.

P186

Molecular nanomachines for in situ reprogramming of innate immunity

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Background

Current approaches to adoptive immunotherapy with reprogrammed natural killer (NK) cells present significant obstacles – they are cumbersome and require manipulation, expansion and cryopreservation. Personalized immunotherapies with NK cells have also proven difficult because in vivo, they are inhibited by the tumor microenvironment's chronic immune suppressive signals, and are resistant to the uptake of exogenous genetic material. [1] We discuss the development of precision molecular tools for the generation of biodegradable, genome-modifying nanomachines that reprogram, in situ, the function of NK cells, without the need for ex vivo expansion, and can be tracked with precision.

Methods

We synthesized novel nanostructures based on cationic oligomers and quaternary-ternary lipids with multivalent headgroups and disulfide linkers, and polymers based on poly(β -amino esters). Incorporation of NKG2D-DAP10-CD3 ζ CARs imparts tumor-targeting ability. Persistent CAR expression is achieved by flanking the gene expression cassette with piggyBac inverted terminal repeats. Continued stimulation is achieved by co-delivering IL-15 and IL-18 genes. The nanomachines also carry transcription activator-like effector nucleases (TALENs) to genomically impart cells with resistance to TGF- β inhibition via a dominant-negative TGF- β type II receptor, DNT β R11.

Results

The structure-function relationships between nanoparticle composition and intracellular

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trafficking pathways have uncovered structural features driving intracellular motion. On the lipid construct side, we investigated whether lipid headgroups with cationic amine combinations favor Brownian diffusion-driven nucleic acid trafficking, while ester-linked quaternary amines favor cytoskeletal delivery. Improved genetic trafficking was obtained with multivalent cationic lipid-based carriers, while the membrane surface charge was critical in directing motion. Incorporation of a hydroxyalkyl chain capable of hydrogen bonding to neighboring headgroups increases hydration. Serum-resistant nanoparticles with positively-charged poly(β -amino ester) and derivatives are also being studied as biodegradable vehicles, and to generate dual-construct nanostructures. "Coats" of serum-resistant amphiphiles are added to avoid off-target degradation. Ex vivo and in vivo studies are uncovering the functional extent of TALENs-carrying nanomachines.

Conclusions

Taking cues from the efficiency with which viruses infect healthy cells, we are developing nanomachines that self-assemble and disassemble when needed and have the capacity to selectively reprogram NK cells. In doing so, we are challenge the traditional notions of nanoparticle-mediated NK cell engineering and molecular imaging to change the clinical paradigm for personalized in situ immunotherapies. [2]

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P187

Efficient non-small lung cancer targeted therapy with TCR-T cell transfer engineered for TGF β blockade

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Background

Adoptive T cell therapy (ACT) has showed promising clinical responses for patients with advanced cancer, though more work needs to be accomplished to enhance the effector functions of T cells, as well as counter the immunosuppressive tumor microenvironment (TME). The cancer testis antigen NY-ESO1 is expressed in many cancer types, including Non-Small Lung Cancer (NSLC). NY-ESO1 targeted T Cell Receptors (TCR) have been successfully used to engineer T cells for ACT. We hypothesize that incorporating a dominant-negative TGF β receptor II (dnTGF β RII) into the NY-ESO-1 TCR construct to transduce T cells will render these cells insensitive to the immunosuppressive effects of TGF β , and augment their effector function in the TME.

Methods

We constructed retroviral vectors with an MHC I HLA-A2.1 NY-ESO1 TCR with and without a second transgene, dnTGF β RII interspaced with a P2A self-cleaving element. NSG mice were inoculated with HLA-A2.1+, NY-ESO+ lung cancer cells subcutaneously. PBMCs from healthy volunteers were activated with OKT3 antibody plus IL-2 for two days. The activated PBMCs were then transduced with NY-ESO1 TCR with/without dnTGF β RII. The transduced cells were analyzed by flow cytometry to confirm NY-ESO1 TCR expression before injection. Equal numbers of TCR+ cells were injected 17 days after tumor inoculation. Mice were then followed for tumor growth. The spleens and tumors were collected for further analysis, including

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Immunophenotype and functional assays at the endpoint.

Results

We have consistently obtained more than 70% retroviral vector transduction efficiency as assessed by surface expression via clonotypic V β staining. The transduced NY-ESO1 TCR with dnTGF β RII cells were able to resist the progression of inoculated tumor for 49 days ($p < 0.01$). We found that adoptively transferred NY-ESO1 TCR with dnTGF β RII cells were effectively infiltrated into tumor microenvironment in higher numbers than in control and eliminated lung cancer cells. Tumor harvest analysis demonstrated that regulatory T cells were significantly lower in NY-ESO1 TCR with dnTGF β RII cells transferred group and characteristic memory phenotypes were observed in these mice. We also obtained data that indicate that epigenetic modulation of NSLC lines can efficiently upregulate NY-ESO1 and therefore augment the effect our ACT platform.

Conclusions

Our results strongly support that T cells engineered with NY-ESO1 TCR with dnTGF β RII more efficiently target NSLC tumors *in vivo*, and this provides a rationale to implement our platform towards clinical translation for this difficult to treat cancer.

P188

A universal killer T cell for adoptive cell therapy of cancer

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Background

T cell-mediated immunotherapy of cancer has achieved remarkable results in hard-to-beat cancers. The main challenge of adoptive T cell transfer (ACT) is its labour intensive and costly production and logistics as well as its dependency on the quality of the patient's T cells

Methods

To overcome these hurdles we have designed a universal cell line for TCR expression by modifying the FDA-approved NK cell line, NK-92. Advantages of using this cell line is that it is easy to expand and can readily be genetically engineered. However, tumor cell recognition and killing by NK-92 is not antigen specific. This can be controlled by introducing an antigen receptor, such as a chimeric antigen receptor (CAR) or, as in the current work, a TCR. We herein present evidence that NK-92 can be modified to become a T cell-like lymphocyte which we named UK-92 cell (Universal Killer).

Results

UK-92 expressing a therapeutic TCR showed conserved binding capacity to the cognate pMHC. Phosphoflow cytometry results indicated that the introduced TCR was able to mediate intracellular signaling upon either crosslinking or cognate pMHC binding. Our data showed that both early and late TCR signalling players were activated in a TCR-specific manner (anti-CD3/anti-CD28 stimulation) and further in a pMHC specific manner. *In vitro* functional assays using TCRs isolated from both CD8 and CD4 T cells demonstrated that UK-92-TCR could be stimulated in a pMHC-specific manner and, importantly, could kill tumor cells specifically. We have now shown *in vitro* that UK-92 cells are as specific and potent as redirected T cells to kill target cells. Finally, encouraging *in vivo* data showed that mice receiving UK92 cells expressing a therapeutic TCR experienced reduction in tumor load and enhanced survival compared with control mice.

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Conclusions

If confirmed, the use of UK92 as a universal cell line might pave the way to truly off-the-shelf therapeutic effector cells for cancer immunotherapy and leading to drastic reduction of cell production time, logistic and cost.

P189

Cytotoxic dendritic cells have efficient anti-tumor capacity of in a murine T cell lymphoma model

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Background

Murine bone marrow-derived dendritic cells are known to possess cytolytic activity that can be directed against various tumor targets. We tested whether this activity can be used to treat tumors in several murine models.

Methods

We used bone marrow-derived dendritic cells as effectors and the solid tumors B16, MC38, CT26 as well as the novel T cell lymphoma line SJ3 as target cells in vitro. For in vivo studies, mice carrying B16, MC38 or SJ3 were treated with combinations of dendritic cells that had been amplified from bone marrow in culture, GM-CSF and the TLR ligand R848.

Results

Co-cultures with tumor cells showed a strong cytolytic capacity of dendritic cells directed against B16, MC38, CT26 or SJ3 when activated with interferon-gamma and/or TLR ligands. MHC mismatches between DCs and tumor cells did not appear to change the outcome.

We attempted to translate these findings into in vivo settings of murine tumor models. Survivals of mice that carried intravenously injected B16 or MC38 were increased by treatments with GM-CSF and the TLR7/8 ligand R848 but an additional

therapy with bone marrow-derived dendritic cells did not change the outcome. In contrast, the survivals of mice that carried the SJ3 T lymphoma line were significantly prolonged in response to DC cell injections.

Conclusions

Our study adds to our understanding of dendritic cell physiology. It points to the possibility to target their cytotoxic capability in the treatment of T cell lymphomas regardless of MHC compatibility.

P190

Patient specific automated dendritic cell generation in a closed system

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Background

Dendritic cells (DCs) are an attractive vehicle for therapeutic manipulation and are used in antigen-pulsed autologous DC therapies and DC-stimulated autologous T cell therapies [1, 2]. Given the low abundance of DCs in blood, they are typically generated *ex-vivo* from monocytes or stem cell precursors using a time-consuming, labor-intensive method that is subject to user-variability. We have developed an automated closed system, MicroDEN (Figure 1), for generating DCs that overcomes these challenges. Similar to the standard plate culture used for dendritic cell generation, MicroDEN uses plastic adherence to enrich monocytes from a mixed PBMC population and, in addition, uses medium perfusion to supply fresh cytokines throughout the duration of monocyte differentiation to DCs.

Methods

Peripheral blood mononuclear cells were seeded into the culture chamber of MicroDEN at a density of 690,00 cells/cm². Monocytes were enriched by

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plastic adherence and cultured for 7 days in the presence of 500 U/mL IL-4 and 800 U/mL GM-CSF in RPMI medium containing 10% FBS, which was perfused continuously throughout differentiation. Simultaneous cultures were set up in 6-well plates as a control. Cells were harvested at day 7.

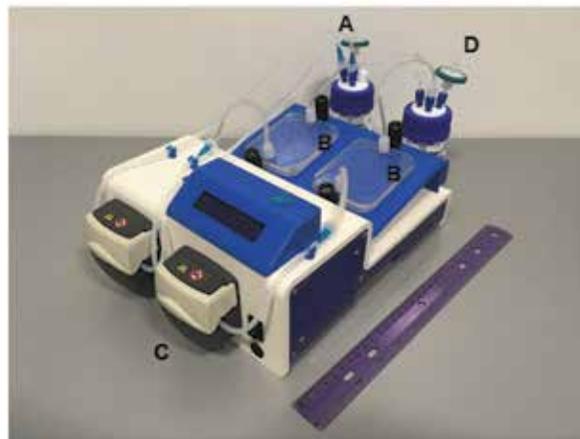
Results

Flow cytometry data indicated that the iDCs from MicroDEN and 6-well plates were phenotypically comparable with regard to expression of DC-SIGN (CD209), CD80, CD83, CD86 and CD14 (Figure 2). Allogeneic T cell proliferation assays and an antigen-specific assay demonstrated that iDCs generated via MicroDEN are functionally competent and can successfully generate a T cell response (Figure 3). A higher number of viable iDCs were harvested from MicroDEN (~2 million iDCs) compared to 6-well plates (~0.3 million iDCs). Moreover, the iDC yield relative to input PBMCs was higher for MicroDEN at 8.4% compared to 4.8% for 6-well plates, indicating that MicroDEN can generate iDCs at a higher efficiency than well plates.

Conclusions

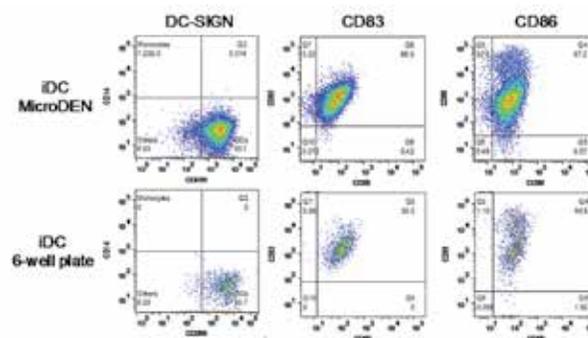
DCs generated by MicroDEN are functionally equivalent to DCs generated by the standard well plate culture. The larger surface area of MicroDEN cartridge allows generation of a higher number of dendritic cells in single run when compared to the standard 6-well plate, thereby reducing time and labor involved in harvesting cells from multiple plates along with variability.

Figure 1. MicroDEN device



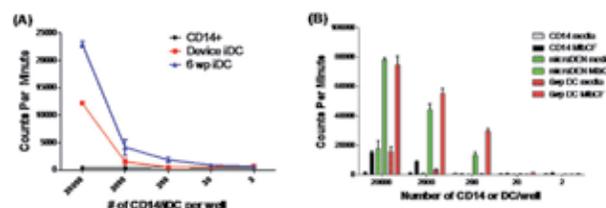
The inlet reservoir (A) containing medium is connected to the culture chamber (B) via a pump (C) to ensure continuous medium perfusion. The effluent medium is collected in outlet reservoir (D).

Figure 2.



Immature dendritic cells generated by MicroDEN (top row) have comparable surface marker profiles to those generated by standard 6-well plate culture (bottom row).

Figure 3.



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T cell response to immature dendritic cells generated in MicroDEN is equivalent to DCs generated in a 6-well plate as measured by (A) Allogeneic T cell proliferation assay and (B) Antigen-specific assay.

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P191  **Abstract Travel Award Recipient**

Limiting antigen escape in multiple myeloma by dual antigen-targeting of BCMA and TACI using the natural ligand APRIL

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Background

Recent clinical trials of CAR T cells directed against B cell maturation antigen (BCMA) have led to responses including complete remission in patients with multiple myeloma. However, treatment failure due to antigen-loss of BCMA has already been described. The transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) is thought to have a redundant role to BCMA in maintaining cell survival, and is highly expressed on multiple myeloma cells. In this study, we utilized the natural ligand for BCMA and TACI, a

proliferation inducing ligand (APRIL), as a CAR binding moiety. With this approach we aim to prevent disease relapse due to antigen-escape by dual targeting of multiple surface antigens.

Methods

We generated CAR constructs with scFv-based anti-BCMA, and APRIL-based CARs bearing different hinge and transmembrane domains (CD8 or 4-1BB), all fused to 4-1BB and CD3 zeta. Human primary T cells were lentivirally transduced with anti-BCMA-CAR or APRIL-based CARs. Cytotoxicity, proliferation and cytokine production was evaluated in vitro against a panel of cell lines with varying expression levels of BCMA and TACI and in vivo in a xenograft model of multiple myeloma.

Results

Activation in response to BCMA+ or TACI+ target cells, were seen for APRIL-based CARs. Anti-BCMA-CAR was only activated in response to BCMA+ target cells. Both BCMA and APRIL-CD8 hinge/transmembrane CARs displayed antigen-specific cytotoxicity. Interestingly, we found lower levels in cytokine production for APRIL-based CARs compared to anti-BCMA-CAR. This observation is likely to reflect the difference in binding affinity between using APRIL or an scFv as CAR binding moiety. Altering the hinge/transmembrane domain to 4-1BB in the APRIL-CAR led to a reduction in cytotoxicity. Ongoing studies, using a xenograft model have shown complete tumor remission in some mice treated with anti-BCMA-CAR or APRIL-CD8 hinge/transmembrane CAR.

Conclusions

In this study, we designed a CAR, based on the natural ligand APRIL, able to recognize both BCMA and TACI in order to limit potential antigen-escape in multiple myeloma. We found that inclusion of the CD8 hinge and transmembrane region was essential for APRIL CAR function; this region could not be replaced with 4-1BB, despite the fact that both APRIL and 4-1BB are members of the TNF receptor superfamily and bear greater similarity to each other than to CD8. Despite the cytotoxic efficacy of

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the APRIL CAR against tumor cells, lower levels of effector cytokine production was seen. This is an important finding, since CAR T cell therapy can lead to cytokine release syndrome.

P192

Oncolytic viruses effectively deliver chimeric antigen receptor targets to triple negative breast cancers

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Background

Chimeric antigen receptor (CAR) T cell therapy is a promising immunotherapy that has shown impressive clinical responses against CD19+ B-cell hematological malignancies [1]. While significant efforts are underway to translate CAR T cell therapy to solid cancers, the restricted antigen expression of targets such as CD19 is largely absent beyond B-cell malignancies [2]. It is widely appreciated that some solid tumors, like triple-negative breast cancer (TNBC), lung cancer, and liver cancer lack amenable tumor antigens to be effectively and safely targeted by CAR T cells [3]. Thus, novel approaches – particularly ones that are capable of selectively targeting intractable solid tumors – are desperately needed to improve clinical outcomes. Oncolytic viruses (OVs) are a novel and attractive form of immunotherapy for treating tumors since it can target tumor cells selectively – even in the absence of tumor specific antigens.

Methods

We have developed a chimeric OV expressing truncated CD19 (CD19t) to create a targetable antigen on human TNBC cells. PBMCs were isolated from healthy donors, which were lentivirally transduced to express CD19-CAR. Flow cytometric

analysis was used to determine killing and activation of CAR T cells. In addition, supernatants of co-culture assays were collected to determine cytokine levels. As a positive control, TNBC cells were lentivirally transduced to express CD19t. NSG mice were used for *in vivo* studies with subcutaneous tumors, which were infected with OV followed by treatment with CAR T cells.

Results

OV effectively delivered CD19t in triple negative breast cancer cells in a time and multiplicity-of-infection dependent manner. OV infected tumor cells expressing CD19t were bona fide targets of CD19-CAR T cells. OV delivery of CD19t in triple negative breast cancer cells induced functional activation and antigen specific tumor targeting by CD19-CAR T cells.

Conclusions

Our studies have harnessed the exquisite capability of OV to selectively deliver expression of genes in tumors, in our case CD19t, such that TNBC now become de-novo targets for CD19-CAR T cells. By using this combinatorial strategy, we have broadened the utility of CD19-CAR T cells to otherwise target-less tumors, which we anticipate can be applied to a wide array of solid cancers as an effective immunotherapy approach.

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TRuC™-T cells: a novel class of engineered T cells that power T cells through the entire T cell receptor complex without MHC restriction

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Background

T cells expressing chimeric antigen receptors (CARs) have demonstrated remarkable clinical benefit in certain hematological malignancies but so far have struggled to show efficacy in patients with solid tumors. This could be because CARs bypass the complex TCR signaling by utilizing only the CD3 ζ chain in combination with a co-stimulatory domain and therefore fail to initiate a complete TCR signaling cascade that is necessary to overcome the immunosuppressive tumor microenvironment. Here, we present the preclinical evaluation of a novel T cell engineering platform, which is based on T Cell Receptor Fusion Constructs (TRuCs) that are integrated into the TCR and target CD19⁺ tumors in a MHC non-restricted fashion.

Methods

TRuC™ variants were constructed by recombinant fusion of an anti-CD19 scFv (FMC-63) to various TCR subunits via a flexible linker sequence. Likewise, CD19-specific CD28 ζ and 41BB ζ CARs were generated for side-by-side comparison. After stimulation and lentiviral transduction, human primary T cells were analyzed for TRuC™ or CAR surface expression. TRuC™ TCR complex integration was assessed by immunoprecipitation and Western blot analysis. *In vitro* functional assays were conducted using both a luciferase based cytotoxicity assay and an impedance-based cytotoxicity assay,

and cytokine release was measured by Luminex. To evaluate the constructs *in vivo*, NOD-SCID-IL2Rg^{null} mice were treated with T cells four days after subcutaneous injection of the Raji tumor cell line.

Results

Of the five CD19-TRuC™ variants tested, the ϵ -TRuC™ variant consistently demonstrated highest surface expression and T cell activation. Biochemical and signaling pathway analysis revealed that TRuC™ variants incorporated into TCR complex and utilized the natural T cell receptor signaling machinery. *In vitro* anti-tumor activity of the CD3 ϵ TRuC was comparable to that of the CD28 ζ and 41BB ζ CARs. However, ϵ -TRuC™ T cells released significantly less cytokines compared to CAR T cells. Intriguingly, TRuC™ T cells were more potent than CD28 ζ or 41BB ζ in clearing solid tumors in a subcutaneous Raji mouse model.

Conclusions

We demonstrate that TRuC™ variants can effectively reprogram T cells to recognize tumor surface antigens in a non-MHC-restricted fashion. TRuC™s are distinct from CARs in their ability to activate T cells through the entire TCR without and do not require additional costimulatory domains. While TRuC™ T cells were equally potent as CAR T cells in eliminating tumor cells *in vitro*, they induced less cytokine release by T cells. We believe that these features make the TRuC™ platform a superior approach to treat cancer with engineered T cells.

P194

Studies of key quality attributes for TIL product, LN-144

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Background

Adoptive T cell therapy with autologous tumor infiltrating lymphocytes (TIL) has demonstrated clinical efficacy in studies conducted by other institutions in patients with metastatic melanoma and cervical carcinoma. Most clinical study reports include exploratory analyses of the infused TIL products intended to identify quality attributes that could relate to product efficacy or safety. We describe progress in the development of a quality control platform for use in the commercial manufacturing of TIL products based on process development studies conducted at Iovance and our experience with the production of investigational TIL products.

Methods

The process of generating an autologous TIL product includes a pre-Rapid Expansion Protocol (pre-REP), in which tumor fragments of 1-3 mm³ size are placed in media containing IL-2. During the pre-REP, TIL emigrate out of the tumor fragments and expand in response to IL-2. To further stimulate TIL growth, TIL are expanded using a secondary culture period termed the Rapid Expansion Protocol (REP) that includes irradiated PBMC feeders, IL-2 and anti-CD3. Pharmaceutical products are required to meet analytical criteria that uniquely and appropriately reflect key features of identity, purity, and potency of the product. The identity and purity of cell populations present in TIL products are extensively characterized through flow cytometry analyses of cell surface markers. Flow cytometry is also used to screen for the potential presence of residual melanoma tumor cells in REP TIL products through quantitative assessment of Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP) expression. Functional analyses relevant to product potency include the measurement of IFN- γ secretion in the presence of beads coated with monoclonal antibodies (CD3, CD28, and CD137).

Results

TIL products manufactured by Iovance are composed of greater than 97% CD45⁺CD3⁺ cells. Non-T cell population including B cells and NK cells

represent fewer than 3% of cells within the final TIL product. Residual melanoma cells in TIL products were below the limit of detection (< 5 cells/10⁶) TIL using a specialized assay developed for this assessment. IFN- γ secretion by the TIL product following anti-CD3/CD28/CD137 re-stimulation was consistently > 200 pg/10⁵ TIL.

Conclusions

Commercial manufacturing of TIL products will require a robust analytical platform to ensure consistent delivery of products meeting the critical quality attributes of cellular therapeutics. Our ongoing research in the development of analytical methodology to fully characterize product identity, purity, and potency guides the establishment of formal release criteria to be implemented in commercial production of TIL products at Iovance.

P195  Abstract Travel Award Recipient

Anti-CD37 chimeric antigen receptor T cells: a new potential therapeutic option for B-cell malignancies

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Background

CD37 is a tetraspanin expressed on mature B cells but absent on early progenitors or terminally differentiated plasma cells. CD37 is highly expressed on malignant B cells in non-Hodgkin lymphomas (NHL), including mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), Burkitt lymphoma and B-cell chronic lymphocytic leukemia (CLL); thus, CD37 represents a promising target for B-cell malignancies, particularly for variants that escape existing therapies targeting the common B cell antigens CD19 and CD20

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Methods

We designed the first anti-CD37 CAR (CAR-37) for the treatment of B-cell malignancies. Specifically, we designed a second-generation CAR, encoded by a lentiviral vector and bearing a 4-1BB costimulatory domain. We tested two different orientations of a humanized murine antibody-derived single-chain variable fragment (V_L - V_H or V_H - V_L) and have generated a pre-clinical data panel to select the most stable and efficacious format

Results

In vitro cytotoxic activity of CART-37 cells was evaluated by co-culturing CART-37 cells with CD37-expressing human tumor cell lines (RAJI, OSU-CLL and JEKO-1) at different effector to target ratios. CD37-directed CAR T cells demonstrated antigen-specific activation, proliferation, cytokine production, and cytotoxic activity in vitro in multiple models of B cell malignancy. Next, we assessed the anti-lymphoma efficacy in vivo in a mantle cell lymphoma model. CAR-37 treatment eliminated the tumor cells within 2 weeks, and mice maintained durable remissions. We were able to detect CAR T cells in the blood of mice after 7 days of injection. Ongoing studies are evaluating the long term persistence of CAR T cells in mice

Conclusions

Taken together these results show that T cells expressing anti-CD37 CAR have substantial activity in vitro and in vivo against B cell malignancies. These findings indicated that CD37-CAR T cells are a novel potential therapeutic agent for the treatment of patients with CD37 expressing tumors

P196

FGFR4 specific chimeric antigen receptor (CAR) T cell therapy against rhabdomyosarcoma

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Background

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in childhood and adolescence with an annual incidence of 4.5 cases per 1 million children. Patients with high-risk metastatic disease have dismal prognosis and require newer therapeutic approaches. Whole genome and exome sequencing have shown that RMS has a low mutational burden with few directly actionable somatic targets. The fibroblast growth factor receptor 4 (FGFR4) cell surface protein is an attractive therapeutic target as the *FGFR4* gene is overexpressed in RMS and FGFR4 protein has been shown to be critical for cell survival, proliferation, and metastasis. In addition, activating mutations in the kinase domain of FGFR4 leads to aggressive tumor growth and RMS metastasis. Here, we hypothesize that FGFR4 provides a specific target for immune-based therapy of RMS. In particular, we are developing T cells genetically modified to express chimeric antigen receptor (CAR) targeting FGFR4.

Methods

To verify specific expression of FGFR4 protein we performed both immunohistochemistry (IHC) and electrochemilluminescence (ECL) ELISA assays. To construct CAR-T cells targeting FGFR4, we first screened a single-chain variable fragment (scFv) cDNA library, identified ten specific human anti-FGFR4 scFv binders and cloned into prokaryotic expression vector containing the human IgG1 Fc region. In addition, we have scFv derived from mouse monoclonal antibodies (mAbs) generated from hybridoma technology that is also being tested in CAR format.

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Results

We confirmed a significantly increased staining for FGFR4 protein on RMS primary tumors, compared to normal tissues by IHC. FGFR4 expression measured using quantitative ECL assay shows an average of 10 fold higher expression in RMS cell lines compared to normal tissue.

ScFv-Fc binders and the mouse mAbs developed against FGFR4, were first assayed for binding to cell surface FGFR4 on RMS cell lines. Anti-FGFR4 binders also showed positive binding on the cell surface of RMS cell lines. Finally, these anti-FGFR4 scFv FGFR4 CAR constructs were further assessed in human T cells. Four out of ten FGFR4 CAR constructs tested showed cell-mediated cytotoxicity against RMS cell lines. Two lead FGFR CAR, BT53 (murine) and M410 (human), are highly potent in inducing gamma interferon, TNF alpha and cytotoxicity when the FGFR4-CART are co-cultured with RMS cells. Preliminary *in-vivo* testing of M410 has shown to be effective in eliminating RMS cells in xenograft models.

Conclusions

We demonstrated that FGFR4 is a novel target for immune-based therapy. FGFR4 CAR-T cell therapy offers a potential novel therapeutic intervention for high-risk, refractory and relapsed RMS patients.

P197

Withdrawn

P198

Bispecific antibody armed activated T cells can target drug resistant pancreatic cancer cells and cancer stem like cells effectively.

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Background

Pancreatic cancer has the worst survival rate of all cancers. The multidrug resistance (both intrinsic and acquired) is thought to be a major reason for chemotherapeutic ineffectiveness of pancreatic cancer. The multidrug resistance (MDR) phenotype is mostly contributed by the members of the ATP-binding cassette (ABC) transporter superfamily and have been shown to be key mediators of drug efflux and drug resistance in many tumor types. Another player to the refractory pancreatic cancer is the presence of CD44⁺/CD24⁺/EpCAM⁺ cancer stem like cells (CSCs) that may contribute to the high recurrence rate after clinical remission. Improved and novel therapeutic strategies are needed for pancreatic cancer. This study was designed to investigate whether bispecific antibody armed activated T cells (BATs) can target drug resistant pancreatic cancer cell lines.

Methods

We used two chemotherapeutic drugs, gemcitabine and cisplatin for generating drug-resistant pancreatic cancer cell lines. MiaPaCa-2 and L3.6, were exposed step-wise to increasing concentrations of gemcitabine and cisplatin ranging from 0.05 to 1.0 μ M. When the cells were adapted to a dose of gemcitabine or cisplatin, the concentration was increased gradually. After 3-4 months of selection, MiaPaCa-2 and L3.6 cells were able to survive at 1.0 μ M of gemcitabine and 0.5 μ M of cisplatin for both lines.

Results

Flow cytometry data showed increased proportion of CD44⁺/CD24⁺/EpCAM⁺ cancer stem like cells as well as increased number of ABC transporter ABCG2 positive cells in drug resistant cell lines compared to the parental cell lines. Bispecific antibody (OKT3 x anti-EGFR [EGFRBi] or OKT3 x anti-HER2 [HER2Bi]) armed activated T cells from 6-7 normal donors were as effective in targeting gemcitabine or cisplatin resistant MiaPaCa-2 (Median cytotoxicity was 18% compared to 13% of parental cells) and L3.6 (Median cytotoxicity was 44% compared to 30% of parental cells) cell lines as parental non drug

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treated cell lines, produced Th₁ cytokines, IFN- γ and TNF- α , and chemokines, MIP-1b and RANTES.

Conclusions

These data suggest that BATs mediated killing of chemoresistant tumor cells and release of Th₁ cytokines may modulate the tumor microenvironment to enhance anti-tumor immune responses.

Trial Registration

NA

P199

IDO1-mediated tryptophan depletion potently inhibits CAR-T functionality as part of a CAR-T driven adaptive immune resistance response in the tumor microenvironment

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Background

Chimeric antigen receptor T cells (CAR-T) are engineered antigen-specific T cells that are poised to kill tumor cells and release inflammatory cytokines, including interferon- γ (IFN γ), into the tumor microenvironment (TME). Unlike endogenous intratumoral T cells, CAR-T generally are highly functional upon infusion and may produce a robust pro-inflammatory milieu in the TME. In a mechanism termed adaptive immune resistance [1], tumor cells can upregulate immunosuppressive pathways in response to inflammatory cytokines. One such pathway, indoleamine 2,3-dioxygenase (IDO1), can limit effector T cell activity through the catabolism of tryptophan, resulting in local amino acid deficiency and accumulation of tryptophan catabolites [2]. Here we evaluated the effect of IDO1 induction in response to multiple CAR-T products.

Methods

Anti-CD19 and anti-ROR1 CAR-T, containing 4-1BB co-stimulatory endo-domains, were generated using healthy donor peripheral blood samples, and exemplary full-scale manufacturing processes. IDO1-mediated effects on CAR-T proliferation and cytolytic activity *in vitro* were evaluated in the presence of IDO1-inducible, target antigen-expressing cell lines and the IDO1-specific inhibitor, epacadostat. *In vivo* studies utilized a xenograft mouse model with subcutaneous tumors expressing CD19, and capable of upregulating IDO1. Anti-CD19 CAR-T were administered to animals in combination with epacadostat or vehicle.

Results

Antigen-stimulated CAR-T induced IDO1 expression in tumor cell lines, likely through secretion of IFN γ , resulting in tryptophan depletion and accumulation of kynurenine. Activation of the IDO1 pathway substantially inhibited CAR-T proliferative capacity, which appeared to be restored in the presence of epacadostat. Studies with tryptophan-free medium indicated that the observed inhibition of CAR-T cells was primarily driven by tryptophan depletion. Mouse models demonstrated that CAR-T may derive a benefit *in vivo* when administered concomitantly with epacadostat.

Conclusions

It was observed that CAR-T could induce quiescent immunosuppressive mechanisms in the TME through secretion of IFN γ . Upon upregulation of IDO1, tryptophan was depleted and the integrated stress response appeared to be activated in CAR-T, limiting proliferation. The IDO1-specific inhibitor epacadostat blocked IDO1 activity and restored CAR-T function. Combination of epacadostat with current CAR-T therapies may be useful across multiple indications, including hematologic and solid tumors. Such a combination may counteract a suppressive mechanism of tryptophan starvation limiting CAR-T performance in the TME, and ultimately, therapeutic efficacy.

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P200

The use of *ex-vivo* stimulation to generate a neoantigen specific T cell product for adoptive T cell therapy

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Background

A growing body of evidence supports the role of neoantigens as important targets for anti-tumor immune responses. Adoptive T cell therapy is a powerful modality to treat patients with solid cancers and has demonstrated success in the treatment of melanoma. Current approaches largely focus on products generated from the non-specific expansion of tumor infiltrating lymphocytes (TILs) or the genetic modification of T cells with T cell receptors (TCRs) that recognize tumor associated antigens such as NY-ESO1. Here, we describe an approach to generate neoantigen specific T cell therapies that are specific to and personalized for each individual patient. We have developed an *ex vivo* method to generate a neoantigen specific T cell product for use as an autologous adoptive T cell therapy. The goal of this method is two-fold: (i) to broaden the tumor-specific T cell repertoire by inducing neoantigen specific T cells from the naïve T

cell compartment and (ii) to expand pre-existing, neoantigen-specific memory T cell responses.

Methods

The model antigens chosen for these studies mimic the type of neoantigens present within a patient's tumor. Naïve T cell responses were induced and studied using neoantigens previously identified from the literature and memory T cells were expanded and studied using viral epitopes against Cytomegalovirus (CMV), Epstein-Barr Virus (EBV), and Influenza. For T cell stimulations, autologous dendritic cells (DCs) were generated from healthy donor apheresis products, loaded with model antigens, and used to stimulate autologous T cells. Induced T cells were harvested and analyzed at different timepoints throughout the stimulation protocol to assess the following: antigen-specificity, functionality, phenotype, and fold expansion.

Results

We demonstrate here the successful induction of both naïve and memory T cell responses from healthy donor material. These T cell responses are:

- Preferentially responsive to mutant epitopes and not their wild-type counterparts
- Have multiple functions (cytokine production and degranulation)
- Show a mostly central memory phenotype.

Can be completed in a timeframe that is therapeutically appropriate for patients with advanced or metastatic cancer.

Conclusions

Finally, supporting data will be presented demonstrating successful induction of personalized neoantigen specific T cell responses in patient donor material.

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P201

Targeting heterogeneous glioblastoma using chlorotoxin-redirected CAR-T cells

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Background

Chimeric antigen receptor (CAR)-modified T cells have shown indications of clinical promise against B cell malignancies yet inconsistent efficacy on solid tumors. One of the challenges of CAR therapy for treating solid tumors is the heterogeneity of tumor cells, which allows for antigen escape and tumor recurrence. Glioblastoma (GBM) is one of the most lethal cancers, displaying considerable heterogeneity across patients as well as among intratumoral subpopulations. Therefore, future progress when applying CAR therapy to GBM requires targeting the majority of tumor cells, which would potentially limit opportunities for antigen escape. Chlorotoxin (CLTX) is a peptide component of scorpion venom which, given its demonstrated capability to bind with a wide-range of brain tumors, has been used for identifying tumor cells and for drug delivery. These results open the potential to exploit CLTX for directing T cells against GBM.

Methods

First, we used a fluorescence-conjugated CLTX to verify its specific binding to patient-derived GBM cells and to mouse GBM cells over normal cells from various human tissues. Then T cells were engineered with a CAR utilizing CLTX as the tumor-binding domain. The CLTX-CAR T cells were tested for antitumor efficacy against GBM neurospheres derived from different patients through *in vitro* co-culture and *in vivo* orthotopic GBM models. To evaluate the safety of CLTX-CAR T cells, we tested

their cytotoxicity against normal cells and evaluated potential pathological alterations of CAR treated mice.

Results

We showed that CLTX binds to a panel of primary GBM cells and low-passage GBM spheres with negligible binding to normal cells derived from neural and other tissues. T cells expressing CLTX-directed CAR were activated when exposed to GBM cells as suggested by T cell degranulation, production of cytokines, and formation of immunological synapses. Moreover, these CAR-T cells were able to eliminate GBM cells with varying TCGA subtypes and antigen expression patterns during *in vitro* co-culture, as well as to eradicate established orthotopic GBM tumors *in vivo*. In contrast, CLTX-CAR T cells showed minor cytotoxicity against normal human cells. Consistently, despite the CLTX binding to mouse GBM cells, CLTX-CAR T cells caused no pathological alterations of normal mouse tissues, indicating that these cells have few or no off-target effects.

Conclusions

Here, we report the development of a new class of peptide-based CAR exploiting the GBM-binding potential of CLTX. Our study supports the potential utility of CLTX-CAR T cells to effectively target GBM while overcoming heterogeneity and limiting antigen escape.

P202

New therapeutic approach for central nervous system lymphoma by CD19CAR T cells

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Background

Central nervous system lymphoma (CNSL) can start in the brain, spinal cord, eye, and/or meninges or result from metastasis of systemic disease. About 90% are B cell lymphomas. Compared with systemic lymphoma, CNSL carries a worse prognosis. Only a few chemotherapeutic drugs can cross the blood-brain barrier, limiting treatment. T cells genetically engineered with chimeric antigen receptors (CARs) targeting CD19 have shown tremendous potential in the treatment of systemic lymphoma. Although CD19CAR T cell trafficking in cerebrospinal fluid (CSF) is frequently reported, most (if not all) protocols exclude patients with active CNS involvement. Here we investigate the feasibility and efficacy of CD19CAR T cells to treat CNSL.

Methods

Human B cell lymphoma Daudi cells were injected intracranially into NSG mice and allowed to engraft for 5 days. T cells genetically modified with CD19CAR lentivirus were administered via three delivery routes: intracranial local infusion (ic, 1×10^6 cells); intracerebroventricular (icv, 1×10^6 cells); and intravenous injection (iv, 3×10^6 cells).

Results

We observed in separate experiments that both a single i.c infusion and a single i.c.v delivery of CD19CAR T cells were able to completely eradicate CNS lymphoma in all mice by day 14 post CAR T cell infusion; and that a single dose of i.v infusion induced significant anti-CNSL activity with a slightly delayed response as compared to i.c and i.c.v treatment and all mice achieved complete remission 21 days post T cell infusion. CAR T cells were detected in peripheral blood obtained from retro-orbital bleeding, not only in the i.v treated mice, but also in i.c.v treated mice 28 days after CAR T cell infusion, suggesting that i.c.v not only controls CNSL but may also play a role in immune surveillance for systemic tumors. To confirm this, we established an NSG CNS B cell lymphoma model by also inoculating *subcutaneous tumors* on the animal's flank, 3 weeks prior to i.c tumor injection into the same mouse. CD19CAR T cells were delivered via i.c.v 5 days after

i.c. tumor injection. CAR T cell injection resulted in complete remission of both the brain tumor and the flank tumor 14 days after CAR T cell administration. All mice survived more than 300 days. We also found that i.c.v. delivered CD19CAR T cells were resistant to tumor re-challenge.

Conclusions

In conclusion, intracerebroventricular delivery of CD19CAR T cells is a promising and feasible therapeutic approach for both primary central nervous system lymphoma and systemic lymphoma with concurrent CNS involvement.

P203

A cryopreserved TIL product, LN-144, generated with an abbreviated method suitable for high throughput commercial manufacturing exhibits favorable quality attributes for adoptive cell transfer

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Background

Existing methods for generating clinical TIL products involve open operator interventions followed by extended incubation periods to generate a therapeutic product. IOVANCE Generation 1 process takes approximately 6 weeks and yields a fresh product. To bring TIL therapy to all patients that may benefit from its potential, IOVANCE has developed an abbreviated 22-day culture method, Generation 2, suitable for centralized manufacturing with a cryopreserved drug product capable of shipment to distant clinical sites [1]. Generation 2 represents a flexible, robust, closed, and semi-automated cell production process that is amenable to high throughput manufacturing on a commercial

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scale. Drug products generated by this method have comparable quality attributes to those generated by IOVANCE Generation 1.

Methods

Qualified methods to assess proliferation, phenotype, and function were applied to in-process and final drug products generated by both IOVANCE TIL expansion methods to determine fit within the internal target product profile. TIL expansion was assessed by triplicate automated count.

Immunophenotyping was performed to determine identity, purity, as well as relative levels of activation, memory, and exhaustion of the cell product. Cellular function was evaluated as the ability of the cell product to secrete IFN- γ in response to CD3, CD28, and 4-1BB receptor engagement.

Results

TIL cultured with the abbreviated Generation 2 method achieved doses comparable to IOVANCE Generation 1 (Generation 2: mean 4.29×10^{10} nucleated cells $n=12$, Generation 1: mean 5.84×10^{10} , $n=28$). Thawed Generation 2 drug products were similar to Generation 1 in terms of T cell purity, with similar ratios of CD4 to CD8 cells and memory subsets. Generation 2 drug products displayed an increased ability to produce INF- γ upon reactivation relative to Generation 1 ($p < 0.0001$ [MF1]).

Conclusions

The IOVANCE Generation 2 process produces a potentially potent TIL product with comparable quality attributes to Generation 1. Generation 2 products exhibit high levels of co-stimulatory molecules, low levels of exhaustion markers, and retain the ability to secrete cytokine upon reactivation. The abbreviated 22-day expansion platform allows for the rapid generation of clinical scale doses for patients in urgent need of therapy. The cryopreserved drug product introduces critical logistical efficiencies allowing flexibility in distribution. The IOVANCE expansion method

overcomes traditional barriers to the wider application of TIL therapy.

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P204

Reenergizing the tumor infiltrating T cells by intratumoral delivery of BCG to treat bladder cancer and prevent recurrence

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Background

Bladder cancer remains one of the most common malignancies, which is difficult to treat with high rates of recurrence. Given the recent astonishing clinical results in treating hematological malignancies, the adoptive cell transfer (ACT) of tumor-reactive T cells holds great promise to address this urgent need [1]. However, the efficacy of such approach is curtailed when treating solid tumors, such as bladder cancer. The primary hurdles which must be overcome for immunotherapy to be effective against bladder cancer include 1) clonal types and quantity of tumor-specific T cells adoptively transferred into tumor-bearing hosts are too low to mount adequate responses, 2) a large portion of adoptively transferred anti-tumor T cells fail to migrate to the tumor and 3) the immunosuppressive microenvironment within the tumor induces a rapid loss of T cell effector function.

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Methods

To overcome these challenges, we designed an innovative approach that capitalized the synergistic effect between pathogen-based immunotherapy and ACT for treating bladder cancer (Figure 1). The tumor-reactive CD8 T cells were genetically engineered with a secondary bacterial TCR to generate dual-specific CD8 T cells, that can recognize both a tumor associated antigen (TAA) and a bacterial antigen (BA). In this study, the bladder tumor bearing mice were treated with ACT intravenously using a small number of these dual-specific CD8 T cells and accompanied by intratumoral injection (I.T.) of a low dose of BCG.

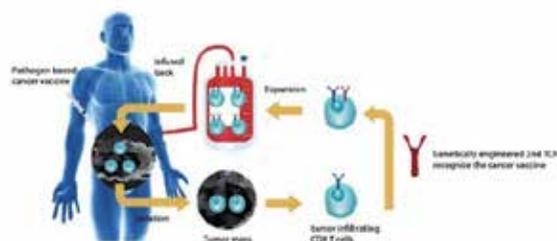
Results

The BCG infection alone sufficiently reduced the number of Treg and MDSC in the tumor microenvironment. In addition, the dual-specific CD8 T cells expanded robustly and migrated to the tumor bed in response to the infection. At the same time, these tumor-reactive CD8 T cells recognized the tumor antigen and executed killer functions, which resulted site-specific tumor regression and protection against recurrence.

Conclusions

Our approach not just breaks the tumor mediated immunosuppression, but also provokes a strong anti-tumor immune reaction that lead to primary tumor eradication and long-term protection. Overall, this proof-of-principle study demonstrated the feasibility of this strategy, and open new avenues to treat bladder cancer.

Figure 1. The conceptual model of ReACT in treating bladder cancer.



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P205

Chimeric antigen receptor (CAR) T cells incorporating linker and/or transmembrane domains derived from TNFRSF19 demonstrate superior anti-tumor activity

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Background

Chimeric antigen receptor T cells redirected to the B cell antigen CD19 (CAR19) are an emerging treatment for a range of B cell malignancies. The functional relationship between the amino acid sequence of the CAR linker, the trans-membrane domain, and CAR T function is, however, poorly understood. We replaced the extracellular linker or trans-membrane domains from previously reported CD8 constructs with novel sequences derived from the tumor necrosis factor superfamily member

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TNFRSF19, with an aim to enhance the anti-tumor activity of CAR19.

Methods

Human T cells were transduced with CAR-encoding lentiviral vectors featuring linker and trans-membrane domains of various lengths derived from CD8, TNFRSF16, or TNFRSF19. CARs expression and activity was assessed by flow cytometry, CTL activity and cytokine production.

Results

CART comprised of both linker (long or short) and trans-membrane domains of TNFRSF19 were the most potent in overnight *in vitro* killing and cytokine release assays (IFN γ , TNF α and IL-2); however, the expression of CAR molecules on the surface of transduced T cells, as measured by flow cytometry, was low or undetected. When CART and Raji tumors were co-incubated at low E:T ratio (1:1) for up to 9 days, all constructs demonstrated a surprising cyclic pattern of CAR surface up-regulation. Constructs comprised of TNFRSF19 linker domains had very low CAR expression on day 1, strong up-regulation on days 4-7, and down-regulation by day 9, as tumors were being eliminated. By contrast, the expression of CD8 linker – containing CART cells remained prominent through day 9, regardless of tumor elimination. The expression of exhaustion markers Tim-3, Lag-3 and PD-1 on the co-incubated T cells was similar between CARs with CD8 or TNFRSF19-derived linkers. CAR constructs with both linker and trans-membrane domains derived from TNFRSF19 cleared the tumors faster than CAR19 variants containing the CD8 linker and/or trans-membrane domain.

Conclusions

Here we demonstrate that direct surface detection of CAR expression may not best represent the most active CAR species, unless co-incubated with tumor target cell lines. Furthermore, TNFRSF19 derived-domains appear to be superior to CD8 linker and trans-membrane domain in *in vitro* anti-leukemia cell assays.

P206

Tumor-penetrating recombinant protein anti-EGFR-iRGD enhance the extravasation and tumor penetration of lymphocytes in gastric cancer

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Background

Efficient trafficking of T cells to the tumor site is a critical step for success of cancer immunotherapy. Cancer patients with tumors which are highly infiltrated with lymphocytes have shown enhanced survival rates. Although the gene transfer technology such as CAR-T is effective in redirecting T cell activity against tumors, the lymphocytes infiltrating in solid tumor tissues is still limited by the poor tumor penetration of adoptive cells due to the complicated tumor microenvironment such as immunosuppression and aberrant vasculature. The tumor-penetrating peptide iRGD contains both a RGD domain which can target the integrin expressing on activated T cells and a CendR motif increasing the vascular and tissue permeability. We have previously constructed a protein of bispecific targets and high permeability named anti-EGFR-iRGD, which could improve tumor penetration of antitumor drugs into extravascular tumor tissue in a tumor-specific and neuropilin-1-dependent manner.

Methods

Recombinant protein anti-EGFR-iRGD consisting of an anti-EGFR VHH fused to iRGD were expressed in *E. coli* BL21 and purified by nickel-nitrilotriacetic acid affinity chromatography. We use gastric cancer cell lines and tumor-bearing mice to examine the synergy antitumor effect of adoptive immunotherapy combine with anti-EGFR-iRGD. In

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In addition, the ability of recombinant protein anti-EGFR-iRGD to improve lymphocytes infiltration into tumors were analyzed in xenograft mouse model.

Results

In this study, no influence on cell viability, proliferation, phenotype and function was observed when lymphocytes co-cultured with anti-EGFR-iRGD in vitro. Furthermore, the recombinant protein anti-EGFR-iRGD exhibited synergy antitumor activity with adoptive cell therapy (ACT) in tumor cell lines and mice. In adoptive transfer studies, we found increased tumor extravasation and tumor penetration of our lymphocytes by the systematic co-administration with anti-EGFR-iRGD for the first time.

Conclusions

Our results provide new insights for effectively target a higher frequency of lymphocytes to the tumor microenvironment, this will be an important clinical translation to improve immunotherapy outcomes using co-administration ACT of and anti-EGFR-iRGD.

References

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Clinical Trials (Completed)

P249

A phase 1 study of the safety, tolerability, and pharmacokinetics (PK) of MGA012 (anti-PD-1 antibody) in patients with advanced solid tumors

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Background

MGA012 is a humanized, IgG4k monoclonal antibody (mAb) that recognizes human programmed cell death protein 1 (PD-1). MGA012 binds to PD-1 expressing T cells, inhibits PD-1 and PD-L1/PD-L2 interactions, and disrupts the negative signaling axis to restore T cell function. The biological activity of MGA012 is comparable to replicas of approved anti-PD-1 mAbs when assessed in vitro, including blockade of PD-1 and PD-L1/PD-L2 interactions, inhibition of PD-1 signaling, and enhancement of T cell effector function.

Methods

This phase 1, dose escalation study will characterize the safety, tolerability, PK/PD, immunogenicity, and preliminary anti-tumor activity of MGA012 administered IV every two or four weeks in patients with advanced solid tumors. MGA012 has been evaluated in sequential dose escalation cohorts (1-10 mg/kg) of 3 to 6 patients each, using a 3+3 design. Four tumor-specific expansion cohorts will be treated at the maximum tolerated dose of MGA012. Selective cohort expansion was allowed during escalation to gather further safety and PK/PD data.

Results

At the data cutoff, 33 patients (12M/21F, median age 63 years) with diverse tumor types were treated at doses from 1-10 mg/kg, including 21 patients on treatment at the time of data cutoff. MGA012 has demonstrated acceptable tolerability with no dose-limiting toxicities (DLTs). Treatment-related adverse

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events (AEs) occurred in 20/33 (61%) patients, most commonly fatigue (n=9) and nausea (n=5).

Treatment-related Grade ≥ 3 AEs occurred in 3/33 (9%) patients and include increased lipase (n=2) and vaginal ulceration/inflammation (n=1). A single treatment-related serious adverse event (SAE) has been reported (aphasia occurring in conjunction with the emergence of new brain metastases). Immune-related AEs (irAEs) were limited to rash (n=3), infusion-related reaction (n=1), and vaginal ulceration/inflammation (n=1). MGA012 has PK features consistent with other IgG4 monoclonal antibodies, as well as full and sustained receptor occupancy at all dosing levels tested, consistent with its known binding characteristics. Twenty-two patients were response evaluable at the data cutoff. Three patients have experienced unconfirmed partial responses (including ovarian, MSI-high colorectal and uterine papillary serous carcinoma), and 4 additional patients experienced stable disease as a best response. Others had radiographic progressive disease or clinical progression.

Conclusions

MGA012 has demonstrated an acceptable safety profile, predictable PK/PD, and early evidence of anti-tumor activity. Subsequent to dose escalation, patients will be enrolled on tumor-specific monotherapy expansion cohorts. Future trials also are planned for combination testing of MGA012 with T cell directed, CD3-based DART[®] molecules.

Trial Registration

NCT03059823

P207

Phase II study of anti-CD3 x anti-HER2/*neu* (HERBi) armed activated T cells (ATC) after neoadjuvant chemotherapy in patients with HER2/*neu* (0-2+)-negative stage II-III breast cancer

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Background

Patients with ER/PR negative, Her2/*neu* negative triple negative breast cancer (TNBC) have dismal outcomes as compared to other subtypes. Based on previous studies, pathological complete remission (pCR) after neoadjuvant chemotherapy is a good surrogate of long-term survival. Many studies have attempted to improve the efficacy of chemotherapy and increase the pCR rate. Currently, anti-Her2 targeting antibodies and chemotherapy are the only approved therapeutic options for primary systemic treatment. We conducted a phase II trial to evaluate safety and efficacy of infusions of anti-CD3 x anti-Her2/*neu* bispecific antibody (HER2Bi)-armed activated T cells (ATC) after neoadjuvant chemotherapy, surgery, and/or radiation for women with stage II-III operable TNBC who had residual disease at the time of surgical resection

Methods

After informed consent was obtained, patients received standard neoadjuvant chemotherapy followed by surgery. Eligible patients underwent pheresis after completion of standard therapy. T cells collected during pheresis were activated with anti-CD3 and expanded in 100 IU/ml of IL-2 to generate ATC. After culture, ATC were harvested, armed with HER2Bi, washed, and cryopreserved in 8 aliquots (~10-15 billion Her2Bi-armed ATC/ aliquot) for bi-weekly infusions for 4 weeks in combination with low dose IL-2 and GM-CSF (Figure 1). Patients then received anthracycline and/or taxane based standard regimens. Following chemotherapy, surgery, and/or radiation, patients were given ~10-15 billion Her2Bi-armed ATC twice per week for 4 weeks beginning starting no sooner than 3 weeks (\pm 1 week) after completion of standard breast cancer treatment.

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Research reported in this abstract was supported by NIH under award number R01 CA 140314.

Results

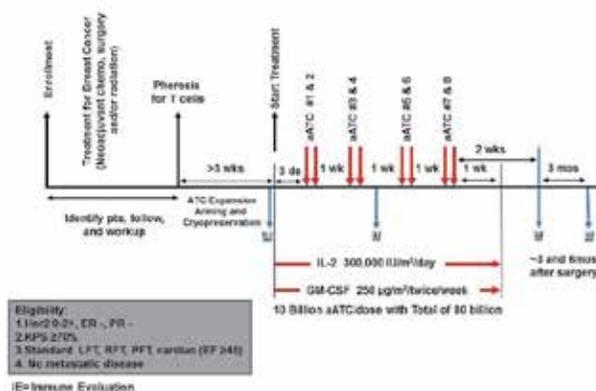
Eight patients were enrolled on the protocol. Median age was 44.5yrs (range, 38.5-52). Four patients (50%) were African American (AA). Two had T2N0, 3 T2N1, 2 T3N1 and 1 T3N2. Everyone received 8 infusions except 1 patient withdrew after 1 infusion due to a grade 3 toxicity experience.

Most common infusion associated adverse events were chills/rigors (87%), fatigue (83%), nausea (75%), fever (75%), anemia (62%), vomiting (50%), hyperglycemia (50%), neutropenia (50%), rash (38%). No dose limiting toxicity was identified in our study. After median follow up of 4.2 years, 3 of 8 patients had disease progression with median time to progression in these pts being 2.1 years.

Conclusions

We conclude that Her2Bi-armed ATC infusions in these patients were safe and it may improve progression free survival in this high risk group. This immunotherapeutic modality warrants further investigation.

Figure 1.



P208

Safety, efficacy and biology of the gp100 TCR-based bispecific T cell redirector, IMCgp100 in advanced uveal melanoma in two Phase 1 trials

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Background

Uveal melanoma (UM) is characterized by low PD-L1 expression, low mutational burden and limited efficacy with checkpoint inhibition. IMCgp100 is a bispecific T cell redirector with an affinity-enhanced TCR recognizing gp100 and an anti-CD3 scFV.

Methods

Two phase 1 trials evaluated safety, pharmacokinetics, pharmacodynamics and efficacy for IMCgp100 administered IV weekly in HLA-A2 patients (pts): a first in human (FIH) study enrolling pts with melanoma including a cohort with advanced UM (n=16, NCT01211262) and a second study of an intra-patient dose escalation (IE) regimen for pts with advanced UM (n=19, NCT02570308). Endpoints included overall response rate (ORR) by RECISTv1.1, progression free survival (PFS), and overall survival (OS). The IE Phase 1 allowed treatment beyond progression assessed by modified irRC if specific clinical criteria were met.

Results

The safety profile of IMCgp100 was consistent between trials with the most frequent adverse events (AE, any grade) including rash (90%), pruritus (90%), and edema (63%). The most frequent grade 3/4 AE was hypotension (16%, IE study, 9% FIH study). The recommended phase 2 dose (RP2D) in the FIH trial was 50 mcg (DLT of hypotension). The IE schedule was designed to mitigate toxicity, with reduced doses of 20 mcg and 30 mcg administered at weeks 1 and 2, respectively, and dose escalation at Day 15. The RP2D was 68 mcg (DLT of transaminase elevation). The ORR in the FIH and IE trials was 20% (3/15 evaluable) and 11% (2/19 evaluable), respectively. Five pts (26%) in the IE trial achieved minor responses (10-29% SLD reduction), including pts treated with prior checkpoint inhibition and with an elevated LDH. Median PFS in the FIH and IE trials (RECISTv1.1) was 3.7 months and 5.6 months, respectively. The 1 year PFS rate by irRC in the IE study was 62%. The 1-year OS rate in the FIH and IE trials is 73% (95% CI [52, 99]) and 79.5% (95% CI [55, 93]), respectively. Median OS has not been reached with a minimum of 16 and 8

months follow-up in the FIH and IE trials, respectively. Within 3 doses of IMCgp100 (day 16), immunofluorescence studies reveal an influx of PD-1+/CD8+ T cells in the tumor bed with PD-L1 expression. Peripheral cytokines indicate activation of immune responses within 24 hours of the first dose.

Conclusions

These studies demonstrate preliminary immune biology, safety and promising efficacy in advanced UM. The pivotal trial with IE dosing is open in advanced UM (NCT03070392).

P209

Randomized trial of autologous dendritic cell vaccines versus autologous tumor cell vaccines in metastatic melanoma

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Background

Metastatic melanoma cells contain high numbers of non-synonymous mutations, making this cancer a prime target for immunotherapies including patient-specific vaccines that utilize autologous tumor associated antigens (TAA). In a 74-patient trial metastatic melanoma patients treated with autologous tumor cell vaccines (TCV) consisting of autologous irradiated tumor cells (ITC) had a median overall survival (OS) of 20.5 months and 5-year OS of 28%. [1] In a subsequent 54-patient trial similar patients treated with a dendritic cell vaccine (DCV) consisting of autologous dendritic cells loaded with TAA from autologous ITC had a median OS greater than 5 years and a 5-year OS of 50%. [2] An

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open-label randomized phase 2 trial was initiated to compare TCV and DCV.

Methods

Short-term autologous tumor cell lines were established from resected metastatic lesions and served as TAA sources. Vaccines were injected with 500 micrograms of granulocyte-macrophage colony stimulating factor (GM-CSF) weekly for 3 weeks, then at weeks 8, 12, 16, 20, and 24. Key objectives were OS, adverse events (AE), and delayed type hypersensitivity (DTH) reactions to injections of intradermal tumor cells.

Results

Forty-two patients were enrolled and all treated as randomized. An interim analysis showed superiority of DCV, but median follow up was less than 2 years and minimum follow up was only 6 months. [3] As of the final analysis, all patients have been followed for 5 years or until death with no patients lost to follow up. DCV was associated with longer OS: median 43.4 versus 20.5 months (95% CI, 18.6 to >60 versus 9.3 to 32.3 months). Cox regression analysis identified tumor burden at the time of randomization and treatment arm as significant independent variables. Cox proportional hazards model revealed a 70% reduction in the risk of death in the DCV arm (hazard ratio=0.304, p=0.0053, 95% CI, 0.131 to 0.702). There was no association between efficacy and DTH reactions to tumor cell skin tests. The most common treatment-related AE were mild to moderate local injection site reactions and flu-like symptoms; grade 2 treatment-related AE were more frequent in the TCV arm.

Conclusions

As a therapeutic vaccine, DCV appears to be superior to TCV for autologous TAA presentation. Autologous DCV is associated with minimal toxicity and extended long-term survival benefit in patients with metastatic melanoma.

Trial Registration

ClinicalTrials.gov (NCT00436930)

References

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P210

Pilot trial of sipuleucel-T, with or without pTVG-HP DNA vaccine, in patients with metastatic, castration-resistant prostate cancer (mCRPC)

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Background

Sipuleucel-T is an anti-tumor vaccine approved by FDA for the treatment of advanced metastatic prostate cancer. Sipuleucel-T targets the prostate-specific antigen prostatic acid phosphatase (PAP). We have investigated PAP as a target antigen using a DNA vaccine, pTVG-HP, in patients with earlier stages of prostate cancer, and have demonstrated that this vaccine can elicit Th1-biased PAP-specific immunity. The current trial was designed to evaluate these two vaccines together, in a prime-boost fashion, to determine whether booster immunizations with pTVG-HP could augment PAP-specific effector and memory T cells following treatment with sipuleucel-T.

Methods

18 patients with asymptomatic, metastatic, castration-resistant prostate cancer were treated in a single-institution trial from 2013 to 2016 in which they were randomized to receive sipuleucel-T alone, as per standard of care, or sipuleucel-T followed by pTVG-HP administered as an intradermal injection with 200 µg GM-CSF as adjuvant every 2 weeks for 4 immunization, and then quarterly at months 6 and 9. Serial blood draws were obtained for immune

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analyses up to one year. CT scans and bone scans were performed at 3-month intervals.

Results

10/18 patients completed treatment per protocol, with 8 experiencing disease progression requiring discontinuation. No unanticipated adverse events were observed, and there were no treatment-associated adverse events greater than grade 2. Th1-biased PAP-specific T cell responses (defined as significant antigen-specific IFN γ or granzyme B ELISPOT responses that were at least 3-fold over baseline, with a frequency of at least 1:100,000 peripheral blood cells, and detectable at least twice post-treatment) were detected in 11/18 individuals, and not statistically different between study arms. Antibody responses to PAP were significantly higher in patients receiving pTVG-HP booster immunizations. Median time to progression was less than 6 months and not statistically different between study arms; median overall survival was 31 months.

Conclusions

Prime-boost vaccination with sipuleucel-T and a DNA vaccine encoding the same antigenic target is feasible, and without obvious toxicity. Th1 immunity to PAP was elicited with sipuleucel-T and was not significantly augmented with booster immunization. Antibody immunity to PAP was augmented with pTVG-HP DNA immunization, a finding that has not been observed in subjects treated with pTVG-HP alone.

Trial Registration

NCT01706458

P211

Phase 2 trial exploring allogeneic vaccine therapy in late-stage, second-line non-small cell lung cancer

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Background

Lung carcinoma is the leading cause of cancer in the United States, and non-small cell lung cancer (NSCLC) is the most common form [1]. Long-term outcome for patients with late-stage NSCLC receiving front-line chemotherapy is poor with few patients alive more than 30 months after therapy. Recent approaches using checkpoint inhibitor therapy (CPI) have shown promise, although tumors with specific genetic mutations may not respond to CPI. [2] Vaccine treatment has the potential of inducing immune responses in patients with lung cancer who poorly respond to CPI. Previous Phase 1 results in treating second-line, late-stage NSCLC with an allogeneic whole-cell vaccine transfected with B7.1 and HLA (PT 107) were promising. [3]

Methods

We randomized in a 2:1 format 94 patients with advanced (stage IIIB/IV) NSCLC to receive intradermal injection of an irradiated whole cell allogeneic lung cancer vaccine that had been engineered to express HLA-A1 and B7.1 (PT 107 arm) or placebo (control arm). Patients in the drug-treated arm received up to 9 intradermal vaccinations of PT 107 every 2 weeks.

Results

The baseline characteristics of the patients enrolled are shown below (Table 1).

Median overall survival for the PT 107 arm was 14.9 months versus 6.7 months for the control arm, with a hazard ratio of 0.71 (one-sided p=0.188). Median progression-free survival was 2.8 months (PT 107 arm) versus 2.6 months (control arm), with a hazard ratio of 0.65 (one-sided p=0.074). Median time to progression was 4.0 months (PT 107 arm) versus 2.5 months (control arm), with a statistically significant hazard ratio of 0.45 (one-sided p=0.016). Safety results were consistent with previous clinical

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experience, with no drug-related serious adverse events reported (Table 2).

Conclusions

While not powered to detect highly significant differences in outcome, this study demonstrated that treatment with PT 107 was associated with an improvement in overall survival and a significant improvement in time to progression compared to placebo therapy. These data provide strong rationale for a larger phase 3 clinical trial, as well as approaches to combine PT 107 with standard of care CPI.

Trial Registration

CTRI/2010/091/001348

Table 1. Summary of Patient Baseline Characteristics

	n	(%)	n	(%)	p-value
ECOG					0.587
ECOG Performance Status 0	17	31.9%	11	24.0%	
ECOG Performance Status 1	27	50.2%	10	22.0%	
ECOG Performance Status 2	4	7.6%	4	8.8%	
Site					0.875
University of Colorado	2	3.6%	0	0.0%	
University of Iowa	20	36.3%	15	32.0%	
University of Colorado Health	2	3.6%	4	8.8%	
Non-small cell lung cancer, NCS	14	25.5%	2	4.4%	
epithelial of unknown type	15	27.4%	5	11.0%	
Stage					0.580
Stage III	10	18.2%	11	24.0%	
Stage IV	27	49.3%	14	30.0%	
Smoking Status					0.308
Never	10	18.2%	15	32.0%	
Current	27	49.3%	4	8.8%	
Former	27	49.3%	12	26.0%	
Race					0.767
White	42	75.5%	24	52.0%	
Black	10	18.2%	7	15.0%	
Age					0.553
0-64	45	81.8%	33	72.0%	
65-74	22	39.6%	5	11.0%	
75-84	15	27.3%	5	11.0%	

Table 2. Summary of Adverse Events

Adverse Events (AE)	Subjects With Grade 1-3 AE	15 (24%)	12 (26%)	45 (98%)
AE Related to Study Drug				
Definite	0	0	0	
Probable	2 (4%)	0	2 (4%)	
Possible	2 (4%)	1 (2%)	3 (6%)	
Unlikely	1 (2%)	2 (4%)	3 (6%)	
Unrelated	26 (43%)	4 (8%)	15 (32%)	
Serious Adverse Events (SAE)				
Subjects With Grade 1-3 SAE	15 (24%)	5 (11%)	10 (21%)	
SAE Related to Study Drug				
Definite	0	0	0	
Probable	0	0	0	
Possible	0	1 (2%)	1 (2%)	
Unlikely	0	0	0	
Unrelated	15 (24%)	4 (8%)	10 (21%)	
Grade 3+ Toxicity				
Subjects With Grade 3+ Toxicity	25 (44%)	13 (28%)	16 (34%)	
Grade 3+ Toxicity Related to Study Drug				
Definite	0	0	0	
Possible	2 (4%)	0	2 (4%)	
Probable	1 (2%)	1 (2%)	2 (4%)	
Unlikely	1 (2%)	1 (2%)	2 (4%)	
Unrelated	23 (39%)	12 (26%)	14 (30%)	

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P212

Tumor immune infiltration in patients with early stage breast cancer after preoperative treatment with IRX-2.

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Background

The IRX-2 biologic is an injectable cancer immunotherapy containing multiple cytokines derived from stimulated lymphocytes. In preclinical models, it activates T cells, natural killer cells and differentiates immature dendritic cells into mature antigen-presenting cells. In a previous phase II trial, neoadjuvant IRX-2 increased tumor-infiltrating lymphocytes (TILs) and shrank tumors in resectable head/neck squamous carcinoma (SCC).¹ Since stromal TILs (sTILs) are associated with improved survival and neoadjuvant chemotherapy response in early stage breast cancer (ESBC), we conducted a phase Ib trial to evaluate the feasibility of preoperative IRX-2, and its effect on TIL recruitment and immune priming within breast tumors, regional lymphatics, and blood.

Methods

Patients with early stage (I-III) breast cancer indicated for standard-of-care surgical lumpectomy/mastectomy were enrolled in this phase Ib trial. Twenty-one days prior to surgery, all patients received a single low dose of cyclophosphamide (300 mg/m²) to facilitate T-regulatory (T-reg) cell depletion, followed by 10 days of subcutaneous peri-areolar injections of IRX-2 into the affected breast (1 mL × 2 at tumor axis and at 90°). The primary endpoint was feasibility. The secondary endpoint was blinded assessment of sTILs by the 2015 San Antonio working group criteria² (measured as % of total area); exploratory endpoints included comprehensive immune monitoring of TILs and blood.

Results

As of August 2017, 12 patients are enrolled and evaluable. Each enrollee received all planned injections with no treatment-related surgical delays, complications, or grade III/IV toxicities. Treatment was associated with increases in sTILs in 6/12 subjects, corresponding with a mean 52% relative increase (range: -25% to +166%) and 6% absolute increase in sTILs (range: -5% to +23%, p=0.02 paired t-test). Increases in T cell activation markers (ICOS, HLADR, and CD38) and decreases in peripheral T-reg

quantity, proliferation (Ki67), and function (GzmB) were observed in blood during and 1 month following IRX-2.

Conclusions

Peri-lymphatic IRX-2 was well tolerated with preliminary evidence of TIL recruitment, peripheral lymphocyte activation, and T-reg depletion. Further assessment by Nanostring analysis, TCR sequencing, and multispectral immunohistochemistry is underway. Study enrollment is ongoing, including patients for a pre-neoadjuvant-chemotherapy triple-negative ESBC cohort.

Trial Registration

NCT02950259

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P213

Efficacy and tolerability of tremelimumab in locally advanced or metastatic urothelial carcinoma

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Background

The efficacy of anti-CTLA-4 antibody monotherapy in metastatic urothelial carcinoma (UC) is not yet known. Tremelimumab was evaluated in patients with advanced solid tumors in a phase 2 multicenter, open label study. We report a planned analysis of safety and efficacy in a cohort of patients with locally advanced/metastatic UC.

Methods

Eligible patients were adults with histologically or cytologically confirmed UC who had progressed on, were ineligible for, or refused prior chemotherapy. Patients received tremelimumab 750 mg intravenously every 4 weeks (Q4W) for 7 doses, followed by 750 mg Q12W for 2 doses, for up to a total of 12 months or until disease progression, initiation of another anticancer therapy, or unacceptable toxicity. The primary endpoints were safety (evaluated by Common Terminology Criteria for Adverse Events v4.0) and confirmed objective response rate (ORR).

Results

As of April 5, 2017, 32 patients from 8 sites in 5 countries had received treatment and were eligible for efficacy analysis. All had stage IV disease and the majority had received prior platinum-based treatment. Median follow-up for overall survival (OS) was 4.5 months (range, 0.2–12.0). Objective response was observed in 6 patients (ORR 18.8%; 95% CI, 7.2%–36.4%), including 2 with a complete

response. Responses occurred as early as 8 weeks after initiation of treatment (median time to response, 3.3 months) and were durable (median duration of response not reached). For the available PD-L1 dataset, response was observed in both PD-L1 high and PD-L1 low/negative patients (PD-L1 expression assessed using the Ventana SP263 assay; PD-L1 high = staining on $\geq 25\%$ of tumor or immune cells). Median progression-free survival and OS were 3.7 months (95% CI, 2.1–9.0) and 9.6 months (95% CI, 7.2, not estimable), respectively. Treatment-related adverse events (AEs) occurred in 53.1% of patients; grade ≥ 3 treatment-related AEs and treatment-related serious AEs occurred in 18.8% of patients each. Four patients (12.5%) discontinued treatment due to an AE. There were no treatment-related deaths. Biomarker analysis will be presented.

Conclusions

Tremelimumab monotherapy showed favorable clinical activity and an encouraging and manageable safety profile in patients with locally advanced/metastatic UC.

Trial Registration

NCT02527434

P214

T cells transfer and boost anti-breast cancer immunity after stem cell transplant

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Background

We investigated whether cellular and humoral anti-breast cancer immunity induced by infusions of OKT3 x HER2 bispecific antibody armed activated T cells (BATs) can be transferred after high dose chemotherapy (HDC) and autologous stem cell

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transplant (SCT) by immune activated T cells (ATC) obtained after BATs infusion.

Methods

Eight metastatic breast cancer (MBC) patients received 8 infusions of BATs, low dose IL-2, and GM-CSF. Seven to 14 days after the last BATs infusion, the patients were leukapheresed for immune T cells. Immune T cells were expanded and cryopreserved for 8-15 booster infusions after SCT.

Results

Six of 8 MBC patients were evaluable. There were no dose-limiting toxicities or delays in engraftment. One patient had sepsis after SCT and was treated successfully. Four of 6 patients exhibited increases in anti-breast cancer (BrCa) cytotoxicity, NK activity, and IFN- γ Elispots after infusions of BATs and these responses were seen 2 weeks after SCT and persisted up to 2 years post-SCT. We verified the specificity of immune responses by multiple tests that provide evidence of T cell and serum antibody reactivity to multiple 9-mer peptides (three HER-2, two pooled EGFR and three CEA peptides) in post IT and post SCT samples. Analysis of V β repertoire and the quantitation of peptide-specific CD8⁺ T cells demonstrated that there were T cell clones that could bind to the HLA-peptide complex in higher frequencies in post IT and of memory T cell clones in ATC boost post SCT. Likewise, there were epitope specific antibody induction after infusions of HER2 BATs and transfer of memory B cells in ATC boost after SCT. A significant correlation ($r=1.0$; $p<0.002$) between immune ATC cytotoxicity directed at BrCa cells and TTP strongly suggests that more robust vaccinations with a Th₁ shift in cytokine profiles can lead to clinical benefit.

Conclusions

Multiple HER2 BATs infusions may result in immunogenic epitope/antigen spreading and development of the broad and durable T- and B-cell memory responses after multiple infusions of HER2 BATs and "transfer of immunity" by immune ATC after SCT that parallels with the clinical responses. This study shows that adoptive transfer

of immune T cells after SCT accelerates reconstitution of cellular, humoral anti-BrCa immunity and may delay TTP.

Trial Registration

NCT00027807

NCT00020722

P215

Randomized phase I/II pilot study using all-trans retinoic acid to target myeloid-derived suppressor cells in melanoma patients undergoing Ipilimumab therapy

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Background

Immune checkpoint inhibitors have improved overall survival rates for many cancers, yet the majority of patients do not respond to treatment and succumb to disease progression [1]. One tumor-related mechanism limiting the efficacy of immunotherapies is the recruitment and expansion of myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immature immunosuppressive myeloid cells, associated with poor outcomes in melanoma patients [2, 3]. Therefore, enhancing the efficacy of current immunotherapies by targeting MDSCs throughout treatment is an attractive strategy to improve response rates and effectiveness.

Methods

Following an IRB approved protocol, a total of ten patients were recruited for this randomized controlled clinical trial. Advanced melanoma patients were randomized into two arms, standard of care Ipilimumab (Arm A), or Ipilimumab plus ATRA (Arm B). MDSC gene expression was

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measured by qRT-PCR. Flow cytometry was used to quantify circulating MDSC populations and T cell activation.

Results

Here we show that *in vitro* treatment with all-trans retinoic acid (ATRA) decreases the expression of immunosuppressive genes (PDL1, IL10, TGFB, NADPH oxidase 1, and indoleamine 2,3-dioxygenase) in human MDSCs. Furthermore, the addition of ATRA to standard of care Ipilimumab therapy is safe and significantly decreases the frequency of circulating MDSCs compared to Ipilimumab treatment alone in advanced-stage melanoma patients ($p = 0.017$) (Figure 1). Additionally, throughout therapy, the frequency of circulating MDSCs inversely correlated with the frequency of activated CD8+ T cells ($p = 0.021$).

Conclusions

These results illustrate the importance of MDSCs in immunotherapy resistance and provide evidence that targeting MDSCs in cancer patients may augment future immunotherapeutic approaches. The strategy of targeting MDSCs in combination with current immunotherapies appears to be a viable and safe therapeutic approach.

Trial Registration

This trial is registered at clinicaltrials.gov as NCT02403778.

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P216

Long-term survival in patients with advanced melanoma, renal cell carcinoma, or non-small cell lung cancer treated with nivolumab

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Background

Nivolumab, a fully human IgG4 antibody that inhibits programmed death receptor-1 (PD-1), is FDA-approved for treating advanced melanoma (MEL), renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), and several other malignancies. Here we report the longest available overall survival (OS) outcomes for any PD-1/PD

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ligand-1 (PD-L1) inhibitor using data from patients with treatment-refractory MEL, RCC or NSCLC who received nivolumab in the phase 1 CA209-003 study.

Methods

Eligible adult patients had progressive cancers after 1-5 previous systemic therapies, with no prior immunotherapy (e.g., anti-PD-1, anti-PD-L1, anti-cytotoxic T-lymphocyte antigen-4 [CTLA-4] antibodies). Patients enrolled in 2008-2012 received nivolumab (0.1-10.0 mg/kg) every 2 weeks in 8-week cycles for up to 96 weeks, unless they developed progressive disease, a complete response, unacceptable toxicity, or withdrew consent. Tumor response was assessed by RECIST v1.0 after each treatment cycle. Patients with stable disease or tumor regression, who experienced off-therapy disease progression within 1-year of stopping nivolumab, could resume nivolumab for ≤ 96 weeks.

Results

270 patients with advanced MEL (N=107), RCC (N=34), or NSCLC (N=129; squamous, n=54; non-squamous, n=74; unknown histology, n=1) received nivolumab. Most patients were heavily pretreated: 194 (71.9%) had received ≥ 2 prior regimens, and 112 (41.5%) ≥ 3 prior regimens. Minimum follow-up for OS was 58.3 (MEL), 63.9 (RCC), 58.3 (squamous NSCLC), and 60.8 (non-squamous NSCLC) months. OS outcomes are shown in the table (Table 1). Long-term safety in these cohorts has been reported previously [1-3]. Long-term (5-year) survivors, patients with durable response, and retreated patients will be characterized by clinical and demographic factors, treatments received, and depth of tumor response.

Conclusions

Nivolumab therapy demonstrates durable survival in some heavily pretreated patients with advanced MEL, RCC, or NSCLC. Characterizing long-term survivors and the potential benefits of retreatment with nivolumab will inform future treatment strategies.

Trial Registration

ClinicalTrials.gov: NCT00730639

Table 1.

	MEL (n=107)	RCC (n=34)	Squamous NSCLC (n=54)	Non-squamous NSCLC (n=74)
Median OS, months (95% CI)	20.3 (12.5-37.9)	22.1 (12.5-48.6)	9.2 (7.1-12.5)	10.1 (5.8-13.7)
3-year OS rate, % (95% CI)	42.3 (32.7-51.6)	40.1 (23.6-56.0)	20.3 (10.5-32.4)	17.0 (5.0-27.2)
4-year OS rate, % (95% CI)	35.3 (26.2-44.5)	37.0 (21.1-53.0)	16.3 (7.6-27.8)	15.3 (7.7-25.3)
5-year OS rate, % (95% CI)	34.2 (25.2-43.4)	27.7 (13.5-43.5)	16.3 (7.6-27.8)	15.3 (7.7-25.3)

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P217

Clinical outcomes for the phase 2, single-arm, multicenter trial of JCAR015 in adult B-ALL (ROCKET Study)

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Background

Promising results have been reported in phase 1 studies of CD19-specific chimeric antigen receptor (CAR)-based cellular therapy for relapsed/refractory (R/R) aggressive B-cell malignancies. Based on a phase 1, single-center trial of a CD19-specific, CD28-CD3z CAR T cell product candidate (19-28z) demonstrating high overall response rate (ORR) with manageable toxicity in adult B-ALL patients (NCT01044069), a phase 2, single-arm, multicenter trial evaluated a similar CD19-CAR T cell product candidate (JCAR015) [ROCKET; NCT02535364]. ROCKET was stopped due to unexpected fatal neurotoxicity (NTX), involving cerebral edema. Herein we report clinical outcomes from this trial.

Methods

ROCKET evaluated the safety and efficacy of two JCAR015 infusions (10^6 and 3×10^6 cells/kg separated by >14 days), in ECOG 0-2 adult patients with morphologically-R/R CD19 positive B-ALL. Primary endpoint was ORR defined as CR/CRi >28 days post-last dose. Patients with R/R disease were eligible, including prior-allo-transplant (HSCT), extramedullary disease and prior CNS leukemia.

Bridging chemotherapy was permitted during manufacturing. Prior to first infusion, patients were restaged for morphologic disease and received lymphodepleting chemotherapy (LD) with either fludarabine (Flu) ($25\text{mg}/\text{m}^2 \times 3\text{d}$) and cyclophosphamide (Cy) ($30\text{-}60\text{mg}/\text{kg} \times 1\text{d}$) or single-dose Cy-only ($1\text{-}3\text{gm}/\text{m}^2$).

Results

ROCKET enrolled 82 patients, 57 underwent apheresis; 38 toxicity-evaluable patients received >1 infusion. Median age was 39 (19-69 years) with a male preponderance (74%), median prior lines of therapy was 2 (1, 7), 37% had previously undergone HSCT and 50% had received prior blinatumomab (Table 1). Primary efficacy analysis included 32 patients with morphologic disease (>5% blasts) prior to LD with Flu/Cy (8/32) or Cy-only (24/32). ORR per investigator assessment was 14/27 (52%, 95%CI: 32-72%) among response-evaluable patients. Median overall survival for all patients was 7.3 months (95% CI: 5.2, 12.7) and 12.7 months (7.3, NR) among responders with median follow-up of 12.6 and 11.9 months, respectively. Median relapse-free survival was 4.4 months (2.1, NR) and 9/14 (64%) responders were alive at last follow-up.

Severe (Gr3-5) NTX and Gr3-4 CRS occurred in 20/38 (52%) and 8/38 (21%) patients, respectively. Notably, vascular leak in five cases of cerebral edema was restricted to the CNS, except for one patient with incidental radiographic findings compatible with interstitial pulmonary edema.

Conclusions

JCAR015 demonstrated anti-leukemia activity in adult B-ALL that was negatively impacted by fatal NTX. Efficacy compared favorably to standard therapy in this patient population with high unmet need [1].

NR: not reached

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Table 1. Demographics of ROCKET patients

Table. Demographics of ROCKET patients

	All Patients	Morphologic Patients	Molecular Patients
Male	28/38 (74%)	25/32 (78%)	3/6 (50%)
Median Age (range, yr)	39 (19-69)	39 (19-69)	34 (24-47)
Age >30	22/38 (58%)	19/32 (59%)	3/6 (50%)
White	34/38 (89%)	28/32 (88%)	6/6 (100%)
Years since primary diagnosis (range)	1.8 (0.5-21.5)	1.6 (0.5-10.6)	2.2 (0.6-21.5)
No. of prior regimens, median (range)	2 (1, 7)	2 (1, 7)	2 (1, 3)
Philadelphia Chromosome+	4/38 (11%)	4/32 (13%)	0/6 (0%)
Prior blinatumomab	19/38 (50%)	16/32 (50%)	3/6 (50%)
Prior HSCT	14/38 (37%)	11/32 (34%)	3/6 (50%)
Prior CNS Disease	8/38 (21%)	7/32 (22%)	1/6 (17%)
Prior CNS Radiation	12/38 (32%)	11/32 (34%)	1/6 (17%)
Prior IT Chemotherapy	30/38 (79%)	26/32 (81%)	4/6 (67%)
Pre-LD BM blast % (aspirate; if not available, biopsy)	48% (0-98%)	86% (6-98%)	2% (0-4%)
Aggressive Bridging Treatment*	19/38 (50%)	17/32 (53%)	2/6 (33%)

*Hyper CVAD parts A or B, regimens containing fludarabine and/or cytarabine

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Clinical Trials (In Progress)

P218

KEYNOTE-585: randomized, phase 3 study of chemotherapy + pembrolizumab vs chemotherapy + placebo as neoadjuvant/adjuvant treatment for patients with gastric or gastroesophageal junction (G/GEJ) cancer

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Background

In KEYNOTE-012 (NCT01848834) and KEYNOTE-059 (NCT02335411), pembrolizumab demonstrated manageable safety and promising antitumor activity alone or in combination with chemotherapy in patients with advanced G/GEJ cancer. Compared with chemotherapy alone, chemotherapy combined with pembrolizumab in the neoadjuvant/adjuvant setting can provide additional benefit to patients with locally advanced, resectable G/GEJ cancer. KEYNOTE-585 is a phase 3, randomized, double-blind study of chemotherapy combined with pembrolizumab versus chemotherapy combined with placebo as neoadjuvant/adjuvant treatment for locally advanced resectable G/GEJ cancer.

Methods

Key eligibility criteria in KEYNOTE-585 (NCT03221426) include age ≥ 18 years; previously untreated G/GEJ adenocarcinoma (Siewert type 2 or 3 tumors; Siewert type 1 tumor eligibility limited to those for whom planned treatment is perioperative chemotherapy and resection), with no evidence of metastatic disease; planning to undergo surgery after preoperative chemotherapy; Eastern Cooperative Oncology Group performance status 0-1; adequate organ function; no active autoimmune disease. Patients will be randomly assigned 1:1 to receive chemotherapy + pembrolizumab (arm 1) or chemotherapy + placebo (arm 2). Stratification factors are geographic region (Asia vs Non-Asia), primary tumor location (stomach vs GEJ), and tumor stage (II/III vs IVa). All patients will receive

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neoadjuvant (preoperative) chemotherapy + pembrolizumab every 3 weeks (Q3W) for 3 cycles or chemotherapy + placebo Q3W for 3 cycles followed by surgery and then adjuvant chemotherapy + pembrolizumab Q3W for 3 cycles or chemotherapy + placebo Q3W for 3 cycles followed by monotherapy with pembrolizumab or placebo Q3W for 11 cycles; treatment will continue for up to 17 cycles overall. Chemotherapy consists of cisplatin 80 mg/m² intravenously + either capecitabine 1000 mg/m² twice daily orally or 5-fluorouracil 800 mg/m² intravenously (investigator's choice). Pembrolizumab 200 mg was administered intravenously. Adjuvant monotherapy consists of pembrolizumab (arm 1) or placebo (arm 2). Primary end points are overall survival (OS), event-free survival, and rate of pathologic complete response (defined as no invasive disease and histologically negative nodes) per central review. Adverse events (AEs) are graded per National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 and will be monitored for 30 days after treatment end (90 days for serious AEs). Patients are followed up for survival every 12 weeks until death, withdrawal from study, or study termination. Planned enrolment is approximately 800 patients.

Trial Registration

ClinicalTrials.gov, NCT03221426

P219

Phase 1/1b, first-in-human study of the PI3K-gamma inhibitor IPI-549 as monotherapy and combined with nivolumab in patients with advanced solid tumors

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Background

IPI-549 is a potentially first-in-class, oral, potent, selective PI3K-gamma inhibitor being developed as an immuno-oncology therapeutic in multiple cancer indications. Preclinical solid tumor research has shown that PI3K-gamma blockade in tumor-associated macrophages by IPI-549 results in transcriptional reprogramming of the pro-tumor macrophage phenotype (M2) to its anti-tumor counterpart (M1). Within this setting, IPI-549 has demonstrated activity as monotherapy and still greater activity when combined with checkpoint inhibitor therapies. Importantly, the latter approach has been found to overcome checkpoint inhibitor resistance in specific resistant models.

Methods

This study is being conducted to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of IPI-549 to ultimately determine its recommended Phase 2 dose and activity, both as monotherapy and in combination with nivolumab, in patients with advanced solid tumors. As shown in Figure 1, the design includes four parts: 1) dose escalation (DE) of IPI-549 monotherapy; 2) DE of IPI-549 with fixed-dose nivolumab; 3) monotherapy expansion; and 4) combination expansion in specific tumor types, including non-small cell lung cancer, melanoma, and squamous cell carcinoma of the head and neck, with de novo or acquired resistance to checkpoint inhibitors (Figure 1).

Results

This trial is currently in progress. No results are available.

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Progress update: Monotherapy DE enrollment is complete for once-daily (QD) IPI-549 doses up to 60 mg, while monotherapy expansion enrollment has been initiated at 60 mg QD. Combination DE enrollment is complete for QD doses of 20 mg and 30 mg IPI-549 combined with fixed-dose nivolumab (240 mg once every 2 weeks).

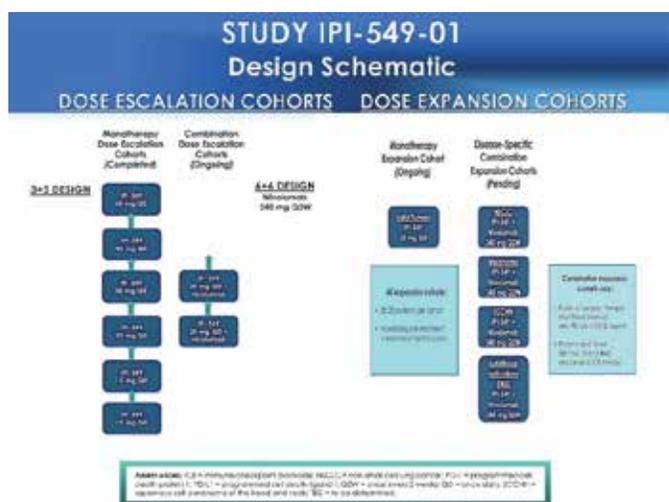
Conclusions

The collective preclinical data highlight the key role of PI3K-gamma in the immuno-suppressive tumor microenvironment and provide a strong rationale for the ongoing clinical study of IPI-549.

Trial Registration

ClinicalTrials.gov: NCT02637531

Figure 1.



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A phase 2, multicenter study to evaluate the efficacy and safety using autologous tumor infiltrating lymphocytes (LN-145) in patients with recurrent, metastatic, or persistent cervical carcinoma

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Background

Adoptive cell therapy (ACT) may be effective in treating immunogenic tumors with high mutational load such as melanoma and virally-associated tumors like cervical cancer with several patients in studies performed by other institutions achieving durable complete response for years. HPV infection increases mutational load, thus providing additional neoantigen targets ideal for the polyclonal nature of ACT. As outcomes for patients with recurrent, metastatic or persistent cervical cancer remain extremely poor, there is an enormous need for novel immunotherapeutic approaches with curative potential such as ACT.

Methods

Clinical trial C-145-04 (NCT03108495) is a prospective, phase 2 multicenter, open-label study evaluating the efficacy of a single autologous tumor infiltrating lymphocyte infusion (LN-145) followed by IL-2 after a non-myeloablative lymphodepletion (NMA-LD) regimen in patients with recurrent, metastatic, or persistent cervical cancer who have failed at least one prior systemic therapy. The clinical trial protocol requires resection of a tumor lesion which is then shipped to a central GMP manufacturing facility for TIL extraction, expansion, and preparation of the final infusion product (LN-145). One week prior to LN-145 shipment and infusion, patients undergo NMA-LD consisting of cyclophosphamide (60 mg/kg) daily x 2 days followed by fludarabine (25 mg/m²) daily x 5 days. LN-145 is infused 24 hours after the last dose of fludarabine followed by up to 6 doses of IL-2 (600,000 IU/kg) every 8-12 hours. Simon's two-stage optimal design with one-sided alpha

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level=0.025 and 80% power will be used to compare an objective response rate (ORR) of 5% vs. 20% in the first stage (n=15 subjects). If two or more ORR are observed, trial will expand to Stage 2 (n=47). The primary endpoint is the ORR per RECIST v1.1. Secondary endpoints include complete response, duration of response, disease control rate, progression free- and overall survival; and the safety summarization of treatment-emergent adverse events (AEs) including serious AEs, AEs leading to discontinuation, and clinical laboratory tests. Patients must have been treated with at least 1 systemic chemotherapy or immunotherapy treatment for recurrent, metastatic, or persistent cervical cancer and, in addition to the tumor targeted for excision and TIL manufacture, must have an additional measurable lesion for assessment of response. Other major eligibility criteria include amongst others: adequate bone marrow, liver, pulmonary, cardiac and renal function; ECOG performance status of 0 or 1. Systemic steroids greater than 10 mg/day prednisone equivalents are prohibited as are a history of serious immunotherapy-related adverse events.

Trial Registration

ClinicalTrials.gov identifier: NCT03108495

P221

A phase 2 study to evaluate the safety and efficacy using autologous tumor infiltrating lymphocytes (LN-145) in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck

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Background

Adoptive cell therapy (ACT) may be effective in treating immunogenic tumors with high mutational load such as melanoma and virally-associated tumors like cervical cancer with several patients in studies performed by other institutions achieving durable complete response for years. Despite the heterogeneity of squamous cell carcinomas of the head and neck (HNSCC), most tumors are either virally-associated (e.g., HPV in oropharyngeal) or carry high mutational load (e.g., tobacco-related) providing an increased diversity of potential targets ideal for the polyclonal nature of ACT. Furthermore, outcomes for patients with recurrent and/or metastatic HNSCC remain poor. Therefore, a clear rationale exists for the potential application of ACT in patients with HNSCC.

Methods

Clinical trial C-145-03 (NCT03083873) is a prospective phase 2 multicenter, open-label study evaluating the efficacy of a single autologous tumor infiltrating lymphocyte infusion (LN-145) followed by IL-2 after a non-myeloablative lymphodepletion (NMA-LD) regimen in patients with recurrent and/or metastatic HNSCC. Study-related therapy begins with resection of a tumor lesion that is then shipped to a central GMP manufacturing facility where TIL are extracted, expanded, packaged, and shipped for administration (LN-145). One week prior to LN-145 infusion, patients undergo NMA-LD consisting of cyclophosphamide (60 mg/kg) daily x 2 days followed by fludarabine (25 mg/m²) daily x 5 days. LN-145 is infused 24 hours after the last dose of fludarabine followed by up to 6 doses of IL-2 (600,000 IU/kg) every 8-12 hours. Simon's two-stage optimal design with one-sided alpha level=0.025 and 80% power will be used to compare an objective response rate (ORR) of 5% vs. 20% in the first stage (n=15 subjects). If two or more ORR are observed, trial will expand to Stage 2 (n=47). The primary efficacy endpoints are the objective

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response rate per RECIST v1.1 and the safety summarization of treatment-emergent adverse events (AEs) including serious AEs, AEs leading to discontinuation, and clinical laboratory tests. Secondary efficacy endpoints include CR, DOR, PFS, and OS. Patients must have been treated with at least one systemic chemotherapy or immunotherapy treatment for recurrent and/or metastatic HNSCC and, in addition to the tumor targeted for excision and TIL manufacture, must have an additional measurable lesion for assessment of response. Additional eligibility criteria include amongst others: adequate bone marrow, liver, pulmonary, cardiac, and renal function; ECOG performance status of 0 or 1. Systemic steroids greater than 10 mg/day prednisone equivalents are prohibited as are a history of serious immunotherapy-related adverse events.

Trial Registration

ClinicalTrials.gov identifier:NCT03083873

P222

Clinical trial in progress: A phase 1b trial of talimogene laherparepvec (T-VEC) in combination with dabrafenib and trametinib in advanced melanoma with an activating BRAF mutation

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Background

Patients with BRAF-mutant advanced melanoma have been shown to respond at high rates to combination BRAF plus MEK inhibition. Response rates to single-agent immune therapies tend to be modest, but are often durable, while response rates

to BRAF plus MEK inhibition tend to be high but transient. While early attempts at combining targeted and immune therapy resulted in dose-limiting hepatic toxicity, more recent attempts with newer agents have shown significant promise. T-VEC is an oncolytic viral immunotherapy which was designed to selectively replicate in tumors resulting in lytic cell death, antigen release, and production of GM-CSF to enhance systemic immune response. In a prior randomized, phase 3 study of T-VEC versus GM-CSF, durable response rate was statistically improved with T-VEC, with a strong trend towards improved overall survival. Dabrafenib and trametinib, inhibitors of BRAF and MEK, respectively, have been shown in combination to result in response rates of up to 75% and improvement in progression-free and overall survival compared to single-agent targeted therapy and chemotherapy. Combining T-VEC with dabrafenib and trametinib may further enhance antitumor immune responses in addition to preserving the targeted effect.

Methods

This is a Phase Ib, prospective, single arm study of T-VEC given in combination with standard doses of dabrafenib and trametinib in advanced melanoma with an activating BRAF mutation. The primary endpoint of the study is tolerability as measured by dose-limiting toxicities seen in the first 5 weeks of treatment. Key secondary endpoints include progression-free survival, objective response rate, change in tumor burden, time to response, and duration of response among responders. Tumor-level responses in injected and uninjected tumors, and characterization of immune markers in pre-study and on-study biopsies will be exploratory endpoints. Key eligibility criteria include unresectable stage IIIB-IV BRAF mutant melanoma, presence of measurable and injectable disease, no active cerebral metastases or autoimmune diseases, and any number of prior lines of therapy but no prior receipt of T-VEC. T-VEC (10⁶ PFU/mL first dose, 10⁸ PFU/mL subsequent doses) will be administered by intralesional injection into cutaneous, subcutaneous, or nodal lesions on week 1 day 1,

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week 4 day 1, and every 2 weeks thereafter until disappearance of injectable lesions, complete response, progressive disease, intolerance of study treatment, or 24 months after starting therapy, whichever occurs first. Dabrafenib and trametinib will be given until progression or intolerance. Twenty subjects are to be enrolled at a single U.S. institution.

Trial Registration

NCT03088176

P223

KEYNOTE-199: Phase 2 nonrandomized study of pembrolizumab in patients with PD-L1+ and PD-L1– metastatic castration-resistant prostate cancer

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Background

Metastatic castration-resistant prostate cancer (mCRPC) treatment has included suppression of androgen receptor signaling, palliative radiation therapy, and chemotherapy. As expression of the programmed death 1 (PD-1) receptor and its ligand PD-L1 is present in a subset of mCRPC lesions, targeting this pathway may be an attractive treatment option. KEYNOTE-199 (NCT02787005) is a nonrandomized, multinational, multicohort open-label phase 2 study to evaluate the anti-PD-1 antibody pembrolizumab in patients with mCRPC.

Methods

Patients must be ≥ 18 years old with histologically or cytologically confirmed prostate adenocarcinoma without small-cell histology, measurable disease (RECIST v1.1) or detectable bone metastases by whole-body bone scintigraphy and no RECIST v1.1 measurable tumors, supplied tumor sample for PD-L1 expression (new or archived), disease progression within 6 months before screening, and ECOG performance status 0-2. As of June 2017, 2 additional cohorts were added called cohorts 4 and 5. For these cohorts patients had to fail or show signs of failure on current prechemotherapy enzalutamide; patients could fail abiraterone treatment before enzalutamide. For cohort 4, patients will be enrolled with RECIST v1.1-measurable disease (n=80) and for cohort 5 patients will be enrolled with bone metastases only or bone-predominant disease (n=40). For both cohorts patients will receive pembrolizumab 200 mg every 3 weeks (Q3W) plus current enzalutamide regimen. For the original cohorts (cohorts 1-3), patients must have been treated with ≥ 1 targeted endocrine therapy (abiraterone or enzalutamide) and ≤ 2 chemotherapy regimens; one must have contained docetaxel. Patients also must be undergoing androgen deprivation with serum testosterone < 50 ng/dL. Patients will be enrolled based on PD-L1 status and RECIST v1.1 measurability and will receive pembrolizumab 200 mg Q3W: PD-L1–

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positive, RECIST v1.1 measurable disease (cohort 1), PD-L1–negative, RECIST v1.1 measurable disease (cohort 2; cohorts 1 and 2 combined, n=200), and bone metastases and RECIST v1.1 nonmeasurable disease (cohort 3, n=50). All patients will continue until documented confirmed disease progression, unacceptable adverse events (AEs), or illness that prevents further treatment. Imaging response will be assessed every 9 weeks for approximately 1 year and every 12 weeks thereafter, per central imaging vendor review (RECIST v1.1) and the Prostate Cancer Clinical Trials Working Group 3 guidelines. AEs will be monitored throughout the study. Primary end points are overall response rate for cohorts 1 and 2 combined and for cohorts 1, 2, and 4. Key secondary end points include safety and tolerability, duration of response, disease control rate, radiographic progression-free survival, and overall survival.

Trial Registration

ClinicalTrials.gov, NCT02787005

P224

Immunological correlates observed in an Interim analysis of the Phase 3 ADAPT Trial evaluating Rocapuldencel-T (AGS-003), for the treatment of patients with metastatic renal cell carcinoma (mRCC)

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Background

Rocapuldencel-T is an investigational patient-specific immunotherapeutic comprised of autologous dendritic cells programmed with RNA from the patient's tumor to express tumor-specific antigens and thereby induce a memory T cell response. Rocapuldencel-T is engineered to secrete IL-12, a critical cytokine for memory-T cell formation, to induce an antigen-specific memory T

cell response. We previously reported results from a Phase 2 study showing a correlation between the magnitude of the induced memory-T cell response and overall survival (OS) in advanced RCC patients. We therefore measured the antigen-specific memory T cell response and the level of secreted IL-12 in patients receiving Rocapuldencel-T to assess the relationship of these parameters to each other and to overall survival (OS).

Methods

The ADAPT trial is designed to evaluate OS in subjects with newly diagnosed mRCC receiving Rocapuldencel-T in combination with standard-of-care (SOC) versus SOC alone. As part of this interim analysis, immune monitoring was performed on patients (N=146) enrolled in the United States and randomized to the combination arm. The change in the number of CD28+/CD45RA- antigen-specific memory T cells present after *in vitro* stimulation of PBMCs with Rocapuldencel-T was measured with multi-color flow cytometry. The amount of IL-12 secreted by each patient's immunotherapeutic was measured by a cytokine bead capture method (N=179). The increase in the number of antigen-specific memory T cells after administration of Rocapuldencel-T and the concentration of IL-12 secreted by each patient's immunotherapeutic were correlated with OS.

Results

Data from this interim analysis revealed a statistically significant increase in the number of CD28+/CD45RA- memory T cells after as few as three doses of Rocapuldencel-T administered three weeks apart. The increase above baseline became a statistically significant correlate with OS after seven doses. Furthermore, the amount of IL-12 each patient's immunotherapeutic produced showed a statistically significant correlation with both the magnitude of the memory T cell response and OS.

Conclusions

This interim analysis demonstrated that administration of Rocapuldencel-T resulted in an increase in antigen-specific memory T cells and that

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the magnitude of the induced memory T cell response after seven doses correlated with OS. Additionally, the amount of IL-12 secreted by each patient's immunotherapeutic correlated with both the magnitude of the induced memory T cell response and OS. Therefore, the level of IL-12 secretion may serve as a predictive biomarker for T cell response to Ropapudencel-T and favorable clinical outcome, warranting further investigation.

Trial Registration

ClinicalTrials.gov identifier-NCT01582672

P225

Functional reversal of Foxp3+ T regulatory activity in patients enrolled in the Phase 3 ADAPT Trial evaluating Ropapudencel-T for the treatment of patients with metastatic renal cell carcinoma (mRCC)

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Background

Ropapudencel-T is an investigational patient-specific immunotherapeutic comprised of autologous dendritic cells programmed with RNA from the patient's tumor to express tumor-specific antigens and induce a memory-T cell response. The Phase 3 ADAPT trial is designed to evaluate overall survival (OS) in subjects with newly diagnosed mRCC receiving Ropapudencel-T in combination with standard-of-care (SOC) versus SOC alone. Current SOC first-line treatment with sunitinib is shown to decrease regulatory T cells (Tregs), thus potentially modulating anti-tumor activity. Therefore, FoxP3+ Treg cell counts in patients receiving Ropapudencel-T were measured *ex vivo* in whole blood samples and the relationship to OS was assessed for each arm of the study.

Methods

For this interim analysis, whole blood samples collected at intervals during the course of the ADAPT trial were stained immediately *ex vivo* by multi-color flow cytometry to determine the number of CD4+/CD25+/CD127-/Foxp3+ Treg cells. The correlation between the number of Tregs present and overall survival was determined for the combination arm subjects (N=177) and the SOC arm subjects (N=80) for whom data was available. We further studied the impact of Ropapudencel-T on Treg phenotype and function after *in vitro* stimulation of PBMCs from treated subjects.

Results

As reported elsewhere, data from this interim analysis showed a decline in the number of Tregs after the first cycle of sunitinib. Surprisingly, the number of Tregs at baseline or at any time point measured, positively correlated with OS in the combination arm, but negatively correlated with OS in the SOC arm. Furthermore, *in vitro* stimulation of autologous PBMCs with Ropapudencel-T resulted in conversion of Tregs to a proinflammatory cell phenotype that proliferates in culture, thus providing a possible explanation for the positive correlation between the number of Tregs and OS in the combination arm.

Conclusions

In this interim analysis, baseline Tregs positively correlated with survival only in the Ropapudencel-T arm and not in the SOC arm. These data suggest that Ropapudencel-T exerts a biologic effect catalyzed by the presence of Tregs, potentially by conversion to T effector cells, resulting in favorable clinical outcome. This is consistent with the *in vitro* observation that Ropapudencel-T can convert Tregs to T effector cells. Therefore, the level of Tregs in whole blood samples may serve as a useful baseline biomarker predictive of favorable long-term clinical outcome in patients treated with Ropapudencel-T while also predicting poor outcome in patients receiving conventional SOC.

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Trial Registration

ClinicalTrials.gov identifier-NCT01582672

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FRACTION-RCC: a randomized, open-label, adaptive, phase 2 study of nivolumab in combination with other immuno-oncology agents in patients with advanced RCC

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Background

Nivolumab, a fully human IgG4 monoclonal antibody (mAb) that targets the PD-1 receptor, is approved for patients with advanced renal cell carcinoma (RCC) after prior antiangiogenic therapy based on superior overall survival vs everolimus (CheckMate 025; Motzer RJ, et al. *N Engl J Med.* 2015). Nivolumab has also shown promising antitumor activity in combination with ipilimumab (a fully human IgG1 mAb that targets cytotoxic T-lymphocyte antigen 4) in patients with metastatic RCC, supporting the rationale that nivolumab in combination with other immuno-oncology (IO) agents or targeted therapies may improve outcomes in patients with advanced RCC. Given the rapid development of novel IO agents, traditional

study designs may not efficiently evaluate all possible IO-IO and IO-targeted therapy combinations. Fast Real-time Assessment of Combination Therapies in Immuno-ONcology (FRACTION) is an innovative clinical trial program with a rolling, adaptive platform design that allows for the addition of new regimens as well as the withdrawal of ineffective regimens. Here we describe the study concept, key design components, and first IO treatment combinations of FRACTION-RCC, a phase 2, randomized, open-label, adaptive study in advanced RCC (NCT02996110).

Methods

FRACTION-RCC is envisioned to accelerate the development of the next generation of IO combinations for patients with metastatic RCC. Patients with advanced RCC with a clear-cell component will be enrolled based on prior IO treatment and randomized to receive nivolumab plus BMS-986016 (a fully human IgG4 mAb that targets lymphocyte activation gene 3) or nivolumab plus ipilimumab. Enrollment is continuous and may offer patients consecutive treatment options based on treatment exposure and response. Primary endpoints include objective response rate, duration of response, and progression-free survival rate at 24 weeks. The secondary endpoint is safety. Biomarker analyses will also be performed. New treatment combinations will be added over time to explore their potential benefits and provide a continuous flow of treatment options for patients whose cancer progresses on existing treatments.

Trial Registration

ClinicalTrials.gov, NCT02996110

P227

A trial to evaluate the safety, immunogenicity and clinical activity of a helper peptide vaccine plus PD-1 blockade

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Background

PD-1 blocking antibodies are standard first line therapy for management of advanced melanoma. Monotherapy response rates to these agents are reported between 30-40%; therefore, the majority of patients require additional therapy [1, 2]. Strategies to improve the benefit of these agents are currently focused on combination with other systemic agents and adjunctive local treatment measures. We are evaluating the combination of pembrolizumab with a vaccine incorporating 6 peptides that induces CD4+ helper T cell (T_H) responses (6MHP) and has proven activity in patients with advanced melanoma [3]. The primary end point is determination of the safety and tolerability of the combination of 6MHP vaccine plus Pembrolizumab and estimation of the CD4+ T cell response rate to 6MHP in the blood and sentinel immunized node. Secondary endpoints include evaluation of the induction of epitope-spreading and the T cell infiltration of the tumor microenvironment.

Methods

This is an open-label, phase I/II study to evaluate the safety, immunogenicity and clinical activity of the 6MHP vaccine and pembrolizumab (MEL64, NCT02515227). Candidates may be resistant or naïve to systemic immunotherapy agents. Patients with prior PD-1 antibody exposure are included if the patient failed to experience a clinical response after 12 weeks or progressed on treatment. All subjects will receive 6MHP on days 1, 8, 15, 43, 64, and 85. Pembrolizumab will be administered intravenously (IV) every 3 weeks for up to 2 years. Biopsy specimens will be collected from the tumor (days 1 and 22) and a sentinel immunized node (SIN; day 22). Biopsy and peripheral blood specimens will be used in the immunologic analyses. Overall target sample size is based upon having sufficient information to assess whether combined treatment with 6MHP vaccine plus pembrolizumab increases the immunogenicity of 6MHP alone in the entire

study population. Maximum target sample size is 40 patients. Trial is currently enrolling patients.

Results

Trial in Progress

Conclusions

Trial in Progress

Trial Registration

NCT02515227

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P228

Pembrolizumab and decitabine for relapsed and refractory acute myeloid leukemia (PD-AML)

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Background

While a variety of different treatment regimens have been studied for patients with relapsed/refractory acute myeloid leukemia (AML) clinical outcomes unfortunately remain dismal. There appears to be no single superior therapeutic

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approach and the current standard of care is referral to an appropriate clinical trial. The unique combination of pembrolizumab and decitabine used in this trial was selected for investigation based on several factors: 1) Largely non-overlapping adverse reaction profiles for these agents. 2) Both agents already FDA approved potentially allowing for rapid translation/adoption. 3) Both agents have previously evaluated dosing schedules that are compatible with one another. 4) Theoretical possibility of synergy given respective mechanisms of action.

Methods

PD-AML (17-H-0026, NCT02996474) is an investigator sponsored, single-institution, single-arm open-label ten subject pilot study to evaluate the feasibility of a novel combination of pembrolizumab and decitabine in adults with relapsed/refractory AML. Secondary objectives will explore efficacy and determine time to first response, best response and duration of best response. Laboratory objectives include investigation of changes in AML clonal composition and disease burden during therapy, and measurement of changes in immune parameters associated with clinical efficacy and/or toxicity. Up to eight 21 day cycles of pembrolizumab are given with decitabine given on days 8-12 and 15-19 on alternative cycles (ie: cycles 1, 3, 5 and 7) (Figure1).

Results

This clinical trial opened in February 2017 and is currently in progress. Currently seven patients have reached response and toxicity assessment time-points. Updated results will be presented at the meeting.

Conclusions

Acute myeloid leukemia is a heterogeneous group of diseases with distinct molecular and phenotypic characteristics. Even within a single patient AML may be polyclonal at any examined time-point, and this clonal composition can change over time with the clone predominant at presentation not necessarily the one responsible for relapse and

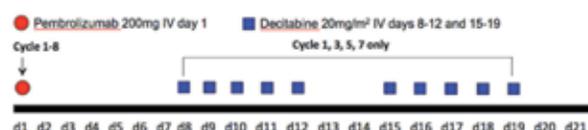
death. We hypothesize that effective pembrolizumab therapy for refractory/relapsed AML may be associated with changes in the leukemic clonal composition due to differences in immunogenicity between clones. The oligoclonal nature of AML biology, together with a blood and bone marrow distribution highly amenable to repeated sampling of the sites of disease burden, provides a near unique opportunity to investigate fundamental mechanisms underpinning treatment efficacy of this new combination of immunotherapeutic drugs.

Trial Registration

[<https://clinicaltrials.gov/ct2/show/NCT02996474>]

FDA-IND: 131826

Figure 1.



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Withdrawn

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Phase I/II safety and efficacy study of image guided intratumoral CD40 agonistic monoclonal antibody APX005M in combination with systemic pembrolizumab in metastatic melanoma patients

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Background

Checkpoint blockade has become a major modality in the treatment of metastatic melanoma (MM). However, long-term survival and durable remission rates remain low and new treatment options are needed. CD40 activation on antigen presenting cells (APCs) initiates their maturation and ability to prime and activate CD8⁺ T cells through upregulation of co-stimulatory molecules (CD80, CD86, CD70, 4-1BBL, OX40L, and GITR-L) as well as expression of cytokines such as IL-12. Furthermore, CD40 activation cause macrophages to develop a more tumoricidal phenotype and tumor cells to increase MHC I expression.

Direct intratumoral (IT) immune modulation utilizes the tumor as a “vaccine site” to generate a tumor specific immune response. We hypothesize that (IT) injection of a CD40 agonist such as APX005M, will “immunize” patients against melanoma neoantigens through “licensing” of tumor infiltrating APCs for tumor specific T cell priming and activation. In preclinical mouse models, we have shown that IT administration of the recombinant adenovirus encoding the dendritic cell-activating CD40L induces CD8⁺ T cell-mediated systemic activity against B16 melanoma. Importantly, IT rAdCD40L also augmented the activity of anti-PD-1.

Methods

This phase I/II trial (NCT02706353) evaluates the safety, efficacy, and immunological impact of IT administration of APX005M (CD40 agonistic mAb) in combination with systemic pembrolizumab in pts with MM. An accelerated 3+3 design was used for the phase 1 dose escalation portion of this study. Pts will receive IT APX005M at escalating doses every 3 weeks for a total of 4 doses. Image guidance will allow for injection of visceral, nodal, and soft tissue metastases. The single-arm phase 2 expansion will evaluate the overall response rate (ORR) of this regimen 12 weeks after initiation of treatment. Some key inclusion criteria: confirmed cutaneous or mucosal melanoma; measurable, unresectable stage-III or stage-IV disease, and at

least 2 injectable lesions. Key exclusion criteria include: prior immunotherapy, uveal melanoma, active autoimmune disease, or active immunodeficiency. A sample size of 26 patients will have 75% power to detect an improvement from a null ORR of 33% to 55%, using a one group chi-square test and assuming a one-sided α -level of 5%. Immune analysis will be performed on pre and on-treatment tumor/liquid biopsies including but not limited to quantification of dendritic cells and T cells both in injected and non-injected tumors.

Trial Registration

NCT02706353

P231

KEYNOTE-590: randomized, phase 3 study of chemotherapy + pembrolizumab vs chemotherapy + placebo as first-line therapy for patients with advanced esophageal or esophagogastric junction (E/EGJ) cancer

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Background

No chemotherapeutic regimens or targeted agents are approved specifically for esophageal cancer; available options have limited benefit and substantial toxicity. In the phase 1b KEYNOTE-028 study (NCT02054806), pembrolizumab monotherapy demonstrated manageable safety and

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durable antitumor activity in heavily pretreated patients with PD-L1–positive advanced esophageal carcinoma. In KEYNOTE-059 (NCT02335411), combining chemotherapy (cisplatin and 5-fluorouracil) and pembrolizumab as first-line treatment for patients with advanced gastric or gastroesophageal junction cancer resulted in encouraging efficacy and manageable safety. This suggests chemotherapy plus pembrolizumab as a potential therapeutic strategy for esophageal cancer. KEYNOTE-590 is a randomized, double-blind, multicenter phase 3 study of cisplatin and 5-fluorouracil plus pembrolizumab versus cisplatin and 5-fluorouracil plus placebo in patients with advanced E/EGJ carcinoma.

Methods

Eligible patients are ≥ 18 years of age; have locally advanced unresectable or metastatic adenocarcinoma or squamous cell carcinoma of esophagus or metastatic Siewert type 1 adenocarcinoma of EGJ, measurable disease per RECIST 1.1, ECOG performance status 0-1, adequate organ function; have had no prior therapy for advanced disease, no autoimmune disease, no active infection; and can provide a newly obtained or archival tissue sample. Patients will be randomly assigned 1:1 to cisplatin 80 mg/m² IV every 3 weeks (Q3W) for 6 cycles plus 5-fluorouracil 800 mg/m² continuous IV on days 1-5 Q3W IV plus pembrolizumab 200 mg IV Q3W or cisplatin 80 mg/m² IV Q3W for 6 cycles plus 5-fluorouracil 800 mg/m² continuous IV on days 1-5 Q3W plus placebo Q3W IV. Treatment will continue up to 2 years. Response will be assessed using CT (preferred) or MRI every 9 weeks by central imaging per RECIST v1.1. Adverse events will be graded per NCI CTCAE v4.0 and up to at least 30 days after the end of treatment. Primary end points are PFS per RECIST v1.1 and OS in all patients and in patients with PD-L1 positive or negative tumor expression (combined positive score $\geq 10\%$ or $< 10\%$ using immunohistochemistry). Secondary end points include ORR per RECIST v 1.1, duration of response, safety, and health-related quality of life. PFS and OS will be compared between groups using a stratified

log-rank test; hazard ratios will be estimated using a Cox regression model. The Kaplan-Meier method will be used to estimate event rates within groups. Enrollment is planned for approximately 700 patients.

Trial Registration

ClinicalTrials.gov, NCT03189719

P232

A pilot study to evaluate the clinical and immunological effects of incorporating a CD40-agonistic antibody into the multimodality treatment of resectable esophageal and GE junction cancers

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Background

Targeting CD40, a member of the TNF receptor superfamily found on antigen presenting cells (APCs), represents a promising cancer immunotherapeutic strategy. Activation of this costimulatory molecule results in improved antigen processing and presentation and cytokine release from activated APCs, enhancing T cell responses. Additionally, CD40 is expressed on many tumor cells that, when activated, results in tumor cell apoptosis and inhibition of tumor growth. APX005M (Apexigen, San Carlos, CA) is a humanized IgG1 anti-CD40 agonistic antibody that binds to CD40 with high affinity. In a FIH phase I clinical trial in patients with advanced solid tumors, APX005M was relatively well tolerated, with cytokine release syndrome (CRS) as the DLT at doses above the RP2D. Importantly, correlative studies show that APX005M produces dose-dependent activation of APCs, T cell activation, and increases in circulating cytokine levels.

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Methods

This pilot trial represents the first to evaluate a CD40-agonistic antibody in esophageal/GE junction cancer, a disease in which IO agents (particularly PD-1 mAbs) have demonstrated promising activity. The study is also the first to explore combining IO with chemoradiation in the neoadjuvant setting, as this multimodality approach represents the standard of care for patients with resectable esophageal/GE junction cancer and is optimally conducive for serial tumor tissue acquisition. A total of 16 patients with resectable (uT1-3N0-1) squamous cell or adenocarcinoma of the esophagus or GE junction will be enrolled. Chemoradiation consists of radiation (5040cGy in 28 daily fractions) and low-dose carboplatin plus paclitaxel weekly x 5, as per standard of care. APX005M 0.3 mg/kg (1 dose level below single-agent MTD) is given every 3 weeks for a total of 4 doses, with the first dose administered two weeks prior to the initiation of concurrent chemoradiation. APX005M administration is offset by 2-3 days from chemotherapy to avoid the steroid premedication administered with paclitaxel. Tumor tissue is acquired via endoscopic biopsy at baseline and following the single-dose "run-in" of APX005M; and then again at esophagectomy, which occurs 1-2 months following completion of chemoradiation. Serial blood collections are also performed at multiple pre-defined timepoints. In addition to assessing the feasibility, safety, and preliminary efficacy (as measured by pathologic complete remission rate) of this novel combination, analyses of tumor tissue and blood will be performed, including Tissue Multiplex Immunohistochemistry and flow cytometry for both APC and T cell activation, as well as T cell receptor sequencing for T cell repertoire diversity.

P233

A phase I study to evaluate the safety of multi-antigen stimulated tumor specific cellular therapy (MASCT-I) in patients with advanced solid tumors

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Background

Tumor-specific immune responses are known to be initiated by tumor associated and/or specific antigen-sensitized dendritic cells (DCs), that can effectively process and present tumor antigens to CD4+ and CD8+ T cells. MASCT-I is a sequential immune cell therapy for solid tumor, which included multi-antigen loaded DC vaccines followed by the adoptive transfer of anti-tumor specific T cells. DC vaccines are produced from patients' autologous PBMC-derived DC loaded with multiple tumor associated antigen peptides and are injected into patients to induce active anti-tumor immunity. Anti-tumor specific T cells are stimulated from the same patient by co-cultured with DC vaccines and Anti-PD1 antibody in vitro, are then infused into the patient to target tumor cells.

Methods

This is a single center, three stages phase I study. Stage1 and 2 will enroll (3+3 design) patients with advanced (unresectable) or recurrent bladder cancer or soft tissue sarcoma who have failed all standard therapies, Patients will be treated with MASCT-I. If the dose limitation toxicity (DLT) in the first cycle of MASCT-I is <33.3%, the stage 2 will begin. Patients who have advanced recurrent or metastatic bladder cancer with Gemcitabine and Cisplatin (GP) chemotherapy achieving clinical benefit (group 1) will be treated with MASCT-I as maintenance therapy. Also, patients who have

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advanced recurrent or metastatic sarcoma with achieving clinical benefit after MAID or CAV/IE (predominant Doxorubicin regimens) (group 2) will be treated with MASCT-I combined with Ifosfamide as maintenance therapy too. During the stage 2, if, in the first cycle of MASCT-I treatment, the DLT is < 33.3%, stage 2 will extend to stage 3. Approximately additional 24 patients will be enrolled in group 1 and 2. The primary objective is safety and tolerability. Secondary objectives include DCR, PFS, TTP, OS. As of 17 May 2017, Three patients were enrolled and completed Stage 1 without any DLT and treatment related SAEs. Five AEs are related with treatment, they are pain on the injection site, fatigue, pruritus and arthralgia, which were all grade 1. Recruitment is ongoing for dose expansion (stage 2). Clinical trial information: NCT030343

P234

AST-VAC2: An allogeneic dendritic cell cancer immunotherapy entering clinical trials in patients with lung cancer in the advanced and adjuvant setting

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Background

Primary lung cancer is the most common malignancy after non-melanocytic skin cancer with deaths exceeding those from any other type of malignancy worldwide. In advanced (TNM stage IIIA, IIIB and IV) disease, only palliative therapy is available which carries a high risk of toxicity and limited potential to extend life. Intradermal delivery of dendritic cells (DCs) carrying an immunogenic

cargo offers a novel approach to address malignancy mediated through upregulated telomerase expression and decreased cell death. AST-VAC2 is a mature, allogeneic DC vaccine derived by differentiating H1 human embryonic stem cells (hESCs) into mature DCs, transfected to express the tumor associated antigen human telomerase reverse transcriptase (hTERT) and lysosomal associated membrane protein 1 (LAMP-1) [1,2]. LAMP-1 fusion proteins enable target hTERT peptides to be directed to HLA (Human Leukocyte Antigen) II and HLA I receptors on the surface of endogenous DCs, invoking dual, antigen-specific CD4+ and CD8+ responses [3]. Preclinical investigations have shown that AST-VAC2 phagocytose, process, and present antigen upon maturation. Furthermore they produce immunostimulatory cytokines, migrate in response to cytokines, and activate antigen specific T cell responses.

Methods

The first-in-human trial will evaluate safety, tolerability, immunogenicity and therapeutic potential in adult NSCLC patients in advanced (metastatic or locally advanced disease) and adjuvant (currently radiologically disease free) settings. Patients who are positive for the HLA-A2 allele (expressed by AST-VAC2) will receive 6 weekly doses of 1×10^7 AST-VAC2 cells and will be monitored for one year as the primary follow-up point and subsequently for up to five years for long-term follow-up. Safety assessments as well as immunological monitoring for the generation and maintenance of hTERT specific T cells will be key endpoints. Results will be used to expand the clinical indications for assessment and support future combination immunotherapy approaches.

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P235

Nivolumab in patients with advanced or metastatic non-small cell lung cancer (Stage IIIb/IV) who have received at least one prior systemic chemotherapeutic regimens

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Background

Nivolumab, a human programmed death 1 (PD-1) immune checkpoint inhibitor antibody, has been shown to increase overall survival in non-small cell lung cancer (NSCLC) patients. This immune checkpoint blockade has been approved in Korea, United States, the European Union, and other countries for the treatment of advanced NSCLC that has progressed after platinum-based chemotherapy. Nivolumab has demonstrated longer overall survival than docetaxel among the previously treated NSCLC patients. We have assessed the safety of nivolumab in 8 patients with previously treated, locally advanced or metastatic NSCLC who were enrolled in the NSCLC Expanded Access Program (EAP) in Korea University Guro Hospital.

Methods

This EAP program included subjects with histologically or cytologically documented NSCLC who have relapsed after systemic treatment with a minimum of 1 prior systemic treatment for stage IIIB/stage IV disease. Subjects were treated with 3 mg/kg of nivolumab IV every 2 weeks for a maximum of 24 months. Each 14-day dosing period constituted a single cycle. Patients included in the analysis had received ≥ 1 dose of nivolumab and were monitored for adverse events (AEs).

Results

As of June 30, 2017, eight patients participated. Median age was 63.0 years. All participants were male. Except for 1 (12.5%) current smoker and 1 (12.5%) never smoker, other 6 (75%) patients were former smokers. The average of smoking period was 27 pack-years. 2 had squamous and 6 had non-squamous histology. At the time of enrollment, 4 had bone, 3 had brain and 3 had brain metastases. Best response rates was 12.5% and disease control rate was 50%. Median progression free survival was 99.5 days (95% CI 50.1-174.7). During the nivolumab chemotherapy, 2 patients had pneumonia and 1 had stroke. Other 5 patients had no critical complication during the treatment.

Conclusions

Our study showed that EAP participants had some lower response rate and more prolonged PFS compared to previously reported in Checkmate-017 and in Checkmate-057. And nivolumab EAP showed good safety profiles. In conclusion, treatment with Nivolumab is safe and effective for patients who have previously received heavily chemotherapy.

P236

Innate immunotherapy of neuroblastoma and PD-1 checkpoint blockade

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Background

Passive immunotherapy of cancer is established for a variety of malignant diseases. Anti-GD₂ antibody (Ab) ch14.18/CHO (dinutuximab beta) showed activity for the treatment high-risk neuroblastoma (NB) patients and received recently marketing approval in the EU. Here we demonstrate that one important mechanism of action in patients is antigen specific Ab-dependent cellular cytotoxicity (ADCC) and we report that ADCC impacts on PD-1/PD-L1 checkpoint regulation that can be targeted by co-treatment with an inhibitor.

Methods

53 patients received 100 mg/m² ch14.18/CHO (d8-17), 6x10⁶ IU/m² sc IL-2 (d1-5; 8-12 and 160 mg/m² oral 13-cis-RA (d19-32) in a closed single center program (53 pts). Polymorphisms in Fcγ-receptor genes 2A (H131R), -3A (V158F) and -3B (NA1/NA2) were determined by real-time PCR. Expression of PD-L1 and PD-1 was analyzed by RT-PCR and flow cytometry. Effect of PD-1/PD-L1 blockade and ch14.18/CHO-mediated anti-NB immune response was evaluated using anti-PD-1 Ab both in vitro (Nivolumab) and in the syngeneic GD₂⁺ NB NXS2 mouse model (anti-mouse PD-1).

Results

We identified 33/53 patients with low affinity FCGR alleles (FCGR2A-H131R/R and/or FCGR3A-V158 F/F). These patients showed lower PFS rates compared to 20/53 patients with high affinity polymorphisms ($p < 0.01$). ADCC levels on day 15 of cycle 1 in pts with high affinity polymorphisms showed an ADCC increase of 20±6% compared to 11±2% in the control. The correlation with functional immune parameter ADCC and clinical outcome confirm its role for clinical efficacy.

Interestingly, tumor specific ADCC in the presence of LA-N-1 neuroblastoma cells, leukocytes and sub-therapeutic ch14.18/CHO concentrations (10 ng/ml)

results in a strong increase of the PD-L1 expression and incubation with IL-2 further enhanced this effect. Blockade with Nivolumab reversed the PD-L1-dependent inhibition of ADCC. Finally, mice treated with ch14.18/CHO in combination with PD-1 blockade showed strongest reduction of tumor growth, longest survival rate as well as the highest level of NB cell lysis mediated by serum and leukocytes of treated mice compared to controls.

Conclusions

Patient studies clearly reveal ADCC as mechanism of ch14.18/CHO against neuroblastoma, and this upregulates the inhibitory checkpoint PD-1/PD-L1. Combination of ch14.18/CHO with PD-1/PD-L1 blockade results in synergistic treatment effects. Similar upregulation of PD-L1 expression by suboptimal ADCC was also seen with the human GD₂⁺ osteosarcoma cell line MG63. This suggests a broader applicability and to consider combinations of passive immunotherapy of cancer with PD-1/PD-L1 checkpoint inhibition.

P237

Phase I study of adoptive transfer of iNKT cells for treating patients with relapsed/advanced hepatocellular carcinoma

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Background

Invariant Natural Killer T (iNKT) cells represent a distinctive subset of T lymphocytes characterized by an invariant receptor Vα24/Jα18 in human, which play critical roles in regulating anti-tumor immunity by bridging innate and adaptive immune responses. We have previously demonstrated that the accumulation of circulating iNKT cells provide a better prognosis for hepatocellular carcinoma (HCC)

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patients, the adoptive transfer of *ex vivo* expanded human iNKT cells in HCC tumor-bearing NOD/SCID mice led to the decrease in tumor size, revealing the iNKT immunotherapy may bring clinical benefits for the HCC patients.

Methods

The phase I trial enrolls patients who have relapsed/advanced HCC tumor relapsed or metastasized through the body after standard treatment or the patients cannot receive standard treatment under current conditions. The purpose of this study is to find the biggest dose of iNKT cells that is safe and tolerance, to see how long they last in the body, to learn the immune-response, the side effects and if the iNKT cells will help people with relapsed/advanced HCC. Key eligibility criteria include age ≥ 18 years, with HCC (BCLC, stage C) proved by histopathology or proved by CT or MRI imaging system, relapsed after previous therapy and no effective therapies known at this time and life expectancy of ≥ 12 weeks. Three different dosing schedules will be evaluated. Three patients will be evaluated on each dosing schedule. The following dose levels will be evaluated: Loading Dose 1: $3 \times 10^7/m^2$; Loading Dose 2: $6 \times 10^7/m^2$; Loading Dose 3: $9 \times 10^7/m^2$. The doses are calculated according to the actual number of iNKT cells. Human Interleukin-2 will be given at a dose of 25,000 IU/kg/day for 5-14 days. Tegafur will be given at a dose of 40~60 mg bis in die (BID) 2 weeks. immune responses were measured by Elispot and ELISA; flow cytometry assays were performed to evaluate the effects on immune cell subsets. Tumor biopsies were evaluated for iNKT cells by immunohistochemistry. Incidence of treatment-emergent adverse events were defined as signs/symptoms, laboratory toxicities, and clinical events that are possibly, likely, or definitely related to study treatment adverse events assessed according to NCI-CTCAE v4.0 criteria 2. HCC progression was evaluated by imaging according to the irRC standard.

Results

To date, one patient has been started on therapy and is in week 3 monitoring period, is tolerating

treatment well, with no significant toxicities thus far. Data regarding the peripheral blood iNKT cells response will be presented.

Trial Registration

ClinicalTrials.gov identifier NCT03175679

P238

Phase I study of adoptive transfer of specific hepatocellular carcinoma antigens CD8⁺ T cells for treating patients with relapsed/advanced HCC

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Background

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers for neoplastic deaths and shows a high recurrence rate. Adoptive T cell therapy, involving the *ex vivo* selection and expansion of antigen-specific T cell clones by MHC/peptide tetramer sorting, provides a method of augmenting antigen-specific immunity against HCC. To generate of a high number of cytotoxic T lymphocytes (CTLs), kind of effective T cells that specific recognizing and killing antigen targeted cells through cloning amplification after receiving antigen information from antigen presented cells, is a promising strategy for adoptive therapy.

Methods

The phase I trial enrolls patients who have HCC tumor relapsed or metastasized through the body after standard treatment or the patients cannot receive standard treatment under current conditions. The purpose of this study is to evaluate the safety and tolerance as well as the potential clinical efficacy of an adoptive transfer of CD8⁺ T cells, sorted with human leukocyte antigen (HLA)-

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peptide multimers and specific for Glypican (GPC)-3 /New York Esophageal Squamous-1 (NY-ESO-1) /alpha-fetoprotein (AFP) antigens and cultured *in vitro*. Key eligibility criteria include age ≥ 18 years, with HCC (BCLC, stage C) proved by histopathology or proved by CT or MRI imaging system, relapsed after previous therapy and no effective therapies known at this time and life expectancy of ≥ 12 weeks. Three different dosing schedules will be evaluated. Three patients will be evaluated on each dosing schedule. The following dose levels will be evaluated: Loading Dose 1: $3 \times 10^7/m^2$; Loading Dose 2: $6 \times 10^7/m^2$; Loading Dose 3: $9 \times 10^7/m^2$. The doses are calculated according to the actual number of GPC3/NY-ESO-1/AFP CTLs. Human Interleukin-2 will be given at a dose of 25,000 IU/kg/day for 5-14 days. Tegafur will be given at a dose of 40~60 mg bis in die (BID) for 2 weeks. Immune responses were measured by Elispot and ELISA, flow cytometry assays were performed to evaluate the effects on immune cell subsets. Tumor biopsies were evaluated for CTLs by immunohistochemistry. Incidence of treatment-emergent adverse events were defined as signs/symptoms, laboratory toxicities, and clinical events that are possibly, likely, or definitely related to study treatment adverse events assessed according to NCI-CTCAE v4.0. HCC progression was evaluated by imaging according to the irRC standard.

Results

To date, 5 patients have been enrolled and 2 of them are in week 2 monitoring period, with no significant toxicities thus far. Data regarding the peripheral blood antigen-specific CTL cells response will be presented.

Trial Registration

ClinicalTrials.gov identifier NCT03175705

P239

Phase 3 study of Pembrolizumab plus Chemoradiation (CRT) vs CRT alone for locally advanced head and neck squamous cell carcinoma (LA-HNSCC): KEYNOTE-412

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Background

CRT with cisplatin is the standard of care for patients with LA-HNSCC not treated by surgery. Preclinical data in murine cancer models show improved tumor growth control and survival when RT is combined with a programmed death 1 (PD-1) inhibitor. Pembrolizumab has been found to be effective for treating recurrent/metastatic HNSCC,

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and initial results from a phase 1b study suggest that pembrolizumab plus CRT is tolerable in patients with LA-HNSCC. KEYNOTE-412 (NCT03040999) is a phase 3, randomized, placebo-controlled, double-blind trial to determine the efficacy and safety of pembrolizumab given concomitantly with CRT and as maintenance therapy versus placebo plus CRT in LA-HNSCC.

Methods

Eligibility includes patient age ≥ 18 years; newly diagnosed, treatment-naive, oropharyngeal p16 positive (any T4 or N3), oropharyngeal p16 negative (any T3-T4 or N2a-N3), or larynx/hypopharynx/oral cavity (any T3-T4 or N2a-N3) SCC; evaluable tumor burden (RECIST v1.1); ECOG performance status 0-1; results available from local testing of human papillomavirus status for oropharyngeal cancer; eligible for definitive CRT and not considered for primary surgery per investigator decision; tissue from a core or excisional biopsy for programmed death ligand 1 (PD-L1) biomarker analysis. Patients will be randomly assigned (1:1) to receive pembrolizumab 200 mg every 3 weeks plus CRT, including radiotherapy (RT; accelerated [70 Gy, six 2 Gy fractions/wk] or standard [70 Gy, five 2 Gy fractions/week] fractionation) plus cisplatin 100 mg/m² Q3W for 3 cycles only, or placebo Q3W plus CRT. Treatment will be stratified by RT regimen (accelerated vs standard), tumor site/p16 status (oropharynx p16 positive vs p16 negative or larynx/hypopharynx/oral cavity), and disease stage (III vs IV). A priming dose of pembrolizumab or placebo will be given 1 week before CRT, followed by 2 doses during CRT, and an additional 14 doses after CRT, for a total of 17 pembrolizumab or placebo infusions. Treatment will be discontinued upon centrally confirmed disease progression, unacceptable toxicity, or patient/physician decision to withdraw. Disease status will be assessed by computed topography or magnetic resonance imaging 12 weeks after CRT, every 3 months for 3 years, then every 6 months for years 4 and 5. Safety will be monitored throughout the study and for 30 days after treatment. The primary end point is event-free survival. Secondary end points include

overall survival, safety, and patient-reported outcomes. Recruitment will continue until ~780 patients are enrolled.

Trial Registration

ClinicalTrials.gov, NCT03040999

P240

Phase 1b trial of cabozantinib in combination with atezolizumab in patients with locally advanced or metastatic urothelial carcinoma or renal cell carcinoma

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Background

Cabozantinib is an oral receptor tyrosine kinase inhibitor targeting MET, VEGFR, and TAM family receptors (TYRO3, AXL, and MER). It is approved for use in patients with advanced renal cell carcinoma (RCC) after prior therapy with antiangiogenic/VEGFR-targeted therapy, and has demonstrated clinical activity in urothelial carcinoma (UC). In clinical studies, cabozantinib exposure resulted in an increase in circulating CD8+ T cells and reduction of immune-suppressive monocytes and Tregs. In preclinical tumor models, treatment with cabozantinib resulted in an increase of MHC class 1 expression on tumor cells and a reduction of myeloid-derived suppressor cells. These observations support that cabozantinib may facilitate an immune-permissive tumor environment and may enhance the response to immune

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checkpoint inhibitors. Atezolizumab, an anti-PD-L1 monoclonal antibody, is approved for use in locally advanced or metastatic UC for patients who are either cisplatin-ineligible or have disease progression during or following platinum-containing chemotherapy. It is also approved for use in patients with metastatic non-small cell lung cancer who have disease progression during or following platinum-containing chemotherapy. Here, we present the study design of an ongoing phase 1b study combining cabozantinib with atezolizumab in patients with locally advanced or metastatic UC or RCC.

Methods

This multicenter, phase 1b, open-label study aims to assess safety, tolerability, preliminary efficacy, and pharmacokinetics of cabozantinib in combination with atezolizumab (NCT03170960). The study will enroll patients with advanced UC (including bladder, renal pelvis, ureter, urethra) or RCC. It consists of two stages: a dose-escalation stage and an expansion-cohort stage. In the dose-escalation stage (3+3 design), a recommended cabozantinib dose for the combination will be established. In the expansion stage, four tumor-specific cohorts will be enrolled, and the primary objective is to determine the objective response rate in each cohort. The four expansion cohorts are (1) patients with UC who have progressed on or after platinum-containing chemotherapy; (2) chemotherapy-naïve patients with UC who are ineligible for cisplatin; (3) chemotherapy-naïve patients with UC who are eligible for cisplatin; and (4) previously untreated patients with RCC with clear cell histology. Exploratory objectives include correlation of tumor and plasma biomarkers, and changes in immune cell profiles with clinical outcome. The study has been initiated and enrollment target is up to 120 patients across the 4 cohorts in the expansion-cohort stage.

Trial Registration

ClinicalTrials.gov: NCT03170960

P241

A first-in-human study of ALX148: CD47 blockade to enhance innate and adaptive immunity for advanced solid tumor malignancy and non hodgkin lymphoma

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Background

CD47, a marker of self, is upregulated by tumors to evade the immune system. Blocking the interaction between CD47 and SIRP α , its receptor on myeloid cells, disrupts a key immune checkpoint and may enhance innate and adaptive immunity against cancer. ALX148 is a high affinity, engineered fusion protein containing the N-terminal D1 domain of SIRP α , which binds and blocks CD47, and is genetically linked to an inactive human Fc domain to minimize toxicity. ALX148 enhanced activity of multiple anti-cancer targeted antibodies and checkpoint inhibitors with minimal effect on normal blood cells in nonclinical models. This phase 1 study evaluates the safety, tolerability, pharmacokinetic (PK) and pharmacodynamic profiles of ALX148 in patients with advanced malignancy.

Methods

The primary study objective is to characterize the safety profile of ALX148 first as a single agent and then in combination with established anti-cancer antibodies. Cohorts (3-6 pts) with advanced malignancy receive escalating doses of ALX148, intravenously, once weekly or once every other

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week. Tumor response, PK, and target occupancy (TO) are characterized as secondary objectives. Preliminary single agent data are reported from the data cutoff, July 21, 2017 and will be updated at the time of presentation.

Results

Ten patients received ALX148 (4 males/6 females; 0.3 mg per kilogram (mpk), 3; 1.0 mpk, 4; 3.0 mpk, 3) as of data cutoff. Median age was 63 (37-76) yrs and ECOG PS 0/1: 1/9. Four patients experienced treatment related adverse events (AEs) which were predominantly low grade and included 1 each at 0.3 mpk (G1 Headache, Rash, Fatigue); 1.0 mpk (G3 Anemia, G1 Dysgeusia); and at 3.0 mpk (G2 Decreased Appetite, Hypersensitivity). As of the data cut-off no pts have experienced a dose-limiting toxicity. One patient achieved SD (0.3 mpk; leiomyosarcoma) for 16 weeks. ALX148 initial PK showed increased exposure with increasing dose and noticeable accumulation with repeated dosing, likely driven by target saturation. Dose dependent TO on CD47 by ALX148 was observed on RBCs and T cells. The magnitude and duration of TO increased with repeat dosing.

Conclusions

ALX148 is well tolerated in patients with advanced solid tumors with favorable PK/TO characteristics and no significant hematologic toxicity at doses evaluated. Accrual is ongoing. When the maximum tolerated dose/optimal biological dose of single agent ALX148 is established, patients with advanced malignancy will be evaluated with ALX148 in combination with anticancer antibodies.

Trial Registration

ClinicalTrials.gov identifier NCT03013218.

References

1. Weiskopf K. Cancer immunotherapy targeting the CD47/SIRP α axis. *Eur J Cancer*. 2017;76:100-109.

P242

A phase 1 multicenter, dose escalation study of CBT-501, a novel anti-PD-1 inhibitor in subjects with select advanced or relapsed/recurrent solid tumors

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Background

Programmed death-1 (PD-1, CD279) is an inhibitory co-receptor expressed on antigen-activated and exhausted T and B cells. PD-1/PD-L1 axis inhibition by targeted-antibodies, increases the T cell proliferation and cytotoxicity. This represents a promising mechanism to stimulate the anti-tumor activity of the immune system. CBT-501, genolimzumab (GB226) is a novel humanized IgG4 monoclonal antibody targeting the PD-1 membrane receptor on T lymphocytes and other cells of the immune system. CBT-501 demonstrated highly specific binding to PD-1 of human (Kd=505 pM) and cynomolgus (Kd=7.2 nM). CBT-501 efficiently inhibited the binding of PD-L1/L2 to PD-1 for both human and monkey and enhanced human T cell activation in the Mixed Lymphocyte Reaction (MLR) assay. CBT-501 has demonstrated anti-tumor activity in the in vivo animal model and no abnormal drug-related toxicity has been observed in the GLP toxicology studies. Data from all pre-clinical pharmacodynamics and toxicology studies of CBT-501 indicate pharmacological activity at effective doses with a wide margin of safety. Based on these findings, a Phase 1 study has been initiated with CBT-501 in Australia.

Methods

CBT-501-01 is a Phase 1, multicenter, dose escalation study of CBT-501 in subjects in select advanced or relapsed/recurrent solid tumors. The primary study objective is to identify the overall

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safety and tolerability, including any dose limiting toxicities (DLT), and determine the recommended Phase 2 dose (RP2D) in subjects with advanced solid tumors. Secondary objectives include assessing efficacy by overall response rate (ORR), best overall response rate (BOR) per RECIST v1.1 and irRECIST, time to response, duration of response (DOR), disease control rate (DCR) by RECIST v1.1 and irRECIST, progression free survival (PFS), and determining the pharmacokinetic (PK) parameters. Exploratory objectives involve the assessment of PD-1 and PD-L1 expression, receptor occupancy and the host immune response (immune modulation) in blood peripheral-blood mononuclear cells (PBMCs) or formalin-fixed paraffin-embedded (FFPE) samples. This is a 2-part study with a dose-escalation segment and dose and disease expansion cohorts of CBT-501. In Part 1, dose escalation (3+3 design) will occur among 3 cohorts to determine the RP2D. The tumor type(s) with the most robust clinical signal relative to response rate and safety/tolerability will be selected for further evaluation in the expansion cohort (Part 2). Approximately 32 subjects will be enrolled in the dose and disease expansion and treated at the RP2D, as determined in Part 1.

Trial Registration

Clinical Trial Registry Number: NCT03053466

P243

An Open-Label Perioperative Pilot Study Evaluating Nivolumab Alone Versus Nivolumab Plus Ipilimumab in Patients with Resectable and Potentially Resectable HCC

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Background

Surgical resection or liver transplantation are the only potentially curative treatment modalities for localized hepatocellular carcinoma (HCC) in select patients. Unfortunately, the 5-year tumor recurrence rate following surgical resection is up to 70%. Moreover, no FDA-approved therapies exist in the adjuvant setting after surgical resection. A recent Phase I/II trial with anti-PD-1 antibody nivolumab in 42 patients with advanced or unresectable HCC demonstrated a 19% ORR with many durable responses and 2 complete responses. Both preclinical data and clinical data in other tumor types demonstrate synergy in targeting the PD-1 and CTLA-4 pathways the combination of nivolumab and ipilimumab induces even greater anti-tumor responses than either agent alone in multiple solid tumor types. We hypothesize that nivolumab alone or in combination with ipilimumab can be safely administered and may induce augmented immunological and clinical responses in patients with resectable or potentially resectable HCC.

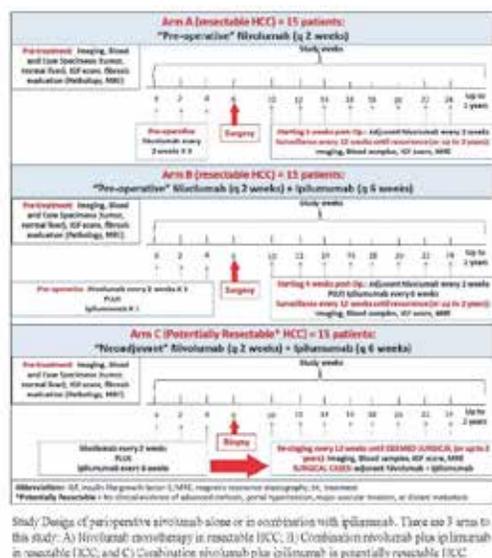
Methods

This is a pilot, randomized, open-label study of perioperative nivolumab alone (Arm A) or in combination with ipilimumab (Arm B) in patients with resectable HCC and perioperative nivolumab plus ipilimumab (Arm C) in patients with potentially resectable HCC (Figure 1). 45 patients will be enrolled with 15 patients in each arm following screening by a surgeon who will determine resectability and a pretreatment biopsy confirming diagnosis. Nivolumab will be administered 240 mg IV q2 weeks and ipilimumab 1 mg/kg q6 weeks. Resection will be performed on week 7 day 1 in arm A and B and treatment will continue adjuvantly until disease progression or for 2 years, whichever is sooner. Patients in Arm C will undergo resection if deemed resectable and will continue therapy until progression or for 2 years, as well. The primary objective is to evaluate safety and tolerability of therapy with nivolumab alone or in combination with ipilimumab in resectable and potentially resectable HCC. The secondary objective is to assess

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objective response rates, time to progression, and progression free survival with perioperative nivolumab alone or in combination with ipilimumab therapy in HCC and estimate the conversion rate to surgery for potentially resectable patients. Immune analysis will be performed on pre and on-treatment tumor/liquid biopsies, as well. Some key inclusion criteria include histologically confirmed HCC who are eligible or potentially eligible for liver resection with curative intent, measurable disease, and ECOG PS ≤ 1 . Key exclusion criteria include prior checkpoint inhibitor therapy, organ transplantation, autoimmune disease, and active immunodeficiency.

Figure 1. Study Design



Study Design of perioperative nivolumab alone or in combination with ipilimumab.

P244

A phase 1, first-in-human, open-label, dose escalation study of MGD013, a bispecific DART[®] protein binding PD-1 and LAG-3 in patients with unresectable or metastatic neoplasms

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Background

Lymphocyte-activation gene 3 (LAG-3) is a membrane protein in the immunoglobulin superfamily that binds to major histocompatibility complex class II (MHC-II). LAG-3 engagement negatively regulates T cell proliferation and differentiation. Blockade of PD-1 and LAG-3 in animal tumor models enhanced antitumor immunity via distinct, non-redundant signaling pathways that fostered the accumulation of functionally competent CD8⁺ T cells in mice [1]. Dual targeting of PD-1 and LAG-3 may help reverse effector cell exhaustion and increase the response rates and/or effectiveness of immunotherapy beyond that observed with single agents alone. MGD013 is an Fc-bearing bispecific tetravalent (bivalent for each antigen) DART[®] protein engineered as a hinge-stabilized immunoglobulin G4 molecule and designed to concomitantly bind PD-1 and LAG-3, thereby contributing to sustain or restore the function of exhausted T cells. MGD013 may enhance T cell activation in a synergistic fashion beyond that observed with the anti-PD-1

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and anti-LAG-3 monoclonal antibodies alone or in combination. A bispecific format for target engagement may confer biologic advantages that may translate to clinical advantages over antibody combinations.

Methods

This is an open-label, dose escalation / cohort expansion phase 1 study (NCT03219268) designed to characterize the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of MGD013. Patients with unresectable, locally advanced or metastatic solid tumors of any histology are enrolled in the dose escalation phase. Sequential escalating flat doses ranging from 1 mg to 1600 mg every 2 weeks are evaluated in successive cohorts of 1 to 6 patients each. A single patient dose escalation design is utilized in the lower dose cohorts. The escalation approach transitions to a conventional 3+3 design after the first three cohorts. Occurrence of a drug-related Grade 2 adverse event in a single patient cohort will lead to enrollment of 3 additional patients at that dose level. Occurrence of a DLT in a single patient cohort will trigger transition to a conventional 3+3 design. Response is first determined at 8 weeks. Patient management is guided by response assessment according to irRECIST. MGD013 dosing may continue up to 2 years based on response. Cohort expansion phase will start after maximum tolerated dose is determined and will be restricted to 5 tumor types, including solid tumors and hematological malignancies.

Trial Registration

Clinicaltrials.gov- NCT03219268

References

1. Grosso JF, Kelleher CC, Harris TJ, Maris CH, Hipkiss EL, De Marzo A, et al. LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. *J Clin Invest.* 2007;117(11):3383-92.

P245

CAPRA: A Phase 1b study of intratumoral Cocksackievirus A21 (CVA21) and systemic pembrolizumab in advanced melanoma patients.

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Background

Cocksackievirus A21 (CVA21) is a novel bio-selected oncolytic, immunotherapeutic agent. Intratumoral (i.t.) CVA21 injection can induce selective tumor-cell infection, immune-cell infiltration, IFN-g response gene up-regulation, increased PD-L1 expression, tumor cell lysis and systemic anti-tumor immune responses. A clinical trial evaluating combination CVA21 and pembrolizumab in patients with melanoma was initiated and preliminary data on a pre-established futility endpoint are presented here.

Methods

This is a single-arm, multi-institutional open-label phase 1b clinical trial of i.t. CVA21 and i.v. pembrolizumab for treated or untreated unresectable Stage IIIB-IVM1c melanoma. Subjects with injectable disease receive up to 3×10^8 TCID₅₀ CVA21 i.t. on Days 1, 3, 5, 8, and then every 3 weeks for up to 19 injections. Subjects also receive pembrolizumab (2mg/kg) i.v. every 3 weeks starting on Day 8. The primary endpoint is safety/tolerability by incidence of dose-limiting toxicity. Secondary endpoints include best ORR by immune-related response criteria, progression-free survival, overall survival, quality of life.

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Results

To date, 22 subjects have started on protocol therapy. Overall, the adverse events have been low-grade constitutional symptoms related to CVA21 and expected pembrolizumab-related side effects. One subject had Grade 3 increased hepatic enzymes that was considered related to pembrolizumab. No DLT's have been reported. Currently, 19 patients are evaluable for investigator response assessment. Among the evaluable subjects (n=19), the ORR was 63% (12/19). The DCR (CR+PR+SD) is currently 84% (16/19). In subjects with stage IVM1c disease, the ORR is 78% (7/9). The study has met its primary statistical futility endpoint of achieving ≥ 2 confirmed objective responses (CR or PR) in the first 12 patients enrolled. One of the 12 responders displayed early pseudo-progression and later developed a partial response.

Conclusions

Based on these initial results, the sample size has now been expanded to enroll up to 50 patients including subjects refractory to anti-PD1 therapy. Combination therapy of CVA21 and pembrolizumab may represent a new approach for the treatment of patients with injectable advanced melanoma.

Trial Registration

NCT02565992

Consent

Written informed consent was obtained from all of the patients for participation in the study and use of the data for publication.

P246

Phase 1/2 study of in situ vaccination with tremelimumab + intravenous (IV) durvalumab + poly-ICLC in patients with select relapsed, advanced cancers with measurable, biopsy-accessible tumors.

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Background

Immunotherapy has demonstrated promising antitumor activity in various advanced cancers. Combined tumor targeting from multiple drugs with unique mechanisms may provide further improved outcomes. Tremelimumab (TRE) is a CTLA-4 antibody and durvalumab (DUR) blocks PD-L1. Poly-ICLC is a toll-like receptor 3 agonist. Intratumoral (intra-T) injection of poly-ICLC directly alters the tumor microenvironment (TME), and by creating an in situ vaccination, may trigger a clinically effective systemic anti-tumor response when also combined with DUR and TRE.

Methods

This is an ongoing Phase 1/2, open-label, multicenter study (NCT02643303). The study evaluates the use of intra-T administration of TRE and IV DUR + poly-ICLC (intra-T and intramuscular [IM]) to determine the safety, preliminary efficacy and immune activity of this regimen in patients with advanced, measurable, biopsy-accessible tumors: head and neck squamous cell carcinoma, breast cancer, sarcoma, merkel cell carcinoma, cutaneous T cell lymphoma, melanoma, genitourinary cancer, and other solid tumors. Phase 1 determines the recommended combination dosing (RCD) for the regimen with dose de-escalation based on dose limiting toxicities (DLTs) and standard 3 + 3 rules. Starting doses are: DUR, 1500 mg IV; TRE, 75 mg IV; TRE, 10 mg intra-T; poly-ICLC, 1 mg intra-T/IM. Phase 1 starts with Cohort 1A (DUR + poly-ICLC). Upon demonstration of tolerability, enrollment proceeds with Cohort 1B (DUR + IV TRE + poly-ICLC) and Cohort 1C (DUR + intra-T TRE + poly-ICLC). The RCD is the highest dose at which < 2/6 patients have DLTs. In Phase 2, up to 66 evaluable patients are

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treated using the RCD regimen, with enrollment of 6 patients per tumor type initially, and enrollment of 6 additional patients per 3 tumor types contingent upon at least 1 response among the initial 6 patients. Study endpoints are RCD and safety, objective response rate, progression-free survival, and overall survival. Exploratory endpoints are biological activity, including effects on the TME and immunological responses. Enrollment opened on 28 Dec 2016.

Results

Trial in Progress

Conclusions

Trial in Progress

Trial Registration

Clinicaltrials.gov: [NCT02643303](https://clinicaltrials.gov/ct2/show/study/NCT02643303)

P247

Study Design: Phase 1 dose escalation, multi-tumor study to assess safety, tolerability and antitumor activity of genetically engineered MAGE-A4 SPEAR T cells in HLA-A2⁺ subjects with MAGE-A4⁺ tumors

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Background

MAGE-A4 is a cancer/testis antigen that has been identified in 13-48% of non-small cell lung cancer (NSCLC), urothelial, melanoma, head and neck,

ovarian, gastric and esophageal tumors. This study (NCT03132922) will evaluate the safety and tolerability of genetically engineered autologous specific peptide enhanced affinity receptor (SPEAR) T cells (MAGE-A4^{c1032}T cells) directed towards a MAGE-A4 peptide expressed on tumors in the context of HLA-A 02. Antitumor activity will also be assessed.

Methods

This first-in-human T cell dose escalation study utilizes a modified 3+3 design to evaluate safety, including dose limiting toxicities (DLT). Secondary objectives include anti-tumor activity (overall response (per RECIST v1.1), duration of response, time to response, progression-free survival, overall survival) and translational research assessments. Patients are screened under a separate protocol (NCT03132922). Those who are HLA-A02 positive (with the exception of A02:05) and have inoperable or metastatic (advanced) NSCLC, urothelial cancer, melanoma, or squamous cell head and neck, ovarian, gastric or esophageal tumors with MAGE-A4 expression and meet all other entry criteria are eligible for treatment. Subjects must have prior treatments as described in the table (Table 1). Patients must have received standard of care therapies and have measurable disease.

Following leukapheresis, the T cells are isolated, transduced with a lentiviral vector containing the MAGE-A4^{c1032} TCR, and expanded with CD3/CD28 beads. Subjects are given lymphodepleting chemotherapy (fludarabine 30 mg/m²/day and cyclophosphamide 600 mg/m²/day, on days -7, -6 and -5) prior to infusion of transduced cells. Groups 1, 2 and 3 will consist of 3-6 subjects, and the transduced cell doses will be as follows, respectively: 0.1×10^9 ($\pm 20\%$), 1×10^9 ($\pm 20\%$), and 5×10^9 (range: $>1.2 - 6 \times 10^9$). The DLT observation period will be during the first 30 days following the infusion of MAGE-A4 SPEAR T cells for each patient in all groups. Following the dose escalation, up to 10 patients will be enrolled at the target dose.

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Disease assessments will be conducted at week 6, 12, 18 and 24, and then every 3 months until confirmation of disease progression or at 2 years post-infusion. On study tumor biopsies and blood samples will be evaluated to compare the pre- and post-T cell infusion immune profile for association with treatment outcome.

Trial Registration
 NCT03132922

Table 1. Eligibility Criteria

Tumor Type	Requirement
Multiple Myeloma	<ul style="list-style-type: none"> Requires relapsed/refractory disease Not eligible if patient has received autologous stem cell transplant (ASCT) or has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT
Multiple Myeloma	<ul style="list-style-type: none"> Requires relapsed/refractory disease Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT
Multiple Myeloma and Plasma Cell Leukemia	<ul style="list-style-type: none"> Requires relapsed/refractory disease Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT
Multiple Myeloma	<ul style="list-style-type: none"> Requires relapsed/refractory disease Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT
Multiple Myeloma	<ul style="list-style-type: none"> Requires relapsed/refractory disease Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT
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Multiple Myeloma	<ul style="list-style-type: none"> Requires relapsed/refractory disease Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT Not eligible if patient has received ≥ 2 cycles of ASCT

P248

Study Design: An open-label randomized pilot study of NY-ESO-1 SPEAR T cells alone or in combination with pembrolizumab in HLA-A2+ subjects with relapsed and refractory multiple myeloma (NCT03168438)

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Background

NY-ESO-1 and LAGE-1a are cancer/testis antigens that are expressed frequently in multiple myeloma (MM) and are often associated with poor prognosis. This two-arm randomized study will evaluate the safety and efficacy of genetically engineered autologous specific peptide enhanced affinity receptor (SPEAR) T cells (NY-ESO-1^{c259}T cells) directed towards a NY-ESO-1/LAGE-1a peptide expressed on tumor cells in the context of HLA-A02, alone and in combination with pembrolizumab.

Methods

This open label randomized pilot study will evaluate safety, efficacy (using the International Myeloma Working Group Uniform Response Criteria), and translational research endpoints. Patients must meet these criteria: ≥ 18 yrs old; HLA-A02:01, A02:05 or A02:06 positive; have histologically confirmed diagnosis of MM with either primary refractory or relapsed/refractory disease expressing NY-ESO-1 and/or LAGE-1a; prior therapies including IMiD and a proteasome inhibitor as separate lines or a combined line of therapy; and adequate organ function. Subjects who have relapsed after autologous hematopoietic cell transplantation (HCT) or are unable to receive autologous HCT are eligible. Target enrollment for this study is 20 subjects, with 10 in each arm; patients will be randomly assigned to a treatment arm. Eligible subjects who do not receive the T cell infusion may be replaced.

Following apheresis, the T cells are isolated and expanded with CD3/CD28 beads, transduced with a lentiviral vector containing the NY-ESO-1^{c259} TCR, and 1– 8 × 10⁹ transduced T cells are infused intravenously on day 1 after lymphodepletion with fludarabine 30 mg/m²/day and cyclophosphamide 600 mg/m²/day on days -7 to -5 and granulocyte-colony stimulating factor support starting on day -4.

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In Arm 1, SPEAR T cell infusion is the only investigational product administered. Subjects in Arm 2 will receive SPEAR T cell infusion followed by an initial 200 mg dose of pembrolizumab on day 22 (week 3). If toxicities preclude week 3 treatment, the first dose may be given at week 6. Subsequent doses of pembrolizumab will be given every 3 weeks up to week 108 post T cell infusion.

In both arms, safety will be assessed at each clinic visit. Disease response is assessed at weeks 1 and 3, every 3 weeks until week 24, every 6 weeks until week 72, and then every 12 weeks until confirmed progression of disease. On study biopsies and blood samples will be evaluated to compare the pre- and post-T cell infusion immune profile for association with treatment outcome.

Trial Registration

NCT03168438

P249

See Clinical Trials (Completed)

P250

LTX-315, an oncolytic peptide converts "cold" tumors to "hot" in a majority of patients with advanced cancer: results from an ongoing phase I study.

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Background

LTX-315 disintegrates cytoplasmic organelles (e.g mitochondria) and induces immunogenic cancer cell death in preclinical in vivo models. Intratumoral LTX-315 increases tumor-infiltrating lymphocytes (TILs) and induces complete tumor regression in several rodent models. Systemic (abscopal) anti-tumor immune responses can be enhanced upon combination with immune checkpoint inhibitors (ICI). Here we report preliminary results of the phase 1 trial which evaluates intratumoral LTX-315 in monotherapy or in combination with ICI.

Methods

Patients with advanced solid tumors were immunologically primed with weekly intratumoral LTX-315 into a single accessible lesion over 6 weeks. Additional injections could be administered thereafter every 2 weeks. ICI combinations included ipilimumab (melanoma cohort) and pembrolizumab (TNBC cohort). Biopsies of injected lesions were taken at baseline and after LTX-315 treatment. Immunoprofiling was performed using immunohistochemistry and Nanostring analysis. Twenty nine patients have been treated with LTX-315 monotherapy. LTX-315 monotherapy was administered at doses of 2-7mg to a median of 1.8 tumor lesions (range 1-6) for a median of 9 weeks (range 1-33).

Results

In 29 patients, all LTX-315-related adverse events were CTC grade 1 or 2, most commonly local erythema, flushing, pruritis and hypotension, usually resolving within minutes of injection. Related grade 3 (3 patients) or 4 (1)

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allergic/anaphylaxis adverse events occurred and resolved without sequelae. Of 44 injected lesions in 20 evaluable LTX-315 monotherapy patients, 2 lesions regressed completely, > 50% regression was seen in 5 lesions, and 20 remained stable. Significant increases in CD8+ TILs occurred in 67% (14 of 21) patients with evaluable biopsies. The HaliDx Immune Gene Signature analysis of LTX-315-treated tumors showed upregulation of genes involved in immune-mediated tumor regression (effector T cells, TH1 orientation, chemokines and cytokines). Regression of distant non-injected tumors (irRC) criteria has been observed in 11 of 30 tumors in 9 patients. Stable disease (SD) of at least 7 weeks duration in non-injected tumors (median duration 11 weeks) by immune-related RECIST criteria (irRC in evaluable patients) occurred in 50% of LTX-315 monotherapy patients (4 melanoma, 3 sarcoma, and 1 breast cancer patients).

Conclusions

This phase 1 study demonstrates that intratumoral LTX-315 is generally safe and tolerable. Intratumoral LTX-315 triggers an increase in TILs as assessed by IHC, and generates a transition from a cold to a hot tumor transcriptome as assessed by Immunosign[®] gene signature. Moreover, local and systemic clinical benefit (achievement of SD in non-injected sites per irRC criteria) was observed.

Trial Registration

Clinical trial information: NCT01986426

P251

A study to evaluate the safety and efficacy of the CD40 agonistic antibody APX005M in combination with nivolumab in subjects with non-small cell lung cancer and subjects with metastatic melanoma

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Background

Blocking immune checkpoint PD-1, PD-L1 and CTLA-4 function enhance antitumor immunity, leading to durable clinical responses for a subset of patients with melanoma, lung cancer and other tumor types. However, the majority of patients with melanoma or lung cancer continue to have short or no response to checkpoint blockade therapies and thus require novel approaches to stimulate the antitumor immune response such as immune stimulatory antibodies. Recently, Zippelius and co-authors [1] showed in preclinical models that CD40 engagement with an agonistic mAb leads to a T cell and IFN- γ dependent upregulation of PD-L1 on tumor infiltrating monocytes and macrophages, thereby promoting a negative feedback loop, which hampers CD40 induced T-cell responses. This resistance mechanism was successfully circumvented by co-administration of PD-1/PD-L1 blocking antibodies. To this end, Apexigen Inc., is developing APX005M a humanized monoclonal IgG1 CD40 agonistic antibody that stimulates both innate and adaptive immune response. APX005M recognizes a unique epitope that overlaps with the CD40 ligand binding sites and uses Fc γ R2b to cluster CD40, thus mimicking CD40L engagement. As a result of antigen presenting cell (APC) activation, APX005M enhances T-cell response to tumor antigens. APX005M combined with antibodies against PD-1 or PD-L1 synergistically enhances T-cell responses. In a phase 1 trial, APX005M was administered IV every 21 days to adult subjects up to 1mg/kg with an acceptable safety profile and has demonstrated a dose-dependent activation of APCs

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and T cells and increases in circulating levels of cytokines.

Methods

Study APX005M-002 is a Phase 1b–2 study of APX005M administered in combination with nivolumab every 21 days to adult subjects with platinum pre-treated immunotherapy naïve non-small cell lung cancer (NSCLC) or metastatic melanoma after failure of anti-PD-1/PD-L1 therapy (MM). The Phase 1b portion will establish the maximum tolerated dose and the recommended Phase 2 dose of APX005M with nivolumab. The Phase 2 portion will evaluate safety and efficacy of the combination in each of the two distinct tumor types. Inclusion criteria include: age \geq 18 years, histologically documented NSCLC or MM, measurable disease by RECIST 1.1, ECOG performance status 0-1, adequate organ function. Exclusion criteria include: concomitant anti-cancer therapy, history of bone marrow transplantation, active coagulopathy, previous immune mediated disorders, active infections or uncontrolled intercurrent illness. Recruitment is ongoing, with a target enrollment of approximately 100 subjects across 7 centers in the United States.

Trial Registration

Clinical trial information: NCT03123783.

References

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P252

Phase 1 study to evaluate the safety and tolerability of the CD40 agonistic monoclonal antibody APX005M in subjects with solid tumors

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Background

APX005M is a humanized monoclonal IgG1 CD40 agonistic antibody developed by Apexigen, which mimics the natural ligand CD154. APX005M binds with high affinity to human CD40 leading to antigen presenting cell (APC) activation and subsequent T cell activation. APX005M enhances T cell proliferation, IFN- γ production and T-cell response to tumor antigens. In comparison with other anti-human CD40 agonistic antibodies, such as CP-870,893/RG7876, SGN-40, and ADC-1013/JNJ-64457107 analogs, APX005M is the most potent CD40 agonist and outperforms all others in many measures of immune activation. In a first in human clinical trial APX005M was administered IV every 21 days and has demonstrated a dose-dependent activation of APCs and T cells and increases in circulating levels of IL-12, IFN- γ , TNF α and IL-6. APX005M was escalated up to 1mg/kg with a good safety profile.

Methods

Study APX005M-001 was originally designed as a multicenter Phase 1 dose escalation study of APX005M administered every 3 weeks to subjects with solid tumors and was amended to introduce two new dosing schedules for APX005M (every 2 weeks and every 1 week). Currently, primary objectives of the study are to evaluate the safety of APX005M administered intravenously (IV) every 2 weeks and every week and to determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of APX005M for the every 2 weeks and every 1 week schedules. Secondary objectives include determining the pharmacokinetics (PK) of APX005M and preliminary assessment of clinical response. Inclusion criteria include: age \geq 18 years, histologically or cytologically documented diagnosis of urothelial carcinoma, melanoma, squamous cell carcinoma of

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the head and neck, non-small cell lung cancer, or any solid tumor with high microsatellite instability status (MSI-high), measurable disease by RECIST 1.1, ECOG performance status 0-1, adequate organ function. Exclusion criteria include: concomitant anti-cancer therapy, history of bone marrow transplantation, active coagulopathy, previous immune mediated disorders, active infections or uncontrolled intercurrent illness. Recruitment is ongoing, with a target enrollment of approximately 20 subjects across 3 centers in the United States.

Trial Registration

Clinical trial information: NCT02482168.

P253

Prioritizing tumor types for treatment with a novel immunotherapy: LYC-55716 a small-molecule ROR γ agonist

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Background

ROR γ is the master transcription factor for Type 17 effector T cell differentiation and function. ROR γ t expression is induced by cytokines such as IL-6, TGF- β , IL-1b, and IL-23 and is activated by endogenous ligands derived from the cholesterol biosynthetic pathway. Synthetic ROR γ agonists augment the activity of this transcriptional regulator by modulating a gene expression program in immune cells, resulting in enhanced effector functions and decreased immunosuppression. LYC-55716 and other ROR γ agonists have shown promise as monotherapy and combination therapy in syngeneic tumor models. During Phase 1 clinical testing of this compound, preclinical and bioinformatics assessments were undertaken to prioritize tumor

types that may respond to ROR γ agonist therapy, for possible inclusion in a Phase 2a trial.

Methods

An ROR γ agonist signature was derived from transcriptional profiling of primary murine and human T cells treated with or without ROR γ agonists. Using a panel of murine syngeneic models and The Cancer Genome Atlas (TCGA) dataset, a series of bioinformatic analyses were conducted to provide information across tumor types on (a) ROR γ expression, (b) ROR γ biology, including sterol mobilizing genes and correlations with prognosis, and (c) general immune parameters. Public data sets were also assessed for correlations of ROR γ signature genes and prognosis. For each assessment, tumor types were prioritized, then compared across categories to determine a final ranking.

Results

Target expression: 15 tumor types were identified, with >20% of samples expressing ROR γ t. However, based on lack of correlation between baseline ROR γ t expression and efficacy of ROR γ agonists in preclinical models, these criteria were extended to include additional tumors that express mRNA for factors known to induce ROR γ expression. Target biology: As a surrogate for endogenous ligand levels, TCGA analysis revealed differentially expressed sterol synthesis and efflux genes across tumor types. ROR γ t expression correlated significantly with patient survival in 5 tumor types. Immune parameters: Consideration of tumors with immune infiltrates, high mutational burden, and reports of prior immunotherapy success highlighted 8 tumor types. After combining these findings, 7 tumor types met all selection criteria: non-small-cell lung; ovarian; stomach adenocarcinoma; and head and neck squamous cell, renal clear cell, hepatocellular, and esophageal carcinomas.

Conclusions

Preclinical data from syngeneic tumor models and bioinformatic analyses of TCGA database prioritized 7 tumors for LYC-55716 monotherapy. Our findings

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support the inclusion of these tumor types in the Phase 2a clinical trial.

P254

A phase 1b/2 study of CD40 agonistic monoclonal antibody (APX005M) together with gemcitabine and nab-paclitaxel with or without nivolumab in untreated metastatic pancreatic adenocarcinoma patients

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Background

Pancreatic cancer is one of the most lethal malignancies of the gastrointestinal tract. While check point inhibitors such as α CTLA4 and α PD-1 have been effective in melanoma and lung cancer, clinical benefit of immunotherapy in the management in pancreatic cancer subjects has not been yet established. A recent study using a genetically engineered mouse model of pancreatic ductal adenocarcinoma (PDA) demonstrated that despite robust expression of PD-1 and PD-L1 in the tumor microenvironment, treatment with α PD-1 with or without α CTLA-4 failed to improve the survival of mice or slow the growth of PDA tumors. However, administration of α CD40, gemcitabine (Gem) and nab-paclitaxel (NP), induces T cell immunity in mice with PDA, controls tumor growth and significantly improves survival in a CD8⁺ T cell dependent manner. In particular, α CD40/Gem/NP

plus α PD-1 nearly double the median overall survival in genetically engineered KPC mice with pre-established spontaneous pancreatic tumors. Moreover, the capability of treated mice to reject second and third subcutaneous tumor challenges in a CD8⁺ T cell-dependent fashion thereby rendering long-term survival suggests the establishment of antitumor immune memory with curative potential.

Methods

This is a multi-center, open label study evaluating the combination of APX005M with Nivolumab and standard chemotherapy (Gem and NP). The Phase 1b will define the recommended Phase 2 dose of APX005M when combined with the standard dose of Gem and NP, with or without Nivolumab. The second part of the study is a 3-arm randomized Phase 2 (35 subjects per treatment arm) aimed to evaluate the activity (overall survival) of APX005M combined with Gem and NP, with or without Nivolumab with Gem and NP. Main inclusion criteria include: age \geq 18 years, documented diagnosis of pancreatic adenocarcinoma with metastatic disease, measurable disease by RECIST 1.1, ECOG performance status 0-1, normal organ function. Main exclusion criteria include: concomitant anti-cancer therapy, previous exposure to CD40, PD-1, PD-L1, CTLA-4 mAbs or any other immunomodulatory agents, previous immune mediated disorders, active infections or uncontrolled intercurrent illness. The study will be exploring different doses of APX005M in combination with approved doses of Nivolumab and chemotherapy. The main safety endpoints include the frequency of DLT and incidence of AEs. The main efficacy endpoints include overall survival, and 1-year OS rate in each treatment arm.

Results

This trial is open for enrollment.

Conclusions

Clinical trial information: NCT02482168.

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Combination Therapy (IO/IO, IO/Standard of Care, IO/Other)

P255

Depleting blood arginine with AEB1102 (Pegzilarginase) exerts additive anti-tumor and synergistic survival benefits when combined with immunomodulators of the PD-1 pathway

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Background

Tumor dependence on specific amino acids for survival and proliferation is well recognized and has been exploited effectively in the clinic through the use of asparaginases for the treatment of acute lymphoblastic leukemia. Sensitivity of tumors to L-Arginine (L-Arg) deprivation results from an impaired ability to synthesize L-Arg, most commonly due to decreased functional expression of argininosuccinate synthase. Native human arginase 1 is not a viable drug candidate due to low activity and low stability in serum. We have developed a novel cobalt substituted, PEGylated human arginase 1 (AEB1102, Pegzilarginase) with enhanced pharmacological properties. We and others have successfully utilized arginase 1 to impart an anti-tumor effect through L-Arg starvation in multiple tumor types *in vitro* and *in vivo* (e.g. AEB1102 single agent efficacy in melanoma, SCLC, sarcoma, large cell NSCLC, Merkel cell carcinoma). Given that arginase 1 has been reported to be immune suppressive, immune neutral (PMID: 23717444), or immune promoting (PMID: 27043409) in different experimental settings and by different groups, we have investigated the impact of systemic depletion of L-Arg on the anti-tumor efficacy of immune checkpoint inhibitors.

Methods

Murine syngeneic models (e.g. CT26, MC38) were dosed with AEB1102 alone and in combination with immunomodulatory anti-PD-L1 monoclonal antibody (mAb).

Results

Combination therapy of AEB1102 with anti-PD-L1 resulted in an additive anti-tumor effect with improved survival benefit (increased life span (ILS) 55-129%) compared to AEB1102 (ILS 29-33%) and anti-PD-L1 (ILS 7-33%) monotherapies. In addition, in the CT26 model, complete tumor regression (non-palpable tumors) was observed in 37% of the mice; importantly, complete responses were observed only in the combination therapy group. When the complete responders were re-challenged with fresh CT26 cells, tumors failed to establish, suggesting the development of an immune memory response as a result of the previously administered combination therapy of AEB1102 and anti-PD-L1. Administration of AEB1102 as a monotherapy or in combination with anti-PD-L1 in the CT26 model was associated with an increase in tumor-infiltrated CD45+ cells, indicating that AEB1102 promotes T-cells accumulation in the tumor microenvironment.

Conclusions

Collectively, these results demonstrate that in addition to tumor growth inhibition, L-Arg depletion in the tumor microenvironment enhances the effectiveness of immunotherapy. AEB1102 is currently in Phase 1 (monotherapy) clinical trials. These data open the possibility of clinical combination of AEB1102 with immunomodulators of the PD-1 pathway to further improve outcomes in cancer patients.

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P256

G100 and ZVex[®]-based combination immunotherapy induces near complete regression of established glioma tumors in mice

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Background

Glioblastoma (GBM) is a malignant brain tumor with an overall survival of < 3.3% at 5 years. Novel immunotherapies are being explored but face limitations due to low infiltration of activated T cells. ZVex is an integration-deficient lentiviral vector-based platform that targets dendritic cells *in vivo* to generate tumor-specific T cells. Intratumoral injections of G100, which contains glucopyransosyl lipid A (GLA, a synthetic TLR4 agonist), modulate the tumor microenvironment through induction of proinflammatory responses. We have previously shown in the B16 model that G100 induces T cell homing chemokines CXCL9 and CXCL10, increases number of T cells in the tumor, enhances intratumoral antigen presentation, and results in antigen spreading of the T cell response. Combined systemic and *in situ* immunization with ZVex/OVA and G100, respectively, completely eradicated established B16/OVA melanomas in mice. Here, we report that, in an orthotopic GBM model, the combined regimen with ZVex/OVA and G100 induced near complete regression of established GL261 syngeneic gliomas in C57BL/6 mice.

Methods

Female C57BL/6 mice were stereotactically cannulated in the left striatum. At study initiation, 2 x 10⁵ GL261 glioma cells expressing ovalbumin (GL261/OVA) were inoculated through the cannula. Tumor growth was monitored via imaging of luciferase activity, twice weekly. On Day 7, mice with tumors (6.8 x 10⁵ avg. radiance) were

immunized by a single subcutaneous injection of ZVex/OVA. G100 (2 μg GLA) was administered through the cannula, also on Day 7, and then once weekly for the duration of the study, for a total of three injections. Animals were sacrificed when displaying signs of cachexia and > 20% weight loss, usually around 25 days after tumor inoculation.

Results

GL261/OVA-bearing mice treated with G100 or ZVex/OVA alone exhibited delayed tumor growth or modest tumor regression, respectively (mean reduction of 17% and 72%, respectively, ranging from none to 83%), while mice treated with ZVex/OVA and G100 combination exhibited significantly greater reduction in tumor size, averaging 98% (74%-99.8%).

Conclusions

This study demonstrates that ZVex/OVA and G100 combination therapy was very successful in controlling growth of GL261 gliomas, presumably in part by chemotactically directing peripheral T cells to the brain, and suggest the potential for treatment of patients with GBM. The precise mechanism of synergy in this GBM model is currently under investigation. Both ZVex-based therapies and G100 are being evaluated in multiple Phase 1 and 2 clinical trials, and their combination is being investigated in a Phase 1 trial in soft tissue sarcoma patients.

P257

Phase II basket study of olaparib and durvalumab: Biomarker analysis in germline BRCA-mutated (gBRCAm) HER2-negative metastatic breast cancer (MBC) and relapsed small-cell lung cancer (SCLC) patients

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Background

Poly(ADP-ribose) polymerase inhibitors (PARPi) trap PARP at sites of single-strand DNA breaks, and generate genomic instability and cell death in homologous recombination deficient tumour cells. Both intrinsic and PARPi-induced DNA repair defects may attract tumour-infiltrating lymphocytes (TILs), upregulate programmed cell death ligand-1 (PD-L1) and release tumour neo-antigens upon cell death. The MEDIOLA study (NCT02734004) assessed the effect of the PARPi, olaparib, alone and in combination with durvalumab (anti-PD-L1), on immune cell pharmacodynamics, and the correlation of candidate predictive markers on disease control rate (DCR).

Methods

Patients with HER2-negative, *gBRCAm* MBC (n=25), or relapsed SCLC (n=38) received olaparib (300 mg PO BID) for 4 weeks, followed by a combination of olaparib and durvalumab (1.5 g IV every 4 weeks) until progression. At 4 weeks after initial olaparib, paired biopsies were conducted for immunology profiling. Primary endpoints were DCR at 12 weeks, safety and tolerability. Biomarker endpoints included archival (Figure 1) and paired biopsy analysis of tumour cell (TC) and immune cell

(IC) PD-L1 expression (SP263), densities of CD3 and CD8 T-cells (cells/mm²), tumour mutation status and peripheral blood immunophenotyping.

Results

Olaparib caused small reductions in circulating T, B and Natural Killer (NK) cells on day 29 and thereafter in 86–93% of MBC and 81–91% of SCLC patients, followed by evidence of post-durvalumab 2-fold or greater increases in Ki67+CD8+ T-cells in 46% of MBC and 50% of SCLC patients on day 57. In MBC (n=25) 5/5 (100%) PD-L1 TC \geq 1% patients had controlled disease at 12 weeks, compared with 9/14 (64.3%) PD-L1 TC<1% patients (no data for 6/25 patients). In SCLC (n=38) PD-L1 TC \geq 1% patients had a DCR of 1/6 (16.7%) compared with a PD-L1 TC<1% DCR of 9/27 (33.3%) at 12 weeks (no data for 5/38 patients). At the time of analysis, 1/2 MBC with paired biopsy data had a partial response (PR) and demonstrated dramatic increases in CD3/CD8 densities and PD-L1 TC positivity 4 weeks post-olaparib monotherapy. 1/3 SCLC paired biopsies had PRs and showed increases in CD3/CD8 densities, but no PD-L1 TC expression, compared with no change in 2/3 patients with stable disease. Updated results will be presented.

Conclusions

Olaparib results in modest reductions in circulating T, B and NK cells: however, this effect does not disrupt durvalumab-based increases in circulating, proliferating cytotoxic T-cells. Preliminary data suggest that olaparib treatment may increase levels of TILs, but more data are required. Data collection and analysis is ongoing.

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Conclusions

Immunotherapies for advanced melanoma, particularly nivolumab+ipilimumab, may lead to a long TFI before starting second-line therapy. The estimated annual cost of managing advanced melanoma is low in the TFI prior to disease progression, especially with nivolumab and nivolumab+ipilimumab. These results require comparison with real-world evidence to measure the impact of bias due to the controlled clinical trial setting.

Table 1.

Stratification	Number of Patients	Median (IQR)	Range	P-value
Overall	100	1.2 (0.8-1.8)	0.1-10.0	0.001
Stratified by Cohort				
1 mg	50	1.1 (0.7-1.7)	0.1-10.0	0.002
6 mg	50	1.3 (0.9-1.9)	0.1-10.0	0.001

Includes costs associated with concomitant medications, laboratory tests, procedures, and consultations.

P259

Pushing the accelerator and releasing the brake: testing the soluble LAG-3 protein (IMP321), an antigen presenting cell activator, together with pembrolizumab in unresectable or metastatic melanoma.

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Background

IMP321 is a recombinant soluble LAG-3Ig fusion protein binding to MHC class II molecules and mediating antigen presenting cell (APC) activation followed by CD8 T-cell activation. The activation of the dendritic cell network and the subsequent T cell recruitment at the tumor site with IMP321 may lead to stronger anti-tumor CD8 T cell responses than observed with pembrolizumab alone. This combination of an APC activator with an immune checkpoint inhibitor (ICI) is aiming to increase efficacy without additional toxicity. We report initial results of the first 2 cohorts of a dose escalation phase I trial (TACTI-mel, NCT02676869) with pembrolizumab and IMP321 at different dose levels.

Methods

In this study, melanoma patients treated with pembrolizumab being without a complete response or fast progression after 3 cycles received pembrolizumab per standard dosing plus either 1 mg (n=6; cohort 1) or 6 mg (n=6, cohort 2) IMP321 injections s.c. (every 2 weeks for 6 months) from cycle 5 onwards. Patients without progressive disease (PD) at the end of the 6 months combination therapy continue on pembrolizumab monotherapy.

Results

In total, 12 patients (11 male, 1 female) with a median age of 62 years (range 48-85) were enrolled between Apr 2016 and Feb 2017. Four patients completed the 6 months combination treatment. One patient discontinued due to serious adverse event (SAE), unrelated to both study drugs. Two patients withdrew consent and five discontinued prematurely due to PD. No dose limiting toxicities for the combination have been reported. No SAE were found related to IMP321. All patients were evaluable according to irRC and 6 showed a reduction of ~50 % after IMP321 initiation. This includes one patient with a confirmed complete response while having PD on pembrolizumab monotherapy before study start.

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Conclusions

One (1) and 6 mg IMP321 given s.c. every 2 weeks in combination with pembrolizumab are safe and testing of the highest dose (30 mg) is underway. The 6 late tumor responses seen after the combination was started may point out to the benefit of adding a systemic APC activator to an ICI.

P260

Glebatumumab vedotin (GV), an anti-gpNMB antibody-drug conjugate (ADC), in combination with varlilumab (V), an anti-CD27 antibody, in advanced melanoma

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Background

gpNMB is an internalizable transmembrane glycoprotein expressed in multiple tumor types. The

ADC GV delivers the potent cytotoxin MMAE to gpNMB+ cells and showed promising activity as monotherapy in advanced melanoma refractory to checkpoint inhibitors (CPI). Preclinical results showed synergistic antitumor activity between ADC with CPI [1, 2], via dendritic cell (DC) activation; ADC-MMAE augmented the immune response induced by V, an agonist monoclonal antibody against the T cell costimulatory molecule CD27. This Phase 2 study was conducted to evaluate the activity and safety of the GV/V combination in advanced melanoma.

Methods

Patients with advanced melanoma, progressive after ≤ 1 chemotherapy, ≥ 1 checkpoint inhibitor (CPI), and if BRAF mutation ≥ 1 BRAF or MEK + BRAF inhibitor, were treated with GV (1.9 mg/kg q3w until progression/intolerance) in combination with V (3.0 mg/kg) on day 1 of weeks 1, 3, 9, 15, 21, and 27. Retrospective analysis on pre-study tumors and skin biopsies include gpNMB expression and infiltrating lymphocytes by immunohistochemistry plus gene expression profiling. Primary objective is to evaluate objective response rate (ORR) (RECIST 1.1).

Results

Thirty-four patients enrolled: median age of 61 years; 59% male; 18% BRAF^{V600} mutated; 59% ≥ 3 lines prior therapy; 76% prior anti-CTLA-4; 100% prior anti-PD-1/PD-L1 inhibitor; 97% Stage IV; 71% M1c. Of 30 response evaluable patients, emerging tumor response data shows 2 confirmed partial responses (PR) (ORR = 7%, CI: 0.8, 22.1), and 1 single time point PR before patient withdrew consent. 48% patients had tumor shrinkage. Median PFS = 2.6 months and median OS = 4.4 months; 3 patients remain on treatment and 18 patients in survival follow up. Most common treatment related toxicities include alopecia, rash, fatigue, neuropathy, nausea, and vomiting. Tumor cells in 84% of patients with samples (n=25) tested to date are 100% gpNMB+.

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Conclusions

The GV/V combination was well tolerated without evidence of additive toxicity. There was no apparent enhanced clinical benefit of GV/V over GV alone in this patient population, perhaps because immune checkpoint molecules remained unblocked and/or of a dearth of antigen presenting cells in tumors. Correlative biomarker analyses are ongoing and will be presented. For further insights into the synergy of ADC and immunotherapy, a cohort evaluating GV with anti-PD-1 in CPI-refractory melanoma is enrolling and a cohort investigating GV with the DC growth factor FLT3L (CDX-301) is planned.

References

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2. Muller P, et al. *Sci Transl Med.* 2015; 7:315rfa188.

P261

Molecular signatures of combination immunotherapy of prostate cancer using a *Listeria*-based PSA vaccine and radiation

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Background

Radiotherapy (RT) has the potential to amplify immune responses triggered by tumor vaccines, including ADXS-PSA, a live-attenuated *Listeria monocytogenes* (*Lm*)-based vector expressing human PSA. Earlier observations suggest that the two treatment modalities cooperatively induce regression of syngeneic mouse prostate cancer cells expressing human PSA (TPSA23), though immune correlates of efficacy and tumor recurrence are poorly understood.

Methods

We compared efficacy of different sequencing regimens of combination RT/vaccine treatments on TPSA23 tumor growth in syngeneic mice. Using the optimal sequencing protocol, tumors were collected post-implantation to assess immune infiltrate and function during initial tumor regression (day 20) and upon resumption of tumor growth (day 38). Correlates of treatment efficacy were determined by transcriptome analysis, phenotypic analyses of infiltrates and TCR sequencing.

Results

We confirmed that combination RT/ADXS-PSA is superior to single modality treatments. Concurrent administration of RT/vaccine was the most effective treatment schedule and was associated with enhanced T cell activation and robust IFN γ signatures in the tumor microenvironment. This was reflected in increased intratumoral CD4 and CD8 T cell infiltration in mice receiving RT/vaccine. TCR β chain sequencing revealed elevated and sustained T cell diversity in tumor tissues of RT/vaccine-treated mice, when compared with mice receiving single modality treatments. In these residual tumors resident and/or memory T cell phenotypic markers were increased. Transcriptome analysis of recurring tumors further revealed induction of PD-L1 as a function of treatment. Targeting of the PD1/PD-L1 axis via a PD1 blocking antibody administered in addition to radiation and ADXS-PSA (triple combination) further amplified tumor growth inhibition in mice receiving dual RT/vaccine therapy.

Conclusions

Combining RT with the ADXS-PSA vaccine leads to effective tumor growth inhibition and induces robust, persistent antitumor immunity within the tumor environment. Transcriptome analysis during treatment revealed increased PD1 expression as a potential resistance mechanism and a PD1 blocking antibody provided further therapeutic benefit. These results support the rationale for combining *Listeria*-based vaccines with radiation in the clinic.

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Phosphatidylserine targeting antibody in combination with tumor radiation and immune checkpoint blockade promotes anti-tumor activity in mouse B16 melanoma

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Background

Phosphatidylserine (PS) is a phospholipid that is exposed on surface of apoptotic cells, viable tumor cells, tumor endothelium and activated immune cells. It has been shown to promote immunosuppressive signals in the tumor microenvironment. In a mouse B16 melanoma model, targeting PS in combination with immune checkpoint blockade promoted greater anti-tumor activity than either agent alone. This combination was shown to enhance CD4+ and CD8+ T cell infiltration and activation in the tumors of treated animals. Radiation therapy (RT) is an effective focal treatment of primary solid tumors, but is less effective in treating metastatic solid tumors as a monotherapy. RT induces immunogenic tumor cell death and enhances tumor-specific T cell infiltration in treated tumors. The abscopal effect, a phenomenon in which tumor regression occurs outside the site of RT, has been observed in both preclinical and clinical trials when RT is combined with immunotherapy.

Methods

Mice were injected intradermally on the hind limb with 10⁵ B16F10 melanoma cells. 7-10 days after implantation, tumors were treated locally with 15 Gy RT. 1 day after RT, mice were given antibodies to PS (mch1N11) and PD-1 (RMP 1-10)

intraperitoneally every 3 days. Tumor surface area and overall survival of mice were used to determine efficacy of the combinations. For FACS analysis, tissues were collected between 1-10 days after RT.

Results

In this study, we show that irradiation of B16 melanoma causes an increase in PS expression on the surface of viable tumor and immune infiltrates. We subsequently examined the effects of combining RT with an antibody that targets PS and anti-PD-1. We found that treatment with mch1N11 synergizes with RT to improve anti-tumor activity and overall survival in tumor bearing mice. In addition, the triple combination of mch1N11, RT and anti-PD-1 treatment displayed even greater anti-tumor and survival benefit. Analysis of the immune response in the tumors of treated animals revealed an increase in M1-like macrophages in the tumors after treatment with RT and mch1N11. In addition, analysis of the systemic immune responses revealed an increase in antigen-specific CD8 T cell infiltration in the tumors as well as increased activation, effector function and differentiation in the triple combination therapy.

Conclusions

This finding highlights the potential of combining these agents to improve outcome in patients with advanced-stage melanoma and may inform the design of future clinical trials with PS targeting in multiple cancers.

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Preclinical development of a Vaccine-Based Immunotherapy Regimen (VBIR) that breaks immunological tolerance and induces high titer and long lived T-cell responses to a tumor-associated self-antigen

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Background

A successful therapeutic cancer vaccine will activate the immune system to rapidly induce potent, balanced and durable CD8 and CD4 T-cell responses to selected tumor antigens that can reduce tumor growth, lead to tumor regression or prevent/delay the onset of new lesions.

Methods

The magnitude and quality of T-cell responses against self (rhesus Prostate Specific Membrane Antigen; rhPSMA) and non-self (human PSMA; hPSMA) antigens delivered by various vaccine platforms in non-human primates (NHPs) given with and without a check point inhibitor, anti-CTLA4 monoclonal antibody (tremelimumab; anti-CTLA4), were evaluated.

Results

Assessment of T-cell responses against self (rhPSMA) and non-self (hPSMA) antigens delivered by various vaccine platforms in NHPs, demonstrated that the AdV vector was the most potent delivery platform for breaking of immune tolerance to a self-antigen.

Many humans have immunity to adenovirus that could blunt the ability of an adenovirus vaccine to effectively prime an immune response. This limitation can be overcome by utilizing an AdV of a different serotype for which humans do not have pre-existing immunity. However, even with a low/no seroprevalent AdV, an AdV boost vaccination following an AdV prime vaccination can only be effective when the neutralizing immunity to the vector wanes in between the vaccinations, making the regimen less suitable for patients with high tumor burden or fast progressive disease.

Therefore, plasmid DNA boost vaccinations delivered intramuscularly by electroporation were evaluated for durable expansion of AdV primed T-cell responses. Administration of anti-CTLA4 delivered subcutaneously to achieve high local concentrations in the vaccine draining lymph nodes, given concurrently with the AdV prime and DNA boost vaccinations was shown to induce and expand durable and polyfunctional (IFN γ ⁺, TNF α ⁺ and/or IL-2⁺) T-cell responses to a tumor-associated self-antigen in NHPs.

Conclusions

In summary, we have developed VBIR that consists of a heterologous prime-boost vaccine approach given in combination with local delivery of tremelimumab to maximize vaccine potency while minimizing the negative impact of neutralizing antibodies to the AdV vector. These results have encouraged clinical development of this unique immunotherapeutic regimen.

P264

Immune modulation by low dose sunitinib combined with a cancer vaccine based immunotherapeutic regimen provides therapeutic benefit to tumor bearing mice

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Background

Sunitinib is a receptor tyrosine kinase (RTK) inhibitor approved as a monotherapy for the treatment of

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advanced renal cell carcinoma (RCC), gastrointestinal stromal tumors and advanced pancreatic neuroendocrine tumors. It inhibits angiogenesis and targets RTKs (PDGFR, VEGFR, KIT, FLT3, and RET) involved in tumor cell growth. Sunitinib also has been shown, in preclinical studies and patients, to have immunomodulatory properties that reduce myeloid derived suppressor cells (MDSCs) and restore a Th1 cytokine profile. The approved dosing regimens for RCC have a safety profile that is overall manageable with the most commonly reported sunitinib-related grade 3 adverse events including hypertension, fatigue, diarrhea and hand-foot syndrome. Although the tolerability of sunitinib as monotherapy was acceptable in advanced stage, metastatic cancer patients, the drug's side effect profile could potentially pose challenges for use in patients with earlier stage cancer and/or when combined with other oncology therapies. Thus, lowering the dose of sunitinib might improve the safety of combinations, including combinations with cancer vaccines, thus enabling treatment of cancers of all stages.

Methods

The anti-tumor efficacy of lowered doses of sunitinib as a monotherapy, and in combination with a cancer vaccine, was investigated in immune-competent mouse tumor models. To understand the mechanism of such efficacy, the impact of the treatments on MDSC profiles in the periphery and tumors, T cell IFN γ production, and antigen-specific immune responses was evaluated. Additionally, adoptive immune cell transfer experiments were conducted to monitor survival of MDSCs and T cells in mice treated with sunitinib.

Results

At doses below the maximum biologic efficacy dose in mice (< 40 mg/kg q.d.), sunitinib selectively and significantly reduced the survival and frequency of MDSCs in the periphery and tumors, which resulted in increased IFN γ production by T cells. Importantly, when low dose sunitinib was given concurrently with a rat HER2 (rHER2)-based heterologous prime-

boost vaccine, it significantly reduced the growth of subcutaneous tumors and prolonged survival of tumor-bearing rHER2 transgenic mice compared to mice receiving only monotherapies. Addition of an anti-CTLA4 monoclonal antibody (anti-CTLA4) significantly enhanced the vaccine-induced immune response and prolonged survival.

Conclusions

The combination of the vaccine, sunitinib, and anti-CTLA4 further increased efficacy and survival. These results indicate that low dose sunitinib, alone or together with either a cancer vaccine or a cancer vaccine plus anti-CTLA4, maintains its immune modulatory properties and may be a valuable component of a safe and efficacious vaccine-based immunotherapy regimen.

P265

Optimizing targeted therapy and immune checkpoint blockade therapy in Kras mutant lung cancer

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Background

KRAS is the most commonly identified driver oncogene in lung cancer. However, to date there is no effective therapy available for KRAS mutant lung cancers. To identify the most effective therapy, we studied the impact of Kras signaling targeted therapy (MEK inhibition) on the immune microenvironment, in order to formulate a combinatorial therapy using targeted therapy and immunotherapy, with a goal of optimally enhancing tumor apoptosis and promoting long-term immune response simultaneously. MEK signaling is a downstream of Kras signaling pathway and critical

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for tumor growth and T cell activation. Conventionally, MEK inhibitor is treated daily/continuously. However, to enhance tumor apoptosis and promote T cell activation on MEK inhibition, we hypothesized intermittent administration of MEK inhibitor will confer T cells temporal release from MEK signaling inhibition allowing T cells activated, while tumor growth is suppressed and immunotherapy can be combined based on T cell activation/inhibitory markers change on this treatment schedule.

Methods

We have treated T cells from Kras lung cancer bearing mice or tumor bearing mice with Selumetinib or Trametinib in a pulsatile/intermittent or continuous way, then analyzed T cell phenotype changes, tumor progression, and survival.

Results

Ex vivo T cell study showed that pulsatile treatment of MEK inhibitor, Selumetinib and Trametinib, showed highly increased CTLA4 expression and mild increase of PD1 in CD8+ T cells and CD4+Foxp3- T cells, compared to continuously treated group which is a standard regimen. This result was confirmed in intermittently treated KrasG12D/+; p53-/- transplantable mouse model and GEMM as well. Moreover, CD8+ T cells from pulsatile group showed increase of Ki67 and 4-1BB expression, suggesting that CD8+ T cells are more activated in pulsatile group. In vivo tumor study using Kras mutant lung cancer animal model showed intermittent treatment suppressed tumor growth better than continuous treatment. Better response, more activated phenotype of CD8+ T cells including increased CTLA4 expression in intermittent group lead us to test combination of intermittent MEK inhibitor treatment with anti-CTLA4 antibody to maximize anti-tumor T cell activity and it is currently under investigation.

Conclusions

In summary, we found that pulsatile/intermittent treatment with MEK inhibitor showed better

response and more activated phenotype of T cells including increased CTLA4 expression compared to continuous treatment. This study suggests that optimized intermittent schedule of MEK inhibitor treatment is essential to maximize T cell mediated anti-tumor activity in combination with anti-CTLA4 therapy and this will benefit Kras mutant lung cancer patients.

P266

Significant enhancement of expanded natural killer cells against GD2 pediatric solid tumors (ST) in combination with ALT-803 (IL-15 superagonist) and dinutuximab

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Background

Children with recurrent and/or metastatic osteosarcoma (OS), neuroblastoma (NB) and glioblastoma (GBM) have a dismal even-free survival (EFS) (<25%). Dinutuximab is an anti-GD2 monoclonal antibody that has significantly increased EFS in children with GD2⁺ neuroblastoma [1]. ALT-803 is a superagonist of an IL-15 variant bound to an IL-15R α Su-Fc fusion with enhanced biological activity [2]. Our group has successfully expanded peripheral blood Natural Killer cells (exPBNK) with irradiated feeder cells [3].

In this research, we aim to determine if the combination of ALT-803 and dinutuximab significantly enhances exPBNK cell *in vitro* cytotoxicity against GD2⁺ OS, NB and GBM.

Methods

PBMCs were expanded with lethally irradiated K562-mbIL21-41BBL cells [4]. ExPBNK cells were isolated using Miltenyi NK cell isolation kits as we previously described.³ ALT-803 was generously

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provided by Altor BioScience Corporation. NK proliferation, NK receptors expression and cytotoxicity were assessed as we previously described [3]. Dinutuximab (generously provided by United Therapeutics) was used for antibody-dependent cellular cytotoxicity (ADCC) assays. IFN- γ and perforin levels were evaluated by ELISA assays. GD2⁺ OS, NB and GBM cell lines were used as target cells.

Results

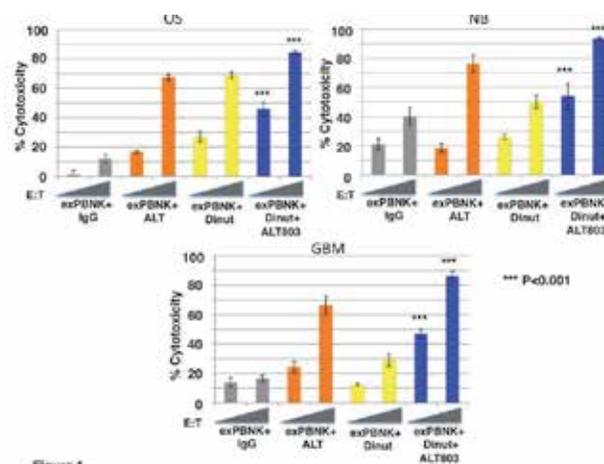
ALT-803 significantly promoted exPBNK *in-vitro* proliferation by increasing the phosphorylation of Akt, Stat3/5 and p38 MAPK. ALT-803 increased NK activating receptors expression: NKG2D, NKp30, NKp44, and NKp46.

ALT-803 significantly enhanced exPBNK mediated ADCC with dinutuximab in a E:T dependent manner ($p < 0.001$) against OS, NB and GBM cells (Figure 1). ALT-803 significantly enhanced IFN- γ ($p < 0.001$) and perforin ($p < 0.001$) release from exPBNK when it was combined with dinutuximab against OS, NB and GBM cells compared to exPBNK, ALT-803+exPBNK, or dinutuximab+exPBNK.

Conclusions

ALT-803 significantly enhanced exPBNK ADCC and IFN- γ and perforin release with dinutuximab against GD2⁺ OS, NB and GBM cells. *In vivo* studies using NOD/SCID human solid tumor xenografts are under investigation.

Figure 1.



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Withdrawn

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Targeting the tumor microenvironment with first-in-class Semaphorin4D MAb for combination immunotherapy.

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Background

Mechanistic findings in preclinical studies demonstrate that antibody blockade of Semaphorin 4D (SEMA4D, CD100) reduces expansion of MDSC and shifts the balance of immune cells within the TME to facilitate tumor rejection. Efficacy is further enhanced when combined with various immunotherapies.

Methods

Anti-SEMA4D antibodies were evaluated in combination with other immunotherapies in preclinical models. Anti-tumor activity and immune response was characterized by immunohistochemistry, flow cytometry, functional assays, and cytokine, chemokine and gene expression analysis. These data support clinical combination trials of VX15/2503, a humanized IgG4 antibody targeting SEMA4D, with immune checkpoint inhibition.

Results

SEMA4D restricts migration of monocytes and promotes expansion of suppressive myeloid cells *in vitro*. Strong expression of SEMA4D at the invasive margins of growing tumors *in vivo* restricts the

infiltration and modulates polarization of leukocytes in the TME. Antibody blockade of SEMA4D in preclinical models facilitated recruitment of activated DCs and T lymphocytes with concurrent reduction in M2 macrophage and Treg within TME [1]. MDSCs were significantly reduced in tumor and blood following treatment and new data characterizing MDSC function will be described. This significant shift in the immune contexture is associated with durable tumor rejection and immunologic memory in murine colon, breast, HNSCC, and melanoma models. New translational data characterizing expression of SEMA4D and its receptors in human tumors will be shown. Importantly, anti-SEMA4D treatment can further enhance activity of immunotherapies and chemotherapy. For example, combinations with immune checkpoint inhibitor anti-LAG3 or anti-CTLA-4 cause complete tumor regression in 90% or 100% of mice, as compared to ~20% with monotherapy ($p < 0.01$). New preclinical data include synergistic activity of combinations of anti-SEMA4D with anti-LAG3 and additional studies combining with epigenetic modulators, including treatment of established tumors.

Conclusions

SEMA4D blockade represents a novel mechanism to promote functional immune infiltration into the tumor and enhance immunotherapy. Treatment with VX15/2503 was well tolerated in a Phase I trial in patients with advanced refractory solid tumors [2]. Plans for several clinical trials will be presented, including a Phase 1b/2 of combination therapy with avelumab in immunotherapy naïve NSCLC, and combination with anti-PD-1 or Ipilimumab in various indications. A neoadjuvant trial of VX15/2503 with anti-PD-1 in patients with metastatic colorectal and pancreatic cancers will be described, as well as introduction of a Phase 1/2 trial of VX15/2503 in pediatric and osteosarcoma patients.

Trial Registration

NCT01313065

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P269

Epigenetic reprogramming of the tumor microenvironment increases tumor sensitivity to multivalent immunotherapy combinations with an IL-15 superagonist plus vaccine or immune checkpoint blockade

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Background

The clinical promise of cancer immunotherapy relies on the immune system recognizing and eliminating tumor cells identified as non-self. However, solid malignancies evade host immune surveillance by multiple mechanisms, including epigenetic deregulation and promoting a tumor microenvironment (TME) that suppresses infiltration and function of immune effector cells. The TME hampers T and NK cell maturation, recruitment, and function, ultimately causing their functional exhaustion via numerous immunosuppressive pathways, including upregulation of immune checkpoints such as PD-L1. Epigenetic silencing of genes involved in antigen processing and tumor immune recognition has been associated with worse prognosis in a wide spectrum of malignancies. Hence, there is an unmet clinical need to develop effective therapeutic strategies

that can reprogram the TME to restore tumor immune recognition and reverse immune evasion. We recently demonstrated that entinostat, a class I histone deacetylase (HDAC) inhibitor reverses carcinoma immune escape to T cell-mediated lysis.

Methods

We hypothesize that the immune-mediated tumor elimination promoted by the IL-15/IL-15Ra superagonist ALT803 in combination with PD-L1 checkpoint blockade or a therapeutic adenoviral vaccine targeting CEA (Ad-CEA) will be augmented by the epigenetic reprogramming of the TME induced by entinostat.

Results

In preclinical studies, ALT803 has been shown to exhibit potent antitumor activity in multiple murine models of cancer through the expansion of NK and CD8+ T cells with high effector function. Here, we demonstrate that entinostat modulates the cell-surface phenotype of murine colon and breast carcinoma cells to become more amenable to immune-mediated elimination. In the MC38-CEA murine model of colon carcinoma, proper scheduling of entinostat administration significantly augmented the antitumor activity promoted by ALT803 plus Ad-CEA, resulting in increased survival. Further, in the 4T1 murine model of triple-negative breast cancer, the combination of entinostat with ALT803 and a monoclonal antibody (mAb) targeting PD-L1 significantly reduced primary tumor weight relative to ALT803 plus anti-PD-L1 therapy. Treatment with the triple therapy in the neoadjuvant setting resulted in significant reduction of the number of 4T1 tumor-forming cells in the lung, with over 85% of animals cured. Current studies aim to elucidate the immune mechanisms associated with the antitumor effects observed with these therapeutic interventions.

Conclusions

Overall, these studies examine the rationale for combining entinostat with multivalent immunotherapy combinations, including cytokines,

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mAbs targeting PD-L1, and therapeutic cancer vaccines.

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Simultaneous PD-1 blockade is detrimental to the anti-tumor effects mediated by the agonist OX40 antibody

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Background

The checkpoint inhibitor antibodies (Abs) have improved the anti-tumor response although in a limited number of patients [1]. The combination of these Abs has enhanced the efficacy but with increased adverse events [1]. Since immune-stimulatory agonist Abs like anti-OX40 significantly increase the immune response [2], their combination with the anti-PD-1 Abs is being tested in clinical trials [3] with at least one clinical trial demonstrating lack of benefit [4] and the rest are not yet reported. Moreover, the potential immune outcome of such a combination are currently lacking. Therefore, purpose of this study was to determine the therapeutic and immune outcome of combining anti-PD-1 with anti-OX40 in an immune-primed environment.

Methods

Effects of adding PD-1 blockade to anti-OX40/vaccine treatment on tumor growth and survival were evaluated in a TC-1 tumor mouse

model using different treatment schedules and immune responses were assessed. *In vitro* mechanistic studies were carried out in pMel-1 CD8⁺ T-cells.

Results

Anti-OX40 treatment resulted in significant enhancement of antigen-specific CD8⁺ T-cells tumor-infiltration leading to strong anti-tumor response and prolonged survival of mice. Interestingly, we found that simultaneous addition of anti-PD-1 to anti-OX40 completely abrogated these effects. Despite an increase in IFN γ -producing E7-specific CD8⁺ T-cells in the spleens of mice treated with the combination, these cells underwent significant apoptosis in both the periphery and the tumor. Consistent with increased apoptosis, immunoSequencing analysis of the tumor and spleen T-cell population showed reduction in T-cell fraction and clonality following the addition of anti-PD-1. We further showed that delay in anti-PD-1 addition resulted in no significant change in T-cell apoptosis, however, it did not add to the anti-tumor response of the anti-OX40 treatment.

Conclusions

These results indicate that anti-PD-1 added at the initiation of therapy exhibits a detrimental effect on the positive outcome of anti-OX40. This may provide an important insight into why some of the immune combination clinical trials are not producing the intended outcome, demonstrating the need to rigorously test the combination partners and sequencing before employing them in the clinic.

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Phase 1b/2 dose-escalation study of ARRY-382, an oral inhibitor of colony-stimulating factor-1 receptor (CSF1R), in combination with pembrolizumab for treatment of patients with advanced solid tumors

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Background

CSF1 regulates tumor-associated macrophages and myeloid-derived suppressor cells, which are critical tumor microenvironment modulators of immune response. Combining a programmed death 1 (PD-1) inhibitor with a CSF1R inhibitor in preclinical models shows enhanced antitumor activity. ARRY-382 is a highly selective oral inhibitor of CSF1R. Studies of ARRY-382 monotherapy identified the maximum tolerated dose (MTD) as 400 mg once daily (QD), with biologic activity at doses \geq 200 mg QD. This 3-part, phase 1b/2 study evaluates ARRY-382 in

combination with pembrolizumab, a humanized monoclonal antibody targeting PD-1.

Methods

Phase 1b (part A) determined the MTD and recommended phase 2 dose (RP2D) of ARRY-382 plus pembrolizumab in patients with selected advanced solid tumors. Patients were enrolled in 3 successive cohorts of ARRY-382 at doses of 200, 400, or 300 mg QD plus pembrolizumab 2 mg/kg every 3 weeks (Q3W). The primary endpoint was the incidence of dose-limiting toxicities (DLTs). Secondary endpoints included safety, pharmacokinetics of ARRY-382, and objective response rate.

Results

Twenty patients enrolled in part A; 19 were treated: 200 mg QD, n=6; 400 mg QD, n=7; 300 mg QD, n=6. Median age was 59 years; tumor types were pancreatic (n=6), colorectal (n=5), ovarian (n=3), gastric and melanoma (n=2 each), and triple-negative breast (n=1). The mean number of prior therapies was 2.9 (range, 1–5). DLTs of grade 3 increased aspartate aminotransferase (AST)/alanine aminotransferase/bilirubin and grade 3 increased creatine phosphokinase (n=1 each, 400-mg dose) and grade 3 pancreatitis (n=1, 300-mg dose) were observed. The most common (>2 patients across doses) grade 3/4 adverse events (AEs) by dose cohort (200, 300, and 400 mg) were increased AST (1, 1, and 2 patients), increased lipase (0, 3, 0), and rash (1, 1, 1), respectively. One patient in the 200-mg cohort discontinued treatment because of an AE of pneumonitis in cycle 5. ARRY-382 pharmacokinetics was unaffected by pembrolizumab. Preliminary efficacy data will be presented.

Conclusions

ARRY-382 plus pembrolizumab has a manageable safety profile. The RP2D of ARRY-382 plus pembrolizumab 2 mg/kg Q3W is 300 mg QD. This combination is currently being evaluated in the study in patients with melanoma and non-small-cell lung cancer.

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Trial Registration

ClinicalTrials.gov: NCT02880371

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The anti-tumor effect of radiation therapy is enhanced with the addition of TTI-621 (SIRP α Fc), an immune checkpoint inhibitor blocking the CD47 “do not eat” signal

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Background

CD47 is an immune checkpoint that binds to signal regulatory protein alpha (SIRP α) and delivers a “do not eat” signal to suppress macrophage phagocytosis. Tumor cells frequently overexpress CD47 to evade macrophage-mediated destruction. TTI-621 (SIRP α Fc) is an immune checkpoint inhibitor consisting of the CD47 binding domain of human SIRP α linked to the Fc region of human IgG1 designed to both: 1) block the CD47 “do not eat” signal, and 2) engage macrophage Fc γ receptors with IgG1 Fc to enhance phagocytosis and antitumor activity.

Radiation therapy (RT), a primary mode of cancer treatment, induces immunogenic cell death, and is associated with release of tumor antigens, induction of pro-inflammatory cytokines, and enhanced migration and infiltration of immune cells, including macrophages, to tumor sites. Thus, the combination of RT and TTI-621 may improve the therapeutic effect over either treatment modality alone. Herein, we report the efficacy of the combination of RT and the CD47-blocking agent TTI-621 in xenograft tumor models.

Methods

The in vivo efficacy of RT, TTI-621, and RT+TTI-621 was evaluated in B cell lymphoma (SU-DHL-6) and solid tumor xenografts, including the radio-insensitive A549 lung adenocarcinoma. TTI-621 (10

mg/kg) or vehicle were administered intratumorally 30 min prior to RT, 3 times per week for 4 weeks. Tumors were locally irradiated using an image-guided small animal irradiator (225 kVp, 13 mA) at a dose of 4-6 Gy for 3 fractions. Tumor volumes were monitored using standard caliper measurement. Systemic toxicity of the treatments was evaluated by body weight change. Tumor-associated macrophages were quantitatively assessed using flow cytometry and immunohistochemistry.

Results

RT+TTI-621 had a more profound effect on tumor control in both lymphoma and solid tumor models than RT alone. In SU-DHL-6, 88% (7/8) of mice treated with both TTI-621 and radiation were tumor-free at the end of the study whereas none were tumor-free when treated with RT or TTI-621 alone, although there was tumor growth delay with each individual therapy compared to vehicle. RT+TTI-621 led to an increased infiltration of macrophages at the tumor sites in SU-DHL-6. Significant tumor control was also observed in A549 tumor bearing mice treated with RT+TTI-621 compared to either treatment alone. No toxicity was observed for any of the treatments.

Conclusions

The current study demonstrates the combination of TTI-621 and radiation therapy is superior to either treatment alone and provides supportive evidence for dual modality therapy in a variety of tumors.

P273

Pembrolizumab and afatinib for recurrent or metastatic head and neck squamous cell carcinoma

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Background

Head and neck squamous cell carcinoma (HNSCC) is an important malignancy in Taiwan. Anti-PD-1, including nivolumab or pembrolizumab, have shown the efficacies against head and neck squamous cell carcinoma. Afatinib, an irreversible EGFR tyrosine kinase inhibitor (TKI), had showed its activity against HNSCC. The role of afatinib for cancer immunotherapy have not been explored. In animal model, afatinib can suppress the carcinogenesis by inhibiting the function of macrophage [1]. EGFR targeted therapy can increase the MHC expression, enhance dendritic cell function, and increase the infiltrating T cell in the tumor [2]. In syngeneic mice cancer models, EGFR TKI can suppress the glycosylation of PD-L1 and sensitize the mice to anti-PD-1 therapy [3]. We hypothesized that adding afatinib with pembrolizumab may improve the treatment efficacy for patients with recurrent or metastatic HNSCC.

Methods

Patients with locally advanced or metastatic HNSCC were retrospectively reviewed in a university hospital in Taiwan. For patients taking pembrolizumab, the combination with afatinib will be discussed between the physician and the patient. For patients taking pembrolizumab and afatinib (P+A), the medical records were reviewed.

Results

From Nov 2016 to Jul 2017, there were 35 patients taking P+A. The median age was 59 years, and 32/35 pts were men. Oral cavity cancer is the most common type of HNSCC in the cohort (29/35 pts). The treatment were: pembrolizumab 200mg: 30pts; 2mg/kg: 5pts; afatinib 40mg QD: 34pts; 30mg QD: 1 pt. The maximal cycles of pembrolizumab per patient is four cycles. Sixteen patients took P+A as first line therapy. Until Jul 30, 2017, 29 pts had evaluable outcomes. The ORR (CR+PR) is 48.2% (14/29), DCR (CR+PR+SD) was 69.0% (20/29), and the median PFS: 184 days (32-332). The common toxicities were: diarrhea 40%; skin rash 37%; mucositis 29%; hand-foot-skin reaction 23%; weight loss 17%; and elevated AST/ALT 9%. Grade \geq 3

toxicities happened in 8.6% patients. There was no treatment related pneumonitis in the cohort.

Conclusions

The addition of afatinib with pembrolizumab showed good efficacy (ORR: 48.2%, DCR: 69.0%, PFS: 6.0 months) and tolerable toxicities. Further confirmatory prospective trial is indicated.

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P274

Combination of NKTR-214 and radiotherapy (RT) to reverse anergy and expand tumor-specific CD8 T Cells

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Background

We investigated therapeutic and mechanistic synergy between single-dose RT and systemic administration of NKTR-214. The effects of NKTR-214 with and without RT on anergic, tumor-specific CD8 T cells were characterized. NKTR-214 is a CD122-biased cytokine agonist conjugated with releasable chains of polyethylene glycol. NKTR-214 provides

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sustained signaling through the IL2 receptor pathway (IL2R $\beta\gamma$) to preferentially activate and expand effector CD8 T and NK cells over regulatory T cells. Preclinical models demonstrated NKTR-214 preferentially expands effector CD8 T and NK cells in the tumor resulting in marked tumor growth suppression as a single-agent and in combination with checkpoint inhibitors. RT can induce antigen-release and epitope spreading, while NKTR-214 activates and expands antigen-specific effector populations. We hypothesized the combination of systemic NKTR-214 and RT would generate better therapeutic responses than either treatment alone. A phase I/II trial is in progress to evaluate NKTR-214 safety and efficacy in an outpatient setting as monotherapy and in combination with nivolumab.

Methods

NKTR-214 was dosed 0.8 mg/kg alone or together with high-dose RT (20 Gy x 1) in multiple murine models, including an established CD8 T cell anergy model using Nur77-GFP reporter CD8 T cells. Activation markers on CD4, CD8 and NK cells in blood, lymph and tumor were evaluated by flow cytometry and gene expression (mRNA) profiling of the irradiated and non-irradiated tumors. In addition, immunohistochemistry was utilized to visualize immune infiltration.

Results

Consistent with prior observations, NKTR-214 induced activation marker expression by CD4, CD8 T and NK cells in the blood, lymph nodes and tumor. The combination of RT and NKTR-214 resulted in unique effects including increased absolute lymphocyte counts, increased expression of CD8 activation markers (CD25, PD1) in the blood and tumor, increased intratumoral NK cells, and a significant survival benefit over monotherapy. Evaluation of tumor infiltrating lymphocytes (TIL) indicates combination therapy reverses tumor-associated T cell anergy and results in a higher frequency of recently activated CD8 TIL.

Conclusions

The combination of NKTR-214 and radiotherapy is synergistic, provides significantly better anti-tumor responses and reverses T cell anergy.

P275

Harnessing the innate and adaptive immune system to eradicate treated and distant untreated solid tumors

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Background

NKTR-262 is a novel therapeutic, which delivers sustained intratumoral engagement of the TLR 7/8 pathway, promoting an immune stimulatory environment and tumor antigen release. When NKTR-262 is administered in combination with NKTR-214, a CD122-biased cytokine agonist currently in clinical trials as a monotherapy and in combination with nivolumab, the combined effect of innate immune stimulation and enhanced antigen presentation with sustained T cell activation leads to systemic tumor immunity.

Methods

Balb/c mice were implanted s.c. with bilateral CT26 colon carcinoma tumors. Once established, one tumor was treated with a single peritumoral dose of NKTR-262, while NKTR-214 was administered i.v. on q9dx3 schedule. Regression of treated tumors and the abscopal effect in contralateral tumors was assessed by tumor size measurements. Immune cell activation in both tumors was assessed by flow cytometry. Cytokine induction was measured in

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plasma, blood cells and treated tumors by MSD and qRT-PCR.

Results

Combination treatment with NKTR-262 and NKTR-214 eliminated both tumors in up to $\geq 95\%$ of mice. Single agent NKTR-262 or NKTR-214 treatment led to complete responses in $\leq 30\%$ of mice. Combination treatment of NKTR-262 plus NKTR-214 induced a two-step immune response in treated and untreated tumors. At early timepoints, accumulation of activated neutrophils correlated with tumor cell death and dendritic cell activation. The innate response was followed by increased CD8+ T cells and a reduction of immunorepressive cells. Single agent treatment showed only a subset of the cellular changes observed in the combination.

Conclusions

We present a designed combination therapy that mimics a natural immune response by activating a broad immune cell network. Combining NKTR-262 and NKTR-214 engages the entire immune activation cascade required for systemic tumor clearance from local tumor antigen production to a sustained systemic T cell response. Unlike treatments that stimulate downstream components of select immune pathways without eliciting systemic tumor immunity, a comprehensive anti-tumor immune activation by coordinated engagement of innate and adaptive immune cells may increase the success of immune therapy for patients

P276

Immunoswitch particles target activation of anti-tumor CD8+ T cells to inhibit tumor growth

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Background

Recent advances in cancer immunotherapy have led to the advent of treatments that boost an anti-tumor response, such as checkpoint blockade and co-stimulatory molecules. However, these approaches are effective for only a subset of patients and target non-specific molecules, thus the large doses required result in off-target toxicities. Here, we have developed a nanoparticle that synergizes checkpoint blockade and T cell costimulation on a single injectable therapeutic. Nanoparticles are conjugated with antibodies against 4-1BB, expressed by T cells, and anti-PD-L1, expressed by tumor cells and target these pathways while also physically linking the effector and target cell to increase efficacy. These dual targeting particles, termed “immunoswitch particles”, localize treatment to the tumor site and are effective at 10-100X lower doses than systemically injected drug and thus have the potential to reduce cost and off-target toxicities.

Methods

Immunoswitch particles were synthesized by conjugating 80nm iron-dextran nanoparticles with agonistic anti-4-1BB and antagonistic anti-PD-L1 antibodies. *In vitro* efficacy was assessed by co-culturing PD-L1^{hi} tumor cells with 4-1BB/PD-1^{hi} CD8+ T cells and measuring cytotoxicity, cytokine secretion, and effector-target cell conjugation. *In vivo* efficacy was measured by comparing intratumoral immunoswitch and soluble antibody treatment in multiple murine tumor models.

Results

Immunoswitch particles were demonstrated to increase CD8+ T cell cytokine secretion when co-incubated with T cells and cognate B16 tumor cells compared to an equivalent amount of soluble antibody *in vitro*. Immunoswitch particles also increased effector-target cell conjugation both *in vitro* (Figure 1) and *in vivo*. *In vivo*, immunoswitch treatment, but not an equivalent amount of soluble antibody, delayed/reversed tumor growth in both a B16-SIY and MC38-OVA tumor treatment model in

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the absence of adoptively transferred cells (Figure 2). This was caused by a change in biodistribution, altered CD8+ T cell repertoire, and an increase in cytokine secretion by tumor-specific CD8+ T cells (Figure 3A). Despite local intra-tumoral treatment, new data shows that MC38-OVA cured mice have systemic immunity against the OVA antigen. These mice have a systemically circulating repertoire of tumor-specific cells, demonstrated by increased *in vivo* killing of intravenously injected OVA-expressing cells (Figure 3B) and protection against a re-inoculation of MC38-OVA.

Conclusions

Here, we have demonstrated the efficacy of a new dual-targeting immunoswitch particle for cancer immunotherapy. Immunoswitch particles synergize checkpoint blockade with co-stimulation by confining the antibodies to a rigid nanoparticle surface. This new approach can be extended to different disease models and new checkpoint or co-stimulatory target molecules.

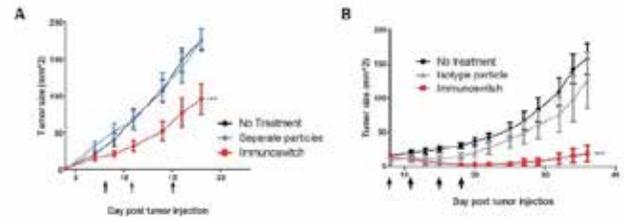


Figure 3.

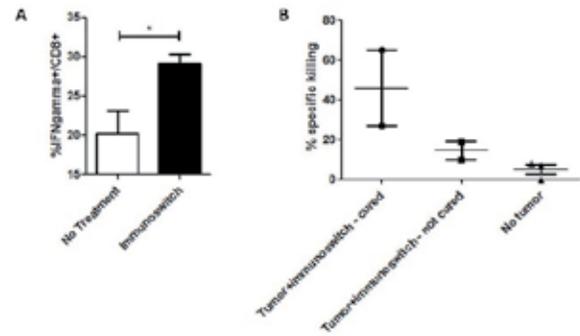


Figure 1.

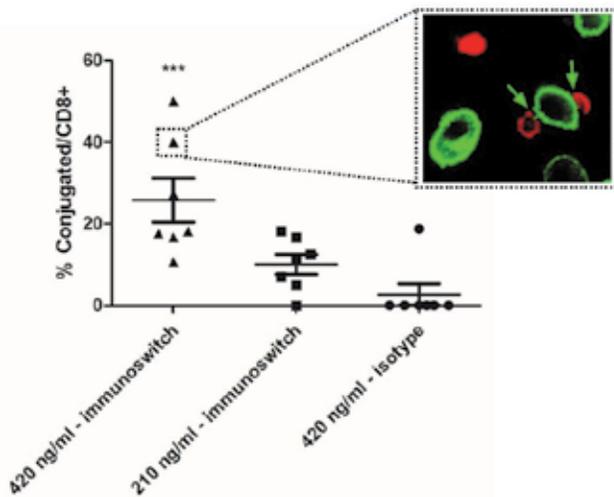


Figure 2.

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P277

STING agonist treatment increases response to chemotherapy and immune checkpoint blockade therapy in a syngeneic murine model of high-grade serous ovarian cancer

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Background

Background: High Grade Serous Carcinoma of the ovary is mostly diagnosed at late stages and primarily treated with surgery followed by platinum/taxane chemotherapy. Unfortunately, majority of the patients exhibit resistance to chemotherapy and ultimately succumb to the disease. We previously reported that tumours from patients with early recurrence show an immunosuppressed pre-existing tumour immune microenvironment with decreased expression of genes involved in Type I Interferon (IFN1) and T helper type 1 response [1, 2, 3]. We thus hypothesized that response to chemotherapy and overall survival of HGSC patients can be improved by stimulating the IFN1 response in the TME post chemotherapy.

Methods

In this pre-clinical study we tested the efficacy of a novel "*Stimulator of Interferon Genes*" agonist in immunocompetent mice implanted with ID8-TRP53^{-/-} mouse ovarian cancer cells. Post treatment tumour immune cell profiles were measured using a combination of flow cytometry and CyTOF based profiling. Tumour immune transcriptomic alterations were measured using the NanoString mouse pan cancer immune profiling panel. Log-rank test based survival analysis was performed to determine significant differences in overall survival post treatment.

Results

Flow cytometry and NanoString based analysis of tumours collected at endpoint showed higher intra-tumoural PD-1⁺ and CD69⁺CD62L⁻, CD8⁺T cells, increased expression of IFN response, antigen presentation and MHCII, genes, respectively, in tumours from STING agonist treated mice compared to those from vehicle treated mice. In addition to significantly decreased ascites accumulation and decreased tumour burden, survival of mice treated with a combination of Carboplatin + STING agonist + anti-PD-1 therapy was significantly longer compared to Carboplatin + STING agonist, Carboplatin only, STING only and vehicle treated mice.

Conclusions

Findings from this study are foundational to future clinical trials aimed at combinatorial immunomodulatory therapies to improve chemotherapy response and overall survival of HGSC patients.

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P278

A rational combination of standard of care and immunotherapy increases survival against glioblastoma

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Background

Glioblastoma (GBM) is a fatal primary brain tumor that, even after maximum surgical resection, radiotherapy (RT) and chemotherapy, is associated with a median overall survival (OS) of 14.6 months. Combinatorial treatment approaches that simultaneously address tumor growth, as well as the immunosuppressive microenvironment, may prove to be more effective than current clinical strategies. This work aims to determine the therapeutic efficacy of combining the novel, pharmaceutical-grade IDO1 enzyme inhibitor, BGB-5777, with PD-1 blockade and/or whole brain radiation, in a syngeneic, immunocompetent mouse glioblastoma model.

Methods

Mice were intracranially-engrafted with 2×10^5 GL261 or CT-2A murine GBM cells, then treated, beginning day 14 post-tumor cell implantation, with 500 μ g loading dose IgG control mAb followed by three 200 μ g doses, administered every third day (n=7), or 2 Gy WBRT for 5 days, PD-1 mAb (J43) administered the same as IgG control mAb, and 100mg/kg BGB-5777 BID for 4 weeks (n=9).

Results

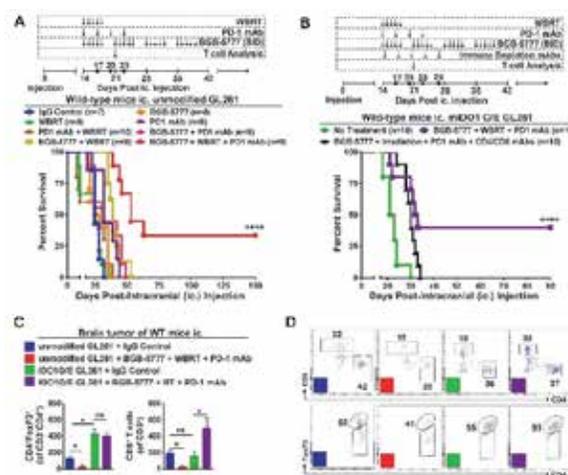
Mice engrafted with the GL261 tumors and treated with the combination therapy showed a significant increase in overall survival (53 days) compared to IgG treated mice (25 days). This therapy regimen also significantly increased durable survival (>120 days) ($P < 0.0001$) in mice ic. unmodified GL261 or CT-2A cells. (Figure 1A) Paradoxically, mice with GBM cells overexpressing mouse IDO1 cDNA and

treated with triple therapy showed a similar proportion of durable survivors (40%) vs. mice with similar tumors treated with IgG control ($P < 0.0001$). (Figure 1B) Interestingly, the survival effects seen in engrafted with unmodified GL261 tumors correlated to a significant decrease in GBM-infiltrating Treg levels ($P < 0.01$), however this same effect was not seen in mice with GL261 mIDO1 O/E tumors. (Figure 1C) These results suggest that effectiveness of triple therapy is independent of tumor IDO1 expression level and Treg accumulation. Results from the analysis of WT and IDO1^{-/-} mice with intracranial tumor indicate that in the presence of non-tumor IDO1, IDO1 inhibition is necessary to confer survival benefit ($P < 0.01$). (Figure 2)

Conclusions

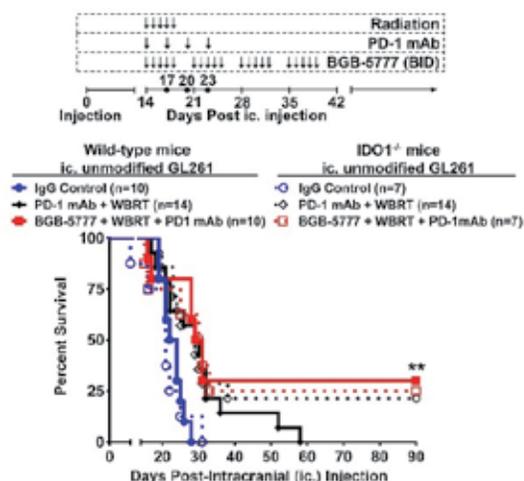
Ultimately, the data suggest that using radiation to induce potential immunogenicity and/or inflammation in GBM, while co-inhibiting immunosuppression, is a rational and potentially clinically-beneficial pursuit.

Figure 1.



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Figure 2.



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An oral small molecule combination therapy targeting PD-L1, VISTA and Tim-3 immune inhibitory checkpoints exhibits enhanced anti-tumor efficacy in pre-clinical models of cancer

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Background

Antibodies targeting immune inhibitory checkpoint pathways have transformed cancer therapy. However, the majority of patients fail to respond to therapies targeting single immune checkpoints due, in part, to the compensatory activity of alternative immune suppressive pathways. A therapeutic strategy that targets multiple immune checkpoints may significantly improve the anti-tumor response

rate, however this strategy may also increase the risk of severe treatment related immune mediated side effects. Orally bioavailable, small molecule immune checkpoint antagonists with short *in vivo* half-lives are ideal candidates for combination cancer immune therapy due to a short drug washout time which may improve safety and flexibility in scheduling which may increase efficacy.

Methods

The anti-tumor efficacy of orally administered CA-170 (a PD-L1/2 and VISTA/PD-1H antagonist), CA-327 (a PD-L1/2 and Tim-3 antagonist) or the combination of CA-170 plus CA-327 was studied in the mouse syngeneic CT26 colon carcinoma tumor model. Compound dosing was initiated within 1-3 days of tumor implantation or in mice bearing established tumors (90-170 mm³). Populations of immune cells were measured by flow cytometric analysis in tumor or blood. *Ex vivo* functional assays were performed on tumor derived CD8⁺ T cells to evaluate effector cell function and gene expression profiling was performed on tumors.

Results

Significant anti-tumor efficacy was observed in CT26 tumor bearing mice treated with either CA-170 or CA-327 compared to vehicle treated animals. Flow cytometric analysis of the tumors taken from these mice revealed that both compounds significantly increased the total number of CD45⁺ immune cells within the tumor relative to vehicle treated animals. Tumor growth inhibition positively correlated with increased numbers of activated CD8⁺ T cells and IFN- γ ⁺ CD8⁺ effector T cells within the tumor. A significant increase in anti-tumor efficacy was observed when CA-170 and CA-327 were administered together as an oral combination therapy, when compared to animals treated with either compound alone.

Conclusions

These non-clinical data demonstrate a proof-of-concept showing that a combination therapy consisting of oral small molecules that antagonize the PD-L1/2, VISTA and Tim-3 pathways significantly

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enhance anti-tumor efficacy. CA-170 is currently undergoing Phase I clinical testing and CA-327 is in pre-clinical development. The results of our study provide a strong rationale for the continued development of combination therapies using small molecule immune checkpoint antagonists for the treatment of advanced cancers.

P280

Combination lymphoma immunotherapy using intratumoral virus-like particles containing CpG TLR9 agonist combined with checkpoint blockade

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Background

Checkpoint blockade of immune inhibitory pathways is an exciting approach to cancer immunotherapy, however there remains considerable room for improvement through the use of unique immunotherapeutic combinations. We explored the combination of intratumoral injection (in situ immunization) with a virus-like particle (VLP) combined with anti-PD1 antibody therapy. The VLP is designated CMP-001 and is composed of an immunostimulatory CpG oligodeoxynucleotide TLR9 agonist encapsulated in the Qb bacteriophage protein. CMP-001 was previously evaluated clinically in over 700 subjects in non-cancer trials where it stimulated a strong Th1 cytokine response. The current studies were based on the hypothesis that intratumoral CMP-001 can augment the development of a tumor-specific T cell response that can be maintained by anti-PD-1 therapy.

Methods

The impact of CMP-001 with and without anti-PD1 was assessed in both human (in vitro) and mouse (in

vitro and in vivo) systems. In vivo therapy studies in mice included the B16F0 melanoma and A20 B cell lymphoma models. CMP-001, or saline control, was delivered intratumorally starting one week after tumor challenge. Anti-PD-1, or isotype control, was administered systemically starting one week after tumor challenge. Tumor growth and survival was followed. In some experiments, tumor inoculation was done bilaterally while a unilateral tumor was injected with CMP-001 to allow for assessment of response of both the treated and untreated tumor.

Results

In vitro, CMP-001 stimulated IFN α production, as well as other pro-inflammatory cytokines, from human and mouse mononuclear cells (from the peripheral blood and spleen respectively). This was only seen when anti-Q β antibody was present.

Intratumoral CMP-001 enhanced survival, and reduced tumor growth of treated tumors in the B16F0 model, and of both treated and untreated tumors in the A20 lymphoma model. Anti-PD-1 enhanced this effect in the A20 model. Depletion of T cells in mice eliminated the anti-tumor effect in both the treated and untreated tumors.

Conclusions

We conclude CMP-001 can induce a robust Th1 response in both murine and human systems in a manner that is dependent on opsonization of the VLP with anti-Qb antibody. The combination of intratumoral CMP-001 and systemic anti-PD-1 leads to development of a systemic anti-tumor T cell response in two murine tumor models and is a promising immunotherapy combination worthy of clinical evaluation. A Phase 1 clinical trial of the combination of anti-PD-1 and CMP-001 is underway in advanced melanoma. These results will be presented separately.

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Treatment with a VEGFR-2 antibody results in intra-tumor immune modulation and enhances antitumor efficacy of PD-L1 blockade in

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syngeneic murine tumor models

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Background

The activation of VEGFR-2 by its primary cognate ligand VEGF has been principally implicated in tumor angiogenesis. However, emerging data has suggested that the VEGF/VEGFR-2 axis also mediates a suppressive effect on the anti-tumor immune response. Inhibition of this pathway has demonstrated the potential to facilitate T cell migration into the tumor and to reduce the direct immune inhibitory activity promoted by tumor endothelial cells. Clinical studies that combine anti-angiogenic antibodies to immune checkpoint inhibitors have shown promising results in a number of solid tumors. However, the mechanisms underlying immunomodulatory activity of agents that target the VEGF/VEGFR-2 axis remain incompletely understood.

Methods

Ramucirumab is an approved VEGFR-2 targeted antibody currently undergoing clinical trials in combination with PD-1/PD-L1 inhibitory antibodies. The emerging data from these early trials has prompted us to undertake non-clinical studies with potential to uncover mechanisms that mediate the enhancement of anti-tumor immune response by the blockade of VEGFR-2. Using murine EMT6 breast and MC38 colon carcinoma models we investigated the immunomodulatory effects of DC101, a surrogate antibody that blocks mouse VEGFR-2, and its ability to increase the anti-tumor efficacy of checkpoint inhibition. Intra-tumor immune-related changes were evaluated by flow cytometry,

immunohistochemistry (IHC) and nCounter gene expression analysis (NanoString).

Results

Anti-VEGFR2 monotherapy resulted in increased T cell infiltration into the tumors. Combination of anti-VEGFR-2 with anti-PD-L1 resulted in greater anti-tumor efficacy compared to anti-PD-L1 monotherapy in both MC38 and EMT6 tumor models. Mice achieving complete tumor regressions after the combination treatment resisted tumor rechallenge demonstrating the development of immunologic memory. Analysis of changes in the tumor microenvironment by flow cytometry during combination therapy showed increased myeloid and T cell infiltration. nCounter gene expression analysis confirmed that anti-VEGFR-2 treatment enhanced inflammation and immune activation gene expression signature in monotherapy, and this effect was much more pronounced after the combination treatment. Pathway analysis highlighted that the combination effect could be attributable to enhanced innate immune response (e.g. dendritic cell maturation, antigen presentation) and T cell activation. These results were corroborated by flow cytometry, showing increased MHCI and MHCII expression on DCs and macrophages, along with increased PD-L1 expression during anti-VEGFR-2 monotherapy.

Conclusions

Taken together, these results highlight the potential of anti-VEGFR-2 antibodies to partially ameliorate intra-tumor immune suppression, providing insights into the mechanisms by which combined VEGFR-2/PD-L1 antibody therapy leads to increased anti-tumor efficacy.

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Immunostimulatory CD40L/4-1BBL Gene Therapy Enhances aPD-1 Antibody Therapy in Experimental Models

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Background

Lung cancer is a deadly disease responding poorly to conventional therapy. Checkpoint blockade antibodies inhibiting PD-1/PD-L1 have shown clinical benefit. Since the success of PD-1 blockade therapy prerequisites the presence of tumor-reactive T-cells, we hypothesized that an activating immunotherapy with high potency to stimulate T-cell responses would be an ideal candidate to combine with anti-PD-1 antibody treatment. LOAd703 is a replication-restricted adenovirus serotype 5/35. It is armed with two immunostimulatory transgenes, 4-1BB ligand (4-1BBL) and a trimerized human CD40 ligand (TMZ-CD40L). Both molecules are co-stimulatory molecules in the dendritic cell (DC)/T-cell synapse. This study aims to evaluate if immunostimulatory gene therapy, such as LOAd703, is a good enhancer of checkpoint blockade therapy.

Methods

The capacity of LOAd703 was tested using the lung cancer cell lines A549 and H727. Tumor viability was evaluated in vitro using MTS viability assay. Co-culture experiments were analyzed in real time using Incucyte technology. Cells were phenotyped with multicolor flow cytometry and cytokine patterns investigated by MesoScale. A20 cells that can be infected with LOAd703 virus were used in a syngeneic model in BalbC mice.

Results

Tumor cells were killed by oncolysis post infection with high dose virus (100MOI) in vitro and in vivo in multiple xenograft mouse models (lung, ovary, pancreas, colon, bladder). In tumor cell/PBMC co-

culture, addition of anti-PD-1 antibody did not increase tumor cell apoptosis. If OKT3/IL2 was added apoptosis was confirmed in the cultures peaking at day 5. Combination of OKT3/IL2 with anti-PD-1 antibody significantly enhanced the effect. LOAd703 was added to tumor cell/PBMC co-cultures at a low MOI to delay oncolysis. LOAd703 induced tumor cell death, starting already at day 2 and the activity was continuously rising throughout the experiment. Combining LOAd703 with anti-PD-1 antibody significantly increased apoptosis induction of tumor cells while a control virus was less effective. At endpoint, LOAd703 plus anti-PD-1 was twice as effective as OKT3/IL2 plus anti-PD-1. The T cells were increased in the same groups as tumor cell killing was previously noted. IFN γ , TNF, IL2 were all enhanced by LOAd703 and OKT3/IL2. In line with in vitro data, LOAd703 could enhance in vivo tumor control of anti-PD-1 therapy.

Conclusions

In conclusion, LOAd703 is a potent stimulator of T cell responses and potentiates the effect of aPD-1 therapy.

P283

Differential impact of chemotherapy on tumor-associated antigen-specific immunogenicity in cynomolgus macaques

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Background

Pfizer's vaccine-based immunotherapy regimen (VBIR) is a cancer treatment which combines a tumor-associated antigen (TAA)-specific vaccine, delivered via an AdC68 adenovirus and DNA

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regimen, with the immunomodulatory agent tremelimumab (anti-CTLA4 antibody) for T cell expansion. Various tumor types that are targets for VBIR, including non-small cell lung, triple negative breast, ovarian, and pancreatic cancer, are treated with chemotherapy as the standard of care (SOC). Accordingly, VBIR clinical administration may take place in patients who are currently undergoing chemotherapy. Synergistic effect has been reported between immune therapies and chemotherapy, either due to immunogenic cell death or a direct effect on immunosuppressive cells. However, limited information is known about the impact of chemotherapy on a vaccine-induced *de novo* immune response.

Methods

A nonhuman primate model was developed to most precisely mimic clinical therapy conditions, including pharmacokinetics and dynamics, dosing regimens, and supplemental treatments, allowing evaluation of potential chemotherapy impact on the VBIR-induced immune response. Macaques were dosed with a VBIR targeting a self-version of a human TAA with or without the following SOC treatments: paclitaxel/gemcitabine, paclitaxel/carboplatin, docetaxel/carboplatin, or doxorubicin. Complete blood counts and immunophenotyping were performed to track changes in immune cell profile as a biomarker of response. TAA-specific IFN- γ ⁺ CD8⁺ and CD4⁺ T cell responses and humoral responses were measured throughout the study.

Results

In non-tumor bearing macaques, different chemotherapies impacted VBIR-induced immune responses with varying degrees of severity. TAA-specific IFN- γ ⁺ CD8⁺ T cell responses ranged from unaffected with doxorubicin, to moderately reduced with carboplatin-based doublet chemotherapies, to almost entirely ablated with paclitaxel/gemcitabine. TAA-specific IFN- γ ⁺ CD4⁺ T cell responses, however, were generally unimpaired by chemotherapy; indeed, in many instances chemotherapy boosted CD4⁺ T cell responses. TAA-specific humoral responses were superior in animals treated with

carboplatin-based doublet chemotherapies, while paclitaxel/gemcitabine continued to preclude an effective immune response. Doxorubicin treated animals demonstrated a strongly diminished humoral response, attributed to B cell depletion. The impact of chemotherapy on the VBIR-induced immune response was also dependent on antigenic strength, with responses to a weaker antigen more dramatically reduced than were responses to a more immunogenic antigen.

Conclusions

Careful clinical trial planning is critical for vaccine-based immunotherapy regimen and chemotherapy combinations that will not only permit robust immune response activation and maintenance but also provide the adequate conditions for optimal synergistic anti-tumor effect.

P284

T cell priming by Toca 511 and 5-FC coupled with T regulatory cell depletion by α CTLA-4 synergistically enhances anti-tumor immune memory in a mouse model of glioma

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Background

Toca 511 (vocimagene amiretrorepvec) is a gamma retroviral replicating vector that selectively infects cancer cells *in vivo* and encodes cytosine deaminase. In combination with the prodrug, 5-fluorocytosine (5-FC), Toca 511 produces 5-fluorouracil (5-FU) locally in the tumor microenvironment. Prior work has demonstrated a reduction in immunosuppressive myeloid cells and an increase in CD4 and CD8 T cells in tumors while T regulatory cells remain unchanged with treatment with Toca 511 and 5-FC.

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Methods

This work, in a mouse model of glioma, aimed to determine if the addition of a checkpoint inhibitor, α CTLA-4, would provide therapeutic benefit to Toca 511 and 5-FC.

Results

Initially, we noted that Toca 511 and 5-FC was highly efficacious and that it provided little room for further improvement and therefore combination with α CTLA-4 did not show additive benefit against the primary cancer. However tumor associated Regulatory T cells were significantly reduced with α CTLA-4 treatment and long term memory was significantly improved with the combination as shown in adoptive transfer studies. Adoptive transfer of immune cells from animals that cleared their primary tumor through Toca 511, 5-FC, and α CTLA-4 showed 100% survival benefit to animals bearing orthotopic gliomas, significantly greater than the ~50% survival seen with transfer from animals that cleared primary tumor through Toca 511 and 5-FC alone. Further, α CTLA-4 treatment during clearance of primary tumors resulted in a marked reduction of memory T regulatory cells in secondary tumors after adoptive transfer. To determine if α CTLA-4 in combination with Toca 511 and 5-FC could reduce primary tumor burden we developed a model using a submaximal infection level of Toca 511. Specifically, restricting Toca 511 infection to only 2% of tumor cells limited the tumor growth arrest activity of 5-FC and the loss of efficacy with 2% infection was rescued when 5-FC treatment was combined with α CTLA-4.

Conclusions

These data suggest that α CTLA-4, and other compounds that target T regulatory T cells, should be evaluated in patients receiving Toca 511 and Toca FC to determine if the combination confers additional clinical benefit.

P285

Antibody and T cell response profiling of pancreatic cancer patients before and after chemotherapy reveals increased recognition of antigens suitable for immunotherapy

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Background

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal cancer, both for lack of effective screening method and for resistance to chemotherapy (CTX) and radiotherapy. At present surgical resection is the only potentially curative option. Once diagnosed, CTX, radiation or combination therapy regimens are used to treat patients, but responses remain poor. However, some chemotherapeutic agents have an immune modulating effect and a combination of CTX and immunotherapy could increase therapeutic efficacy. Thus, more immunogenic antigens can be induced by CTX and targeted by passive or active immunotherapy. To discover TAAs that might be selected for immunotherapy, antibody response in PDA patients' sera were analyzed before and after CTX. TAAs selected based on their increased recognition after CTX were used to evaluate whether PDA patient autologous T cells have an increased TAAs specific response after CTX.

Methods

Antibody response in sera of 29 PDA patients, before and after CTX treatments, has been analyzed by Serological Proteome Analysis (SERPA). The production of IFN-g and IL-10 by PBMC stimulated *in vitro* with recombinant TAAs was evaluated by ELISA.

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Results

CTX increases the number of TAAs recognized by PDA patients. Specifically, the set of TAAs we identified are proteins whose mRNA expression

have been found up-regulated in PDA patients. We observed a positive correlation between patients survival and the increased antibodies production against alpha-enolase (ENO1), glyceraldehyde-3-phosphate dehydrogenase (G3P), tubulin (TUBB5), and keratin, type II cytoskeletal 8 (K2C8). Of note, 48% of PDA patients sera after CTX treatment showed an increase of Complement Dependent Cytotoxicity (CDC) against human cell lines. Moreover in about 50% of the same PDA patient cohort the *in vitro* specific T cell response to recombinant TAAs that were mostly recognized by antibodies after CTX (ENO1, G3P, K2C8 and Far Upstream Binding Protein 1 (FUBP1) switched from a pro-tumor regulatory (low IFN-g/IL10 ratio) to anti-tumor effector (high IFN-g/IL10 ratio) phenotype after one or two rounds of CTX.

Conclusions

Data indicated that in PDA patients CTX induces an increase of IgG antibody to ENO1, G3P, TUBB5 and K2C8 whose expression is upregulated in PDA that may have a prognostic role. CTX also increases the ability of IgG kill PDA cells by CDC. Finally, CTX switches the T cell response to ENO1, G3P, K2C8 or FUBP1 from regulatory to effector and thus renders these TAAs promising targets for the design of new immunotherapeutic approach to improve the efficacy of CTX.

P286

Efficacy of interleukin 2 and interleukin 15 for in situ vaccination in a mouse melanoma model.

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Background

We have previously shown that a direct intratumoral (IT) injection of immunocytokine (IC), an anti-GD2 antibody linked to interleukin 2 (IL-2), can serve as an in situ vaccine and synergize with anti-CTLA-4 antibody to induce T cell-mediated antitumor effects. We have also shown a synergy of this approach with activation of innate immunity with an agonistic anti-CD40 monoclonal antibody (anti-CD40) and CpG (Rakhmievich et.al. Journal of Immunology 2017). We used these two immunotherapeutic approaches to test whether IT treatment with a mixture of IL-2 and anti-GD2 antibody, hu14.18K322A, will be effective against subcutaneous melanoma. In addition, we hypothesized that IL-15, a cytokine having several activities similar to those of IL-2 except induction of T regulatory cells (Tregs), may be as or more effective than IL-2 for in situ vaccination.

Methods

GD2⁺ B78 mouse melanoma cells were injected subcutaneously in C57BL/6 mice. Anti-CD40 and anti-CTLA-4 were given intraperitoneally, and CpG, IL-2, IL-15 and K322A were given IT. IL-2 and IL-15 were given at the dose of 7.5×10^4 units per injection daily for 5 days. Tumor growth and survival were followed. In some experiments, tumors were analyzed by flow cytometry.

Results

Flow cytometric analyses showed that IL-2 treatment increased Tregs within a tumor whereas IL-15 did not. When the treatments were started on day 6 post tumor cell implantation, IL-2 and IL-15 combined with K322A induced similar reductions of B78 tumor growth, and complete tumor regression with the addition of anti-CTLA-4. When the treatments were started on day 22 post tumor cell

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implantation, IL-2 and IL-15, combined with K322A, anti-CTLA-4, anti-CD40 and CpG, also induced comparable antitumor effects resulting in survival of 40-60% of mice.

Conclusions

In two therapeutic approaches, IL-2 and IL-15, combined with other immunotherapeutic agents, induced similar antitumor effects.

P287

Immunological effects of checkpoint blockade plus galectin-3 inhibition with GR-MD-02 in a first-in-human phase I clinical trial

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Background

Immunosuppression of tumor-infiltrating lymphocytes (TIL) is a major obstacle to creating effective therapies for patients with metastatic cancer. Galectin-3 (Gal3), a lectin family member, is expressed by numerous cancers and immune cell subsets. Serum Gal3 expression is higher in patients with metastatic disease and is associated with reduced survival in patients with metastatic melanoma. Furthermore, Gal3 has been implicated in disease progression via the promotion of angiogenesis and metastasis. Interestingly, extracellular Gal3 induces immune suppression via inhibiting TIL function, promoting M2 macrophage polarization and mobilizing myeloid cells from the bone marrow to promote a metastatic niche within the tumor. We hypothesized that Gal3 inhibition in conjunction checkpoint blockade immunotherapy would improve TIL function while inhibiting tumor growth and metastasis. Preclinical studies revealed that Gal3 blockade with GR-MD-02 and agonist anti-OX40, anti-CTLA-4 or anti-PD-1 mAb therapy

enhanced tumor-specific immunity and improved survival in tumor-bearing mice.

Methods

We initiated 2 phase I clinical trials at our Institute to evaluate the safety and immunological effects of GR-MD-02 plus checkpoint blockade immunotherapy. These studies are investigating dose escalations of GR-MD-02 with the standard therapeutic dose of anti-CTLA-4 (ipilimumab/ipi) or anti-PD-1 (pembrolizumab/pembro) in patients (pts) with advanced melanoma (ipi; pembro), HNSCC (pembro), or NSCLC (pembro) (NCT02117362, NCT02575404).

Results

The GR-MD-02 + ipi study has completed enrollment. Three patients received GR-MD-02 at 1 mg/kg, 3 at 2 mg/kg and 2 at 4 mg/kg. There were no DLTs for GR-MD-02; however there was 1 grade 3 AE from ipilimumab (diarrhea) that occurred after dose 4 of ipi. The GR-MD-02 + pembro study is ongoing. Six patients were enrolled at the 2 mg/kg dose level to gain more data from the immunological monitoring assays. Three patients were treated at 4 mg/kg. Eight of the 9 enrolled patients have melanoma and 1 has HNSCC. There have been no DLTs related to GR-MD-02 or pembro, but there one patient experienced transient grade 3 tumor-related pain. Two pts in cohort 1 (2 mg/kg GR-MD-02) had objective responses (1 PR, 1 CR) and two pts in cohort 2 (4 mg/kg) have PR at the first response assessment. All responses have been observed in patients with melanoma.

Conclusions

These data demonstrate that the Gal3 inhibitor GR-MD-02 can be combined safely with checkpoint blockade in patients with metastatic disease and that melanoma regression was observed in multiple patients following combination therapy. Comprehensive immunological monitoring is being conducted to provide insight into potential mechanisms of action.

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Trial Registration

NCT02117362, NCT02575404

P288

KY1044, a novel anti-ICOS antibody, elicits long term in vivo anti-tumour efficacy as monotherapy or in combination with immune checkpoint inhibitors.

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Background

The inducible co-stimulator molecule (ICOS/CD278) is a member of the CD28/CTLA-4 family that is up-regulated upon T cell activation. In the tumour microenvironment, ICOS expression levels vary in different immune cell subtypes, with expression on highly immunosuppressive regulatory T cells (Treg: CD4+/FOXP3+) significantly higher than that on effector CD8+ T cells. The high expression levels on Tregs highlights the potential of targeting ICOS to deplete these cells and enhance the anti-tumour immune response when used in combination with immune checkpoint blockers.

Methods

Using the Kymouse™ platform, we have identified a novel, fully human antibody called KY1044. KY1044 is an anti-ICOS subclass G1 kappa monoclonal antibody that selectively binds to dimeric ICOS (Fc fusion) with an affinity of less than 2nM as measured by SPR using Fabs of KY1044. This antibody, which binds ICOS from human, cynomolgus monkey, rat and mouse with similar affinity, was used in several in vitro and in vivo assays to refine its mechanism of action and assess the anti-tumour efficacy in pre-clinical models.

Results

Using in vitro reporter and primary cell assays, we have demonstrated that KY1044 can affect the immune context via a dual mechanism of action. On the one hand, KY1044 is extremely potent (low pM EC50) at depleting ICOS^{high} Tregs and on the other hand KY1044 was shown to stimulate ICOS^{int} effector T-cells by increasing their IFN γ and TNF α production. Since KY1044 is cross reactive to the rodent orthologue of ICOS, a mouse effector enabled version of KY1044 (mIgG2a) was generated and tested in syngeneic tumour models. Using this antibody, we have confirmed strong anti-tumour efficacy as monotherapy or in combination with surrogates of “approved” immune checkpoint blockers. Noteworthy, and confirming the in vitro data, pharmacodynamic studies demonstrated a long-term depletion of Tregs and a significant increase in the effector T cell to Treg ratio in response to KY1044.

Conclusions

Altogether, the in vitro and in vivo properties of this novel, fully human anti-ICOS antibody support the continued development of KY1044 as a treatment option to activate an anti-tumour immune response.

P289

Dual cIAP1/XIAP inhibitor ASTX660 synergizes with radiation therapy and PD-1 blockade to enhance anti-tumor immunity

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Background

Head and neck squamous cell carcinomas (HNSCCs) frequently harbor genomic mutations in cell death pathways. Nearly 30% of HNSCC overexpress Fas-Associated Death Domain (FADD), with or without BIRC2/3 genes encoding cellular Inhibitor of Apoptosis Proteins 1/2 (cIAP1/2), critical

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components of the Tumor Necrosis Factor (TNF) Receptor signaling pathways. ASTX660 is a novel dual cIAP1/XIAP antagonist in clinical trials for advanced solid tumors and lymphomas.

Methods

Murine oral cancer 1 (MOC1) cells were used *in vitro* and *in vivo* to investigate the anti-tumor activity of ASTX660 alone and in combination with tumor necrosis factor receptor (TNFR) superfamily ligands, radiation, cisplatin chemotherapy, and anti-PD-1 checkpoint blockade. OT-1 T cells were used to investigate the effects of ASTX660 on antigen-specific T cell killing of ovalbumin-expressing MOC1 (MOC1ova) cells.

Results

ASTX660, at nanomolar concentrations, sensitized MOC1 cells to TNF α and stimulated cytotoxic T lymphocyte (CTL) killing of MOC1ova. CTL killing was found to be predominantly mediated by perforin/granzyme B during the earliest stages of killing and release of death ligands TNF α , TRAIL, and FasL as a sustained mechanism of killing. Using MOC1 cells *in vivo*, ASTX660 synergized with radiation therapy (XRT), cisplatin chemotherapy, and PD-1 blockade to significantly delay or eradicate MOC1 tumors. These combination therapies significantly increased CD8 $^+$ T cells and dendritic cells, as well as T cell activity. Depletion of CD8 $^+$ T cells and NK cells *in vivo* revealed both to be important components of the anti-tumor response enhanced by ASTX660+XRT.

Conclusions

These findings serve to inform future studies of IAP inhibitors and support the potential for future clinical trials investigating ASTX660 with XRT and immunotherapies such as PD-1/PD-L1 blockade in HNSCC.

P290

Agonist redirected checkpoint (ARC), TIM3-Fc-OX40L, for cancer immunotherapy

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Background

Current attempts at combination immunotherapy with bispecific antibodies, linked scFv's or T cell engagers have not demonstrated that both checkpoint blockade and TNF receptor activation (agonism) can be achieved with a single molecule. This is likely due to the fact that these molecules lose target avidity when engineered to bind multiple targets with monovalent antigen binding arms. Fusion proteins incorporating the extracellular domain (ECD) of type I membrane proteins (eg. Enbrel, Orencia) or type II membrane proteins (eg. OX40L-Fc, GITRL-Fc), linked to the hinge-CH2-CH3 domain of antibodies are both functional, despite the fact that the ECDs are in opposite orientation. Here we report the generation of a two-sided fusion protein incorporating the ECD of TIM3 and the ECD of OX40L, adjoined by a central Fc domain.

Methods

Shattuck synthesizes both murine and human versions of ARC proteins, and assesses them using a litany of biochemical assays to determine molecular weight, subunit composition & binding affinity; molecular assays to characterize *in vitro/ex vivo* binding, *in vitro* functional activity; and anti-tumor efficacy in multiple syngeneic tumor model systems. The human TIM3-Fc-OX40L has advanced into cell line development and early manufacturing.

Results

The TIM3 end of the fusion protein binds GAL9 and phosphatidylserine (PS) on the surface of human

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tumor cells. The OX40L end of the fusion protein binds OX40 on the surface of primary T cells. TIM3-Fc-OX40L activates NFkB signaling in cells engineered to overexpress OX40 and an NFkB-luciferase reporter. Additionally, the TIM3-Fc-OX40L ARC added to primary human PBMCs along with the super-antigen Staphylococcal enterotoxin B, induced robust secretion of the cytokines IL2 and TNFa. *In vivo*, TIM3-Fc-OX40L stimulates significant expansion of antigen-specific CD4 and CD8 T cells in mice adoptively transferred with OT-I/OT-II cells and vaccinated with ova/alum. Finally, the therapeutic activity of TIM3-Fc-OX40L in established murine MC38 and CT26 tumors was significantly superior to either TIM3 blocking antibody, OX40 agonist antibody or combination antibody therapy. Importantly, a pharmacodynamic biomarker of tumor rejection was identified by coordinated elevations in serum IFN γ , IL-2, IL-4, IL-5, IL-6 and IL-17A.

Conclusions

These data demonstrate feasibility and functional activity of a novel chimeric fusion protein platform, providing checkpoint blockade and TNF superfamily costimulation in a single molecule, which is uniquely advantageous because the construct links those two signals in the same microenvironmental context, at the time in which T cells are engaging cognate tumor antigen.

P291

Agonist redirected checkpoint (ARC), SIRP α -Fc-CD40L, for cancer immunotherapy

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Background

Current attempts at combination immunotherapy with bispecific antibodies, linked scFv's or T cell

engagers have not demonstrated that both checkpoint blockade and TNF receptor activation (agonism) can be achieved with a single molecule. This is likely because these molecules lose target avidity when engineered to bind multiple targets with monovalent antigen binding arms. Fusion proteins incorporating the extracellular domain (ECD) of type I membrane proteins (eg. Enbrel, Orencia) or type II membrane proteins (eg. SIRP α -Fc, GITRL-Fc), linked to the hinge-CH2-CH3 domain of antibodies are both functional, despite the ECDs being in opposite orientation. We report the generation of a two-sided fusion protein incorporating the ECD of SIRP α (CD172a) and the ECD of CD40L, adjoined by a central Fc domain.

Methods

Shattuck synthesizes both murine and human versions of ARC proteins, and assesses them using a litany of biochemical assays to determine MW, subunit composition & binding affinity; molecular assays to characterize *in vitro/ex vivo* binding & functional activity; and anti-tumor efficacy in syngeneic tumor models. The human SIRP α -Fc-CD40L has completed cell line development and single cell cloning and is in late-stage manufacturing.

Results

The SIRP α end of the ARC binds immobilized CD47 at 3.59 nM affinity and binds CD47 on the surface of human tumor cells both *in vitro* and *in vivo*, but does not bind human platelets or RBCs. Importantly, no hemolytic activity has been observed with the human SIRP α -Fc-CD40L ARC; where significant hemolysis has been reported with comparative CD47 mAbs. The CD40L end of the ARC binds immobilized CD40 at 756 pM affinity and binds CD40 on primary macrophages. The SIRP α -Fc-CD40L ARC stimulates functional activity in NFkB-luciferase reporter cells (CD40 driven activation of NFkB) and when added *ex vivo* to human PBMCs primed with the super-antigen SEB; increases secretion of IL2 and TNFa. Furthermore, when activated human macrophages were co-cultured with CD47 positive human tumor cells, SIRP α -Fc-CD40L was shown to

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enhance phagocytosis of human tumor cells. Finally, the therapeutic activity of SIRP α -Fc-CD40L in established murine MC38 and CT26 tumors was superior to either CD47 blocking antibody, CD40 agonist antibody or combination antibody therapy.

Conclusions

These data demonstrate feasibility and functional activity of a novel chimeric fusion protein platform, providing checkpoint blockade and TNF superfamily costimulation in a single molecule. Signal replacement of CD47 by CD40L may uniquely poise macrophages in the tumor microenvironment for activation and cross-presentation of tumor antigens following enhanced tumor cell phagocytosis.

P292

Pre-clinical activity of a novel immunotherapy combination of CAVATAK (Coxsackievirus A21), anti-PD1 blockade and an IDO inhibitor in melanoma

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Background

Coxsackievirus A21 (CAVATAKTM) is a bio-selected oncolytic immunotherapy virus. A Phase Ib trial of i.v. CAVATAK (NCT01227551) in advanced cancer patients has displayed viral tumor targeting and initial indications of antitumor activity in some lesions. Intratumoral CAVATAK injection of melanoma lesions can induce selective tumor-cell infection, immune-cell infiltration, IFN-g response gene up-regulation, increased PD-L1 and IDO (indoleamine-pyrrole 2,3-dioxygenase) expression, tumor cell lysis and systemic anti-tumor immune responses. Blockade of programmed death protein-1 (PD1) and/or IDO inhibition in many cancer patients has resulted in substantial tumor responses. We investigated anti-tumor activity in a B16-ICAM-1 melanoma immune competent mouse

model of a novel combination of CAVATAK, an anti-PD1 mAb and an IDO inhibitor.

Methods

Palpable flank tumor of murine melanoma B16-cells expressing human ICAM-1 were propagated to assess the antitumor activity of CAVATAK, an anti-mouse PD1 (mPD1) mAb and an murine IDO inhibitor in an immune competent mouse model. CAVATAK was administered i.v, while anti mPD1 mAb was delivered via the i.p route and the IDO inhibitor in drinking water.

Results

Notable single agent antitumor activity against the B16-ICAM-1 tumors was only observed in mice treated with anti-PD1 blockade relative to saline controls. Significant survival benefits were only observed in mice treated with the CVA21-anti-mPD1 doublet or the CVA21, anti-PD1 and IDO inhibitor triplet combinations. While not significant, we observed a positive trend in both reduction of overall tumor burden and survival in mice treated with the CVA21, anti-mPD1 doublet and the CVA21, anti-mPD1 and IDO inhibitor triplet compared with mice receiving anti-mPD1 blockade and IDO-inhibition. All combinations of CVA21, anti-mPD1 and IDO blockade appeared to be generally well tolerated.

Conclusions

The notable anti-tumor activity and survival benefit mediated by the combination of CAVATAK, PD1 blockade and IDO inhibition observed in the presented melanoma model supports potential clinical evaluation of such a novel immunotherapeutic combination treatment regimen.

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P293

Talimogene Laherparepvec combined with anti-PD-1 based immunotherapy for unresectable stage III-IV melanoma: a case series

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Background

Checkpoint inhibitors have become standard of care for treating advanced melanoma. By blocking key inhibitory pathways, checkpoint inhibitors reinvigorate effector T cells to overcome an immunosuppressed microenvironment. A key preceding step to effective tumor killing is the release and presentation of tumor antigen. Talimogene Laherparepvec (T-VEC) is an oncolytic virus recently approved as an intratumoral therapy for treating unresectable stage III-IV metastatic melanoma. As monotherapy, it confers modest efficacy in controlling disease progression in patients with locoregional lesions. T-VEC is used to directly lyse tumor cells and promote anti-tumor immunity via the release of tumor antigens and virus-encoded GM-CSF. The mechanisms of action for T-VEC and checkpoint inhibitors are highly complementary, raising the possibility of synergistic benefit from combining these two therapies.

Methods

We reviewed 10 consecutive cases of stage IIIc to stage IVm1b melanoma patients that received T-VEC plus either pembrolizumab or ipilimumab/nivolumab, treated between January 2016 and July 2017 at the Cleveland Clinic with a median follow-up of 7 months (range: 4 to 13 months). Responses of injected (on-target) and

uninjected (off-target) lesions were evaluated according to RECIST 2.0.

Results

The overall response rate for on-target lesions was 90%, with 5 patients experiencing a complete response of injected lesions. Two patients had off-target lesions, both of whom experienced complete response of their uninjected distant metastases. Overall survival of this cohort was 80%. Of the two patients who died, one died of causes unrelated to melanoma. Checkpoint inhibitor therapy was interrupted for 2 patients due to adverse events, with one patient experiencing Grade 3 nephritis and the other patient experiencing Grade 3 diarrhea. There were 2 patients who experienced progression of disease. Taking this into account with the 2 patients who died, overall progression-free survival for this cohort was 60%.

Conclusions

There may be potential synergistic benefit in combining checkpoint inhibitors with T-VEC injection in patients with unresectable melanoma. Although this study is limited by the lack of randomization and short follow-up time, the data revealed positive signals in the responses of both on-target and off-target lesions to the combination of T-VEC and checkpoint inhibitor treatment. In addition, the results show a better response rate than previous publications using T-VEC. Ongoing clinical trials will elucidate the true clinical benefit of this combinational therapy.

P294

Cost of adverse events associated with immunotherapy monotherapy versus targeted therapy in elderly metastatic melanoma patients

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Background

The National Comprehensive Cancer Network guidelines recommend the use of immunotherapy (IO) or targeted therapy (TT) (if BRAF-mutated) for the first-line treatment of metastatic melanoma (MM). Both IO and TT can lead to treatment-related adverse events (AEs). The objective of the study is to estimate and compare the cost of treatment-related AEs between elderly MM patients receiving IO monotherapy and those receiving TT.

Methods

A retrospective cohort study was conducted using Medicare research identifiable files from 2006 to 2014. Eligible patients had ≥ 1 MM diagnosis and ≥ 1 prescription for an IO (ipilimumab or pembrolizumab) or TT (dabrafenib, trametinib, vemurafenib, or dabrafenib+trametinib) that was FDA-approved during the study period. Patients were assigned to the IO or TT cohort based on the most recent therapy used, and had a minimum of 3 months follow-up within the study period. 10 categories of AEs were identified based on a review of IO or TT package inserts, as well as AEs that were common among MM patients. Cost per AE was calculated as the average per-episode cost (inpatient + outpatient) incurred in the 30 days after the first occurrence of the AE. Costs were inflated to 2017 USD and compared between the cohorts using the Wilcoxon rank-sum test, with a significance level of 0.05.

Results

The study included 266 IO patients and 159 TT patients. The TT cohort was younger (72.8 ± 14.3 vs. 73.7 ± 12.6 years; $p=0.04$) and less likely to be male (57.2% vs. 63.2%; $p=0.01$) compared with the IO cohort. The 30-day AE costs for each cohort are summarized in Table 1. The average costs associated with gastrointestinal, respiratory, and pain-related AEs were significantly higher in the IO cohort than the TT cohort (difference: \$9270, \$2654, and \$2155, respectively; $p<0.01$). In

contrast, the average costs associated with pyrexia and/or chills, and other AEs (including decreased appetite/anorexia, fatigue, or infections) were significantly lower in the IO cohort (difference: - \$5761, -\$598 respectively; $p<0.05$). There was no significant difference in the costs associated with hematologic/lymphatic, central nervous system/psychiatric, metabolic/nutritional, skin/subcutaneous tissue, or cardiovascular AEs.

Conclusions

The costs associated with treatment-related AEs among elderly MM patients were substantial. Patients treated with IO incurred higher costs after gastrointestinal, respiratory, and pain-related AEs, while patients treated with TT incurred higher costs after pyrexia/chills, decreased appetite/anorexia, fatigue, or infections.

P295

Economic burden of adverse events associated with immunotherapy and targeted therapy for metastatic melanoma in the US elderly population

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Background

The National Comprehensive Cancer Network guidelines for the first-line treatment of metastatic melanoma (MM) recommend the use of immunotherapy (IO) or targeted therapy (TT) (if BRAF-mutated), both of which are associated with treatment-related adverse events (AEs). The occurrence of AEs is associated with increased healthcare resource use and costs. The objective of this study is to estimate the incremental costs of experiencing treatment-related AEs among elderly MM patients receiving IO monotherapy or TT.

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Methods

A retrospective cohort study was conducted using Medicare research identifiable files from 2006 to 2014. Eligible patients had ≥ 1 MM diagnosis, ≥ 1 prescription for an IO (ipilimumab or pembrolizumab) or TT (dabrafenib, trametinib, vemurafenib, or dabrafenib+trametinib) that was FDA-approved during the study period, and a minimum of 3 months of follow-up. 10 categories of AEs were identified based on a review of IO or TT package inserts, as well as AEs that were common among MM. The incremental cost for each AE category was determined by comparing the 30-day expenditures (inpatient + outpatient) between patients with the AE versus patients without the AE using a generalized linear model with a log-link function and gamma distribution, adjusting for baseline covariates. All costs were inflated to 2017 USD.

Results

A total of 425 patients were included. The mean age was 73.4 years ($SD \pm 13.8$), 61% were male, 94% were white, and the mean baseline Charlson comorbidity index was 8.5 ($SD \pm 2.3$). Table 1 summarizes the adjusted 30-day incremental cost of each AE. The adjusted 30-day incremental cost was highest for respiratory AEs (\$24,150; 95% confidence interval [CI] \$17,630–30,671), followed by central nervous system/psychiatric disorders (\$21,932; 95% CI \$16,011–27,854), metabolic/nutritional disorders (\$19,776; 95% CI \$14,239–25,314), skin/subcutaneous tissue AEs (\$19,183; 95% CI \$14,195–24,170), fever (pyrexia) and/or chills (\$18,976; 95% CI \$13,663–24,289), pain (\$18,406; 95% CI \$13,805–23,008), cardiovascular AEs (\$16,393; 95% CI \$12,131–20,655), hematologic/lymphatic AEs (\$15,850; 95% CI \$11,888–19,813), gastrointestinal AEs (\$13,699; 95% CI \$10,138–17,261), and other AEs (e.g. decreased appetite/anorexia fatigue, infections (including folliculitis)) (\$9,754; 95% CI \$7,315–12,192).

Conclusions

Among elderly MM patients in the United States, the incremental costs of treatment-related AEs are substantial. These findings may help to inform comparisons between treatments, and thereby aid in clinical and budgetary decision-making in this population.

P296 Abstract Travel Award Recipient

Exosomes shuttle TREX1-sensitive IFN-stimulatory dsDNA from irradiated cancer cells to dendritic cells.

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Background

Radiotherapy (RT) used at immunogenic doses leads to accumulation of cytosolic dsDNA in the cancer cells, which activates interferon type I (IFN-I) via the cGAS/STING pathway. Cancer cell-derived IFN-I is required to recruit BATF3-dependent dendritic cells (DCs) to poorly immunogenic tumors and trigger anti-tumor immune responses in combination with immunotherapies. Importantly, we have recently demonstrated that TREX1 regulates radiation immunogenicity by degrading cytosolic dsDNA (Vanpouille-Box et al., 2017 Nat Commun). Tumor-derived dsDNA has also been shown to be critical for cGAS/STING-mediated IFN-I by tumor-infiltrated DCs (Woo et al., 2014 Immunity). Here we hypothesized that activation of DCs by tumor-derived dsDNA is modulated by RT and regulated by TREX1. Exosomes are secreted by cancer cells and can carry dsDNA. Thus, we also tested whether tumor-derived exosomes (TEX) can deliver dsDNA to DCs.

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Methods

TEX were purified from supernatants of mouse carcinoma TSA or TSA knock-in for *Trex1* (TSA^{KI}*Trex1*) cells that were mock-treated (UT-TEX), or irradiated with 3 doses of 8Gy (RT-TEX). Protein composition and content of dsDNA was analyzed. TEX were incubated in vitro with primary DCs, or used to vaccinate BALB/c mice (n=6) by 3 s.c. injections followed by challenge with TSA cells to evaluate the development of protective anti-tumor immunity. Tumor-specific CD8⁺ T cells were identified using H2-L^d/AH1 peptide pentamers.

Results

Double-stranded DNA content of RT-TEX was significantly higher than that of UT-TEX. In vitro, RT-TEX but not UT-TEX induced the upregulation CD40, CD80 and CD86 on DCs, and the production of IFN β , which was dependent on STING expression by DCs. When TREX1 is upregulated in TSA^{KI}*Trex1* cells, dsDNA amount of RT-TEX was markedly reduced, indicating that it is largely derived from cytosolic dsDNA present in the irradiated parent cells. Most importantly, enforced TREX1 expression abrogated the ability to RT-TEX to induce IFN-I in recipient DCs. In vivo, vaccination with UT-TEX led to 100% tumor outgrowth, while 2/6 mice vaccinated with RT-TEX were protected from tumor development. Remaining tumors grew significantly slower compared to UT-TEX treated animals. Tumor-specific CD8⁺ T cells were significantly increased in the tumors and spleen of RT-TEX vaccinated mice.

Conclusions

Overall these results identify RT-TEX as a mechanism whereby IFN-stimulatory dsDNA is transferred from cancer cells to DCs. Importantly, they also demonstrate that TREX1 in the irradiated cancer cells regulate the production of IFN-I by DCs. Findings further support the use of RT doses that do not induce TREX1 to achieve in situ vaccination by RT.

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Predicting the efficacy of combination immunotherapy in animal models using tumor microenvironment immune cell profiling.

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Background

Due to the complex nature of the tumor microenvironment, combinations of immune modulating compounds are likely required for maximal clinical benefit in advanced cancer patients. In our previous work, we have demonstrated that a therapeutic cancer vaccine combined with metronomic cyclophosphamide (mCPA) can enhance T cell infiltration of the tumor. This treatment can be added to treatments such as anti-PD-1 to provide enhanced immune infiltration and better tumor control in murine models. A part of this increased efficacy was due to the increased levels of these molecules in the tumors of treated animals. In this study, we have conducted a systematic flow cytometry survey of checkpoint molecules expressed on T cells within the tumor microenvironment after immune therapy, and utilized this to predict the potential of combining vaccine therapy with other clinical stage antibodies targeting the checkpoint system.

Methods

HPV16 E7-transformed C3 cells were implanted subcutaneously into C57BL/6 mice, and then treated with mCPA (20mg/kg/day PO), followed by vaccination with HPV16 E7₄₉₋₅₇ peptide in a DepoVaxTM platform (DPX-R9F). Additional mice were also treated with anti-PD-1 (200 mg) or anti-OX40 (100 mg). Tumors were collected for analysis 10 days post-vaccine treatment, and digested for staining for flow cytometry to observe differences in infiltrating immune cells and markers on tumor infiltrating immune cells.

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Results

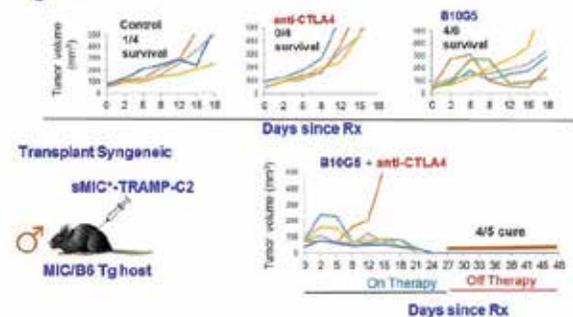
We found that expression of the inhibitory checkpoint markers such as PD-1 and TIM-3 were increased, but activating checkpoint molecules GITR and OX40 were not increased, on T cells after treatment with DPX/mCPA, and no checkpoint molecules are altered further with either anti-PD-1 or OX40 antibody. We performed a tumor challenge study with the C3 model and antibodies targeting either PD-1, GITR or OX40 to determine whether targeting a molecule upregulated by the vaccine would have greater effect than targeting a molecule that is not altered. Antibodies targeting PD-1, GITR or OX40 had significant synergistic activity with DPX-R9F and mCPA treatment, but anti-PD-1 antibody was most potent in tumor growth inhibition.

Conclusions

In conclusion, we have found that an extensive tumor immune profile of treatment regimes in animal models can accurately predict the efficacy of combination immunotherapy, and activating checkpoint molecules do not necessarily enhance the efficacy of treatment in our tested models. Current studies are exploring additional checkpoint molecules in this model that were shown to be elevated in tumor infiltrating CD8⁺ T cells with vaccine treatment to maximize combination immunotherapy potential.

Figure 1.

Optimal dosing of B10G5 combination therapy induces regression of established tumors



Emerging Models and Imaging

P298

Utilization of murine breast cancer models in preclinical immuno-oncology pharmacology

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Background

The field of immuno-oncology is persistently challenged by the need for more syngeneic mouse models in any given tissue type. The most prevalently used syngeneic model for breast cancer is the 4T1 mammary cancer cell line. The 4T1 cell line has useful traits for immuno-oncology research including a highly metastatic phenotype that leads to extensive lymph node and lung metastasis. The tumors have a highly immunosuppressed microenvironment with regulatory T cells (Tregs) and large numbers of granulocytic myeloid derived suppressor cells (G-MDSCs). Radiation can induce changes in an immunosuppressive microenvironment, and focal beam radiotherapy remains an important therapeutic strategy for the treatment of breast cancer. For this reason, we established a radiation dose response on established 4T1 tumors for the purpose of guiding future immunotherapy combinations.

Nonetheless, there are some important challenges with using the 4T1 model, in particular with studying the activity of T cell checkpoint inhibitors and costimulatory antibodies. Mice bearing 4T1 tumors develop a fatal pulmonary hypersensitivity upon repeated treatment with rat antibodies to PD-1, PD-L1 or OX40. 4T1 tumors are completely resistant to PD-1 pathway blockade and the hypersensitivity reaction makes it challenging to perform combination studies with novel test agents.

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Methods

We have characterized two mammary cancer alternatives to 4T1, EMT-6 and E0771. The in vivo growth characteristics and baseline immune cell profiling have been completed for these models with some notable immune profile differences. Further, we have completed an efficacy study of E0771 using an array of immunotherapies.

Results

All three models have a similar proportion of CD4+ T cells and Tregs. In contrast, 4T1 has a much larger proportion of G-MDSCs while E0771 is almost entirely lacking in G-MDSCs with EMT-6 in between the two. The content of monocytic MDSCs (M-MDSCs) is nearly reciprocal with E0771 having a proportionately high population and 4T1 having a minimal number. These immune profile characteristics can be factored into model selection decisions.

An efficacy study in the E0771 model showed that it is highly sensitive to several checkpoint inhibitors, including anti-PD-1, anti-PD-L1, and anti-CTLA-4. E0771 shows a more modest response to anti-LAG3. Costimulatory agonist antibodies to OX40, CD137 (4-1BB) and glucocorticoid induced TNFR-related protein (GITR) were highly active. The indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor epacadostat had no impact on the growth of E0771.

Conclusions

These data enable rational combination strategies and provide alternatives to breast cancer studies in 4T1.

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Cell therapy - TRacking, Circulation, & Safety (CT-TRACS): The Health and Environmental Sciences Institute (HESI)'s new collaborative effort to address the challenges of cell therapies translation

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Background

Cell therapies show great therapeutic promise in the field of immunotherapy. To realize their full clinical potential there is a need for greater understanding of their mode of action, migration after administration, delivery, persistence at sites of action, and whether their localization or distribution may cause safety issues. There are several existing and emerging tools available to develop pharmacokinetics data on cell-derived therapies to improve our understanding but adoption by investigators has been limited. Furthermore, the regulatory landscape is not clearly defined for these emerging therapeutics.

Methods

The Health and Environmental Sciences Institute's Emerging Issues Committee recently launched a multi-sector collaborative sub-committee to identify key needs for assessing the safety of cell therapies and identify opportunities to meet these needs. This program, the Cell Therapy - TRacking, Circulation, & Safety (CT-TRACS) sub-committee, provides a platform for developers, researchers, regulators, imaging specialists and other stakeholders to interact, discuss challenges and identify best practices to ensure that therapies are safe and effective. The sub-committee aims to bring awareness on how existing cell tracking technologies, methods, and best practices can benefit the clinical translation of these new therapies.

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Results

Since its inception in December 2015, CT-TRACS gathered more than 60 members from 25 organizations across the United States, Europe and Japan. The sub-committee has convened monthly and the focus of the group has been narrowed to Cell Fate, i.e., distribution, survival/engraftment and phenotype, post-administration, *in vivo*, as well as evaluating the tumorigenic potential of cell-based therapies. Our initial goals have been to: 1. evaluate current cell-based therapies safety assessment practice and tools; 2. develop best practices for application of available tools for safety assessment of cell therapies and/or identify gaps in safety assessment; 3. organize a workshop to present findings of the sub-teams and develop recommendations for next steps; and 4. initiate a manuscript describing the needs and gaps identified, to build confidence in safety assessment approaches for clinical applications. To date, we have held our first scientific session at an international cell therapy meeting and are compiling the results of a survey of therapeutic stakeholders' needs and wishes in the imaging of cellular therapeutics, survey shared herein.

Conclusions

The CT-TRACS project is open to all current HESI members as well as new participants with relevant technical expertise. The program encourages inquiries by those with interest in providing support for these innovative efforts.

P300

PSMA-associated PET imaging of CAR T cells

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Background

Chimeric antigen receptor (CAR) T cells have demonstrated clinical benefit in numerous hematological malignancies and hold promise for application in solid tumors. As a "living drug", CAR T cells traffic throughout the body with dynamic expansion and contraction kinetics. This dynamic nature limits the utility of standard blood pharmacokinetic analyses where the temporal and spatial distribution of CAR T cells is only partially captured. Therefore, a non-invasive imaging technique that allows tracking of CAR T cells may enhance the understanding of *in vivo* CAR-T behavior and inform associations with safety and efficacy. Previous attempts to track gene modified T cell therapies with PET imaging have been limited by immunogenicity of PET reporters, poor tissue penetration of large molecular weight PET ligands and/or limited sensitivity.

Methods

To improve the ability to track CAR T cells, we engineered CD19-directed CAR T cells to express a truncated form of human prostate-specific membrane antigen (tPSMA) as a reporter. We then used the PSMA-directed small molecule PET ligand [¹⁸F]DCFPyL for *in vitro* and *in vivo* CAR T visualization.

Results

We demonstrate high level surface co-expression of tPSMA and the CD19-directed CAR. The addition of tPSMA to the CAR T cells did not impact *in vitro* or *in vivo* anti-tumor functionality. Phantom imaging studies demonstrated that PET with [¹⁸F]DCFPyL could reliably detect as few as 4000 CD19-tPSMA CAR T cells in a 50uL volume. We developed a spontaneous metastatic acute lymphoblastic leukemia model by subcutaneously injecting CD19+ Nalm6-ffLuc-eGFP tumor cells into immunodeficient NSG mice. Metastatic lesions were observed in liver, spleen and bone marrow, providing an opportunity to evaluate CAR T infiltration into primary subcutaneous and secondary disseminated tumor sites. CD19-tPSMA CAR T cell treatment eradicated Nalm6 tumors from mice up to 90 days post-

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treatment. PET/CT with [¹⁸F]DCFPyL visualized infiltration of CAR T cells at primary and metastatic tumor sites, which was confirmed by immunohistochemical analyses.

Conclusions

These preclinical results establish a new technology for whole body, non-invasive tracking of CAR T cells and support translation into the clinic.

P301

A dual *in vivo* and *in silico* system to model tertiary lymphoid structure formation and anti-tumor immune response in the murine tumor microenvironment

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Background

Tertiary Lymphoid Structures (TLS) are highly organized foci of lymphocytes and antigen-presenting cells that predict increased survival across multiple solid tumors, and are associated with a previously identified gene expression signature composed of twelve chemokines (12-CK-GES) [1]. These chemokines likely result from chronic lymphotoxin-beta receptor activation on resident stromal cells or by paracrine production involving subsequent infiltrates. The exact nature and timing of this induction is unclear due in part to insufficient models of TLS formation. Here, we build upon insights gained from the 12-CK-GES using an implantable three-dimensional bioscaffold to study the interaction of developing murine tumor, endogenous infiltrate, and implanted stroma. Concurrently, we use this system to parameterize an integrated mathematical model of the microenvironment, which can then re-inform our three-dimensional bioscaffold model.

Methods

Injectable bioscaffolds were prepared using primary lymph node reticular fibroblast cells implanted in either Matrigel or chitosan hydrogels loaded with recombinant Lymphotoxin- α 1 β 2 (LT) or individual chemokines from the 12-CK-GES, with or without murine MC-38 colon carcinoma cells. In some experiments, recombinant factors were loaded in lipid-coated silica microparticles to delay release. Preparations were then injected subcutaneously in C57BL/6 mice. Implants were resected at later time points and dissociated for flow cytometry or cryosectioned for histology and immunofluorescent staining. Resulting data, along with those from *in vitro* chemotaxis assays, were used to parameterize an *in silico* model of TLS formation simulated in two dimensions using fixed stromal cells, three types of immune cells, and discrete chemokine fields.

Results

LT, when implanted with stromal cells, can induce organized aggregates of endogenous lymphocytes, significantly increase infiltration of T cells, B cells, and dendritic cells (all $p < 0.05$), and prevent the growth of MC-38 tumors in resected implants ($p < 0.001$). *In silico* model runs predict polarized activation of stromal cells and subsequent production of CCL19, CCL21, and CXCL13 is sufficient to compartmentalize lymphoid aggregates into discrete B and T cell zones and promote anti-tumor activity.

Conclusions

This dual-model system has identified an important role for LT activation of stromal cells in the induction of TLSs and suggests components of the 12-CK-GES are vital for TLS organization.

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P302

Characterizing immunotherapy-induced lymphocyte infiltration at the single patient level using CANscript™, an *ex-vivo* human tumor model

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Background

The presence and activity of lymphocytes within the tumor is critical for clinical response to cancer immunotherapy, such as immune checkpoint blockade. Poor lymphocyte infiltration into the tumor, known as a 'cold' phenotype, is associated with modest clinical response. High baseline infiltration of effector lymphocytes is considered 'hot', and patients are predicted to respond more favorably to treatment. Despite these fundamental predictive biomarkers, patient-to-patient response and durability remains highly variable. There is an urgent gap in available methods to study lymphocyte infiltration, trafficking and spatial heterogeneity induced by different cancer immunotherapies in individual patients.

Methods

Here, we used CANscript™, an *ex-vivo* human tumor model that recapitulates and preserves the native, patient-autologous tumor microenvironment, including peripheral blood mononucleated cells (PBNC). Utilizing tissue from breast cancer patients classified as either 'cold' (N=5) or 'hot' (N=5), we studied lymphocyte infiltration under pressure of immune checkpoint blockade for 72h using pembrolizumab (a-PD-1) and avelumab (a-PDL-1). Using flow cytometric analysis, we characterized infiltrating lymphocytes, and coupled these data with multiplex immunohistochemistry (CD3⁺, CD4⁺,

CD8⁺, CD56⁺, CD25⁺) to map proximity of tumor cells to lymphocytes before and after treatment, *ex-vivo*.

Results

We determined that immune checkpoint blockade induced unique patterns of migration and infiltration of effector T-cells (T_{eff}), T-regulatory (T_{reg}) cells and natural killer (NK)-cells in 'hot' vs 'cold' tumors. Furthermore, we determined that, in some instances, 'cold' tumors can be driven towards a 'hot' phenotype characterized by trafficking of active immune lymphocytes following treatment, which corresponded to differential ratio of T_{eff} to T_{reg} compared to baseline.

Conclusions

Taken together, these data demonstrate the utility of CANscript™ as a platform to characterize response to immunotherapy in a spatial context. Such an advance in our preclinical methods to study immuno-modulators at the individual patient level can help guide treatment decisions for clinicians while simultaneously functioning as a platform to study and discover mechanisms of clinical efficacy for emerging drugs.

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P303

Immune checkpoint inhibitor responses in humanized mouse melanoma models using patient-derived xenografts.

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Background

Melanoma patients develop resistance to both chemo- and targeted-therapy drugs. Promising pre-clinical and clinical results with immune checkpoint inhibitors using antibodies directed against CTLA-4 and PD-1 have re-energized the field of immune-based therapies in melanoma. However, only a third of melanoma patients respond to immune checkpoint blockade. Currently available mouse xenograft and transgenic mouse melanoma models have several short comings and are unable to address the basis of drug resistance and immune non-responsiveness that are frequently observed in melanoma patients. Thus, there is an urgent need to establish an *in vivo* model with a human immune microenvironment that can address issues of therapy resistance.

Methods

For this, our laboratory has developed a humanized mouse melanoma model using patient-derived xenografts (PDX). Immunodeficient NSG mice are reconstituted with human CD34+ cells and after 7-9 weeks, mature human CD45+ cells are observed in circulating blood. Humanized mice were then challenged with HLA-matched melanoma PDX and the functional ability of human immune cells to restrict tumor growth is monitored.

Results

Delayed tumor growth was observed in humanized mice indicating *in-vivo* sensitization of human immune cells to melanoma. This was confirmed by *in-vitro* demonstration of human lymphocytes from tumor-bearing mice showing enhanced cytokine expression after stimulation with melanoma antigen peptides. Further, cytotoxic T-cells derived from melanoma peptide stimulation could functionally lyse tumor cells *in vitro*. In preliminary therapy studies, most tumor-bearing humanized mice treated with anti-PD-1 antibody showed restricted tumor growth. Anti-PD-1 antibody therapy resulted

in enhanced infiltration of CD4+ and CD8+ T-cells that correlated with tumor response.

Conclusions

Our results suggest that humanized mouse melanoma model can be explored further to understand the causes of therapy resistance and immune non-responsiveness.

P304

Predicting pre-clinical tumour response to anti-PD-1 immunotherapy with computational modelling

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Background

Majority of patients fail to respond to anti-programmed death-1 (anti-PD-1) immunotherapy and the reasons for this remain largely unknown. The aim of this study was to develop a computational model, able to predict antitumour response to anti-PD-1 on the basis of key biological properties that might serve as predictive biomarkers of response.

Methods

Interplay between tumour cells and tumour infiltrating lymphocytes (TILs) is described with deterministic population model. It incorporates intrinsic tumour parameters (growth rate (k)), as well as other biological parameters, such as major histocompatibility complex (MHC) class I and PD-1 ligand (PD-L1) appearance on tumour cells, PD-1 appearance on TILs, anti-PD-1 pharmacodynamics, dosing and scheduling regimen, etc. Most of the

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parameters were taken from literature. The remaining free parameters (k, TILs infiltration rate, tumour cells-TILs interaction rate) were fitted to experimental data, where B16-F10 melanoma was treated with anti-PD-1 [1]. Predictive ability of the model was tested on independent experiment from literature, namely B16-OVA treated with anti-PD-1 [2]. Simulated tumour growth curves were compared to experimental data and sensitivity study of key parameters was performed.

Results

Simulated tumour growth curves are in good agreement with experimental data. Maximum deviation of simulated control tumour volume (solid line – blue) to experimental data (blue squares) is –25% (day 13). On the other hand, the model slightly underestimates the effect of treatment with anti-PD-1. Maximum deviation of simulated anti-PD-1-treated tumour volume (solid line – red) to experimental data (red squares) is +40% (day 17). Sensitivity study reveals that dosing and scheduling regimen does not importantly affect treatment outcome (data not shown). The most sensitive parameter of the model is MHC class I appearance. By modulating MHC class I appearance from 1% to 100%, while keeping other parameters fixed, we are able to simulate responders as well as non-responders to anti-PD-1.

Conclusions

Model predictions of antitumour response to anti-PD-1 are within expectations. The predictions might be even more reliable if model parameters were, due to their inter-patient variability and presumably dynamic nature, actually measured for every specific experiment/patient. The emphasis should be on MHC class I appearance as it might be one of the predictive biomarkers of response to anti-PD-1.

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CD137 and anti-PD-1 monoclonal antibodies requires BATF3-dependent dendritic cells. *Cancer discovery*. 2016; 6.1:71-79.

P305

Unravelling the immune contexture of pre-invasive lesions of the lung by multispectral imaging

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Background

Lung cancer is the leading cause of cancer deaths world- wide and despite advances in therapy, the overall survival rate for lung cancer patients remains only 15%.

As most of sporadic cancers, lung cancer emerges from pre-neoplastic lesions characterized by morphological and molecular changes. If morphological changes of pre-invasive bronchial lesions are well characterized, the cause and effect relationship between those changes and the immune response is still un- known. Though, we identified gene expression alterations that suggest a role of the innate and adaptive immunity in the transformation towards carcinoma.

Methods

In order to characterize the evolution of the immune response in pre-invasive bronchial lesions, we have optimized different multispectral 7 colors immunofluorescence panels, by using the Tyramide Signal Amplification (TSA) technology.

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Results

We have performed multiplex staining on FFPE human bronchial biopsies (N=114) at 8 successive morphological stages of lung squamous carcinogenesis, from normal, to low grades dysplasia, high grades, to carcinoma. Images of each biopsy have been acquired multispectrally and digitally analyzed to identify and quantify the density and the tissue distribution of different immune cell types. We aimed to characterize the immune infiltrates at different stages of carcinogenesis and elucidate the role of different cell subtypes in tumor development and progression and the possible causal relationship between the immune phenotypes in pre-neoplastic lesions and tumor progression and patient prognosis.

Conclusions

Evaluation of the immune contexture and prognostic assessment of precancerous lesions of the lung may identify promising new biomarkers for early detection and targets of novel therapeutic strategies for lung cancer.

Immune Modulation, Cytokines, and Antibodies

P306

A protein extract from fermented wheat germ promotes NK cell-mediated lymphoma eradication in mouse xenografts.

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Background

Proteomic and genomic data has allowed for the development of promising targeted agents for NHL [1]. Most have acute and chronic toxicities that limit

efficacy. The use of complementary and alternative medicines has increased during the last decade. However, scientific evidence of their efficacy is scarce. Fermented wheat germ extract (FWGE) has been claimed to have anti-cancer properties in many tumor types. FWGE therapeutic activity has been attributed to its content of benzoquinones [2].

Methods

A protein fraction (FWGP) was isolated by FPLC and proteins identified by mass spectrometry. Direct cytotoxic was studied *in vitro* using NHL cell lines. Immunomodulatory properties were evaluated *ex vivo* by measure immune cell activation in human PBMCs and isolated NK cells. *In vivo* experiments used nu/nu NHL xenografts with or without NK cell depletion; endpoints were tumor volume and toxicity. *In vivo* immunomodulatory effects were evaluated by treating tumor-free BALB/c mice with FWGP and measuring NK cell killing activity and degranulation.

Results

FWGP was cytotoxic in 17 cancer cell lines (IC₅₀ = 20-171 µg/ml in NHL, 12-27 µg/ml in colon and 70-144 µg/ml in lung) and induced apoptosis by increasing levels of caspase-3, PARP, BAK, BAD and p53, while reducing levels of AKT. FWGP increased % NK cells, production of IFγ and GrB, and NK-mediated killing. *In vivo* efficacy was confirmed, with no toxicity, in pre-emptive and established models. *In vivo* treatment with FWGP+rituximab was as effective as R-CHOP, with 90% complete remission. NK depletion resulted in no response to FWGP. These results support the hypotheses that FWGP augments NK-mediated tumor killing. Proteomic profiling identified 844 proteins. An active fraction consisted of 169 proteins.

Conclusions

FWGP represents a promising immunomodulatory agent with anti-tumor activity, minimal toxicity and low cost. Our results suggest FWGP has direct lymphomacidal activity by inducing apoptosis and indirect anti-tumor efficacy by enhancing NK-mediated tumor eradication. Further experimental

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validation will allow translation of al “alternative” product into mainstream medicine.

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P307

Expression and function of PD-1 and TIM-3 in non-small cell lung cancer (NSCLC)

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Background

The use of anti-programmed cell death protein 1 (PD-1) and PD-Ligand 1 agents in the treatment of non-small cell lung cancer (NSCLC) has been well established but many patients are either intrinsically resistant or become refractory during therapy. One potential resistance mechanism is the upregulated expression of additional checkpoint receptors such as T cell immunoglobulin and mucin domain 3 (TIM-3), a transmembrane receptor that binds multiple putative ligands, and that has been shown to negatively regulate the function of T cells that co-express PD-1 [1].

Methods

We examined the immunophenotype and checkpoint receptor expression of over 100 NSCLC samples from primary surgical resections. From a subset of these samples, we evaluated T cell functional status by gene expression analysis on sorted PD-1+ and TIM-3+ CD8+ T cells as well as *ex vivo* stimulation assays to evaluate cytokine

production. Furthermore, we used *ex vivo* and *in vivo* studies to assess the effect of blockade of PD-1 and TIM-3 alone and in combination on T cell activation and anti-tumor activity.

Results

We showed that primary NSCLC samples display heterogeneity in both their baseline immune infiltrate and also PD-1 and TIM-3 checkpoint receptor expression. We examined mRNA expression of multiple immune genes on sorted PD-1+ and TIM-3+ CD8+ T cells, and found that PD-1/TIM-3 double positive cells express reduced interleukin-2 (IL-2) and tumor necrosis factor alpha (TNF α), but similar mRNA levels of interferon gamma (IFN γ) when compared to double negative cells. This phenotype is recapitulated in CD8+ T cells derived from patient samples stimulated with PMA and ionomycin, where we found PD-1 and TIM-3 double positive cells to be significantly deficient in IL-2, but not IFN γ production. Importantly, in addition to their expression being associated with T-cell dysfunction, we also found that blockade of PD-1 and TIM-3 was associated with increased T cell activation and anti-tumor activity in *ex vivo* and *in vivo* models, suggesting a potential functional role for the inhibition of TIM-3, in addition to PD-1, in the enhancement of anti-tumor immunity.

Conclusions

Taken together, these data provide further evidence that TIM-3 may play a role in intrinsic resistance to single agent anti-PD1 therapy in NSCLC and support evaluating the combination of anti-PD-1 and anti-TIM-3 agents in the clinic.

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A PD-1 x CTLA-4 bispecific DART[®] protein with optimal dual checkpoint blockade and favorable tolerability in non-human primates

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Background

Immunotherapy with the combination of monoclonal antibodies that block PD-1 and CTLA-4 has shown clinical benefit beyond that observed with either mAb alone. A PD-1xCTLA-4 bispecific DART protein was designed to induce antitumor immunity through simultaneous targeting of both checkpoint pathways via administration of a single molecule. The DART protein increases checkpoint blocking activity on PD-1/CTLA-4 dually expressing cells, while displaying distinct immunological effects of CTLA-4 blockade *in vivo* absent evidence of toxicity.

Methods

A PD-1xCTLA-4 DART protein was engineered as a tetravalent bispecific molecule from humanized anti-PD-1 and anti-CTLA-4 mAb sequences in a human hinge-stabilized IgG4 backbone. PK, PD and toxicology studies were performed in cynomolgus monkeys.

Results

The PD-1xCTLA-4 DART molecule demonstrated binding to immobilized PD-1 protein and PD-1-expressing cells lines, inhibition of PD-1 interaction with PD-L1 or PD-L2, as well as reversal of PD-1-mediated T-cell signal inhibition in gene-reporter assays comparable to that supported by a replica of

nivolumab. Similarly, binding, ligand blocking and rescue of CTLA-4-mediated T-cell suppression was comparable to that supported by a replica of ipilimumab. The DART molecule demonstrated activation properties comparable to the combination of ipilimumab and nivolumab replicas in a variety of human primary T-cell assays and showed enhanced B7-ligand binding blockade over that mediated by the ipilimumab replica on PD-1/CTLA-4 double-positive cells. In the cynomolgus monkey, the PD-1xCTLA-4 DART molecule exhibited a PK profile consistent with that of an IgG4 and was well tolerated, with no mortality or significant adverse findings up to 75 mg/kg QWx3, the highest dose tested. T-cell expansion in the peripheral blood and lymphoid organs was observed, which was attributable to the CTLA-4 blocking arm, since no such finding was observed with similar or higher doses of the anti-PD-1 constituent of the bispecific molecule.

Conclusions

- PD-1xCTLA-4 DART protein binds and blocks its targets, with increased activity on dual PD-1/CTLA-4-expressing cells.
- The DART molecule enhances T-cell responses *in vitro* to the level achieved by a combination of nivolumab and ipilimumab replicas.
- PD-1xCTLA-4 DART protein was well tolerated in cynomolgus monkeys, with a safety profile similar to that observed with PD-1 blockade alone, while demonstrating biological effects of CTLA-4 antagonism.

The favorable safety and tolerability profile of the PD-1xCTLA-4 DART molecule combined with its enhanced activity on PD-1/CTLA-4 double-positive cells suggest a potential for an improved therapeutic window for PD-1/CTLA-4 co-blockade strategies, with the administration of a single molecule providing dosing convenience and ease of incorporation into additional therapeutic regimens.

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Treatment with heterodimeric IL-15 promotes effector T cell infiltration into tumors

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Background

The presence of tumor-infiltrating effector T cells is considered the most predictive biomarker for clinical benefit in response to immunotherapies. IL-15 is a cytokine important for the proliferation, activation and mobilization of lymphocytes, including natural killer and CD8⁺T cells. We have previously shown that bioactive IL-15 in vivo comprises a complex of the IL-15 chain with the IL-15 receptor alpha chain that are together termed heterodimeric IL-15 (hetIL-15). Several preclinical models have indicated the ability of IL-15 to enhance the response of the immune system against cancer, and based on these results hetIL-15 has advanced to clinical trials.

Methods

We have produced hetIL-15 and tested its anti-tumor activity in several murine cancer models. Analysis of lymphocytes in lymphoid organs and in tumors was performed by flow cytometry and multi-color immunohistochemistry. Chemokine and cytokine levels were determined using electrochemiluminescence (MSD) and ELISA assays.

Results

Repeated injections of hetIL-15 in mice were effective in delaying tumor growth in the MC38 colon carcinoma, TC-1 cervical carcinoma and B16 melanoma models. The combination of hetIL-15 and adoptive cell transfer of melanoma specific Pmel-1 cells showed anti-tumor efficacy in B16-bearing

mice in absence of lymphodepletion. The E0771 orthotopic breast cancer model showed delay in tumor progression and significantly reduced lung metastasis upon hetIL-15 treatment. A significantly reduced onset of lung metastasis was also observed in 4T1 breast cancer-bearing mice. Flow cytometry and multi-color immunohistochemistry assays showed increased trafficking and persistence of CD8⁺ T cells, including tumor specific T cells, into the tumors and an increased CD8⁺/Treg ratio, upon hetIL-15 administration. Importantly, hetIL-15 treatment led to preferential enrichment of adoptively transferred tumor-specific CD8⁺T cells in the B16 tumor in an antigen-dependent manner. Tumor infiltration by CD8⁺T cells was accompanied by increased plasma levels of CXCL10. Tumor-resident CD8⁺ T cells showed features of activated effector cells with enhanced proliferation (Ki67⁺) and high cytotoxic potential (Granzyme B⁺). Upon ex-vivo stimulation, an increased frequency of both CD8⁺ and CD4⁺ T cells producing IFN γ was observed in the tumors of mice treated with hetIL-15.

Conclusions

Our results show that hetIL-15 administration may be a general method to enhance T cell entry in non-inflamed tumors, increasing the success rate of immunotherapy interventions. Preclinical cancer studies support the use of hetIL-15 in tumor immunotherapy approaches to promote the development of anti-tumor responses by favoring effector over regulatory cells. The effect of hetIL-15 on metastasis establishment in orthotopic models may provide synergies against metastatic disease.

P310

CSF1/CSF1R signaling blockade triggers release of matrix-degrading proteases in mouse models

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Background

The heterotypic interplay between cancer cells and their microenvironment provides an opportunity for therapeutic targeting. The abundant tumor-associated macrophage (TAM) infiltrate can be substantially reduced in mouse tumor models and cancer patients by colony-stimulating factor 1 receptor (CSF1R) signaling blockade [1]. Notably, TAM depletion provides marked clinical benefits in diffuse-type tenosynovial giant cell tumors [2,3]. However, facial edema is reported as the most common adverse event of TAM elimination in patients [2,4]. We here sought to gain insight into the molecular mechanisms mediating edema formation. To this aim, we characterized antibody exposure, CSF1 levels and an array of extracellular matrix-degrading and restructuring metalloproteinases (MMPs) in tumor-bearing and tumor-free mice treated with an anti-CSF1R antibody.

Methods

Western Blotting and multiplex assays of tumors and sera of anti-mouse CSF1R mAb (clone 2G2)-treated mice showed an association of TAM elimination with an early intratumoral and systemic release of a specific set of MMPs, including MMP-2, -3 and -8, in multiple transplant (MC38, E0771, KPL-4 and PyMT) and de novo (MMTV-PyMT) tumor models.

Results

In addition, we found that the early increase of MMPs in the face of CSF1/CSF1R pathway blockade was independent of tumor burden and was accompanied by a significant increase of body weight in tumor-free mice following long-term exposure to the antibody. The body weight gain may therefore indicate the enhanced retention of

body fluids. Discontinuation of the CSF1R antibody reinstated unaltered body weight and MMP levels. We excluded platelets and neutrophils as sources of MMP release, even if they accumulated in tumor-bearing mice during CSF1R inhibition. We also examined the effects of blocking the CSF1R ligand CSF1, which is one of the two known ligands of the CSF1R [2,4]. CSF1 antibodies had no impact on the binding of IL-34 (the second CSF1R ligand) to CSF1R. Similar to CSF1R blockade, an anti-mouse CSF1 antibody (clone 5A1) provoked an early systemic surge of a subset of MMPs.

Conclusions

Collectively, our data suggest that CSF1 and CSF1R blocking antibodies induce the release of a distinct set of matrix-degrading proteases (MMP-2,-3 and -8), but not metastasis-promoting proteases (MMP-9 and -12), which may be potentially causative of edema formation. Further studies may inform optimized dosing schedules of CSF1/CSF1R targeting regimens for cancer patients.

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Clinical outcomes of PD-1 inhibition by PD-L1 expression level across malignancies in 204 consecutive patients in a real world oncology setting

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Background

The utility of the PD-L1 biomarker in predicting response to anti-PD-1 agents has been inconsistent

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across malignancies. In this study, we describe outcomes of patients treated with anti-PD-1 agents by PD-L1 expression level.

Methods

Molecular profiling of tumors in patients with advanced cancer was performed at West Cancer Center using Caris Molecular Intelligence Profile testing, which includes PD-L1 percentage assessed by immunohistochemical staining. Patients were included in this retrospective analysis if they were treated with a PD-1 inhibitor and had available PD-L1 results between November 2014 and May 2017. Patients were assessed by PD-L1 expression level, defined as negative (0% expression) or positive (>1% expression), regardless of staining intensity. PD-L1 positive samples were further subclassified into PD-L1 low (1-4%), intermediate (5-49%) and high (≥50%). Best overall response using RECIST 1.1 criteria was retrospectively assessed using 2-physician review of radiologic data. Progression free survival (PFS) and overall survival (OS) were assessed using the Kaplan-Meier method.

Results

204 patients with quantifiable PD-L1 expression were treated with PD-1 inhibitors. Primary tumors included 125 non-small cell lung cancers, 31 melanomas, 12 renal cell carcinomas, and 36 others. 110 (54%) tumors were PD-L1 negative and 94 (46%) were PD-L1 positive (22 [11%] low, 37 [18%] intermediate, and 35 [17%] high). ORR was 39% for PD-L1 positive versus 17% for PD-L1 negative ($p < 0.001$). Best response for each PD-L1 level is shown in Table 1. The estimated median PFS was 6.4 months for PD-L1 positive versus 3.0 months for PD-L1 negative (HR 0.59; $p = 0.001$; 95% CI, 0.43 to 0.81). The estimated median OS was 17.3 months for PD-L1 positive versus 6.9 months for PD-L1 negative (Fig. 1; HR 0.64; $p = 0.021$, 95% CI, 0.44 to 0.94). Increasing PD-L1 expression was associated with a statistically significant improvement in PFS and OS. There was a statistically significant difference among PFS and OS with increasing PD-L1 levels (PFS $p = 0.002$; OS $p = 0.026$). Multivariate

analysis did not identify tumor type as a predictor of response.

Conclusions

PD-L1 staining of any level predicted for improvements in ORR, PFS, and OS with PD-1 inhibitors across multiple malignancies. The higher the PD-L1 staining, the greater the likelihood of benefit. These data provide important real world confirmation for the potential utility of global PD-L1 testing in clinical practice, regardless of malignancy.

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AGEN2034, a novel anti-PD-1 antibody that combines effectively with CTLA-4 pathway blockade to enhance T cell activity

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Background

PD-1 (or CD279) is a co-inhibitory receptor that suppresses T cell function upon binding to its ligands, PD-L1 or PD-L2. PD-1 signaling functions cooperatively with CTLA-4 to limit T cell activation during priming by antigen presenting cells, leading to reduced proliferation, cytokine and chemokine production and cell survival. Anti-PD-1 antibody therapies that block the interaction between PD-1 and its ligands have shown durable clinical benefit both as single agents, but particularly in combination with antibodies that antagonize CTLA-4.

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Methods

AGEN2034 (anti-PD-1; IgG4) was discovered using a proprietary mammalian display technology, Retrocyte Display™. Binding kinetics and affinity to PD-1 were characterized by surface plasmon resonance. Cell-based potency was determined in a Jurkat PD-1⁺ NFAT reporter-based assay where luciferase activity was measured as an endpoint of PD-1/PD-L1 antagonism. The pharmacological effects of AGEN2034 alone or in combination with CTLA-4 pathway blockade using a novel high affinity anti-CTLA-4 IgG1 antibody, AGEN1884, was assessed *in vitro* using peripheral blood mononuclear cells (PBMC) from healthy donors. Prior to human clinical trials, pharmacokinetic and pharmacodynamic profiling of AGEN2034 were performed in cynomolgus monkeys both alone and in combination with CTLA-4 blockade.

Results

AGEN2034 selectively binds to human and cynomolgus PD-1 with high affinity ($K_d < 1\text{nM}$) and sub-nanomolar EC_{50} . The Fc interactions of AGEN2034 are minimized *via* selection of a human IgG4 Fc region. In primary human immune cell assays, AGEN2034 showed a dose-dependent increase in T cell cytokine and proliferative responses. Notably, AGEN2034 combined effectively with AGEN1884 in a dose-dependent manner to further enhance T cell responsiveness. AGEN2034 was well tolerated, and no-observed-adverse-effect level (NOAEL) could be established up to 40 mg/kg in non-human primates. Furthermore, the combination of AGEN2034 and anti-CTLA-4 blockade promoted a dynamic pharmacodynamic response in cynomolgus monkeys, including a transient increase in proliferation and ICOS (inducible co-stimulator molecule) expression in a subset of central memory and effector memory T cells.

Conclusions

The functional attributes of AGEN2034 combined with the favorable pharmacokinetic and pharmacodynamic profile in cynomolgus monkeys are ideally suited for clinical development.

Moreover, AGEN2034 combined effectively with CTLA-4 blockade in a range of preclinical assays to enhance antigen-specific T cell responsiveness and produced a dynamic pharmacodynamic response in non-human primates. AGEN2034 is currently under evaluation in a Phase 1/2 study in subjects with advanced tumors and cervical cancer (NCT03104699) and clinical studies to evaluate AGEN2034 in combination with AGEN1884 are planned.

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In vivo effect of albumin binding domains attached to immune modulators

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Background

Recombinant therapeutic proteins < 50Kd (eg., receptor ligands, cytokines) exhibit short circulation half-lives (mins/hour vs. days for IgGs) which limit their therapeutic utility. A specific way to increase the pharmacokinetic half-life of these agents is via conjugation to circulating albumin. Here we describe the creation of an albumin binding single chain fragment antibody (ScFv-ABD) that binds albumin in circulation, is recycled by binding to the FcRn (similar to IgG's) and then recycled after cellular uptake resulting in increased half-life of the appended therapeutic protein. A second advantage to linking a therapeutic protein to an ScFv-ABD is improved tumor target delivery as numerous studies have shown that albumin accumulates in tumors and inflamed tissues.

Methods

Sonnet BioTherapeutics, using a XOMA phage library, has developed scFv ABD fusion constructs with several different small therapeutic proteins (recombinant interleukin proteins and scFvs

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targeting relevant immune-oncology receptors). These various ABD constructs have high binding affinity to mouse, human & cyno circulating serum albumin thereby preventing renal clearance and retaining benefits of FcRn mediated recycling of albumin for extended PK. Early studies to investigate improved tumor accumulation translates into anti-tumor efficacy in vivo and have shown that ScFv-ABD enhances tumor targeting.

Results

Our characterized scFv-ABD constructs have demonstrated that;

1. biologic activity is retained when the therapeutic protein is attached via the N- or C-terminus suggesting utility for delivering more than one therapeutic protein/scFv;
2. half-life in mouse serum in vivo was extended from minutes to hours/days for three different recombinant proteins and scFv with MWs of 10-80Kd;
3. in an established B6F10 melanoma model these ABD constructs have demonstrated markedly superior reductions in tumor growth and improved overall survival compared to the same constructs without the ABD. Superior efficacy was observed with lower doses and with a single dose of the ScFv-ABD constructs vs free recombinant protein

Conclusions

We will describe several examples of improved half-life, tumor accumulation and efficacy using our albumin linkage approach that is leading to the selection of drug candidates for clinical development.

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Glioblastoma stem-like cell targeting antibodies identified using yeast display biopanning

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Background

BACKGROUND: Glioblastoma stem-like cells (GSCs) are hypothesized to evade current therapies and cause tumor recurrence, contributing to poor patient survival. Existing cell surface markers for GSCs are developed from embryonic or neural stem cell systems; however, currently available GSC markers are suboptimal in sensitivity and specificity. We hypothesized that the GSC surface proteome could be mined with a yeast display antibody library to reveal novel immunophenotypes.

Methods

MATERIALS AND METHODS: A naïve yeast expression library of single-chain human antibodies (scFv) was mined using biopanning against patient-derived GSCs. Discovered unique clones were characterized for qualitative binding affinity against 5 patient-derived GSC lines, 5 matched non-GSC/GBM lines, and 2 normal cell lines. Presumptive GSC-specific antibodies were purified from yeast, and GSC specificity and affinity determined by confocal microscopy and flow cytometry. GSC targeting in vitro was evaluated using flow cytometry, and in vivo after intravenous administration of near infrared fluorescent tagged scFv to immunodeficient mice harboring orthotopic GBM xenografts.

Results

RESULTS: Nine rounds of positive selection against patient-derived GSCs enriched for GSC-binding scFv, with selected pools also negatively screened against normal human astrocytes, neural stem cells, and serum-cultured GBM (i.e. non-GSC). We identified 62 unique scFv clones from ~600 candidates by differential PCR and restriction analysis. Clone 9.7 (heavy chain only, termed VH-9.7) demonstrated specificity against 5 patient-derived GSC lines, with

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minimal binding to non-GSC and normal controls. Purified VH-9.7, produced predominantly in a monomeric form, had a GSC binding affinity (Kd) of 74.3 ± 9.85 nM. Flow cytometry using 5 GSC lines verified VH-9.7 specific GSC labeling: 17-115-fold higher compared to normal astrocytes and 10-65-fold higher compared to non-GSC/GBM lines. After intravenous injection, VH-9.7 significantly localized to GSC-derived GBM xenografts in mice [92 ± 11 relative fluorescent units (RFI), $n=3$, $p<0.05$], compared to control non-targeting scFv (11 ± 7.8 RFI).

Conclusions

CONCLUSIONS: Rapid screening via yeast antibody library biopanning identified human-specific antibodies that demonstrated GSC specificity compared to both non-GSC (i.e. bulk of GBM) and normal neural cells. Identified antibodies could potentially be developed into immunotargeted diagnostics and therapeutics in brain cancer.

P315

A bispecific fusion protein, ACDCI_x, can selectively activate T cells against glioblastoma cells *in vitro*

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Background

Glioblastoma (GBM) is a highly invasive and fatal form of brain tumor with a median survival of approximately 15 months. Treatment for GBM is hampered by the blood-brain barrier (BBB) and small populations of cells that resist conventional therapies. There is an urgent need for innovative new therapies to target all GBM cells within a patient. Chlorotoxin is a small peptide derived from

the venom of the deathstalker scorpion that has been shown to be highly selective for all GBM cells, does not bind healthy tissue, is non-toxic to humans, and has been demonstrated to cross the BBB. To this end, we have designed a T cell engaging molecule, anti-CD3/chlorotoxin (ACDCI_x), composed of the variable heavy (V_H) and light (V_L) fragments of an anti-CD3 antibody (2C11) tethered to chlorotoxin. Here, we show that T cells can be selectively activated against GBM cells, only in the presence of ACDCI_x.

Methods

The gene sequence encoding for His-tagged ACDCI_x was cloned into a deconstructed and improved geminiviral vector and introduced into agrobacteria for needleless infiltration into leaf tissue of *Nicotiana benthamiana*. ACDCI_x was extracted from tissue 4 days post-infiltration and purified via nickel affinity chromatography. T cell activation was evaluated via calcium flux assay (Fluo-4) using ionomycin as a positive control and CD69 expression using full length anti-CD3 antibody as a positive control. Activity was measured using freshly isolated mouse splenocytes and mouse GBM cells (GL261-LucNeo). For flow cytometry, lymphocytes were gated from the splenocyte population and further gated based on CD4 and CD8 markers.

Results

Expression and purification from *N. benthamiana* resulted in a yield of 614ug ACDCI_x per gram of leaf tissue with greater than 99% purity (Figure 1A). Expression of CD69 was observed when splenocytes and GBM cells were incubated with ACDCI_x or anti-CD3 antibody, but not with media alone (Figure 1B). T cell activation as measured by an increase in calcium flux over baseline was observed when ACDCI_x was added to a mixture of splenocytes and GBM cells (Figure 1C). Flux was not observed when mock isolated protein from mock-infiltrated plants was added to splenocytes and GBM, nor with splenocytes and ACDCI_x alone.

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Conclusions

Our results indicate that ACDC1x can be expressed to high levels in plant tissue and has the capacity to selectively activate T cells *in vitro*. These results provide further motivation for our current studies of ACDC1x *in vitro* and in an immunocompetent model of GBM *in vivo*.

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Impact of anti- PD1 on TIL phenotype and function

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Background

Therapeutic antibodies targeting PD-1 have demonstrated efficacy in several solid tumor types with durable responses in a subset of patients. However most patients will either not respond to therapy or progress after an initial response. It is not fully understood how this treatment may alter the phenotype or function of Tumor-infiltrating Lymphocytes (TIL). In this study, methods have been optimized to derive TIL from a single core biopsy and applied to growing TIL from pre-treatment and on-treatment biopsies in patients receiving anti-PD-1 to investigate drug-induced changes in TIL phenotype and function.

Methods

Tumor samples are obtained from an ongoing Phase II clinical trial of anti-PD-1 in cohorts of patients with rare solid tumor types (NCT02721732). Mandatory core biopsies are taken at baseline and on day 15-21 after the first cycle of anti-PD1 (Pembrolizumab, 200 mg). TIL are propagated *ex*

vivo utilizing IL-2 and an agonistic anti-4-1BB antibody (Urelumab, BMS), with or without anti-CD3 (clone OKT3). TIL phenotype and function are evaluated after 2 or 3 weeks of culture.

Results

TIL cultures were first initiated by mincing one core biopsy into 3-6 tumor fragments and cultured in media containing IL-2 and the anti-4-1BB mAb. Observed TIL growth was poor with only 11% of all samples yielding over 2 million TIL per fragment after 3 weeks (2 /17 samples). The addition of anti-CD3 to the regimen dramatically improved TIL outgrowth, with 78% and 80% of the baseline biopsy and on-treatment biopsy samples, respectively, growing over 108 million TIL (n=38/49 baseline; 24/30 treatment, range 10 million to 357.7 million). Phenotypic analysis of the expanded TIL showed an effector memory differentiation status at both time points. However TIL grown from samples obtained after anti-PD-1 dosing showed enhanced proportion of CD8⁺ TIL and enriched CTLA-4 expression in both CD4 and CD8 TIL subsets. PD-1 expression on expanded CD8⁺ and CD4⁺ TIL was maintained or elevated after therapy. Analysis of 4 patients' paired baseline and on-treatment TIL show that CD4⁺ and CD8⁺ TIL grown post anti-PD-1 treatment secrete significantly more of the effector cytokines IFN- γ , IL-13, and TNF- α following anti-CD3 re-stimulation.

Conclusions

Our study highlights phenotypical and functional differences of TIL after a single dose of anti-PD-1. Additionally we demonstrate that it is possible to grow TIL in numbers that would be sufficient to proceed with rapid expansion and adoptive cell transfer from one core biopsy.

Trial Registration

NCT02721732

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***In vivo* efficacy and mechanism of action of anti-TIGIT monoclonal antibody CASC-674**

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Background

TIGIT is a coinhibitory immune checkpoint receptor expressed on regulatory T cells (Tregs), cytotoxic T cells and NK cells. TIGIT ligands include CD155 and CD112, which are expressed on antigen presenting cells and a variety of tumors. These ligands also bind the activating receptor CD226, often co-expressed with TIGIT, creating a network that modulates adaptive and innate immune response in a manner analogous to the CD28-CTLA4-CD80-CD86 network. We previously reported on the characterization of a panel of fully human antagonistic monoclonal antibodies that bind with subnanomolar affinity to mouse, NHP and human TIGIT and block ligand-receptor interactions and signaling in T cells (AACR, 2017). Here we report on the *in vivo* mechanism of action and efficacy of CASC-674 in a number of syngeneic tumor models.

Methods

A lead candidate antibody (CASC-674) was selected and produced as mouse IgG2a and tested in syngeneic *in vivo* mouse tumor models. To explore the mechanism of action *in vivo*, CT26 tumor bearing mice were treated with CASC-674 alone or in combination with anti-PD1/anti-PD-L1 and their tumors and spleens isolated and evaluated by flow cytometry for various lymphoid and myeloid cell subsets.

Results

CASC-674 as single agent has significant anti-tumor activity in the anti-PD1/anti-PD-L1 insensitive CT26 colorectal tumor model, resulting in 8/10 complete regressions (CR). Combination treatment with anti-PD-1/PDL-1 did not enhance the anti-tumor effect

compared to CASC-674 alone. Tumor free mice from this study were re-challenged 8 weeks after the last treatment with CT26 cells in the opposite flank of the original inoculum and no CT26 tumor growth was observed in any of the mice after 30 days, demonstrating that previous treatments resulted in an immune memory response. In addition to the CT-26 model, CASC-674 demonstrated significant anti-tumor effects in EMT6, H22 and MBT-2 syngeneic mouse tumor models.

In CT26 tumors, treatment with CASC-674 but not an IgG1 version of the same variable domains, shifted the immunosuppressive phenotype to an inflammatory anti-tumor phenotype. CASC-674 alone and in combination with anti-PD1 significantly reduced the % of Tregs and exhausted (PD1+ Ki67+) CD8+ cells and increased the % of cytotoxic CD8+ cells (PD-1- Ki67+) and anti-tumor (CD155+) M1 macrophages.

Conclusions

These results combined with the antitumor effect observed suggest Fc functionality is critical for the effect of CASC-674. The observed single agent anti-tumor effect in several different tumor models of CASC-674 and its unique human, non-human primate, and murine cross-reactivity supports consideration of CASC-674 as a therapeutic development candidate.

P318

Anti-tumor efficacy and enhancement of T cell effector functions by EOS084448, an antagonist anti-TIGIT antibody

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Background

: T cell Immunoreceptor with Ig and ITIM domains (TIGIT) is an ITIM domain- containing co-inhibitory receptor preferentially expressed by NK, CD8⁺ and CD4⁺ T cells as well as by regulatory T cells (Treg). Several ligands are described to bind to TIGIT with PVR (CD155) showing the highest affinity. CD226 (DNAM-1), a co-stimulatory receptor also expressed on NK and T cells compete with TIGIT for PVR binding but with a lower affinity. Co-expression of TIGIT and CD226 receptors on T and NK effector cells suggests a role in the fine control of their activation

Methods

Antagonistic anti-TIGIT antibodies were selected by Adimab, LLC. using a synthetic library of human antibodies presented on the surface of yeast. Anti-TIGIT mAbs were characterized for affinity to recombinant human TIGIT (Biacore), for binding to human primary T cells, for competition to CD155 binding as well as for functional activity on cells engineered to express TIGIT or on human primary T cells. An anti-TIGIT mAb cross-reactive to mouse TIGIT was used to evaluate the anti-tumor efficacy of anti-TIGIT in the CT26 syngeneic model

Results

Anti-TIGIT mAb EOS084448 affinity for human TIGIT was 0.25nM, translating into potent binding to human primary T cells with an average EC₅₀ of 0.09nM. EOS084448 inhibited CD155 binding to TIGIT at the surface of TIGIT-expressing cells with an EC₅₀ of 0.16nM. EOS084448 antagonistic activity was evaluated in a TIGIT:CD155 reporter bioassay that resulted in activation of the IL-2 promoter and by measuring IFN γ secretion by minimally stimulated human primary CD8⁺ T cells; EOS084448 potency in these 2 assays was 8nM and 0.4nM. A surrogate anti-TIGIT mAb with a 26,7nM Kd for mouse TIGIT was evaluated in established CT26 mouse tumors. Anti-TIGIT monotherapy delayed CT26 tumor growth and achieved complete response in few mice. Complete tumor regression occurred in most of the animals receiving the anti-TIGIT mAb combined with anti-PD-1 mAb. Anti-

tumor efficacy was associated with an increased CD8⁺ T cell : Treg ratio, an increase TH1/TH2 cytokine ratio and a transcriptional signature indicating an increased cytolytic T cell activity

Conclusions

In vitro and in vivo data demonstrate the potential for EOS084448 to promote antitumor immunity and efficacy and supports the rationale for its clinical evaluation

P319

Long-term disease-free survival (DFS) of metastatic melanoma (mM) and renal cell cancer (mRCC) patients following high-dose interleukin-2 (HD IL2)

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Background

HD IL2 treatment produces durable complete responses (CRs) and surgical CRs. Patients achieving partial response (PR) and stable disease (SD)

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demonstrate improved survival compared with patients who progress.

Methods

11 HD IL2 treatment centers identified patients with survival > 5 years after HD IL2. DFS was from end of IL2 to June 2017. Treatment courses generally consisted of 2 1-week cycles of HD IL2, 600,000-720,000 U/kg IV every 8 hours. We collected data on patients treated with HD IL2 alone, or HD IL2 plus local therapy (surgery or radiation (SRS) leading to CR) with survival > 5 years after HD IL2.

Results

99 patients are reported: 46 mRCC (male 32, female 10, unknown 4) and 53 mM (male 31, female 22). Median age at HD IL2 treatment of mRCC patients is 54 years (range, 39-73 years) and of mM patients is 53 years (range, 24-76 years). Sites of metastatic disease for mM patients were lymph nodes (LN), lungs, bone, liver, brain, and other organs, and for mRCC patients were lung, LN, adrenal, bone, and other organs. The majority of patients received 2-3 courses of IL2 (63 of 99 patients) and 18 received 1 course, with the overall number of IL2 courses ranging from 0.5 to 4 courses. Among the 46 mRCC patients, there are 38 CRs, 5 surgical CRs and 3 PRs with no further treatment. Among 53 mM patients, there are 42 CRs, 2 near CRs, and 9 Surgical/SRS CRs without further treatment. DFS in these patients after HD IL2 ranges from 5+ years to 30+ years, median 10+ years. 27 mRCC and 31 mM are alive > 10 years after IL2. Long-term toxicity among these 99 patients includes hypothyroidism-5 patients, arthralgias/arthritis-6 patients, vitiligo-3 patients, and 1 patient each: neuropathy, PVCs, and normal pressure hydrocephalus. Additional patients may be added as records become available.

Conclusions

We document long-term DFS (>5 years) after CR or PR from HD IL2 alone. Surgical or SRS conversion of PR to CR can produce durable CRs. Long-term DFS was observed in patients with visceral and bone metastases, not only those with LN or lung sites of metastases. Neither age, sex, nor number of

courses of IL2 predicted long-term DFS. Chronic toxicity due to IL2 is uncommon in long-term survivors.

P320

Targeting CD38 beyond haematological malignancies: a panel of anti-CD38 antibodies with unique functional properties

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Background

Because of its high level of expression on haematological cancers, depleting antibodies targeting CD38, an ectoenzyme with hydrolase and cyclase activity, have been generated and showed clinical benefits in particular against multiple myeloma. Interestingly, CD38 is not restricted to haematological cancer cells but also expressed on many different immune subsets including NK and effector T cells, exhausted PD-1⁺ T cells, suppressive myeloid cells, and regulatory T and B cells. Anti-CD38 antibodies will therefore not only impact CD38-expressing tumour cells but also both effector and suppressive immune cells, as illustrated by the increased interest for CD38 as a target in Immuno-Oncology.

Methods

An antibody production and screening campaign has been initiated resulting in a panel of fully human CD38-binding antibodies. All antibodies have been screened for their potential to induce ADCC, apoptosis, ADCP, and CDC. The potential to deplete CD38-expressing cells has been verified *in vivo* in lymphoma engrafted mice. Additionally, all antibodies have been evaluated for their ability to influence effector T cell and NK cell activation.

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Results

We have produced a portfolio of antibodies targeting distinct epitopes of CD38. These antibodies exert ADCC when directed against CD38-overexpressing targets, while showing differential capacity to induce ADCP and CDC. This translated into differential inhibition of *in vivo* tumour growth of human lymphoma tumours in SCID mice. Most interestingly, some of these antibodies augment TCR-induced proliferation and activation of human T cells *in vitro*, with an activity ranging from strong T cell activators that increase proinflammatory cytokine release, to medium or weak activators resulting in low or no cytokine release. In addition, in tumour coculture models, anti-CD38 antibodies increased NK cell activation and proliferation. Regarding CD38 enzymatic function, i.e. hydrolase and cyclase activity, our antibodies display differential profiles in terms of blockade or augmentation of both activities. Effects of anti-CD38 antibodies on immune effector cells, suppressive immune cells as well as tumour cells will be further explored in patient-derived *ex vivo* tumour models.

Conclusions

We present a portfolio of CD38-targeting antibodies with distinct activity profile. The broad expression and multiple functions described for CD38 underline the importance of being able to choose from a range of antibodies that can address the different functionalities depending on the most prominent role of CD38 in each disease setting. The new class of anti-CD38 antibodies presented here will be further explored for their potential to improve response rates, especially in solid tumours.

P321  **Abstract Travel Award Recipient**

Ibrutinib in combination with agonist α OX40 mAb and CTLA-4 blockade induces Eomes^{hi} CD8 T cells and promotes tumor regression

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Background

Antagonist monoclonal antibodies (mAb) targeting T cell checkpoints such as CTLA-4 or PD-1 have shown efficacy in treating a subset of patients with metastatic disease. In preclinical models, CTLA-4 blockade synergized with an agonist anti-OX40 mAb to enhance the expansion and effector function of tumor-specific T cells. Previous studies demonstrated that combined aOX40/aCTLA-4 therapy also induced the generation of CD8 T cells expressing high levels of Eomesodermin (Eomes), a transcription factor known to regulate CD8 T cell differentiation and memory. Eomes expression is negatively regulated in T cells by the T cell signaling kinase ITK (interleukin-2 inducible T cell kinase). Importantly, the FDA-approved Bruton's tyrosine kinase inhibitor ibrutinib also blocks ITK, thus providing a potential means of modulating Eomes expression. We sought to characterize these Eomes^{hi} CD8 T cells and investigate the mechanisms regulating the generation of this novel subset.

Methods

Wild-type C57BL/6 mice were challenged by 1×10^6 TRAMP-C1 tumor cells in the right flank. Wild-type BALB/C mice were challenged by 5×10^4 4T1 tumor cells orthotopically in the mammary fat pad. Mice were treated starting day 7 with 200ug aCTLA-4 (clone 9D9), 200ug aOX40 (Clone OX86), or 150ug Ibrutinib. All treatments were injected i.p. Tumor growth (area) was assessed with microcallipers every 2 to 3 days. Mice were killed when tumors exceeded 175mm^2 for experiments tracking tumor growth and survival. Lymph nodes and tumors were harvested on day 14 to assess and characterize T cell responses by flow cytometry.

Results

Our data revealed that these Eomes^{hi} CD8 T cells expressed significantly less PD-1 on their surface compared to Eomes^{lo} CD8 T cells (97% vs. 57%,

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respectively), while maintaining high levels of IFN- γ production within the tumor. Additionally, we confirmed that clinical concentrations of ibrutinib do not inhibit T cell receptor signaling in CD8 T cells. Next, demonstrated that the combination of ibrutinib/aOX40/aCTLA-4 therapy enhanced the frequency of Eomes^{hi} CD8 T cells in 4T1 tumor-bearing mice, a model of triple negative breast cancer. Additionally, this triple therapy significantly enhanced IFN- γ and TNF- α expression by CD4 and CD8 T cells in the tumor and draining lymph nodes, which was associated with tumor regression and enhanced survival in TRAMP-C1 and Myc-CaP models of prostate adenocarcinoma.

Conclusions

Taken together, these data demonstrate that combined ibrutinib/aOX40/aCTLA-4 therapy induced a robust population of Eomes^{hi} CD8 T cells with enhanced effector function capable of mediating tumor regression in multiple pre-clinical tumor models.

P322

Antitumor effects of radiation combined with intratumoral anti-Tem8 mAb and IL2

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Background

Tumor Endothelial Marker 8 (Tem8) is overexpressed on endothelial cells that line the tumor vasculature. Chaudhary et al. [*Cancer Cell*, 2012] showed in several tumor models that the anti-Tem8 monoclonal antibody, L2mAb, augmented the activity of anti-cancer agents

leading to slowed tumor growth. Tem8 is also overexpressed on tumor cells themselves in several tumor types, including melanoma. We have recently shown that radiation (RT) given in combination with intra-tumoral injection (IT) of a tumor-specific antibody and interleukin-2 (IL2) can elicit an *in situ* vaccine effect for mice bearing a B78 syngeneic melanoma. Here, we show that RT+L2mAb+IL2 treatment resulted in improved outcome for mice bearing a Tem8+ B78 melanoma.

Methods

B78 melanoma cells were transduced to overexpress Tem8 and injected into C57BL/6 mice. Mice bearing a ~200mm³ tumor received external beam RT (12Gy), followed by IT injection of L2mAb (2mg/kg) and/or IL2 (150,000U) on days 5-9 post-RT. Mice were monitored for tumor growth and overall survival. Statistical differences in tumor volume were determined by Two-Way ANOVA followed by Tukey's test for multiple comparisons, and Log-rank/Mantel-Cox test was used for overall survival. Chi-Square test was used to compare response rates.

Results

Treatment with RT+L2mAb resulted in slower tumor growth as compared to RT alone ($p < 0.001$). By including IL2 to RT+L2 (i.e. RT+L2mAb+IL2), tumor growth was significantly reduced as compared to mice treated with either RT alone ($p < 0.001$) or with RT+L2 ($p < 0.001$). In our preliminary study, only the combination of RT+L2mAb+IL2 yielded any mice that were tumor free (2 tumor-free mice out of 5); treatment with RT+L2mAb, or RT+IL2 did not generate any tumor-free mice.

Conclusions

Tem8-targeted therapy given in conjunction with other anti-cancer agents has shown promising effects. Prior to this study, anti-Tem8 antibody given in combination with RT or IL2 had not been investigated. Here, we show that RT followed by Tem8-specific antibody significantly improved outcome, and that addition of IL2 further improved outcome. Inhibition of tumor-angiogenesis via anti-

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Tem8 antibodies may block the function of the Tem8 that is expressed on tumor endothelial cells, slowing tumor growth. However, since RT+L2mAb treatment resulted in improved outcome when given in combination with IL2, antibody-dependent-cell-mediated cytotoxicity may also contribute to the mechanism of action of this therapy.

P323

Novel treatment of cutaneous T cell lymphoma: Targeting TNFR2, an oncogene and marker of potent Tregs, with anti-TNFR2 antibodies

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Background

Tumor necrosis factor receptor 2 (TNFR2) is a lymphoid marker of the most potent regulatory T cell (Treg) subtype, which is enriched in the tumor microenvironment, and a commonly expressed oncogene in human tumors. Anti-TNFR2 antibodies can inhibit both Tregs and tumors with specificity for the tumor microenvironment [1], and recent data shows that TNFR2 is a candidate oncogene in cutaneous T cell lymphoma (CTCL) with recurrent point mutations and gain of function alteration of TNFR2, resulting in abnormal expression of TNFR2 on CD4+CD26- tumor cells [2].

Methods

We screened novel TNFR2-directed antagonistic antibodies for their ability to kill leukemic cells in Stage IV CTCL (Sézary syndrome) subjects with failure on diverse drug regimens, as well as their ability to induce killing of tumor-associated Tregs

and unleash effector T cell (Teffector) proliferation. Studies were performed in vitro on sorted CD4+CD26- Sézary cells or V-beta specific populations when a tumor was typed.

Results

At baseline, CTCL blood samples showed significant burdens of tumor cells within the CD26- subset of CD4 cells (averaging 45-95% of this subfraction), vs control blood (18% CD26- cells on average). In CTCL subjects, Treg numbers (CD4+CD25hiFoxp3) were elevated at baseline (11% vs 7% control, $p < 0.05$), Teffectors were depressed (3% vs 8% control, $p < 0.05$), and Treg/Teffector ratios were abnormally elevated (8% vs 1% control, $p < 0.05$).

Regardless of underlying therapy used in vivo, TNFR2 antagonism showed dose responsive killing of tumor cells within the CD26- fraction of peripheral CD4 T cells. TNFR2 antagonism also had specificity for tumor cells vs CD26- cells from paired controls. In vivo treatment of CTCL subjects with anti-proliferative agents such as methotrexate hindered TNFR2 antagonism driven killing, demonstrating the specificity of TNFR2 antagonism for rapidly proliferating cells.

Dose response experiments in vitro showed TNFR2 antagonism also had the desired dual effect of Treg killing combined with unleashing of T effector proliferation at 48-72 hours.

Conclusions

TNFR2 is a recurrent genomic gain alteration in CTCL that can potentially be targeted to directly stop tumor cell growth by antibody-induced cell death, eliminate Tregs of the tumor microenvironment and unleash T effector proliferation.

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P324

Co-targeting of mesothelin and CD47 with bispecific antibodies for efficient elimination of solid tumors

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Background

Mesothelin (MSLN) is a cell surface glycoprotein overexpressed in several human cancers, including mesothelioma, pancreatic-, ovarian-, lung- and gastric cancer. MSLN overexpression is associated with poor prognosis, with serum levels of soluble MSLN a biomarker of disease severity in mesothelioma patients. While a promising target in cancer, monoclonal antibodies (mAbs) targeting MSLN have demonstrated limited efficacy in clinical trials. Current MSLN-targeted approaches in development have thus incorporated novel modalities to enhance tumor-killing potential (e.g., Antibody Drug Conjugates and CAR-T cells). CD47, an immune check-point, interacts with SIRP α providing a 'don't eat me' signal that allows healthy cells to limit elimination by immune cells, in particular macrophages. CD47 upregulation in solid cancers is correlated with poor clinical prognosis, almost certainly by allowing tumor cells to escape immune surveillance by phagocytes. Clinical development of mAbs to CD47 is hindered by the ubiquitous expression of CD47 leading to rapid drug elimination and significant hematological toxicity including anemia. To address these concerns, we have employed a bispecific antibody (biAb) approach that pairs a high affinity anti-MSLN targeting arm to an anti-CD47 arm of an optimized

affinity that drives the efficacious binding only on MSLN-positive cells. This MSLN/CD47 biAb approach, therefore, is designed to target the CD47-SIRP α pathway in the tumor microenvironment to more efficiently harness the immune system for tumor eradication.

Methods

Fully human biAbs targeting both MSLN and CD47 were generated. An array of biAbs coupling MSLN-targeting arms binding to different epitopes on MSLN with a common CD47-targeting arm have been tested in antibody dependent cellular phagocytosis (ADCP) and antibody dependent cellular cytotoxicity (ADCC) assays *in vitro* plus in mouse xenograft experiments. The following human cell lines were used: NCI-N87 (gastric), HPAC (pancreatic), OVCAR3 (ovarian), Caov-3 (ovarian), NCI-H226 (mesothelioma) and MSLN-transfected HepG2 (hepatic).

Results

The MSLN/CD47 biAbs significantly enhanced macrophage-mediated ADCP of NCI-N87, HPAC, OVCAR3 and Caov-3 as compared to the anti-MSLN mAb, Amatuximab. In addition, the biAbs also demonstrated superior ADCC of NCI-N87 cells and NCI-H226. When tested in a xenograft tumor model using MSLN-transfected HepG2 cells, therapeutic treatment with MSLN/CD47 biAbs significantly prevented tumor development, while Amatuximab and B6H12, an anti-CD47 mAb, only slightly delayed tumor growth.

Conclusions

Using a novel biAb approach that focuses the blockade of the innate immune checkpoint receptor CD47 to MSLN-expressing tumors substantially enhances their elimination *in vitro* and *in vivo*. Thus, MSLN/CD47 targeting biAbs are a potentially superior strategy in managing MSLN-positive solid tumors.

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Characterization of the anti-CTLA-4 antibody AGEN1884, including toxicology and pharmacology assessments in non-human primates

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Background

Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an important negative regulator of T cell function. Together with CD28, these receptors exemplify a co-inhibitory and co-stimulatory signaling axis that dynamically sculpts the interaction of antigen-specific T cells with antigen presenting cells (APCs). Preclinical studies have demonstrated that anti-CTLA-4 antibodies can enhance tumor-specific immunity through a variety of mechanisms including: i) blockade of CD80 or CD86 binding to CTLA-4; ii) preventing CTLA-4-expressing regulatory T cells from physically removing CD80 and CD86 from the surface of APCs; and iii) selective elimination of CTLA-4-expressing intratumoral

regulatory T cells by an Fcγ receptor-dependent mechanism.

Methods

Here we describe the pharmacological and toxicological characterization of a novel human IgG1 anti-CTLA-4 antagonist antibody, AGEN1884. Binding, blocking, T cell activation as well as Fcγ receptor-mediated activity of AGEN1884 were evaluated *in vitro*. The activity and tolerability of AGEN1884 was further assessed *in vivo* using a non-human primate model. Our *in vitro* and *in vivo* assessments extended to a direct comparison of AGEN1884 with an IgG2 Fc variant, AGEN2041.

Results

AGEN1884 potently enhanced T cell responsiveness *in vitro*, and combined effectively with other immunomodulatory antibodies targeting co-inhibitory and co-stimulatory receptors on T cells. AGEN1884 was well-tolerated in non-human primates and was confirmed to modulate cellular and humoral immune responses to co-administered reporter vaccines. In addition to the activity of AGEN1884 as a monotherapy, a memory T cell proliferative response was observed in peripheral blood of animals when co-administered with an anti-PD-1 antibody. Finally, we provide a comparison of the *in vitro* and *in vivo* functional properties of an IgG2 variant of AGEN1884, revealing important antibody isotype differences that may have an impact on the design of optimal dosing regimens in patients.

Conclusions

Taken together, the pharmacologic properties of AGEN1884 support its clinical investigation as both a single therapeutic agent and in combination therapies.

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Isoform specific TGF- β inhibition in combination with radiation therapy as a novel immune therapeutic approach to cancer therapy

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Background

TGF- β is a pleotropic cytokine, which has emerged as a potential target in cancer treatment due to its dual role in tumorigenesis and homeostasis. There are three isoforms of TGF- β (TGF- β 1, TGF- β 2 and TGF- β 3), which are secreted by immune and non-immune cells as a latent complex. Depending on the local context, TGF- β adopts opposing roles in carcinogenesis and in modulating the immune system. These dueling roles of TGF- β are dependent on its secretion and activation. Local radiation therapy (RT) can activate TGF- β via reactive oxygen species. Such TGF- β expression is linked to radioresistance and dose-limiting toxicities, reducing the effectiveness of RT. In these studies, we aim to characterize the effect of RT on the temporal and cell-specific expression patterns of TGF- β isoforms in mouse tumor models. This will inform treatment regimens combining isoform specific anti-TGF- β therapy with RT.

Methods

Fluorescence-activated cell sorting (FACS): C57BL/6 mice were implanted on the hind limb with B16-F10 melanoma cells. On day 10, tumors were irradiated locally with 15 Gy. Expression of TGF- β isoforms was measured at 1, 3 and 5 days post-RT by FACS.

In-vivo: C57BL/6 mice were implanted with tumors and irradiated as described. Mice were treated (10/group) with anti-TGF- β 1, anti-TGF- β 3 or a pan-TGF- β antibody beginning 1 day after RT given intraperitoneally (200 ug/mouse) every other day for 8 doses. Tumor growth and overall survival were monitored. A similar experiment was conducted in the 4T1 breast cancer model, in which mice were treated 1 day prior to radiation.

Results

FACS data indicated that TGF- β 1 and TGF- β 3 expression increases on most immune cells in the tumor 1 day after RT, decreases 3 days after RT and reaches a peak 5 days after RT. Preliminary in-vivo studies demonstrate that both α TGF- β 1 and α TGF- β 3 as monotherapies have activity against B16 melanoma. In combination with RT, α TGF- β 3 trends towards inhibiting tumor growth. Similar observations were obtained in a 4T1 breast model; however, α TGF- β 3 alone and in combination with RT as well as α TGF- β 1 + RT showed a significant delay against tumor growth. No significant differences in survival were seen in either tumor model.

Conclusions

TGF- β 1 and TGF- β 3 are expressed on numerous lymphoid and myeloid cells in B16 tumors and spleens. TGF- β isoform expression peaks 5 days post-RT. Anti-TGF- β therapy is effective in delaying tumor growth and may synergize with RT in certain cancers. This demonstrates rationale for the use of anti-TGF- β therapy to enhance the effectiveness of RT in cancer.

P327

Intratumor injection of tumor-specific antibody and IL2 triggers *in situ* vaccination following local radiation therapy

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Background

In murine models of GD2⁺ melanoma, GD2⁺ neuroblastoma, and EGFR⁺ head and neck cancer, we have reported a cooperative interaction between radiation and intratumor (IT) injection of tumor-specific antibody (anti-GD2 hu14.18K322A or anti-EGFR cetuximab). Consistent with a process mediated by antibody-dependent cell-mediated cytotoxicity, this interaction required the Fc portion of the antibody, host Fcγ receptor, NK cells, and tumor expression of the antibody-targeted antigen. In this GD2⁺ melanoma model, combined treatment with RT + IT-hu14.18-IL2 immunocytokine (a fusion protein of hu14.18 antibody and IL2) markedly enhanced response compared to radiation or IT-IC alone, radiation + IT-hu14.18 antibody, or radiation + intravenous-IC. In those studies, radiation + IT-IC induced an *in situ* vaccine effect, resulting in a tumor-specific memory T cell response. Here we test whether IT administration of non-fused tumor-specific antibody and IL2 may elicit an *in situ* vaccination response following local radiation.

Methods

C57BL/6 mice were flank engrafted with syngeneic GD2⁺ B78 melanoma and 5-week tumors (~200 mm³) were treated with single fraction 12 Gy radiation, IT-IL2 (150,000U), and/or IT-hu14.18K322A (50μg). IT injections were given daily on days 6-10 after radiation. Outcomes included tumor response and rates of complete regression, overall survival, tumor-specific memory (tested in disease-free mice by contralateral flank injection with B78 melanoma >90 days after radiation), and immunohistochemistry on tumors resected at day 12 after radiation.

Results

The combination of local radiation + IT-hu14.18K322A + IT-IL2 resulted in greater tumor regression compared to radiation + IT-hu14.18K322A or radiation + IT-IL2 [50% (19/38) aggregate complete tumor regression vs 0% (0/11) and 25% (3/12), respectively, $p < 0.001$]. No mice were rendered disease-free with these IT-treatments in the absence of radiation. Kaplan-Meier analysis demonstrated improved survival with radiation + IT-hu14.18K322A + IT-IL2 compared to radiation + IT-hu14.18K322A and radiation + IT-IL2 (log-rank $p < 0.0001$; 80% alive at day 65 vs 0% and 50%, respectively). Thus far, 100% (6/6) of mice rendered disease-free by combined radiation + IT-hu14.18K322A + IT-IL2 have rejected subcutaneous B78 re-implantation at >90 days after radiation, compared to 0/10 naïve control mice. Preliminary immunohistochemistry analyses suggest that IT-IL2 may increase tumor infiltrating CD8⁺ T cells in this preclinical model.

Conclusions

We present evidence of a cooperative anti-tumor effect with the combination of local radiation and IT injection of both tumor-specific antibody and IL2. Given the widespread availability of tumor-specific antibodies, this may offer a viable approach to pursuing *in situ* tumor vaccination in many diverse types of cancer using off-the-shelf reagents.

P328

Therapeutic application of radiation-induced anti-tumor antibody

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Background

Radiation therapy (RT) has significant immune modulatory properties that, when strategically integrated with immunotherapy, may improve cancer treatment outcomes. It has been previously reported that RT induces tumor-specific antibodies

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[1]; however, this response needs to be better characterized and its therapeutic potential evaluated. Antibodies binding to the Fc receptor of dendritic cells (DC) have been shown to direct DC to the tumor site [2]. We hypothesize that radiation-induced anti-tumor antibodies (RT-Ab) increase dendritic cell (DC) trafficking to the irradiated tumor to increase tumor antigen presentation. Here, we explore the therapeutic application of RT-Ab.

Methods

Multiple syngeneic murine cell lines were used (EG7, B16, LLC, 4T1), along with their respective murine strains. Fluorescence tagged anti-mouse antibodies (eBiosciences) and anti-CD11c antibodies (eBiosciences) were used for flow cytometry.

Results

Multiple syngeneic murine tumor grafts (B16, LLC, 4T1) were grown in the hind legs of respective murine strains and focally irradiated with a single 15Gy RT dose, with post-RT sera (pRTs) obtained at different time intervals. Flow cytometric analysis using anti-mouse antibodies of the pRTs incubated with the respective tumor cell showed a significant increase in RT-Ab that peaked between days 7-13. This increase was more pronounced for a single fraction of 15Gy when compared to 4.5Gy on 5 consecutive days while the tumor growth delay was equal between the two groups. Next, flow cytometry was performed on bone marrow-derived dendritic cells (BMDC) from the respective murine strains co-cultured with pRTs, which showed an increase in the binding of RT-Ab to DCs when compared to normal or non-irradiated tumor bearing mice sera. When an Fc receptor blocking agent was used, this binding was significantly inhibited, which suggested Fc receptor-mediated binding of RT-Ab to the BMDC. Lastly, we demonstrated that RT-Ab-bound-BMDC (RT-DC), when administered as an autologous DC therapy in conjunction with focal RT, increased the therapeutic effect of focal tumor RT significantly compared to multiple control conditions.

Conclusions

RT induces a tumor-specific antibody response that increases DC trafficking to the tumor via Fc receptor-mediated targeting. This likely contributes to RT-mediated tumor antigen presentation and adaptive anti-tumor immunity. Amplification of the RT-Ab response may have therapeutic applications.

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P329

Dual blockade of PD1 and CTLA4 with bispecific antibody XmAb20717 promotes human T cell activation and proliferation

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Background

Treatment of melanoma patients with nivolumab plus ipilimumab increases progression-free-survival compared to each monotherapy. The increase in efficacy of the combination regimen is accompanied by an increase in adverse events. Since PD1⁺CTLA4⁺ tumor-infiltrating-lymphocytes are dysfunctional in the tumor microenvironment, we developed XmAb20717 to selectively target PD1⁺CTLA4⁺ double-positive T cells in an effort to recapitulate efficacy of the combination regimen while reducing toxicity.

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Methods

XmAb20717 component antibodies binding to PD1 and CTLA4 with favorable stability and functionality were assembled in a bispecific antibody platform with substitutions in the Fc domain to suppress effector function. XmAb20717 was evaluated *in vitro* by measuring antibody binding and de-repression of super-antigen stimulated peripheral blood lymphocytes (PBMCs) and *in vivo* by monitoring the engraftment of human PBMCs in NSG mice (huPBMC-NSG) by flow cytometry. To evaluate anti-tumor efficacy we monitored the growth of established cancer cells in huPBMC-NSG following treatment.

Results

Optimized candidate single-chain Fvs were confirmed to bind PD1 and functionally block PDL1 and PDL2 binding to PD1. We also generated optimized anti-CTLA4 Fabs. Anti-PD1 and anti-CTLA4 targeting component antibodies were assembled into XmAb20717, which displayed favorable biophysical and manufacturing properties. XmAb20717 enhanced IL2 secretion *in vitro* 4.1-fold relative to a negative control antibody ($p < 0.01$, $n = 15$ donors) in response to antigenic challenge of previously stimulated T cells, with 1.6-fold superior activity compared to an anti-PD1 bivalent antibody ($p < 0.01$, $n = 15$ donors). XmAb20717 enhanced T cell engraftment (9.6-fold) and IFN γ secretion (2.9-fold) compared to vehicle controls in huPBMC-NSG mice ($p < 0.001$, $n = 10$ /group), with equivalent activity to a combination regimen of anti-PD1 and anti-CTLA4 bivalent antibodies. Blockade of PD1 and CTLA4 with an equimolar mixture of monovalent component antibodies that compose XmAb20717 lead to inferior activity compared to XmAb20717, suggesting that binding selectivity of the bispecific antibody contributes to function. XmAb20717 also exhibited anti-tumor activity in preliminary anti-tumor studies in mice.

Conclusions

Dual blockade of PD1 and CTLA4 with XmAb20717 resulted in T cell activation that is comparable to a combination of bivalent antibodies targeting PD1

and CTLA4. Specific targeting of human lymphocytes positive for both PD1 and CTLA4 with XmAb20717 may promote similar efficacy compared to a combination of bivalent antibodies while reducing adverse events. These data suggest that clinical development of XmAb20717 is warranted for the treatment of human malignancies.

P330

T cell immunotherapies trigger innate immunity and aseptic inflammation leading to potent anti-tumor and off-targets effects

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Background

Mobilizing the immune system to treat advanced cancers is now a clinical reality. Successful immune-based therapies that treat tumors are often accompanied by immune-related adverse events (irAE) including toxicities that can occasionally present with severe and lethal symptoms. The primary immunotherapies currently in clinic include agents that activate T cell responses such as checkpoint blockade of inhibitory pathways and infusion of ex-vivo tumor-derived or T cell receptor (TCR)-transgenic or chimeric antigen receptor (CAR)-modified T cells. While the beneficial and toxic effects of T cell-based immunotherapies in the clinic are being extensively explored, the precise mechanisms of tumor elimination and irAE remain the subject of intense investigation.

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Methods

In the present study, we treated established tumors with melanoma-specific adoptive CD4+ T cell transfer and costimulation via OX40 or checkpoint blockade with anti-CTLA-4.

Results

We found that, in spite of co-opting the adaptive immune response to treat cancer, acute local inflammation, resembling delayed-type hypersensitivity, plays a fundamental role in tumor elimination and related toxicities in a model of irAE. While OX40 or CTLA-4 antibodies stimulated T cells are necessary for initiating a therapeutic response, activation of endogenous neutrophils constitutes an important and necessary effector mechanism of tumor destruction and irAE. Upon closer examination, we found extensive neutrophil extracellular traps (NETs) in ear pinnae of treated mice and in melanoma patients suffering from immunotherapy-induced irAE.

Conclusions

Our results illustrate the involvement of innate immunity in promoting tumor elimination and subsequent side effects with immunotherapies that engage T cells.

P331

Involvement of estrogen and progesterone in the modulation of indoleamine 2,3 dioxygenase - IDO – expression in cultured mammary carcinoma cells of female dog

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Background

Indoleamine 2,3 dioxygenase - IDO is an enzyme that prevents the establishment of an immune response in the microenvironment in which it is expressed by catabolizing the amino acid

tryptophan. The deprivation of tryptophan and the generation of its metabolites, mainly kynurenine, impairs effector T- cells proliferation leading them to apoptosis [1] and, by its interaction with the aryl-hydrocarbon receptor (AhR) in CD4+ T-cells, favors the expansion of T-regulatory cells [2]. It is known that several cancer cells and leucocytes in the tumor microenvironment express IDO and are sensitive to hormones. Steroidal hormones, such as estrogen and progesterone, are capable of altering immune functions in cells and may influence IDO expression; nonetheless, the mechanisms involved are still poorly understood. Our group previous data have shown that the progesterone was directly involved in IDO expression modulation via its receptor in dendritic and CD4+ T cells from the maternal-fetal interface of Wistar rats [3]. Therefore, this study aims to investigate whether this mechanism is present in the female dog mammary carcinoma microenvironment and if it occurs in a similar way with estrogen.

Methods

Cells of mammary carcinoma from bitches were treated with exogenous progesterone and estrogen and their respective receptor antagonists tamoxifen and mifepristone. IDO expression was analyzed by immunohistochemistry, flow cytometry and the mRNA by real-time PCR. IDO quantification was obtained by western blot technique.

Results

IDO quantification exhibited the same pattern as mRNA expression. There was an increase of the enzyme expression and mRNA in the estrogen treated group, in contrast to the decrease observed in the progesterone group. When the cells were subjected to the hormonal inhibitors, an evident decrease of IDO expression percentage and the respective mRNA was verified following the supplementation of tamoxifen and a restoration of IDO expression values and the mRNA after the addition of the progesterone inhibitor, mifepristone.

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Conclusions

These findings strongly suggest that progesterone and estrogen participate indirectly in the modulation of IDO through their membrane receptors.

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P332

Pre-clinical efficacy and tolerability of NKTR-255, a polymer-conjugated IL-15 for immunology

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Background

IL-15 is a cytokine that activates T-cells and NK cells and has long been recognized for its potential as an immunotherapeutic agent for the treatment of cancer. Exploiting this potential has been challenging due to unfavorable pharmacokinetic properties. NKTR-255 is a polymer-conjugated IL-15 that shows improved plasma exposure while retaining potency and high affinity for IL-15R α . Here we investigate the pharmacodynamics, pre-clinical efficacy and tolerability of NKTR-255.

Methods

To assess the pharmacodynamic effects of NKTR-255 in mice and non-human primates NKTR-255 was delivered intravenously and whole blood was collected at the indicated timepoints; flow cytometry was used to measure signaling activity (STAT5 phosphorylation), proliferative status (Ki-67 expression) and absolute frequency of various lymphocyte subpopulations. Cytotoxicity assays were performed by incubating NK cells purified from spleens of NKTR-255-treated mice with dye-labelled YAC-1 target cells and assessing extent of cell-killing by flow cytometry. Efficacy was studied using the CT-26 lung metastasis model; briefly, 10⁵ CT-26 cells were injected into the tail-vein of mice, NKTR-255 treatment was initiated the following day, lungs were harvested on day 13 and metastases were counted.

Results

NKTR-255 induces rapid and sustained signaling in lymphocytes following intravenous administration in mice and non-human primates. This sustained signal results in proliferation of CD8 T-cells and a preferential expansion of the CD8 central memory population. NK cells increase in number and in Granzyme B expression, concomitant with an increase in cytotoxic potential. The robust induction of CD8 and NK cell proliferation is maintained upon repeat dosing. In a mouse model of tumor metastasis to the lungs, NKTR-255 treatment results in an 85% reduction in the number of metastases. This efficacy is dependent on NK cells but not CD8 T-cells, as demonstrated in cell-depletion studies. Toxicology assessments demonstrate that NKTR-255 is well tolerated at efficacious dose levels.

Conclusions

NKTR-255 provides a sustained IL-15 signal, resulting in profound and sustained immune activation and anti-tumor activity. NKTR-255 is well tolerated and its pharmacokinetic properties and pharmacodynamic effects translate well from rodents to non-human primates, supporting further development.

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P333

MPL-5821, an ESM™-p38 MAPK inhibitor, enhances tumor immune response and M1 macrophage polarization in a 3D *Ex Vivo* system utilizing fresh tumor microspheroids of lung cancer patients

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Background

Lung cancer is one of the most common causes of death worldwide. Immunotherapy has demonstrated durable responses and tolerability in subsets of lung cancer patients. Macrophages are a significant component of the tumor microenvironment and the predominant phenotype (M2-like) is frequently associated with one supportive of tumor immune evasion. However, the plasticity of macrophages offers the opportunity for therapeutic intervention to repolarize the phenotype to one that is non-immunosuppressive and supportive of an anti-tumor immune response (M1-like). MPL-5821 is a p38 MAPK inhibitor employing Esterase Sensitive Motif (ESM™) technology [1] to principally target myelomonocytic cells. This study evaluates the immunomodulatory effect of MPL-5821 in a 3D *ex vivo* assay of lung cancer.

Methods

Fresh tumor tissues obtained from consented patients with non-small cell lung cancer at the time of surgical resection were utilized in a proprietary 3D *ex vivo* tumor microsphere assay with intact tumor immune microenvironment. Tumor microspheres were treated with MPL-5821 for 36 hours. At the end of the treatment, flow cytometry analysis was performed to assess M1/M2 plasticity

and TIL proliferation (CD3+/Ki-67+). Additionally, multiplex human cytokine assay was used to simultaneously analyze the differential release of cytokines in culture media and gene expression analysis was performed using the NanoString PanCancer Immune Profiling panel which contains probes to quantitate 770 immune function genes.

Results

MPL-5821 at 10nM and 50nM effectively inhibited the p38 signaling pathway in the tumor samples and led to profound inhibition of the immunosuppressive cytokine IL-10 and pro-inflammatory cytokines TNF α and IL-6. Evidence of enhanced T-cell proliferation and activation was observed in several of the tumor samples. The most statistical significant up-regulated genes were associated with antigen processing/presentation and MHC class II protein binding and included CD4, CR1, CD74 and multiple HLA genes. CD40 and ICOSLG genes were up-regulated in several tumors as were CXCL9 and 10. Chemokine genes CXCL3, 5, 7 and 8, associated with a pro-angiogenic macrophage phenotype, were the most statistically down regulated genes along with the M2 phenotype associated genes CCL13, 23 and 24. Down-regulation of PD-L1 and IDO gene expression was also seen in several tumors.

Conclusions

This lung patient derived *ex vivo* approach indicates that MPL-5821 may alleviate myelomonocytic cell induced immunosuppression and enhance antigen responsiveness suggesting potential clinical implications in the treatment of lung cancer.

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P334

Inhibition of IDO activity by epacadostat (INCB024360) activates tumor infiltrating lymphocytes in a patient-derived 3D *ex vivo* system of lung cancer and alleviates stromal immunosuppression

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Background

Immune evasion is one of the major hallmarks of cancer and identifying mechanisms by which cancer cells evade the immune system have become a major strategy against cancer. IDO (indoleamine 2,3-dioxygenase) is a tryptophan catabolizing enzyme expressed constitutively by tumor cells and different components of immune cells present within the tumor microenvironment. It has been shown that high expression of IDO increases the number of Tregs and blocks the proliferation of effector T cells. Thus, inhibiting the IDO pathway is a promising strategy to restore immune system responses to more easily identify and destroy cancer cells. This study evaluates the immunomodulatory effect of an IDO inhibitor epacadostat (INCB024360) on the immunosuppressive effect of cancer-associated fibroblasts and activation of tumor infiltrating lymphocytes in a 3D *ex vivo* assay utilizing fresh patient tumor samples

Methods

3D *ex vivo* studies were performed with fresh tumor tissue obtained from consented NSCLC patients. Tumor samples were treated with IDO inhibitor at 1 μ M for 48 hours. HPLC analysis on kynurenine and tryptophan was performed to verify target inhibition in the *ex vivo* model. A multiplex human cytokine assay was used to simultaneously analyze the differential release of cytokines in culture

media. Additionally, NanoString PanCancer Immune Profiling platform containing probes to quantitate 770 immune function genes was used to determine positive and negative associations between expression of immune function genes and TIL activation by *ex vivo* treatment. Furthermore, autologous patient-derived cell lines (CAF and TILs) were utilized in an *in vitro* assay to determine the role of IDO inhibition on CAF-mediated immunosuppression.

Results

3D *ex vivo* studies showed a significant decrease in kynurenine demonstrating that epacadostat effectively inhibited the enzymatic activity of IDO in the tumor microenvironment accompanied by increased release of pro-inflammatory cytokines such as IFN γ . Treatment with epacadostat demonstrated decreased expression of genes involved in tumor growth (CCL25) and increased expression of antitumor immune response genes (CXCL14, CCL19 and CCL21). These studies showed epacadostat at an effective concentration of 1 μ M induced specific changes in the microenvironment and increased immune response. Furthermore, the autologous patient derived cell line *in vitro* assay determined that epacadostat overcame CAF induced inhibition of TIL activity.

Conclusions

This patient-derived 3D *ex vivo* approach demonstrated the immunomodulatory activity of epacadostat in NSCLC and indicates that inhibition of IDO activity may overcome stroma-induced immunosuppression in lung cancer. Studies on the effects of epacadostat in combination with anti-PD1 in the same culture systems are currently ongoing.

P335

Characterization of a novel differentiated anti-CTLA-4 antibody (ADU-1604) *in vitro* and *in vivo*

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Background

Targeting the CTLA-4 immune checkpoint with antibodies, either alone or in combination with PD-1/PD-L1 inhibitors shows clinical activity and durable responses in advanced cancer. The use of anti-CTLA-4 may augment other immunotherapies. Indeed, in syngeneic mouse tumor models anti-CTLA-4 strongly enhanced anti-tumor efficacy of live attenuated double-deleted *Listeria monocytogenes* (LADD) immunotherapy and of the stimulator of interferon genes (STING) pathway activator ADU-S100, indicating combination potential. Activity of anti-CTLA-4 is enhanced by Fc receptor engagement and tumor-selective depletion of regulatory T cells in mice, but this is unproven as of yet for cancer patients. Here we present a novel, humanized anti-CTLA-4 antibody originating from Aduro's proprietary B-Select platform.

Methods

Among a panel of mouse anti-human CTLA-4 antibodies, 27A was humanized and functionally characterized on an IgG1 and IgG4 backbone. Both variants, named hCTLA4.27H6L1.i1 and .i4 were characterized *in vitro* for binding to human and cynomolgus CTLA-4, blocking of CD80/CD86 and functional activity. Both antibodies are compared for their capacity to induce tumor rejection in patient-derived tumor graft models in humanized mice.

Results

The IgG1 and IgG4 variants bound recombinant human CTLA-4 with a K_d of 2.7 ± 2.9 nM and 1.3 ± 0.5 nM as measured by Bio-Layer Interferometry, and cell-expressed CTLA-4 with an EC₅₀ of 0.042 ± 0.000 nM and 0.048 ± 0.002 nM, respectively.

Binding to endogenous CTLA-4 was demonstrated by flow cytometry using primary activated human and non-human primate lymphocytes. Both IgG1 and IgG4 variants potently blocked the interaction of CD80 and CD86 with human CTLA-4 (IC₅₀ ranged from 1-2 nM). Using swap mutants of mouse and human CTLA-4 and cross-competition assays by Bio-Layer Interferometry, these antibodies showed a unique binding profile indicating a previously undiscovered epitope on CTLA-4. Functional characterization demonstrated that both antibodies enhanced activation and IL-2 production by human primary T cells or peripheral blood mononuclear cells (PBMCs) co-stimulated by Raji cells or Staphylococcal enterotoxin B, in a dose-dependent manner. The IgG1 variant (in contrast to IgG4) bound to low and high-affinity Fcγ receptors inducing antibody-dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer (NK) cells and CD16+ monocytes towards CTLA-4+ cells. Similarly, complement-dependent cytotoxicity was differentially induced *in vitro*.

Conclusions

Overall, both hCTLA4.27 derivatives compared favorably to benchmarks in their biophysical and functional characteristics. The IgG1 variant, designated ADU-1604, is progressing through preclinical development.

P336

Preclinical characterization of MGA012, a novel clinical-stage PD-1 monoclonal antibody

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Background

Monoclonal antibodies (mAbs) that target immune checkpoint pathways, such as the cytotoxic T lymphocyte-associated antigen-4 and the programmed cell death protein 1 (PD-1) pathways, have demonstrated broad clinical efficacy against a variety of malignancies as monotherapy or in a combination. MGA012 is a novel anti-PD-1 mAb developed to disrupt the PD-1 interaction with PD-L1/PD-L2 to restore or improve T-cell function as stand-alone therapy or in combination with other immune approaches.

Methods

Murine PD-1 mAbs were generated and benchmarked against replicas of the approved mAbs, nivolumab and pembrolizumab. Several mAbs with favorable characteristics were further chimerized or humanized. MGA012, a humanized, hinge-stabilized IgG4κ mAb, was selected based on binding and biophysical properties as well as a functional characterization inclusive of enhanced T-cell activation following superantigen re-stimulation.

Results

MGA012 bound human PD-1 with an affinity equal to or exceeding those of replicas of nivolumab or pembrolizumab. MGA012 bound PD-1-expressing cell lines and chronically-activated T cells, blocked PD-1 interactions with PD-L1/PD-L2, resulting in inhibition of PD-1 signaling and enhanced antigen-driven cytokine secretion to levels comparable to those observed with nivolumab or pembrolizumab replicas. Furthermore, characterization of MGA012 in ex-vivo tumor microenvironment immune models showed activation profiles recapitulating the benchmark PD-1 mAbs. MGA012 showed combinatorial activity in vitro when added to anti-CTLA-4 or anti-LAG-3 mAbs and enhanced the activity of a T-cell redirecting molecule in a mouse tumor model. MGA012 showed no unexpected cross-reactivity in human tissues, with staining observed primarily in lymphocytes and lymphoid

organs. In a repeat-dose (10-150 mg/kg QWx4) study in cynomolgus monkeys, PK was linear with a beta half-life of 11.2 days (± 4.6 SD) and full PD-1 occupancy on circulating T cells at all doses tested. Occupancy of $\geq 80\%$, persisting for 4-7 weeks, was also observed in monkeys receiving a single 10 mg/kg dose. MGA012 was well tolerated in cynomolgus monkeys and demonstrated a favorable safety profile with a no-adverse effect level (NOAEL) of 150 mg/kg.

Conclusions

MGA012 is a novel anti-PD-1 mAb with favorable preclinical characteristics, including PD-1 binding and biophysical properties, PD-1 pathway blockade, the ability to enhance T-cell responses in vitro and in vivo, and a favorable PK and safety profile in cynomolgus monkeys. Clinical trials are ongoing [NCT03059823] or in planning stage to ascertain the safety and preliminary activity of MGA012 alone or in combination with other immune oncology agents, including T-cell redirecting bispecific DART[®] molecules.

Trial Registration

ClinicalTrials.gov NCT03059823

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Preclinical characterization of MGD013, a PD-1 x LAG-3 bispecific DART[®] molecule

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Background

Monoclonal antibodies (mAbs) that target the immune checkpoints, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1), have shown enhanced clinical anti-tumor activity when given in combination, triggering interest in determining whether additional checkpoint inhibitor combinations may afford enhanced clinical benefit. Lymphocyte activation gene-3 (LAG-3) is another immune checkpoint expressed on activated T cells and tumor infiltrating lymphocytes. Recognizing the therapeutic potential of dual checkpoint blockade, we have engineered MGD013, a IgG4k bispecific DART molecule to bind PD-1 and LAG-3 concomitantly or independently and disrupt these non-redundant inhibitory pathways to further restore exhausted T-cell function.

Methods

Proprietary PD-1 and LAG-3 mAbs were generated and selected based on binding characteristics, biophysical properties, the ability to block their respective receptor/ligand axes and to synergize in T-cell stimulation assays. Humanized sequences were incorporated into a tetravalent bispecific DART format and benchmarked against combinations of replicas of the approved PD-1 mAb, nivolumab, and BMS-986016 anti-LAG-3 mAb, which is currently under clinical evaluation. MGD013 biological activity was evaluated in various primary cell-based immune assays. Safety was assessed in cynomolgus monkey toxicology studies performed at MPI (Mattawan, MI) under Institutional Animal Care and Use Committee-approved protocols.

Results

MGD013 bound with high affinity to human and cynomolgus monkey PD-1- and LAG-3-expressing cells and blocked PD-1/PD-L1, PD-1/PD-L2 and LAG-3/HLA (MHC-II) interactions, with resultant signaling blockade. Functional characterization revealed enhanced cytokine secretion in response to antigen stimulation that was greater than that of the combination of individual equimolar amounts of PD-

1 and LAG-3 mAbs. MGD013 was well-tolerated in a repeated-dose (Q1Wx4) cynomolgus monkey toxicology study. Except for the occurrence of watery feces in a few animals, no MGD013-related adverse findings were noted, including hematological or clinical chemistry changes, serum cytokine levels or gross and microscopic histological findings, establishing a no-observed adverse-effect level (NOAEL) of 100 mg/kg.

Conclusions

MGD013 is a bispecific DART molecule capable of simultaneously blocking the PD-1 and LAG-3 pathways, resulting in enhanced T-cell activation compared to single or combination mAb blockade. MGD013 has demonstrated a favorable pre-clinical safety and toxicological profile and is currently initiating clinical testing [NCT03219268].

Trial Registration

ClinicalTrials.gov NCT03219268

P338

Combined IL-6 and CTLA4 blockade enhances CD8+ T cell infiltration via CXCR3 and limits growth of pancreatic cancer in murine models.

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Background

Immune checkpoint blockade has not shown efficacy in pancreatic cancer. We hypothesized the cytokine interleukin-6 (IL-6) enhances immune suppression in this disease, and represents a logical target to augment immunotherapy. Prior publications from our group and others

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demonstrate IL-6 is derived from pancreatic stroma, is inversely associated with overall survival in metastatic patients and enhances efficacy of anti-PD-L1 antibodies in pre-clinical models. This report addresses the efficacy of combined IL-6 and CTLA4 blockade, and identifies a mechanism by which this combination enhances effector T cell infiltration into tumors.

Methods

In vivo efficacy of Ab targeting IL-6 and CTLA4 was tested in mice bearing subcutaneous MT-5 murine pancreatic tumor cells with *Kras*^{G12D} and *TP53*^{R172H} mutations. CXCR3 blocking antibodies were used for *in vivo* mechanistic studies, while immune biomarkers were analyzed using flow cytometry or immunohistochemistry.

Results

Combined blockade of IL-6 and CTLA4 inhibited tumor growth rate as compared to controls ($p < 0.05$). IHC analysis revealed increased T cells in tumors from combination treated mice ($p = 0.035$ vs. anti-IL-6; $p = 0.038$ vs. anti-CTLA4; $p < 0.0001$ vs. iso control). Analysis of systemic immune biomarkers was conducted via flow cytometric analysis of splenocytes. Despite the role for IL-6 in expanding MDSC, no change in monocytic (CD11b⁺Ly6G⁻Ly6C⁺) or granulocytic (CD11b⁺Ly6G⁺Ly6C^{low}) cells were observed. However, analysis of T cell subsets revealed both anti-CTLA4 alone or the combination increased cells with Th1 or Th17, but not Th2 phenotypes ($p < 0.05$). Cells with a CD4⁺CD25⁺FoxP3⁺ phenotype were increased in combination treated mice ($p < 0.05$). We are studying if these cells are suppressive or a byproduct of enhanced activation marker expression. Blockade of IL-6, CTLA4 or the combination altered circulating cells with phenotypic properties of naïve, effector and central memory T cells (based on CD44/CD62L). Notably, circulating CD4⁺CD44⁺CD62L⁺ T cells were significantly higher in the combination ($p < 0.05$). Consistent with T cell infiltration data, sequencing of TCR β within $n = 3$ representative tumors from each group of mice revealed a trend toward increased number of both productive templates and

rearrangements. Finally, *in vivo* administration of Ab targeting CXCR3 limited the efficacy of the treatment combination, and reduced CD8⁺ T cell infiltration into tumors.

Conclusions

IL-6 blockade enhances efficacy of antibodies targeting CTLA4 in pre-clinical models of pancreatic cancer. Mechanistically, this involves CXCR3-mediated intratumoral infiltration of CD8⁺ T cells. These data suggest that clinically-available IL-6/IL-6R targeted agents could be repurposed to enhance immune checkpoint blockade as a novel combination therapy.

P339

Critical role of hematopoietic progenitor kinase 1 in anti-tumor immune responses

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Background

Hematopoietic progenitor kinase 1 (HPK1) was previously reported as a negative regulator of T cell responses due to its ability to attenuate T cell receptor (TCR) signaling. T cells from HPK1 knockout (HPK1^{-/-}) mice demonstrated elevated proliferation and cytokine production in response to TCR engagement and are resistant to prostaglandin E2 (PGE2)-mediated suppression. Additionally, HPK1^{-/-} bone marrow derived dendritic cells (BMDCs) eliminate established Lewis Lung Carcinoma more effectively compared with HPK1^{+/+} BMDCs, suggesting HPK1 is a critical negative regulatory component in key immune cell types involved in anti-tumor immunity. To further investigate the potential value of targeting HPK1 pharmacologically in cancer immunotherapy, we generated HPK1

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kinase dead mice (HPK1 KD). Extensive characterization of these mice was conducted via employing syngeneic tumor models and ex vivo studies. We report here our findings on the blockade of HPK1 kinase activity in modulating anti-tumor immune responses.

Methods

HPK1 KD mice were generated and characterized, including immune cell phenotyping in peripheral blood and secondary lymphoid organs. These mice were then subjected to in vivo stimulation with agonistic anti-CD3 followed by measurement of plasma cytokines. HPK1 WT and KD mice were further interrogated in syngeneic tumor models in combination with a cyclooxygenase inhibitor (celecoxib) or anti-PD1. Anti-tumor efficacy was monitored, immune phenotyping was conducted for draining lymph nodes and tumor tissues, and ex vivo studies were performed to evaluate the enhancement of immune responses.

Results

HPK1 KD mice were grossly normal and immune phenotyping revealed no difference compared with WT mice. HPK1 KD mice demonstrated superior anti-tumor efficacy in a sarcoma model. Significantly better anti-tumor efficacy with 50-70% tumor free was observed in HPK1 KD mice compared to WT mice treated with either celecoxib or anti-PD1, in sarcoma and colorectal tumor models, respectively. Further immune phenotyping and ex vivo studies indicated enhanced CD4⁺ and CD8⁺ T cell populations as well as IFN γ and TNF α secretion along with reduced ratios of CD8/Tregs. Cytolytic activities against syngeneic tumor cells were also augmented with HPK1 KD CD8⁺ T cells compared with WT CD8⁺ cells.

Conclusions

Genetic blockade of HPK1 kinase activity revealed a significant enhancement of immune responses and resulted in improved anti-tumor efficacy in combination with inhibition of PGE2 or PD-1 pathways.

P340

In vitro effects and mechanism of hu14.18-IL2 immunocytokine against GD₂ positive pediatric malignancies

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Background

Passive immunotherapy with anti-GD₂ antibody (Ab) ch14.18/CHO (dinutuximab beta) showed activity for the treatment high-risk neuroblastoma (NB) patients and received recently marketing approval in the EU. Immunocytokines are antibody-cytokine fusion proteins that combine the targeting properties of monoclonal antibodies with the immune stimulatory function of cytokines. Here we demonstrate activity and mechanism of hu14.18-IL2 against a variety of GD₂ positive pediatric tumor cell lines (neuroblastoma, osteosarcoma, Ewing's sarcoma, retinoblastoma) in preclinical models.

Methods

Expression of the target antigen GD₂ on LAN-1 (neuroblastoma), MG63 (osteosarcoma), TC-71 (Ewing's sarcoma) and Y79 (retinoblastoma), and PD-1/PD-L1 checkpoint on LAN-1 was analyzed by flow cytometry. Hu14.18-IL2 mediated ADCC, CDC and whole blood cytotoxicity (WBT) was determined by ⁵¹Cr release assays.

Results

We found expression of antigen GD₂ on all cell lines derived of major neuro-ectodermal malignancies in childhood. The highest level was found in neuroblastoma but all other cell lines were clearly GD₂ antigen positive. Importantly, hu14.18-IL2 was effective in mediating ADCC, CDC and WBT against all cell lines in vitro, and potency was found higher

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than that of ch14.18/CHO in the osteosarcoma and retinoblastoma models. The effects were antigen specific as addition of an anti-idiotypic antibody abrogated the cytolytic activity.

Interestingly, tumor specific ADCC in the presence of LA-N-1 neuroblastoma cells, leukocytes and sub-therapeutic hu14.18-IL2 concentrations (10-100 ng/ml) resulted in a strong increase of PD-L1 expression both on effector and target cells. This effect required cell-cell contact, since separation of effector cells from target cells using a membrane abrogated the activity. This provides a rationale to explore combinatorial approaches with agents that inhibit the PD-1/PD-L1 checkpoint.

Conclusions

Immunocytokine hu14.18-IL2 is effective against GD₂ positive pediatric malignancies derived of neuroectodermal origin. Engaged immune effector functions by hu14.18-IL2 result in a concomitant upregulation of immune checkpoints that suggests a combinatorial approach with checkpoint inhibitors early during clinical development.

P341

Peripheral immunomodulatory changes in metastatic renal cell carcinoma (mRCC) patients treated with nivolumab

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Background

CheckMate-009 was a biomarker-based study in which serial biopsies were conducted for patients receiving nivolumab [1]. Lesser data is available for fluxes in circulating biomarkers during the course of treatment. We hypothesized that immunomodulatory changes in blood would mirror previous observations in tissue.

Methods

Patients with mRCC receiving nivolumab as standard of care were prospectively enrolled. Blood samples were drawn at baseline, week 4 and week 12 during treatment. Analysis of samples for relevant white blood subsets was conducted by immersing peripheral blood mononuclear cells in a mixture of PBS, 2% FCS and 0.1% sodium azide with FcIII/IIR-specific antibody to block nonspecific binding and stain the cells with different combinations of fluorochrome-coupled antibodies. We further collected fluorescence data on Fortessa (Beckton Dickinson) and analyzed them using FlowJo software (Tree Star) [2]. Plasmas were analyzed for human cytokines using Luminex. Changes in cells and cytokines overtime in the overall population were interrogated. We also compared baseline levels of cytokines in responders (R; including partial/complete response and stable disease) and non-responders (P; primary progression).

Results

A total of 10 patients were included, with a total of 29 samples collected. During treatment, we observed a significant decrease in the proportion of CD3+CD4+ T cells and an increase in the CD3+CD8+ T cells in the overall population ($p < 0.05$). In addition, the expression of PD-1 was significantly decreased in both CD4+ and CD8+ T cells overtime, while serum level of IL2R and CXCL9 significantly increased ($p < 0.05$). Compared to P, R group showed a trend towards higher baseline levels of IFN-alpha and IFN-gamma ($p = 0.05$ and 0.11 , respectively) as well as VISTA expression on CD4+ T cells ($p = 0.15$).

Conclusions

Our results emphasize the immunomodulatory changes in the peripheral blood occurring during treatment with nivolumab in mRCC pts. Some dynamics contrast with previous observations in tissue [3], reflecting temporal and spatial heterogeneity during immune system modulation by immunotherapies. Differential baseline levels of cytokines and immune checkpoint expression in the R group may be used as a predictive biomarker for nivolumab response if confirmed in larger series.

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P342

Blood in circulation, with intact cascade systems, as a tool to predict first-infusion reactions and mechanism-of-action of immunotherapeutics

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Background

Since the TGN1412 disaster in London, where an anti-CD28 superagonistic antibody induced a life-threatening cytokine storm in six healthy men during a clinical trial [1], risks of first-infusion reactions are important to study prior clinical trial initiation. These assays can be constructed in different ways and depending on the setup, the sensitivity of the assay depends on the antibody tested. It is therefore of great need to validate a cytokine release assay with the potential to detect first-infusion reactions regardless of the nature of the antibody tested.

Methods

As the assays used today to predict cytokine release syndrome (CRS) are devoid of one or several blood components we set out to investigate if a modified

chandler loop model could be used to predict CRS. The assay makes use of extracorporeal fresh whole blood in circulation, thus it contains blood immune cells, immunoglobulins along with intact cascade systems [2]. The challenge with such systems is to avoid clotting during assay performance, but correctly performed, will allow for both monitoring of cytokine release along with studies of ADCC and CDC.

Results

Uniquely the assessed agonistic antibodies were scored to induce CRS in blood from all tested donors (n=22) after only 4 hours of incubation in the loop assay, whereas non-agonistic antibodies associated with no or low infusion reactions in the clinic neither induce cytokine release nor cause false positive responses. Additionally, the value of an intact complement system in the assay was highlighted by the possibility to dissect out the mechanism-of-action of alemtuzumab and rituximab.

Conclusions

A modified chandler loop system can complement lymph node-like assays or be used as an excellent stand-alone test to investigate drug/blood interactions during preclinical development, or for individual safety screening prior a first-in-man clinical trial.

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P343

Novel immuno-oncology biologics derived via directed evolution of IgSF domains

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Background

Our variant Ig domain (vIgD™) platform creates novel, therapeutically-applicable protein domains with tailored specificity and affinity. These vIgDs are created through directed evolution of immunoglobulin superfamily (IgSF) proteins and have unique biochemical properties including small size, single domain structure, and the capacity to interact with multiple counter-structures. Because many IgSF family members and their counter-structures are widely expressed on immune cells and tumors, the vIgD platform is well positioned for the development of immuno-oncology biologics with potential first-in-class mechanisms of action. Here, multiple therapeutic formats for vIgDs were developed and characterized.

Methods

Novel vIgDs were created with tailored affinities and modulatory activities against PD-1, TIGIT, PD-L1, CTLA-4, CD28, and/or ICOS. These domains were successfully developed into multiple therapeutic formats, including single and multiple domain Fc fusion proteins and vIgD-monoclonal antibody (V-mAb) fusion proteins. In addition to ligand binding and specificity assays, *in vitro* functional activity was characterized in several T cell-based assays including cell-based reporter systems for pathway agonism or antagonism, primary human mixed lymphocyte reactions (MLRs), and costimulation assays utilizing artificial APCs (assessed by proliferation and IFN γ production).

Results

Several functionally active therapeutic vIgD-based molecules were created successfully. (1) Single-domain vIgD-Fc fusion proteins with tailored binding to CD28, CTLA-4, and PD-L1 demonstrated differential activity in T cell activation assays and, depending on their ligand binding profile, resulted in greater or reduced IFN γ production and T cell proliferation in human T cell activation assays. (2) Multi-domain vIgD-Fc fusion proteins demonstrated promising targeting of immunomodulatory pathways in cell-based reporter assays and MLRs as assessed by IL-2 signaling and IFN γ production. Efficacy was comparable to monoclonal antibodies against the individual vIgD targets. (3) V-mAbs demonstrated target-specific T cell proliferation and IFN γ production *in vitro*, using both recombinant target proteins or target-specific cell lines.

Conclusions

The vIgD platform has successfully generated multiple immuno-oncology therapeutic candidates, in various formats including single- and multiple-domain Fc fusion proteins as well as V-mAbs. These varied formats confer, from a single molecule, multiple advantages including the multi-target modulation capability of evolved IgSFs, and, where applicable, tumor localizing capability of partner molecules or domains. This platform may contribute to the next generation of immunotherapeutic proteins in an oncology setting and efforts are ongoing to develop these candidates for human therapeutic use.

P344

Development and functional characterization of COM902, a novel therapeutic antibody targeting the immune checkpoint TIGIT

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Background

TIGIT is a coinhibitory receptor that is expressed on lymphocytes, including effector and regulatory CD4⁺ T cells (Tregs), effector CD8⁺ T cells, and NK cells, that infiltrate different types of tumors.

Engagement of TIGIT with its reported ligands, poliovirus receptor (PVR) and PVR-like proteins (PVRL2 and PVRL3) directly suppresses lymphocyte activation. PVR is also broadly expressed in tumors, suggesting that the TIGIT-PVR signaling axis may be a dominant immune escape mechanism for cancer. We report here the biophysical and functional characterization of COM902, a therapeutic antibody targeting TIGIT. We demonstrate that co-blockade of TIGIT and a new checkpoint inhibitor, PVRIG, augments T cell responses.

Methods

Human phage display and mouse hybridoma antibody discovery campaigns were conducted to generate therapeutic anti-TIGIT antibodies. The resulting antibodies were evaluated for their ability to bind to recombinant and cell surface-expressed human TIGIT with high affinity. Cross-reactivity of the antibodies to cynomolgus and mouse TIGIT was also examined. A subset of antibodies that bound with high affinity to human TIGIT, and cross-reactive to cynomolgus TIGIT were further characterized for their ability to block the interaction between TIGIT and PVR. Blocking antibodies were screened for their ability to enhance antigen-specific T cell activation *in vitro* either alone, or in combination with an anti-PVRIG antibody, COM701.

Results

We identified a lead antibody, COM902, that binds to human TIGIT with high femtomolar affinity. This antibody bound to TIGIT endogenously expressed on human CD8⁺ T cells with higher affinity than tested benchmark antibodies, and was also cross-reactive to both cynomolgus and mouse TIGIT. When tested for *in vitro* activity, COM902 augmented cytokine secretion and tumor cell killing by CMV-specific CD8⁺ T cells with superior or

equivalent potency to the tested benchmark antibodies. Combination of COM902 with an anti-PD1 antibody or COM701 resulted in enhanced CMV-specific CD8⁺ T cell activity. Furthermore, we demonstrated that TIGIT is predominantly expressed on Tregs and effector CD8⁺ T cells from solid tumors compared to peripheral blood, suggesting that these populations will likely be preferentially targeted by COM902.

Conclusions

We describe the development of a high affinity antagonistic TIGIT antibody, COM902, that is currently in preclinical development. We postulate that the femtomolar affinity of COM902 could result in lower and less frequent dosing in patients. COM902 can enhance human T cell activation either alone or in combination with other checkpoint antibodies. Thus, our data demonstrates the utility of targeting TIGIT, PD1, and PVRIG for the treatment of cancer.

P345

Tumor protective effect of anti-MUC1.IgE in pancreatic cancer

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Background

Pancreatic adenocarcinoma remains a highly lethal disease, that has thus far proven to be highly refractory to immunotherapeutic strategies. Hence, new approaches are needed. There is epidemiological evidence that individuals with allergies have a lower incidence of pancreatic cancer [1]. This suggests that distinct immune surveillance (Th2 based) might underlie protection against pancreatic cancer observed in allergic

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individuals. We hypothesized that therapeutic targeting with IgE to enhance FcεRI signaling would trigger an effective anti-tumor immune response against pancreatic cancer. We posited that tumor antigen directed IgE/FcεRI cross-linking on mast cell and controlled release of histamine near intra-tumor blood vessels could be exploited to improve drug delivery in pancreatic cancer.

Methods

We therefore explored the efficacy of human tumor antigen targeted IgE antibody (humanized anti-MUC1.IgE) in combination of anti-PD-L1 (for relieving T cell exhaustion [2]) and PolyICLC (for effective dendritic cell maturation [2]) in a pre-clinical model of pancreatic cancer using mice transgenic for human MUC1 and FcεRI (hMUC1/hFcεRI).

Results

We observed that a combination of anti-MUC1.IgE+anti-PD-L1+PolyICLC induced antigen specific rejection of two different MUC1 expressing pancreatic tumors cell lines (Panc02.MUC1, KPC.MUC1) and prolonged the overall survival of mice challenged with subcutaneous and orthotopic tumors as compared to mice treated with an isotype control antibody (anti-PSA.IgE). Furthermore, anti-MUC1.IgE+anti-PD-L1+PolyICLC combination induced MUC1 specific memory responses as evidenced by antigen specific rejection/delays of tumors in mice re-challenged with Panc02.MUC1 tumors. Importantly, we observed that NK and CD8 T cells were required for the cell mediated anti-tumor responses, as *in vivo* depletion of these subtypes but not CD4 abrogated the tumor protective in mice bearing orthotopic tumors. Additionally, our study demonstrated a time dependent increase in intra-tumor vascular permeability (increased dextran-647 influx) post anti-MUC1.IgE treatment (*iv*) in subcutaneous tumor (Panc02.MUC1) bearing dTg mice.

Conclusions

Taken together, this is the first study to show that cellular immune responses induced by antigen

specific stimulation of the IgE/FcεRI axis in combination with PolyICLC and anti-PD-L1 provided tumor protective benefits against pancreatic cancer. Our study provides evidence for the clinical applicability and rapid translation of anti-MUC1.IgE based combination therapy against pancreatic tumors.

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P346

Inhibition of MMP9 yields improved effector T cell responses in a PD1-Axis refractory model

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Background

Matrix metalloproteinase 9 (MMP9) acts via diverse mechanisms to promote tumor growth and metastasis, and is a key component of the immune-suppressive myeloid inflammatory milieu. We developed a monoclonal antibody (AB0046) that inhibits murine MMP9 and assessed its mechanism of action in immunocompetent mice as a single agent or in combination with a murine anti-PDL1 antibody.

Methods

An orthotopic, syngeneic tumor model of Her2-driven breast cancer was utilized for both efficacy and pharmacodynamic studies involving RNA and T

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cell receptor (TCR) sequencing, flow cytometry, and protein analysis. Enzymatic analyses were performed on T cell chemoattractant CXCR3 ligands (CXCL9, CXCL10, and CXCL11) which were subsequently evaluated in chemotaxis assays.

Results

Anti-MMP9 treatment alone or in combination with an anti-PDL1 antibody decreased primary tumor growth as compared to IgG control-treated animals (56% vs 335% tumor growth increase, $p=0.0005$) or anti-PDL1 alone. Profiling of tumors by RNA sequencing revealed that inhibition of MMP9 resulted in elevated expression of genes associated with immune cell activation pathways (Hallmark Interferon Gamma Response, FDR $p<0.001$). Treatment with anti-MMP9 and anti-PDL1 antibodies decreased TCR clonality, with evidence of a more diverse TCR repertoire ($p=0.005$). Immunophenotyping of tumor-associated T cells by flow cytometry showed that anti-MMP9 and anti-PDL1 co-treatment promoted a 2.8-fold increase in CD3+ cells in tumors ($p=0.01$), which was associated with an increase in CD4+ T cells (3.2-fold increase; $p=0.006$) and CD8+ T cells (2.8-fold increase; $p=0.013$). In contrast, anti-MMP9 and combination treatment resulted in a decrease in tumor-associated regulatory T cells (CD25+ FoxP3+ cells, $p=0.04$). MMP9 cleavage of T cell chemoattractant ligands *in vitro* rendered them functionally inactive for recruitment of activated primary human effector T cells. Luminex analysis of tumor protein lysates showed elevated levels of key immune cytokines IL-12p70, IL-18, and CXCL10.

Conclusions

Inhibition of MMP9 reduces tumor burden and promotes cytotoxic T cell infiltration in a PD1-axis refractory mouse model. The combination of nivolumab and GS-5745, a humanized anti-MMP9 inhibitory antibody, is being evaluated in gastric cancer (NCT02864381).

Trial Registration

ClinicalTrials.gov: NCT02864381

P347

Anti-PD1 x anti-ICOS bispecific antibody XmAb23104 brings together PD1 blockade and ICOS costimulation to promote human T cell activation and proliferation

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Background

Tumor infiltrating lymphocytes (TILs) often express multiple immune checkpoints and costimulatory receptors. Checkpoint blockade has demonstrated increased clinical response rates relative to other treatment options; however, many patients fail to achieve a response to checkpoint blockade. We sought to identify an additional therapeutic modality to stack with checkpoint blockade that could increase patient response rate. We hypothesized that engagement of T cell costimulatory receptors in concert with checkpoint blockade could further increase T cell activation and proliferation. The combination of checkpoint blockade with costimulation could be accomplished using a bispecific antibody format, with the potential benefits of reduced cost and more selective targeting of TILs to improve safety.

Methods

XmAb23104, which binds to immune checkpoint PD1 and T cell costimulatory receptor ICOS, was assembled in a bispecific antibody platform with substitutions in the Fc domain to suppress effector function. XmAb23104 was evaluated *in vitro* by measuring antibody binding to and cytokine release from Staphylococcal enterotoxin B (SEB) stimulated PBMCs. *In vivo* activity was evaluated using a mouse model in which human PBMCs are engrafted into

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NSG mice (huPBMC-NSG) and the extent of T cell engraftment monitored by flow cytometry.

Results

We produced XmAb23104, a PD1 x ICOS bispecific antibody that binds both PD1 and ICOS monovalently. XmAb23104 selectively targeted PD1⁺ICOS⁺ T cells more than monovalent controls, indicating that a single bispecific molecule was capable of avid co-engagement of both PD1 and ICOS. Surprisingly, despite monovalent engagement of ICOS, XmAb23104 promoted strong T cell activation above that expected of PD1 blockade alone, both *in vitro* and *in vivo*. *In vitro*, XmAb23104 enhanced IL2 production in an SEB stimulation assay (n = 16 donors) relative to control, anti-PD1 alone, and anti-ICOS alone (p < 0.001 for all comparisons), indicating productive combination of PD1 blockade plus ICOS costimulation. Analysis of XmAb23104-treated cells revealed hallmarks of ICOS signaling, including AKT phosphorylation and a multi-gene expression signature consistent with ICOS costimulation. *In vivo*, treatment of huPBMC-NSG mice with XmAb23104 promoted superior T cell engraftment to that found for anti-PD1 treatment alone. In a representative study, XmAb23104 induced a 14-fold increase in human CD45⁺ cells, while anti-PD1 treatment alone only promoted a 2-fold increase.

Conclusions

XmAb23104, a PD1 x ICOS bispecific antibody, brings together PD1 blockade and ICOS costimulation and promotes strong T cell activation *in vitro* and *in vivo*. Compelling activity suggests clinical development is warranted for the treatment of human malignancies.

P348

Dual blockade of PD-L1 and LAG-3 with FS118, a unique bispecific antibody, induces T-cell activation with the potential to drive potent anti-tumour immune responses

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Background

Despite advances with therapies targeting the PD-1/PD-L1 pathway, many patients are refractory or relapse following treatment. LAG-3 expression on exhausted T cells and T-regulatory cells (Tregs) in the tumour may be responsible for this resistance and provides a rationale for co-treatment with antibodies targeting LAG-3 and PD-L1. An alternative approach is the development of a bispecific antibody encompassing binding sites for two antigens. FS118 is a bispecific antibody targeting LAG-3 and PD-L1 that provides dual pathway blockade with the potential to drive unique biology via co-binding of PD-L1 and LAG-3.

Methods

A LAG-3/PD-L1 mAb² bispecific antibody, termed FS118, was engineered by introducing a distinct LAG-3 binding capability into the constant region of a human IgG1 molecule and assembled into a bispecific format with anti-PD-L1 Fabs. Additional mutations introduced into the Fc region suppress effector function. FS118 was evaluated *in vitro* for antigen binding and de-repression of LAG-3 and PD-L1 function in both a DO11.10 T-cell activation system and a super-antigen stimulated peripheral blood mononuclear cells (PBMC) assay. Anti-tumour activity of a murine-specific molecule, mLAG-3/PD-L1 mAb², was assessed *in vivo* in the MC38 mouse tumour model and associated immunophenotypic changes were evaluated using flow cytometry.

Results

FS118 is bivalent for both LAG-3 and PD-L1. It is capable of binding to both targets simultaneously and can de-repress the inhibitory function of human PD-L1 and human LAG-3 in an engineered murine T-cell system. FS118 is a potent activator of immune

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cell function generating sub-nanomolar EC₅₀ values in a human PBMC assay as measured by cytokine with at least equivalent activity to a combination of anti-LAG-3 and anti-PD-L1.

In murine *in vitro* assay systems, mLAG3/PD-L1 mAb² recapitulates the function of FS118 in human systems. MC38 tumour growth studies indicated that mLAG-3/PD-L1 could result in significant anti-tumour activity equivalent to a combination of antibodies targeting LAG-3 and PD-L1. Pharmacodynamic assessment demonstrated changes in the immunophenotype of tumour-infiltrating lymphocytes in the tumour of mLAG3/PD-L1 mAb² treated mice.

Conclusions

Dual blockade of LAG-3 and PD-L1 with a bispecific antibody results in T-cell activation at least comparable to a combination of antibodies targeting LAG-3 and PD-L1 in primary T-cell assays and murine tumour models. These data provide evidence to support the rationale for clinical development of FS118, a LAG-3/PD-L1 mAb², for the treatment of human cancer.

P349

Molecular-targeted radiotherapy with an alkyl-phosphocholine analog leads to immunomodulation in a syngeneic murine melanoma model

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Background

Ionizing radiation can influence the immune response to tumors, and the combination of external beam radiotherapy with immunotherapy has increasingly garnered attention in clinical trials.

Little is known about the immunomodulatory effects of molecular-targeted radiopharmaceuticals. 18-(p-iodophenyl) octadecyl phosphocholine (NM404) is a phospholipid ether analog with selective sequestration in cancer cells [1]. Here, we report on the immunomodulatory properties of the radioactive isostere, ¹³¹I-NM404 in a syngeneic murine melanoma model.

Methods

In vitro cell uptake of NM404 was evaluated by flow cytometry using a fluorescent-labeled NM404 analog. Mice, bearing syngeneic B78 melanoma tumors, were injected with a subtherapeutic dose of 2.2 MBq ¹³¹I-NM404 via lateral tail vein. Mice were euthanized and tumor tissue was harvested at 4 consecutive 1-week intervals post injection; the half-life of iodine-131 is ~1 week. To evaluate markers of immunomodulation over time, quantitative PCR and immunohistochemistry (IHC) were performed on the serially collected tumor tissue samples.

Results

In vitro, murine B78 melanoma cells sequestered 3-4 times more NM404 than normal murine splenocytes, and 6-10 times more than primary human fibroblasts over an 18 hour incubation period. The single, sub-therapeutic dose of ¹³¹I-NM404 did not significantly reduce the growth rate of B78 tumors in the treated mice compared to a control cohort injected with equivalent mass dose of nonradioactive NM404 (excipient). Gene expression analysis revealed a marked modulation of various immune and tumor-associated markers during the course of radiotherapy, such as IL-1 β , IFN γ , CXCL1, IL-18, TGF- β 2, B7-H3, PD-L1 and PD-L2. Our preliminary data suggest time-dependent patterns. IHC demonstrated that the number of tumor-infiltrating CD4 and CD8 T cells did not change significantly over time.

Conclusions

Our preliminary results suggest time-dependent immunomodulation induced by ¹³¹I-NM404 in B78 flank tumors. Further research should address the

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influence of variables such as radioactive dose, choice of radionuclide, tumor type and location on immunomodulation. This information has the potential to provide guidance in the design of more effective therapeutic strategies combining molecular-targeted radiotherapy and immunotherapy for cancer patients.

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P350

Breast tumor suppression mediated by therapeutic expression of chemerin, an innate leukocyte chemoattractant

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Background

The infiltration of immune cells into the tumor microenvironment can regulate growth and survival of neoplastic cells. Several studies have shown a correlation between increases in the number of effector immune cells present in a tumor and clinical outcomes in many human tumors, including breast. Current strategies employing checkpoint inhibitors aim to stimulate effector immune cells, however lack of adequate effector cell numbers within the tumor microenvironment can result in suboptimal responses to these agents. Here, we describe a novel strategy employing therapeutic overexpression of chemerin to recruit immune cells into the tumor microenvironment (TME) and suppress tumor growth.

Methods

Publically available whole genome expression datasets were analyzed for *RARRES2* expression, comparing normal breast tissue to invasive breast cancers. Both the JC and EMT6 tumor lines were used in fully immune competent BALB/c mice. Mammary fat pads were used for inoculation, and chemerin-expressing tumors were compared to control tumors. Tumors were measured using standard caliper measurements, and infiltrating leukocytes measured by flow cytometry. Leukocyte depletion studies were performed using specific depleting antibodies.

Results

Our analyses of both TCGA and other public whole genome expression datasets show that *RARRES2* (the gene for chemerin), a widely expressed endogenous chemoattractant protein for innate immune cells, is downregulated in several studies of human breast cancer. Significant downregulation of *RARRES2* is seen in both invasive ductal and lobular breast cancers, compared to normal breast tissue. In mouse models using both the JC and EMT6 tumor lines, we have found that forced overexpression of chemerin within the TME significantly suppressed tumor growth with increased numbers of infiltrating leukocytes compared to controls. Systemic depletion of NK cells using anti-GM1 antibody treatment resulted in significant abrogation of chemerin's anti-tumor effect, suggesting – at least in part – a reliance on the innate response.

Conclusions

We have shown, for the first time, the use of therapeutic overexpression of chemerin is effective at suppressing breast tumor growth in *in vivo* mouse models. This approach has been used successfully in our melanoma models, and may be a broadly applicable approach to increase the number of immune effector cells within the TME. Ongoing studies are looking at the combination of therapeutic chemerin modulation in combination with available checkpoint inhibitors for determination of additive or synergistic effects.

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P351

A novel, individualized xenograft model of cancer immunotherapy and tumor growth inhibition by ALKS 4230

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Background

Recent successes in tumor immunotherapy highlight the curative potential of modulating patient anti-tumor immune responses. However, preclinical *in vivo* modeling of immune/tumor interactions often depend on a limited number of well-established cell lines. Of available treatment strategies, the use of cytokine therapy offers the advantage of using the patient's own immune cells as anti-tumor effectors. ALKS 4230 is a fusion protein of circularly permuted IL-2 and IL-2 receptor α that is selective for the intermediate-affinity IL-2 receptor expressed on NK cells and subsets of memory and effector T cells. ALKS 4230 is currently in a phase 1 trial to evaluate safety and tolerability in the treatment of patients with refractory solid tumors.

Methods

In order to evaluate the ability of ALKS 4230 to promote and enhance anti-human tumor immune responses preclinically, individual xenograft tumor models were established in NOD-*scid* IL2Rgamma^{null} mice (NSG) using tumor cells derived from metastatic melanoma patients following surgical resection. Upon tumor implantation and palpable growth, mice received an adoptive transfer of autologous, unexpanded PBMC from the same patient and treatment with ALKS 4230.

Results

We found that autologous T cells successfully engrafted NSG recipient mice after ALKS 4230 treatment similar to IL-2 and that both treatments induced cellular expansion over vehicle controls.

Following treatment with ALKS 4230 and adoptive transfer of autologous PBMCs, PDX tumor-bearing mice consistently displayed increased numbers of both CD8 and CD4 T cells migrating into tumor tissue, preferential expansion of non-regulatory T cell subsets, and significant delays in tumor growth as compared to vehicle-treated controls.

Conclusions

Together these data support the rationale for ALKS 4230 as a novel immunotherapeutic for the treatment of melanoma and potentially other solid cancers, as well as the strategy of screening individual, patient-specific xenograft models to assess potential treatment efficacy.

P352

A novel CD73 blocking antibody restores T cell function and augments efficacy of Adenosine 2A Receptor (A2AR) inhibitor CPI-444

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Background

Adenosine accumulates in the tumor microenvironment (TME) through degradation of extracellular ATP by ectonucleotidases CD39 and CD73 and suppresses T cell activity through activation of A2AR. CD73 levels are elevated and are prognostic in certain tumors suggesting CD73 is an important immune-suppressive mechanism. Multiple CD73 antibodies are in clinical development that are allosteric CD73 inhibitors that stimulate internalization (type 2 mechanism), leading to incomplete inhibition of cell surface CD73 activity. We describe CPI-006, a novel CD73 antibody that directly inhibits CD73 enzymatic activity (type 1 mechanism).

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Methods

CD73 activity was assayed by Malachite Green. Internalization was measured by flow cytometry. T cell proliferation was assayed by flow cytometry and cytokine levels by ELISA. Tumor CD73 was assessed by immunohistochemistry. Gene expression was determined by Nanostring.

Results

CPI-006 (nM affinity) was compared to CPX-016 (pM affinity), a potent type 2 CD73 antibody. CPI-006 completely inhibited CD73 catalytic activity across a broad range of CD73 expression. In contrast, CPX-016 incompletely inhibited CD73 activity (~60%) and was not effective towards cell lines with high CD73 expression.

CPI-006 was significantly more effective than CPX-016 at blocking suppression of T cell proliferation and cytokine secretion by adenosine (80% restoration compared to 45%). CPI-006 was effective across a broad range of CD73 expression while CPX-016 efficacy was restricted to samples with lower CD73 levels.

CD73 expression was evaluated by IHC in renal cell, melanoma, non-small cell lung and breast cancers (N=70 each). CD73 expression was heterogeneous in the tumor and stromal compartments; however, when expressed, CD73 levels were high, exceeding expression in the high expressing CD73 cell lines that were incompletely inhibited by CPX-016. These data suggest that type 2 antibodies will have limited efficacy in CD73 high expressing tumors.

Tumors with high CD73 expression are more responsive to A2AR inhibition [1] therefore we investigated the combined effect of CPI-006 with A2AR inhibitor, CPI-444. The combination significantly restored T cell proliferation and interferon-gamma production and suppressed adenosine responsive gene expression to levels greater than either agent alone.

Conclusions

CPI-006 is a novel CD73 blocking antibody that directly and completely inhibits CD73 activity, in contrast to type 2 antibodies in development. CPI-006 blocks CD73 activity and improves T cell function independent of CD73 expression levels and augments the effect of CPI-444.

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P353

Discovery and pre-clinical development of an orally available small molecule antagonist targeting the CD47/SIRP α pathway for cancer immunotherapy

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Background

The CD47/signal regulatory protein alpha (SIRP α) axis is a critical regulator of myeloid cell activation and serves as an immune checkpoint for macrophage mediated phagocytosis. Because of its frequent upregulation in several cancers, CD47 contributes to immune evasion and cancer progression. Most of the current approaches in the immunotherapy focus on T-cell axis. Macrophages and other myeloid immune cells offer much promise as effectors of cancer immunotherapy, hence efforts to modulate them for therapeutic benefit are gaining momentum. In this regard disruption of CD47-SIRP α interaction is now being evaluated as a

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therapeutic strategy for cancer to stimulating macrophage mediated anti-tumor immune response. In view of the requirement for the intravenous dosing for all reported CD47 targeting agents, we sought to discover and develop an orally available small molecule CD47 antagonist. An oral CD47 agent potentially offers the convenience, flexibility to adjust the dose and schedule to address any emergent adverse events and ease of combination therapy.

Methods

Series of peptide disrupting CD47-SIRP α interactions were identified by rational design strategy based on CD47/SIRP α interacting interface. An FITC probe based cellular binding assay was established to identify high peptide fragments. The elements of this pharmacophore were incorporated into non-peptidic small molecule scaffolds resulting in lead compounds disrupting CD47/SIRP α interaction. The shortlisted compounds were further screened in a phagocytosis assay. In vivo efficacy was evaluated in A20 B Cell lymphoma syngeneic tumor model.

Results

Initial hits identified in FITC probe based cellular binding assay was further optimised for their activity in binding assay. Lead CD47 antagonists induced phagocytotic activity of human macrophages to a similar extent as commercially available anti-CD47 antibodies. Further optimization of these leads resulted in compounds with desirable physico-chemical properties and good oral bioavailability. An advanced lead CD47 antagonist inhibited primary tumor growth (~90%TGI) in a mouse syngeneic model of B-cell lymphoma upon twice a day oral dosing. Biomarker characterization and efficacy studies in additional tumor models are ongoing.

Conclusions

Rational design based on CD47/SIRP α interacting interface led to the identification of a novel and selective CD-47 antagonist with potent activity in cellular binding assay and phagocytosis assay. The lead compound exhibited desirable metabolic

stability, solubility and oral bioavailability. In in-vivo studies, lead compound demonstrated significant anti-tumor efficacy in A20 B-cell lymphoma tumor model upon oral dosing. The above findings support further development of these orally bioavailable agents for use in the clinic.

P354

An orally bioavailable small molecule antagonist of VISTA and VSIG8 signaling pathways shows potent anti-tumor activity

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Background

Antibody-mediated blockade of immune checkpoint pathways have transformed the outlook for cancer therapy. While PD-1/PD-L1 antibodies primarily focus on T-cells to achieve anti-tumor efficacy, other cells in the tumor microenvironment such as myeloid cells including MDSCs also play a role in immune evasion. To overcome the immune resistance induced by MDSCs, V-domain Ig suppressor of T-cell activation (VISTA) expressed predominantly on myeloid cells and tumor-infiltrating lymphocytes is considered as an ideal target. Recent findings also support the role of VISTA pathway in clearance of apoptotic bodies and prevention of autoimmunity. VISTA is reported to mediate immune suppression through homophilic interaction as well as interaction with V-Set and immunoglobulin domain containing 8 (VSIG8). We sought to discover and develop an orally available small molecule VISTA antagonist targeting both VISTA and VSIG8 pathways. Unlike antibodies an oral agent potentially offers the convenience, flexibility to adjust dose and schedule to address

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any emergent adverse events and ease of combination therapy.

Methods

VISTA belongs to B7 family and shares sequence homology with the B7 family ligands PD-L1, a focused library of compounds mimicking the interaction of checkpoint proteins of B7 family was designed and synthesized. These compounds were tested in VISTA and VSIG8 specific functional assays. In-vivo efficacy was evaluated in mouse syngeneic B16F10 melanoma and CT26 colon carcinoma models. Impact of test agents on various immune populations was measured by flow cytometric analysis in tumor-bearing animals upon repeated dosing.

Results

Screening and analysis of the focused library of compounds led to the identification of hits capable of functional disruption of the checkpoint protein(s) signaling. Further optimization resulted in lead compounds targeting both VISTA and VSIG8 signaling pathways with desirable drug-like properties. Potent functional activity comparable to that obtained with an anti-VISTA or anti-VSIG8 antibody in rescuing effector functions was observed with the lead compound along with selectivity against other immune checkpoint proteins. An advanced lead compound exhibited sustained immune PD in tumor-bearing animals including desirable impact on myeloid and T-cells in both circulation and tumor. The advanced lead compound also exhibited significant efficacy in syngeneic pre-clinical tumor models of melanoma and colon cancers upon once a day oral dosing with excellent tolerability.

Conclusions

Our efforts have resulted in the identification of novel oral antagonists of VISTA and VSIG8 signaling. Desirable drug-like properties and anti-tumor efficacy at well-tolerated doses by the advanced lead compound in pre-clinical models supports its further development towards advancing to the clinic.

P355  Abstract Travel Award Recipient

Epigenetic modulation promotes tumor suppression and improves survival when combined with checkpoint inhibition in murine models of breast cancer

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Background

Checkpoint inhibition is a very successful treatment strategy in cancers that are naturally immunogenic by attracting T cells into the tumor microenvironment (TME) and promoting cytotoxic signaling pathways. Most breast cancers are not highly immunogenic likely due to an immunosuppressive microenvironment. One strategy to transform this TME is to use epigenetic modulation to affect activation and trafficking of myeloid derived suppressor cells (MDSCs), known to alter the immunogenicity of the TME and sensitize tumors to checkpoint modulation. We hypothesize that combinatorial therapy primes the TME by altering infiltration and function of MDSCs leading to a more robust T cell response.

Methods

We use the HER-2/neu transgenic mouse model with challenge of syngeneic cell lines. This model enables us to study the efficacy of different combinations of an anti-HER2 antibody (a-Her2), an epigenetic agent, the histone deacetylase inhibitor entinostat, and checkpoint inhibitors, anti-programmed cell death protein (PD-1) and anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies, on tumor growth and to help identify co-stimulatory and inhibitory factors regulating T cell and MDSC responses. Characterization of tumor infiltrating lymphocytes (TIL) and their functional capabilities are being investigated in primary tumors using fluorescence-activated cell sorting, nanostring

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gene expression profiling, and immunohistochemistry.

Results

We found significant improvement in survival and delay in tumor growth in tumor bearing mice-treated with entinostat, anti-PD1, and anti-CTLA-4 and with a-Her2, entinostat and anti-PD1 or anti-CTLA4. We show that addition of entinostat to checkpoint inhibition leads to significantly increased infiltration of granulocytic-MDSCs and CD8⁺ T effector cells into the TME. Flow cytometric evaluation suggests increased T cell activation, exhaustion, and altered MDSC function. Functional assays of isolated MDSCs from mice treated with combination therapy demonstrates reduced suppressive ability of these cells. Gene expression profiling of isolated MDSCs and TIL is underway to help determine significant changes in immune related pathways that have lead to our observed outcomes.

Conclusions

Addition of entinostat and checkpoint inhibition to a-Her2 therapy significantly increases infiltration of innate and adaptive immune cells into the highly tolerant breast tumors and leads to improved survival and decreased tumor burden. Results suggest that this combinatorial treatment alters the function of the infiltrating cell types that lead to the observed phenotype. We aim to delineate genetic alterations responsible for these observations. It is our hope that our novel findings will provide further rationale for combination therapy and improve the response rate to immunotherapy in patients with breast cancer.

P356

Efficacy and Mechanism of Action of CXCR4 Inhibition in B16-OVA Melanoma Model

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Background

In our previous studies, we demonstrated that combination of a CXCR4 antagonist with an anti-angiogenesis agent axitinib relieved myeloid-derived suppressor cells (MDSCs) mediated immunosuppression and suppressed HIF-2 α expression, which resulted in synergistic antitumor effects in 786-0 and A498 RCC xenografts. To further study the CXCR4 mechanism of action in an immune-proficient background, we investigated the activity of a CXCR4 antagonist in a syngeneic mouse tumor model. As the current murine models for RCC do not share the same genetic alterations with the human disease, the B16-OVA model was selected for this study. The CXCR4 antagonist was tested in combination with axitinib or with checkpoint inhibitors which are part of the SOC in managing melanoma in clinic.

Methods

B16-OVA cells were implanted into C57BL/6 mice. Seven-days post implantation, mice were treated with axitinib, X4-136 (CXCR4 inhibitor) or anti-PD-L1/anti-CTLA4, or the combinations for 16 days. At sacrifice, tumors were excised and flash frozen in liquid nitrogen for Western Blot analysis or treated with collagenase for the analysis of subsets of T-cells by FACS.

Results

Axitinib alone modestly inhibited growth of B16-OVA tumors. X4-136, the CXCR4 inhibitor, alone demonstrated more robust activity than the combination treatment of anti-PD-L1 and anti-CTLA4. The anti-tumor activity of the anti-PD-L1 and anti-CTLA4 regimen was further enhanced in combination with X4-136.

Preliminary analysis of infiltrating immune cells by flow cytometry showed that axitinib treatment led to an increase in immunosuppressive Treg cell population in tumors, while treatment with X4-136 alone or the anti-PD-L1/anti-CTLA4 combination led to a decrease in Tregs. The decrease was more

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pronounced when X4-136 was used in combination with axitinib. Additional analysis of other immune cell subsets will also be presented.

This combination also led to a decrease in the expression of HIF-2 α and cyclin D1 in comparison to vehicle treated mice as demonstrated by western blot analysis of tumors, suggesting an anti-proliferative effect. PARP cleavage was apparent when X4-136 was used in combination with axitinib indicating the role of apoptosis in anti-tumor effect. Additional analysis of tumors after treatment in earlier time points will also be presented.

Conclusions

X4-136 alone exhibited potent anti-tumor activity in the B16-OVA murine melanoma model. Added benefit was observed when X4-136 was added to either axitinib or to anti-PD-L1/anti-CTLA-4 treatments. Treatment benefits were associated with the reduction of Treg population in the tumor microenvironment. Induction of apoptosis was observed in combination treatment of axitinib and X4-136.

P357

The Tt-cell growth factor cocktail IL-2/IL-15/IL-21 enhances expansion and effector function of tumor-infiltrating T cells in a novel process developed by iovance

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Background

Adoptive T cell therapy with autologous tumor infiltrating lymphocytes (TIL) has demonstrated clinical efficacy in patients with metastatic melanoma and cervical carcinoma. In some studies, clinical outcomes have positively correlated with the total number of cells infused and/or percentage of CD8+ T cells. Most current production regimens solely utilize IL-2 to promote TIL growth, although

enhanced lymphocyte expansion has been reported using IL-15 and IL-21-containing regimens. This study describes the positive effects of adding IL-15 and IL-21 to the standard IL-2-alone TIL generation protocol.

Methods

The process for generating TIL includes a pre-Rapid Expansion Protocol (pre-REP), in which TIL emigrate out of tumor fragments of 1-3 mm³ in media containing IL-2. To further stimulate TIL growth, TIL are expanded using a secondary culture period termed the Rapid Expansion Protocol (REP) that includes irradiated PBMC feeders, IL-2 and anti-CD3. In this study, a shortened pre-REP and REP expansion protocol was developed to expand TIL while maintaining the phenotypic and functional attributes of the final TIL product. This shortened TIL-generation protocol was used to assess the impact of IL-2 alone versus a combination of IL-2/IL-15/IL-21. These two culture regimens were compared for the generation of TIL grown from colorectal, melanoma, cervical, triple negative breast, lung and renal tumors. At the completion of the pre-REP, cultured TIL were assessed for expansion, phenotype, function (CD107a+ expression and IFN γ release) and TCR V β repertoire.

Results

Enhanced TIL expansion (>20%), in both CD4+ and CD8+ cells in the IL-2/IL-15/IL-21 culture cocktail was observed across multiple tumor histologies. Preliminary analysis demonstrated a shift towards a predominantly CD8+ TIL population with a skewed TCR V β repertoire in TIL cultured with the IL-2/IL-15/IL-21, versus IL-2 alone. IFN γ release and CD107a expression were also elevated in TIL cultured in the presence of IL-2/IL-15/IL-21 versus those cultured using IL-2 alone.

Conclusions

Rapidly expanding TIL *ex vivo* for adoptive cell therapy is essential in treating patients with cancer. We report an increased TIL-product yield, in addition to potentially beneficial phenotypic and functional differences, when TIL are cultured with

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IL-2/IL15/IL-21 as compared to IL-2 alone. We suggest a more robust process encompassing the use of IL-2/IL-15/IL-21 in TIL culture may provide a means to promote TIL expansion particularly in tumors with poor T cell infiltration.

P358

Development of CDX-1140, an agonist CD40 antibody for cancer immunotherapy

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Background

Current limitations for immunotherapy approaches include poorly functioning events early in the immune response cycle, such as efficient antigen presentation and T cell priming. CD40 signaling in dendritic cells (DCs) leads to upregulation of cell surface costimulatory and MHC molecules and the generation of cytokines, which promotes effective priming of CD8+ effector T cells while minimizing T cell anergy and the generation of regulatory T cells. This naturally occurs through interaction with CD40 ligand (CD40L) expressed on CD4+ T-helper cells. CD40 signaling can also be achieved using specific monoclonal antibodies (mAbs), leading to the development of several agonist CD40 antibodies that have initiated clinical development.

Methods

Our approach for the development of a CD40 agonist antibody was to define a balanced profile between sufficiently strong immune stimulation and the untoward effects of systemic immune activation.

Results

CDX-1140 is a human IgG2 antibody derived from human Ig transgenic mice, that activates DCs as demonstrated by upregulation of costimulatory molecules and increased production of cytokines. This activity is Fc independent as it is maintained using an F(ab')₂ fragment of the antibody. CDX-1140 does not block the binding of CD40L and the addition of soluble CD40L greatly enhances DC activation by CDX-1140, suggesting that CDX-1140 may act synergistically with naturally expressed CD40L. CDX-1140 also activates B cells, and has direct anti-lymphoma activity in xenograft models. However, the antibody does not promote cytokine production in whole blood assays. Importantly, CDX-1140 has shown good pharmacodynamic and safety profiles in a cynomolgus macaque study investigating doses from 0.01 to 10 mg/kg. This study showed a clear dose response with respect to changes in hematologic and circulating cytokine values (IL-12p40) that were expected to result from CD40 activation.

Conclusions

These data support the potential of CDX-1140 as part of a cancer therapy regimen, and the phase 1 dose-escalation clinical study is anticipated to begin this year.

P359

Effect of targeted anti-GD2/-CD16 Bispecific NK cell Engager (BiKE) with or without IL-15 super agonist ALT803 against GD2+ Neuroblastoma (NB) and Ewing Sarcoma (ES)

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Background

GD2 is a surface disialoganglioside that is a well-characterized immunotherapeutic target in NB and Sarcomas [1]. The efficacy of anti-GD2 antibodies depends on engaging functional NK cells to kill GD2-positive targets through antibody dependent cellular cytotoxicity (ADCC). However, NK cell number and function are decreased in most cancer patients at diagnosis, are further reduced by radiation chemotherapy, antigen loss and immunosuppressive tumor microenvironment (TME) which contribute to treatment failure. One of the new approaches to overcome TME resistance and improve NK cell mediated ADCC against tumor cells is using a BiKE [2]. ALT-803 is a superagonist of an IL-15 variant bound to an IL-15RaSu-Fc fusion with enhanced IL-15 biological activity.

To investigate the in-vitro activity of hu-anti-GD2/-CD16 BiKE with or without ALT803 against GD2 expressing NB/ES

Methods

Anti-GD2/-CD16 BiKE was constructed in pBudCE4.1 mammalian expression vector and transfected in to HEK293-EBNA Cells. Stable clone were selected by Zeocin for the secretion and purification of anti-GD2/-CD16 plasmid by ProBond™ Ni column and validated by Western blot. Cytotoxicity was examined against NB/ES cells with or without ALT803 (generously supplied by Altor Biosciences) with K562-mbIL21-41BBL expanded NK cells by DELFIA cytotoxicity assay at 10:1 E:T ratio.

Results

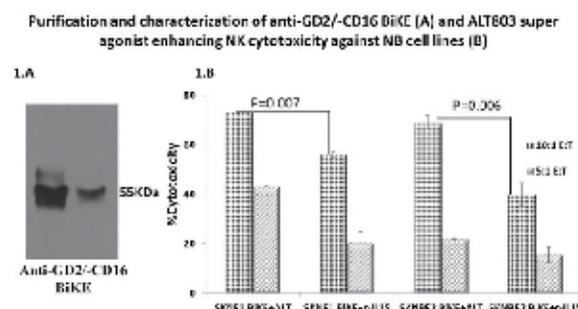
GD2 expressing NB (54.4± 10.61%) and ES (71.93±8.33%) cells were used for functional assays. Anti-GD2/-CD16 BiKE was purified from the transfected culture supernatant and validated by western blot (Figure 1A). BiKE+NK compared to Medium+NK significantly increased NK mediated cytotoxicity against NB: SKNF1 (64.5±5.9% vs. 20.1±1.3%, p=0.002), SKNBE2 (67.4±4.02% vs. 15.1±0.9%, p=0.004), SHS5Y5 (68.9±0.9% vs. 30.2±0.85%, p=0.005) and ES: EWS502 (68.05±3.06% vs. 28.1±4.05%, p=0.004), A673

(66.02±4.05% vs. 20.3±0.8%, p=0.005) respectively. Further, BiKE+NK+ALT803 compared to BiKE+NK+rhuIL15 improved NK mediated cytotoxicity against NB: SKNF1 (73.9±9.3% vs. 56.5±8.5%, p=0.007) and SKNBE2 (63.9±0.6% vs. 40.5±0.49%, p=0.002) respectively, against NB/ES cell lines (Figure 1B).

Conclusions

Our preliminary results demonstrated that the BiKE with ALT803 significantly enhanced NK cytotoxicity against NB and ES. Future studies will investigate the efficacy of this BiKE with ALT803 against GD2 expressing solid tumor in humanized NSG xenografted mouse model.

Figure 1.



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P360

APX005M is a potent CD40 agonistic antibody capable of stimulating both innate and adaptive immune responses against cancer

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Background

CD40 plays an important role in activation and regulation of both innate and adaptive immunity. Using the proprietary APXiMAB™ discovery platform, Apexigen discovered APX005M, a potent immune stimulatory CD40 agonistic antibody currently being developed for cancer immunotherapy. APX005M is a high-affinity humanized IgG1 antibody targeting human CD40. APX005M was engineered to carry an S267E mutation in its Fc region to enhance CD40 agonistic activity via cross-linking to the FcγRIIb.

Methods

PBMCs were obtained from healthy human subjects. Isolated cell subsets were cultured in various conditions with APX005M. The potential synergistic effect of APX005M with immune checkpoint inhibitors (ICI) was assessed by in vitro culture of human dendritic cells (DC) and allogeneic T cells with APX005M and anti-PD-1 or anti-PD-L1 antibodies. The duration of APC activation following exposure to APX005M was determined by culturing PBMCs with APX005M for 24 hours. Cell activation was measured at various times post washout.

Results

APX005M binds with high-affinity to human CD40 (Kd=0.12 nM) and recognizes a unique epitope that overlaps with the CD40 ligand (CD40L) binding domain thus mimicking CD40L-induced activation. APX005M is capable of activating human B cells (EC50=12 pM) and DC (EC50=0.49 nM) and also promotes proliferative responses of tumor-infiltrating T cells. APX005M's CD40 agonistic activity depends on cross-linking of Fc-gamma receptors. Receptor occupancy studies revealed that 10% of CD40 receptor occupancy is sufficient to produce maximum APC and T cell activation. Short-term exposure (24 hours) of PBMC to APX005M induced long-lasting activation of B cells, monocytes

and T cells that remained activated 2 weeks after removal of APX005M. In vitro co-culture of human DC and allogeneic T cells showed that APX005M induces a dose-dependent increase of CD4 and CD8 T cell proliferation and IFN-γ secretion, and the T-cell responses were further enhanced by anti-PD-1 or anti-PD-L1 antibodies suggesting that APX005M combined with ICI can synergistically stimulate T-cell responses.

Conclusions

The immune stimulatory activity of APX005M results from its unique epitope and is dependent on cross-linking by Fc-gamma receptors. The properties of APX005M make it an optimal CD40 agonist for stimulating anti-tumor immune responses while maintaining a good safety profile. Combination of APX005M with ICI enhances T-cell responses providing a rationale for the ongoing clinical studies combining APX005M with anti-PD-1 antibodies in NSCLC and melanoma patients (trials NCT03123783 and NCT02706353).

P361

A novel T-cell engaging bispecific antibody platform: Efficient *In vivo* tumor clearance with minimal cytokine release

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Background

Using transgenic rats and a unique sequence-based discovery approach, we have created a large collection of fully human anti-CD3 antibodies with diverse T-cell agonist activities. We have used these anti-CD3 antibodies to create a unique multi-

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specific platform for T-cell redirected tumor cell killing.

Methods

Our novel discovery platform combines antibody repertoire deep sequencing, high-throughput gene assembly, and recombinant expression and generates a much larger diversity of antibodies than traditional approaches. The CD3 antibodies identified by our platform show diverse *in vitro* T-cell activation profiles measured by CD69 upregulation, IL2, and IFN γ production. Using our discovery platform, we have also generated human domain antibodies targeting tumor antigens that may be combined with our unique CD3 antibodies to create multi-specific antibodies.

Results

As one example, we have created a CD3xBCMA bispecific antibody (TNB-383B) for the treatment of multiple myeloma. TNB-383B kills multiple myeloma cells *in vitro* and *in vivo* in a BCMA-dependent manner, and kills primary patient myeloma cells *ex vivo*. The EC₅₀ for cytotoxicity was in the single-digit nanomolar range for TNB-383B against MM cell lines *in vitro*. TNB-383B showed much reduced (ie IFN- γ) or absent (ie IL-2) cytokine release compared to other anti-CD3 antibodies. *In vitro* results were consistent across 10 healthy huPBMC donors. *Ex vivo*, TNB-383B efficiently lysed primary MM cells in the presence and absence of supplementary T-cells. *In vivo*, TNB-383B mediated clearance of MM tumors from NSG mice at doses as low as 10ng of bispecific antibody.

Conclusions

In summary, we have created a T-cell engaging bispecific antibody platform with tunable T-cell agonism that can be used to optimize the therapeutic index for a variety of tumor antigens.

P362

A novel assay using RNA aptamers to quantitate the fraction of IL2Ra (CD25) receptors occupied by IL2

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Background

RNA aptamers bind to antigens in a manner analogous to antibodies and their binding can be quantitated using simple RT-PCR. Using IL2-IL2Ra as a model, we report a novel assay using IL2-IL2Ra-binding RNA aptamers that allows for quantitation of receptor occupancy by its ligand.

Methods

A modification of whole cell Systematic Evolution of Ligands by EXponential enrichment (SELEX) was used to select T regulatory (Treg) cell-specific RNA aptamers. Aptamers specific for common T cell antigens were precleared using normal donor CD4⁺IL2Ra⁻T cells, followed by enrichment for aptamers specific for CD4⁺IL2Ra⁺ Tregs obtained from the same donor. The process was repeated for eight rounds with each round using T cells from a different normal donor. High-throughput sequencing and bioinformatics analysis was used to select top Treg-binding aptamers. Binding of aptamers to unoccupied IL2Ra and IL2Ra occupied by IL2 was determined by RT-qPCR. Some aptamers bound preferentially to unoccupied IL2Ra while others bound preferentially to IL2Ra occupied by IL2. To determine the fraction of IL2Ra occupied by IL2, aptamers that bound preferentially to unoccupied receptor or to IL2-occupied receptor were mixed in equimolar quantities. Aptamer mix was added to IL2Ra-coated Dynabeads that were pre-incubated with various concentrations of IL2 to create various IL2-occupied receptor fractions. Aptamer binding was quantified by RT-qPCR using a set of primers specific for both aptamers.

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Fluorescent probes specific for the variable region for each aptamer were used to quantify each of the aptamers.

Results

Five of the top 12 Treg-binding aptamers recognized IL2Ra. Some IL2Ra-specific aptamers (e.g. Tr-8) bound preferentially to unoccupied IL2Ra and others (e.g. Tr-7) to the IL2-IL2Ra complex (Figure 1). Standard curves were developed which allowed for determination of the fraction of IL2Ra occupied by IL2 (Figure 2).

Conclusions

A Treg cell-based SELEX was used to select Treg-binding aptamers specific for IL2Ra. Aptamer pairs showing differential binding preferences towards unoccupied IL2Ra versus the IL2-IL2Ra complex were identified. Because the selected aptamer pairs share the same primers, RT-qPCR amplification using a single set of primers allowed for quantitation of the ratio of bound aptamers. The resulting ratio reflects the occupancy of the IL2Ra receptor by its ligand IL2. This technique could be valuable for studying the role of IL2-IL2Ra ligand-receptor complex in anti-tumor immunity. Importantly, modifications of this assay could be used to quantitate other receptor-ligand complexes relevant in the field of cancer immunotherapy.

Figure 1.

Figure 1: Aptamers specific for IL2Ra were tested for their binding to IL2Ra-occupied by IL2. Binding of each aptamer to IL2Ra was compared to binding to IL2-IL2Ra complex using Dynabeads-coated with IL2Ra or IL2-IL2Ra complex.

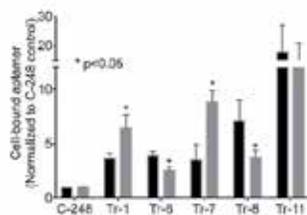
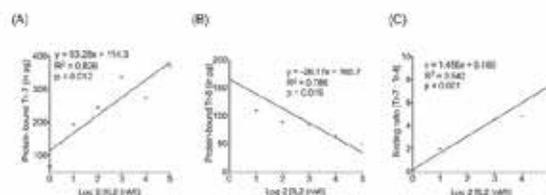


Figure 2.

Figure 2: To determine the fraction of IL2Ra receptors occupied by its ligand IL2, aptamers binding preferentially to unoccupied IL2Ra (Tr-8) or IL2-occupied IL2Ra (Tr-7) were mixed in equimolar quantities and added to IL2Ra-coated Dynabeads pre-incubated with various concentrations of IL2. Aptamer binding was quantified by RT-qPCR assay using primers binding to both aptamers and fluorescent probes specific to the variable region in each aptamer. (A) Tr-7 binding showed a positive correlation with increasing concentrations of IL2 added, while (B) Tr-8 binding showed an inverse correlation with the concentrations of IL2 added. (C) The binding ratios of Tr-7 and Tr-8 showed significant linear correlation to the amount of IL2 added reflecting the fraction of IL2Ra occupied by IL2.



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The OX40-CTLA-4 bispecific antibody, ATOR-1015, induces immune activation and anti-tumor effect

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Background

ATOR-1015 is an OX40-CTLA-4 bispecific immune activating antibody developed for tumor-directed immunotherapy. The compound was generated by fusing a high affinity CTLA-4 binder, derived by FIND[®] optimization of CD86, to an agonistic OX40 antibody derived from the human antibody library ALLIGATOR-GOLD[®].

ATOR-1015 binds both targets simultaneously resulting in cell-cell interactions expected to enhance the immuno-stimulating effect of the compound. The mode of action of ATOR-1015 is thought to be a combination of effector T cell activation and regulatory T cell (Treg) depletion.

Methods

Human primary cells used in *in vitro* assays were isolated from leukocyte concentrates from healthy

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donors. The *in vitro* effects were measured by using either standard cytokine release assays or Promega reporter assays.

Human OX40 transgenic (knock-in) mice were generated by genOway SA and female mice were used for the anti-tumor effect studies using syngeneic mouse models. Tumors and spleens from treated mice were analyzed for Treg and T cell populations by flow cytometry.

Results

ATOR-1015 dependent T cell activation and Treg depletion is supported by cell-based *in vitro* studies and *in vivo* syngeneic tumor models.

The ability to induce ADCC of human Treg was investigated using a FcγR expressing reporter assay demonstrating superior effect of ATOR-1015 compared to the monospecific antibodies. Further, ATOR-1015 has been shown to induce activation of T cells in the presence of CTLA-4 expressing cells.

Treatment with ATOR-1015 reduces tumor growth and prolongs survival in syngeneic tumor models *in vivo* using human OX40 transgenic mice. Further, ATOR-1015 treatment demonstrates an increase in the intratumoral CD8⁺ T cell/Treg ratio which is superior compared to the monospecific counterparts, without affecting systemic T cells.

Conclusions

ATOR-1015 suppresses/depletes Tregs and activates CD8⁺ T cells *in vivo* and is currently in the second phase of production, and process development. ATOR-1015 is planned to enter clinical trials in 2018.

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Discovery and pharmacological characterization of JNJ-63723283, an anti-programmed cell death protein-1 (PD-1) antibody that blocks PD-1 function

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Background

Programmed cell death protein-1 (PD-1), a negative immune checkpoint receptor, suppresses T cell function when bound to its ligands PD-L1 and PD-L2. Tumor cells exploit this pathway to evade immune surveillance; therefore, inhibiting the PD-1 pathway can restore T cell function, stimulating their anti-tumor response. Here we report the characterization of JNJ-63723283 (JNJ-283) to support new combination approaches in the future.

Methods

JNJ-283 was generated by phage panning against human and cynomolgus monkey (cyno) PD-1 extracellular domain followed by affinity maturation. *In vitro* activity was evaluated using cytomegalovirus (CMV) recall, mixed lymphocyte reaction (MLR), and Jurkat-PD-1 nuclear factor of activated T cells (NFAT) reporter assays. *In vivo* activity was assessed using human PD-1 knock-in mice implanted with MC38 tumors and a lung patient-derived xenograft (PDX) model (LG1306) using CD34⁺ cord blood humanized NSG mice. Pharmacodynamic, toxicokinetic, and safety assessments were performed in cyno following single (0.1, 1, 10 mg/kg) and/or repeat (10, 30, 100 mg/kg/wk up to 5 weeks) intravenous (IV) dosing.

Results

JNJ-283 displayed high affinity for human (1.72 ± 0.99 nM) and cyno (0.90 ± 0.08 nM) PD-1, blocked PD-1 binding to PD-L1 (IC₅₀=111.7 ± 22.0 ng/mL) and PD-L2 (IC₅₀=138.6 ± 12.4 ng/mL), and cross-

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competed with nivolumab and pembrolizumab analogs.

JNJ-283 dose-dependently increased T cell-mediated cytokine production in CMV recall and MLR assays and enhanced NFAT activity in a Jurkat-PD-1 reporter assay, indicating functional disruption of the PD-1/PD-L1 interaction. JNJ-283 *in vitro* activity was comparable to that of competitor molecule analogs.

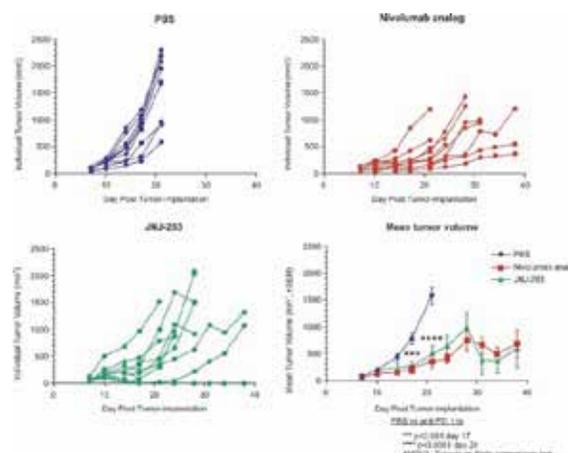
Following intraperitoneal injection with 10 mg/kg JNJ-283 or nivolumab analog (2x weekly; day 7 after tumor implantation), mean MC38 tumor volume in PD-1 knock-in mice was significantly lower at days 17 ($p < 0.001$) and 21 ($p < 0.0001$) compared to control (Figure 1). In the lung PDX model, 10 mg/kg JNJ-283 or pembrolizumab (q5d x 6) reduced ($p < 0.01$) mean tumor volume compared to control.

JNJ-283 was tolerated in cyno following single and/or repeat dosing; mean drug exposures increased in a dose dependent manner. Primary findings attributed to JNJ-283 included proliferation of T-lymphocyte subsets, stimulation-related cytokine expression (*in vitro*), increased IgM and IgG titer secondary to antigen challenge, and decreased cellularity (lymphocytes) in the thymus.

Conclusions

JNJ-283, a high affinity anti-PD-1 antibody, was well-tolerated in toxicology studies and demonstrated robust activity *in vitro* as well as displayed anti-tumor efficacy *in vivo* comparable to that observed with other agents targeting PD-1. These data support the ongoing clinical study of JNJ-283 and combination potential with other modalities.

Figure 1. In vivo anti-tumor activity of JNJ-283 in human PD-1 knock-in mice implanted with MC38 tumors



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Development of JNJ-64158120, an anti-TIM-3 antibody to overcome innate and acquired mechanisms of resistance to PD-1 therapy

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Background

Programmed cell death protein-1 (PD-1) pathway blockade has revolutionized the treatment of a variety of malignancies but has only demonstrated clinical benefit in a subset of patients. Emerging

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preclinical and clinical data have proposed T cell immunoglobulin and mucin domain-3 (TIM-3) as an inhibitory mechanism that is co-regulated with PD-1 to restrict T cell-mediated immune responses. Dynamic upregulation of TIM-3 has been observed in patients in response to anti-PD-1/PD-L1 therapy, implicating TIM-3 as a potential mechanism of immune resistance. These observations suggest that dual blockade of the TIM-3 and PD-1 pathways can broaden the effectiveness of anti-PD-1/PD-L1 therapy as a new immunotherapy for PD-1 responsive malignancies. Here we describe the characterization and functional activity of JNJ-64158120 (JNJ-120), an antibody targeting human TIM-3.

Methods

JNJ-120 *in vitro* activity was assessed by CMV, MART-1, and NK cell activation assays; *in vivo* activity was evaluated using a NY-ESO TCR transgenic model of T cell exhaustion and a human TIM-3 knock-in mouse model bearing MB49 murine bladder carcinoma tumors.

Results

JNJ-120 is a fully human, phage-derived, IgG2 σ monoclonal antibody that binds to human ($K_d=2.5$ nM) and cynomolgus monkey ($K_d=0.75$ nM) TIM-3. JNJ-120 inhibited the binding of TIM-3 to two putative TIM-3 ligands, phosphatidyl serine and galectin-9 and enhanced T cell function in primary antigen-specific T cell assays (CMV and MART-1). In CMV stimulated PBMCs, JNJ-120 triggered expression of IFN- γ and TNF- α and enhanced CD137 expression on CD8 $^+$ and CD4 $^+$ T cells. Furthermore, in MART-1 assays, JNJ-120 enhanced IFN- γ , TNF- α , IL-2, and IL-8 production by CD8 $^+$ T cells. JNJ-120 also enhanced T cell function in combination with anti-PD-1 antibodies in peripheral blood samples derived from melanoma patients. JNJ-120 led to higher NK cell activation, as measured by CD69 expression and cytokine secretion, in IL-2-stimulated PBMCs derived from normal donors and cancer patients.

In vivo, JNJ-120 treatment enhanced the anti-tumor activity of PD-1 blockade in tumor-bearing TIM-3 knock-in mice and in a NY-ESO TCR transgenic model of T cell exhaustion. In the NY-ESO TCR model, JNJ-120 increased T cell infiltration in NY-ESO-expressing A549 tumors and, in combination with anti-PD-1 antibodies, enhanced the ability of infiltrating T cells to kill tumor cells *ex-vivo*.

Conclusions

These data demonstrated that targeting TIM-3 in combination with PD-1 leads to increased tumor T cell infiltration and superior anti-tumor efficacy compared to PD-1 blockade alone. These data support the clinical testing of PD-1 and TIM-3 in tumor types not broadly responsive to current immunotherapy regimens.

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Anti-PD-L1 mAb treatment combined with cisplatin modulates intratumoral immune responses and promotes antitumor effects

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Background

Immune checkpoint inhibitors such as anti-PD-L1/PD-1 agents have shown marked antitumor effect in a range of cancer types, and to further expand their effects, combination treatments with chemotherapeutic drugs have been actively investigated. Although cisplatin is widely used as a standard chemotherapy, the antitumor activity of cisplatin combined with immune checkpoint inhibitors remains largely unknown. Here, we investigated if anti-PD-L1 plus cisplatin combination can augment antitumor immunity in a syngeneic mouse tumor model.

Methods

E.G7-OVA cells, expressing ovalbumin (OVA) gene as a model tumor antigen, were subcutaneously

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inoculated into syngeneic C57BL/6 mouse. After tumor mass was established, anti-mouse PD-L1 mAb (anti-PD-L1; 10 mg/kg, three times a week) and cisplatin (1 mg/kg, once) were administered intraperitoneally on the day of treatment initiation (Day1). The frequency and activation status of immune cells in tumor tissues were evaluated by flow cytometry. For detection of IFN γ -producing cells, the immune cells were stimulated with anti-CD3/anti-CD28 mAbs *in vitro* before staining.

Results

We found that anti-PD-L1 plus cisplatin combination resulted in profound effect leading to tumor shrinkage vs anti-PD-L1 alone or cisplatin alone even though each monotherapy showed significant inhibition of the tumor growth than the control group at Day17. In parallel with the clinical effect, the combination therapy significantly increased tumor-infiltrating CD8+ T cells vs each monotherapy at Day7 even though more tumor-infiltrating CD8+ T cells were observed in mice treated with anti-PD-L1 or cisplatin alone than control. Further, anti-PD-L1 plus cisplatin combination activated tumor-infiltrating CD8+ T cells, characterized by higher frequency of granzyme B-expressing cells than each monotherapy. We also observed increased OVA-specific CD8+ T cells with combination vs monotherapies. Additional analysis of CD4+ T cells showed that anti-PD-L1 plus cisplatin combination or anti-PD-L1 alone, but not cisplatin alone, induced IFN γ -producing CD4+ T cells in the tumor tissues.

Conclusions

Anti-PD-L1 plus cisplatin combination therapy demonstrated marked antitumor effect in this mouse tumor model. This robust therapeutic effects may at least partly be due to the increased tumor-specific CD8+ T cells in tumor tissues. Cisplatin or anti-PD-L1 alone increased tumor-infiltrating CD8+ T cells, however anti-PD-L1 but not cisplatin induced IFN γ + CD4+ T cells at tumor sites. These differences in immune status in response to anti-PD-L1 or cisplatin may lead to the combined

therapeutic effect and provide a rationale for the combination.

P367

X4P-001, an orally bioavailable CXCR4 antagonist, increases T cell infiltration in human metastatic melanoma

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Background

The CXCR4/CXCL12 axis plays a central role in the trafficking of key immune cells in the tumor microenvironment. Enhanced survival is reported in multiple syngeneic mouse models when a CXCR4 antagonist is combined with a check-point inhibitor. X4P-001 is an oral, selective, allosteric inhibitor of CXCR4, and X4P-001 alone demonstrated robust inhibition of murine B16-OVA melanoma growth. We hypothesize that disruption of CXCR4/CXCL12 signaling will result in modulation of the immune cell profile within the tumor microenvironment and ultimately lead to increased CD8+ T-cell infiltration, favoring an improved response to checkpoint inhibitors in metastatic melanoma.

Methods

The primary objectives for this ongoing biomarker-driven Phase Ib trial of X4P-001 alone and with pembrolizumab are to evaluate the safety and tolerability in patients (pts) with metastatic melanoma, and to characterize the effects of X4P-001 alone and with pembrolizumab on tumor immune cell infiltrates. Serial biopsies of cutaneous or subcutaneous metastatic melanoma lesions and peripheral blood mononuclear cells (PBMCs) are

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collected at pre-dose, after 3 weeks of X4P-001 treatment and after 6 weeks of combination treatment. Biopsies are assessed by immunohistochemistry (IHC) for multiple markers including CD3, CD8, FoxP3 and CXCL12, and PBMCs are analyzed by flow cytometry for both lymphoid and myeloid-cells.

Results

As of June 30, 2017, 13 pts have been enrolled and 4 of the 13 pts have completed the study. The median age was 74 years (range 53-91). Most pts (12/13) had not received prior systemic therapy. X4P-001 was well tolerated. Treatment related AEs (related to either X4P-001 or pembrolizumab; >5%) of any grade were: diarrhea (15%), chill, fatigue, headache, ocular hyperemia, photophobia, pruritus, rash, and vomiting (7.7%). Two treatment related G3 SAEs were reported: acute diarrhea and immune-mediated drug-reaction. Three of the 4 pts who completed the study had IHC evaluable tumor samples. All showed an increase in T-cell infiltration in the central region of the tumors following both single agent and combination treatment. The percentage of Ki67 positive CD8+ T cells in PBMC were increased post treatment in all 4 pts. These data along with additional biomarker readouts in all enrolled patients will be presented.

Conclusions

Treatment with X4P-001 as a single agent, and in combination with pembrolizumab, is well tolerated with preliminary evidence of enhanced immune cell infiltration and activation. The enrollment of the study is near completion and further biomarker analysis is on-going.

P368

eFT508, a potent and highly selective inhibitor of MNK1 and MNK2, is an activator of anti-tumor immune response

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Background

eFT508 is a potent and highly selective inhibitor of MNK1 and MNK2, kinases that function to mediate tumor immune evasion downstream of MEK and MAPK signaling. eFT508 treatment establishes a regulatory program that promotes multiple steps in the cancer immunity cycle including antigen presentation and T cell priming, expansion of memory T cells, and prevention of T cell exhaustion.

Methods

The immunological effects of eFT508 have been evaluated in the context of normal human immune cells *in vitro* and in immunocompetent syngeneic and genetically engineered mouse models *in vivo*.

Results

eFT508 treatment of normal donor T cells has no deleterious effect on α CD3/ α CD28 stimulated T cell proliferation or T cell viability in contrast to inhibitors acting upstream of MAPK signaling. eFT508 selectively down regulates key immune checkpoint proteins and the production of a subset of proinflammatory and immunosuppressive cytokines. *In vitro* mechanism of action studies have demonstrated that MNK selectively regulates gene expression at the level of mRNA translation via specific sequence elements in the 5'- and 3'- untranslated regions. In addition, eFT508 activated antigen presenting cells leading to more effective T cell priming. eFT508 also affected T cell memory formation, both in the context of specific peptide antigen stimulation and in a mixed lymphocyte reaction, shifting the distribution of T cells towards a CD62L⁺CD44⁺ central memory T cell population. eFT508 also enhanced the cytotoxic function of T cells from OT-I mice stimulated with SIINFEKL peptide demonstrating a dose-dependent increase of cell killing. Consistent with the mechanisms elaborated upon *in vitro*, eFT508 showed significant

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anti-tumor activity mediated through tumor infiltrating lymphocytes in the CT26 syngeneic tumor model as well as genetically engineered mouse models of NSCLC and HCC.

Conclusions

eFT508 treatment establishes a regulatory program that promotes anti-tumor immunity. eFT508 is currently under evaluation as a single agent in two phase 1/2 clinical trials for patients with advanced solid tumors and patients with advanced lymphoma. A biomarker driven proof of concept study, including mandatory pre- and on-treatment biopsies, to evaluate the immunological mechanism of action of the drug is planned to be initiated later this year. In addition, a phase 2 study evaluating eFT508, alone or in combination with avelumab, a PD-L1 immune checkpoint inhibitor, in microsatellite stable relapsed or refractory CRC patients is planned.

P369

Analysis of the TIGIT/PVRIG axis in human cancers to support indication selection and biomarkers for COM701 and COM902

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Background

PVRIG and TIGIT were identified by Compugen's Predictive Discovery Platform as immune inhibitory receptors and have been reported to inhibit anti-tumor activity. We are pursuing clinical development of antagonistic antibodies to PVRIG (COM701) and to TIGIT (COM902). Here, we analyzed primary human cancer tissues and immune cells to characterize expression in the TIGIT/PVRIG axis to support indication selection and combination strategies for COM701 and COM902.

Methods

COM701 and COM902 were identified based on ability to block the interaction of PVRIG and TIGIT with their cognate ligands (PVRL2 and PVR respectively) and were screened for their ability to enhance antigen-specific CD8 T cell activation in a co-culture with tumor cell lines.

Immunohistochemistry and Flow cytometry were performed to assess receptor/ligand expression in dissociated bladder, breast, colorectal, head and neck, lung, kidney, ovarian, prostate, and stomach tumors.

Results

Among the cancers examined, PVRIG and PVRL2 expression was highest in endometrial, lung, kidney, ovarian, and head and neck cancers compared to normal adjacent tissue. From dissociated tumors, PVRIG expression was detected on T and NK TILs whereas PVRL2 expression was detected on CD45⁺ cells and myeloid cells. A co-expression analysis of PVRIG, TIGIT, and PD1 demonstrated that PVRIG was co-expressed with both TIGIT and PD1 and that PVRIG⁺TIGIT⁺PD1⁺ cells comprised a major proportion of CD8 TILs. In comparison to PD-L1, PVRL2 expression was more prevalent across several cancer types and expression of PVRL2 was detected in PD-L1 negative samples. In vitro, combination of COM701 with PD1 inhibitors or COM902 enhanced CD8 cytokine production and cytotoxic activity, with the triple combination of COM701, COM902, and PD-1 antibody yielding the greatest increase in functional activity. Several immune receptors were induced in response of PVRIG blockade by COM701 on CD8 T cells. Taken together, these data support indication selection and combination strategies for COM701 and COM902 and potential biomarkers that could be indicators of response.

Conclusions

In summary, we demonstrate that PVRIG and PVRL2 are induced in the tumor microenvironment of human cancers, and the potential of COM701 as a cancer therapeutic, either as a monotherapy or as a dual- or triple-combination therapy with antibodies

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targeting TIGIT, and PD-1. These data highlight the potential of this combination approach to expand the immune checkpoint inhibitor responsive cancer patient population, including those who are non-responsive to PD-1 inhibitors.

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Enhancement of tumor specific immunity by activation of CD40 through a bispecific molecule targeting CD40 and a tumor surface antigen

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Background

CD40 activation can bridge the innate and adaptive immune systems during immune activation. Agonistic anti-CD40 monoclonal antibodies (mAbs) have demonstrated anti-tumor activity in clinical studies. Due to the dose-limiting toxicity observed, an alternative approach to developing an anti-CD40 therapy, and potentially reducing systemic toxicity, is to achieve immune activation via a bispecific molecule that is maximally active in the presence of a tumor antigen (TA), but with limited activity in its absence.

Methods

Different bispecific formats targeting both CD40 and a TA were designed and constructed. Bispecific molecules with desired properties were screened and assayed for activation of primary B cells or monocyte-derived dendritic cells (moDC) co-

cultured with TA positive or negative cells. Selected bispecific molecules were tested for anti-tumor activity using PC3 tumor cells with or without TA expression mixed with autologous moDC and T cells, cultured in vitro or inoculated in NSG mice. Syngeneic mouse models of TA positive tumors were used to test bispecific molecules for immune activation, anti-tumor potency, and tolerability.

Results

ABBV-428 is a bispecific molecule with single chain Fv (scFv) domains targeting human CD40 and a TA. ABBV-428 was less potent than a CD40 mAb in stimulating B cells and moDCs when cultured alone or in the presence of cells that do not express TA. However, ABBV-428 exhibited enhanced B cell and moDC activation when cultured with cells expressing TA. T cells were also activated by ABBV-428 when mixed with moDC in the presence of cell-surface TA, as evidenced by a reduction in growth of PC3 cells, both in vitro and in NSG mice. Although expression of the cell surface TA is necessary for immune-mediated anti-tumor activity, ABBV-428 inhibited the growth of both TA-positive and TA-negative PC3 cells as long as the TA was expressed within the tumor environment. This phenomenon was confirmed in a mouse model carrying syngeneic 4T1 tumors expressing the cell surface TA. A surrogate molecule of ABBV-428 elicited T cell responses against both TA-expressing and non-expressing 4T1 cells, where the anti-tumor activity was similar to anti-CD40 mAb, but did not elicit increases in serum cytokines and liver enzyme levels observed in anti-CD40 mAb treated mice.

Conclusions

ABBV-428 exhibits enhanced CD40 activation upon binding to TA-expressing cells, and provides tumor-specific immune stimulation with systemic administration. The enhanced tumor-specific immune activation is hypothesized to maximize anti-tumor potency while limiting systemic toxicity in clinical studies.

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Impact of Diet, Exercise, and/or Stress on Antitumor Immunity

P371

Featuring adipocytes secretion as pharmacological target for adjuvant immunotherapy against breast cancer

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Background

Obesity and adipose tissue have been shown to be associated with low grade inflammation resulting in cellular and humoral inflammatory factors that contribute to carcinogenesis¹. It has been known that inflammation exerts an important role in carcinogenesis and tumor progression. Inflammatory molecules can potentially be used as adjuvant in immunotherapy against cancer. However, little is known about the differential role of brown and white adipocytes against breast cancer. In the present work, we aimed to characterize the differential function of brown and white adipocytes in breast cancer cell death in vitro and investigate the role of NLRP3 and caspase-1/11 in this process.

Methods

Brown and white adipose tissue were isolated from wild type and caspase-1/11 and NLRP3 knockout mice. Conditioned medium from these brown and white adipocytes were used to stimulate breast cancer cells 4T1. Breast cancer cells (I) viability was analyzed by MTT assay; (II) cell death was investigated by annexin V/PI staining and flow cytometry analysis (III) membrane pore formation was observed by PI staining and spectrophotometry analysis and (IV) lipid droplet biogenesis was analyzed by Bodipy staining and flow cytometry analysis.

Results

Our data showed that brown adipocytes conditioned medium triggered significant higher levels of breast cancer cell death and pore membrane formation and lower levels of lipid droplet biogenesis and cell viability compared to white adipocytes conditioned medium.

Conclusions

The absence of caspase-1/11 in brown adipocytes, but not NLRP3, enhanced these cell death and carcinogenic parameters in breast cancer cells. Identification of molecules from these adipocytes secretion is ongoing and can be potentially used as adjuvant immunotherapy against breast cancer.

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Mechanisms of Efficacy or Toxicity

P372

A syngeneic mouse model of CAR-T mediated toxicity and neuroinflammation

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Background

Interrogating the spectrum of systemic effects of CAR-T cell therapy, especially in the context of adverse events (i.e. systemic cytokine release syndrome (sCRS) and neurotoxicity) that have occurred in clinical trials, may provide important insights into factors contributing to the cause and/or progression of such events and potential interventions. Preclinical CAR-T cell efficacy models in immune-compromised mice do not capture the potential role of the host immune system in sCRS and central nervous system (CNS) pathologies that,

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in combination with other patient factors, may lead to severe neurotoxicity. We have developed a novel model system where conditioning immune-competent mice with cyclophosphamide (CPA) followed by transfer of murine CD19-directed CAR-T cells induces acute symptoms including systemic cytokine release in concordance with what is observed in patients that develop CRS and/or neurotoxicity. This systemic toxicity is accompanied by the alteration of gene expression levels in the mouse brain indicating a possible neuro-inflammatory response.

Methods

We examined the effects of murine CD19-directed CAR-T cell therapy in a syngeneic BALB/c model. Following dose optimization for both CPA conditioning and the intravenous transfer of CAR-T cells, mice were evaluated for acute toxicity and systemic and brain-related pathologies including serum cytokine levels, brain and peripheral organ histopathology by microscopy of hematoxylin and eosin-stained sections, evaluation of blood brain barrier (BBB) integrity by extravasation of fluorescently-labeled low-molecular weight dextrans, wet-dry brain weights indicative of cerebral edema, and flow cytometry and gene expression analysis of transcardially-perfused brain tissues.

Results

Conditioning with CPA, followed by administration of murine CD19-directed CAR T cells, but not control CAR-T cells, induced rapid weight loss, peripheral organ pathologies and elevated serum cytokine levels (including a spectrum of cytokines similar to those observed in clinical CRS). In the brain, we observed significant changes in gene expression indicative of neuro-inflammation including genes associated with interferon response pathways, vascular endothelial activation and oxidative stress, accompanied by CAR-T cell infiltrate into the brain. No evidence for overt brain histopathology was observed, nor increased BBB permeability or cerebral edema.

Conclusions

These findings describe a new animal model and highlight its potential use to elucidate the mechanisms underlying CAR-T-cell mediated toxicities and test proposed interventions to reduce neuro-inflammation that may arise from CD19-directed CAR-T cell therapies. Ongoing work seeks to identify and evaluate various pharmacological interventions with the potential to ameliorate systemic and neuro-inflammation in this model, with the goal to translate these learnings to the clinic.

P373

Optimizing anti-OX40 mediated immunotherapy: preclinical exploration of the relationship between antitumor activity and isotype choice, ligand blocking capacity, dose and schedule

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Background

Following the clinical success of checkpoint blockade, the field of cancer immunotherapy is rapidly expanding. Extensive preclinical data have demonstrated that treatment with an agonist OX40 antibody can result in anti-tumor immune responses both alone and in combination with other immune-targeting agents including CTLA-4 and PD-1 blockers. The reported mechanism of action for OX40 includes costimulation of effector T cells as well as reduction in regulatory T cell (T_{reg}) suppression either through depletion or receptor engagement. However, the vast majority of published work utilizes a single, ligand non-blocking antibody to define the role of anti-OX40 in enhancing tumor immunity *in vivo*. We generated an alternative agonistic, ligand-blocking mouse

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OX40 antibody as well as series of isotype variants in order to better define the role of isotype, ligand blocking vs non-blocking epitopes, dose and schedule on the anti-tumor activity of anti-OX40 alone and in combination with PD-1 blockade *in vivo*.

Methods

The activity of OX40 agonist antibodies, OX86 (ligand non-blocker) and OX40.23 (ligand blocker), were tested across a dose range from 0.03 mg/kg to 10mg/kg in the CT26 tumor model. Anti-PD-1 (10 mg/kg) was dosed either concurrently or 5 days after initial OX40 treatment. On-treatment immune monitoring of peripheral and tumor-infiltrating lymphocytes was analyzed by flow cytometry.

Results

Fc-competent agonist OX86 antibodies display potent *in vivo* anti-tumor activity at 10 mg/kg, whereas an Fc inert antibody at the same dose had no effect on CT26 tumor growth. Relative anti-tumor activity was related to their ability to preferentially bind activating FcγR and deplete intratumoral T_{reg}S. Maximal activity of OX40.23 was achieved at 3 mg/kg and 0.3 mg/kg as monotherapy and in combination with anti-PD-1, respectively. Interestingly, administration of OX40.23 at 10 mg/kg in both monotherapy and combination displayed diminished activity, accompanied by a reduction in peripheral T cell activation. Evaluation of OX40 RO demonstrated that peripheral and intratumoral RO was similar as was RO between monotherapy and combination treatment. Maximal anti-tumor activity of the combination was achieved well below 100% OX40 RO. Furthermore, for combination treatment, concurrent dosing resulted in greater anti-tumor activity than a staggered regimen.

Conclusions

Our results demonstrate the importance of isotype choice, ligand blockade capacity, dose and schedule on the *in vivo* anti-tumor activity of mouse OX40 agonist antibodies alone and in combination with PD-1 blockade. These data provide valuable insight

relevant in biomarker, dose and schedule selection for agonist OX40 antibody-containing regimens currently in clinical development.

P374

Activation of 4-1BB on liver myeloid cells triggers hepatitis via an interleukin-27 dependent pathway

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Background

Agonist antibodies targeting the T cell co-stimulatory receptor 4-1BB (CD137) are among the most effective immunotherapeutic agents across multiple pre-clinical models of cancer. In the clinic, however, development of these agents has been stymied by dose-limiting liver toxicity. Lack of knowledge of the mechanisms underlying this toxicity has limited the potential to separate 4-1BB agonist driven anti-tumor immunity from hepatotoxicity.

Methods

The capacity of 4-1BB agonist antibodies to induce liver toxicity was investigated in immune competent mice, with or without co-administration of checkpoint blockade, via measurement of serum transaminase levels, through imaging of liver immune infiltrates, and via qualitative and quantitative assessment of liver myeloid and T cells via flow cytometry. Knockout mice were used to clarify the contribution of specific cell subsets, cytokines and chemokines.

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Results

We find that activation of 4-1BB on liver myeloid cells is essential to initiate hepatitis. Once activated, these cells produce interleukin-27 which is required for liver toxicity. CD8 T cells infiltrate the liver in response to this myeloid activation and mediate tissue damage triggering transaminase elevation. FoxP3+ regulatory T cells limit liver damage and their removal dramatically exacerbates 4-1BB agonist hepatitis. Co-administration of CTLA-4 blockade ameliorates transaminase elevation, whereas PD-1 blockade exacerbates it. Loss of the chemokine receptor CCR2 blocks 4-1BB agonist hepatitis without diminishing tumor-specific immunity against B16 melanoma.

Conclusions

4-1BB agonist antibodies trigger hepatitis via activation of myeloid cells to produce Interleukin-27. Co-administration of CTLA-4 and/or CCR2 blockade may minimize hepatitis but yield equal or greater antitumor immunity.

P375

Evaluation of surrogate endpoints for overall survival in patients treated with immunotherapies

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Background

Surrogate endpoints have not been clearly determined in oncology immunotherapy (IT). Using both arm- and comparison-level data, this study explored the relationship between overall survival (OS) and clinical endpoints (objective response rate [ORR], disease control rate [DCR], and progression-free survival [PFS]) in patients treated with IT. The aim was to assess whether any of these endpoints could function as surrogates for OS.

Methods

A systematic review was conducted in MEDLINE™ and Embase (January 2005–March 2017), and supplemented with conference proceedings (2014–2017). Eligible studies were randomized controlled trials (RCTs) that investigated blocking antibodies targeting programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) or cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4). Studies were included in the arm-level analyses if the treatment arm's absolute effects could be obtained for ORR, DCR, 6- and 9-month PFS, median PFS, median OS, or OS at 12 or 18 months. They were included in comparison-level analyses if the treatment's relative effects (odds ratios [ORs] on ORR and DCR or hazard ratios [HR] on PFS and OS) were reported/could be derived.

Weighted linear regression models were fitted and adjusted R² values estimated, with analyses stratified by treatment regimen (IT mono or IT plus chemotherapy), by type of IT (PD-1/PD-L1 or CTLA-4), and by indication, as data permitted.

Results

29 RCTs involving 66 treatment arms (11,797 patients) were included. In the arm-level analyses, higher PFS rates at 6 months predicted better OS rates at 12 and 18 months, with similar trends across subgroups (Figure 1A–D); results by type of IT revealed stronger correlations for PD-1/PD-L1 than CTLA-4 as potential surrogates for OS (Figure 1A, Figure 1B). Similarly, the comparison-level analyses only found the PFS HR to be moderately correlated with the OS HR (R²=0.372; P=0.002; Figure 1e); this

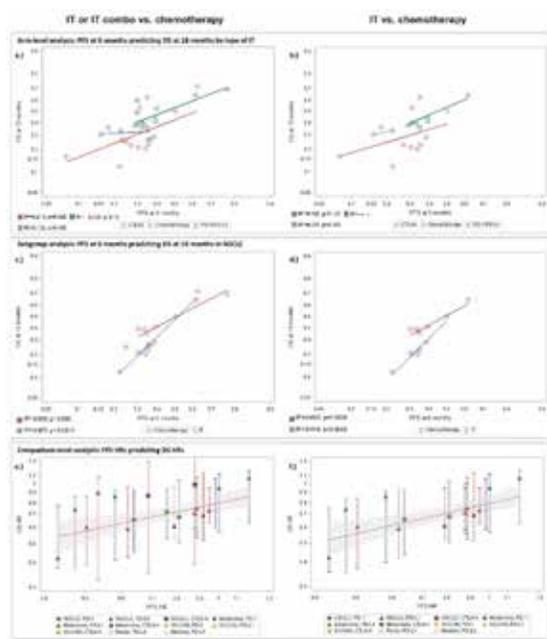
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finding remained consistent when restricted to studies assessing IT monotherapy only ($R^2=0.460$; $P=0.002$; Figure 1F) and in subgroups stratified by treatment type or indication.

Conclusions

Among anti-PD-1/PD-L1 studies, PFS was an imperfect surrogate (low to moderate correlation) for OS, whereas other surrogate clinical endpoints were not correlated with OS. Thus, a new surrogate, such as a biomarker, is needed to better predict the OS benefit for IT.

Figure 1. Abstract Summary



P376

Evaluating CD38 as a therapeutic target in non-small cell lung cancer (NSCLC)

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Background

Daratumumab (DARA), a human CD38-targeted monoclonal antibody, is approved as monotherapy and in combination with standard of care regimens for patients with relapsed or refractory multiple myeloma. DARA demonstrates direct on-tumor and immunomodulatory mechanisms of action. In multiple myeloma patients treated with DARA, CD38⁺ immunosuppressive cells including regulatory T-cells (T_{reg}), myeloid derived suppressor cells (MDSC) and regulatory B-cells (B_{reg}) are depleted. DARA also results in increased CD8⁺ T-cells and T-cell clonality. We hypothesized that DARA could be effective in solid tumors that have elevated levels of CD38 expression on the surface of tumor and/or immunosuppressive cells. Here, we investigated CD38 expression on lung cancer and intra-tumoral immune cells to determine whether NSCLC may be susceptible to the immunomodulatory effects of DARA.

Methods

CD38 RNA expression in lung cancer cell lines and tumor sections from The Cancer Genome Atlas (TCGA) database was investigated using RNA-sequencing. Protein expression on lung cancer cell lines, tumor-infiltrating immune cells, and peripheral blood (PB) immune cells was analyzed by flow cytometry. Primary tumor samples were evaluated by immunohistochemistry. The effect of DARA on lung cancer cell viability was measured in an *in vitro* assay. DARA-mediated depletion of immune cells in the PB of lung cancer patients was assessed *ex vivo*.

Results

CD38 RNA was highly expressed in lung tumor specimens from the TCGA database. Of the lung cancer cell lines investigated, 3/12 (A549, NCI-H2023, and NCI-H2073) showed detectable CD38 receptor. These cell lines demonstrated moderate receptor density (8,830, 20,900, and 58,100 average receptors/cell, respectively) compared to a multiple myeloma cell line (MM.1R; 75,590 average receptors/cell). Detectable CD38 receptor expression was observed on tumor cells from 5/21

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primary NSCLC tumor samples and was highly expressed on intratumoral immunosuppressive cells including: monocytic MDSC, tumor-associated macrophages and T_{reg}. In the PB of lung cancer patients, CD38 receptor was expressed on >50% of NK cells, B-cells, monocytic MDSC, immature MDSC, and monocytes. Significantly, DARA induced cell-mediated killing of CD38⁺ lung cancer cell lines *in vitro*, and depleted monocytic MDSC from the PB of lung cancer patients *ex vivo*.

Conclusions

Tumor cell CD38 expression is observed in a subset of lung cancers and is highly expressed on patient tumor-infiltrating immune cells and immunosuppressive cells in PB samples. Because DARA has demonstrated *in vitro* activity in depleting these cells, DARA may represent a novel therapeutic approach to targeting the immune microenvironment in solid tumors. Clinical trials in NSCLC are underway (NCT03023423) and/or planned.

P377

Cisplatin induces immunogenic cell death in preclinical models of head and neck squamous cell carcinoma

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Background

Immunogenic cell death (ICD) is the process by which stressed cells exhibit translocation of markers including calreticulin and heat shock proteins (HSPs) to the cell surface along with release of HMGB1 and ATP. This activates toll-like receptors and adaptive immunity. Studies in several cancer types suggest that oxaliplatin induces superior ICD compared to

cisplatin. This has never been studied in head and neck squamous cell carcinoma (HNSCC).

Methods

Three HNSCC cell lines were treated with sublethal doses of the platinum chemotherapy drugs cisplatin, oxaliplatin, and carboplatin for 48 hours. Cell surface levels of HSP70 and calreticulin were then assayed by flow-cytometry. Intracellular HMGB1, an indirect measurement of HMGB1 release, was also quantified. Release of HMGB1 was measured in the cell culture supernatants. A syngeneic mouse model was then used to compare the effects of cisplatin vs. oxaliplatin, alone or in combination with anti-PD-1 immunotherapy, on tumor growth and survival. A subset of tumors were analyzed for immune cell infiltrates by flow cytometry.

Results

All three platinum drugs induced translocation of HSP70 and calreticulin to the cell surface, as well as release of HMGB1 in multiple cell lines. Cisplatin was the superior ICD inducer in these cell lines. Cisplatin and oxaliplatin induced similar tumor growth delay when combined with anti-PD-1 immunotherapy in tumor-bearing, immunocompetent mice.

Conclusions

Treatment of HNSCC cells with platinum chemotherapy drugs appears to induce ICD, which may enhance anti-tumor immunity. Cisplatin, which is the standard chemotherapy drug for treatment of HNSCC, appears to be at least as effective as oxaliplatin as an ICD inducer in these preclinical models.

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P378

Improving the efficacy of cancer immunotherapy: An intimate play of CD8 T and NK cells

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Background

The interaction between the innate and adaptive immune components is fundamental for an effective antitumor immunity. Our studies in murine models of mastocytoma, kidney, breast and lung solid tumors showed that productive antitumor effector response relies on functional crosstalk between innate immune effectors—natural killer (NK) cells, and adaptive immune effectors—cytolytic CD8⁺T lymphocytes. We found that this lymphocyte cooperativity between CD8⁺T and NK cells can prevent the development of antigen-escape tumor variants.

Methods

Using a nanofiber matrix engineered to provide lymphocytes a controlled 3D interaction, we found that activated CD8⁺T cells (CD69^{high}CD25^{high}) formed multiple intercellular contacts with several naïve NK cells, while naïve CD8⁺T cells made single or no contact with NK cells.

Results

In lymphocyte coculture (physical contact possible), activated CD8⁺T and NK cells cross-regulated each other's phenotype wherein NK cells polarized activated CD8⁺T cells towards T central memory phenotype and activated CD8⁺T lymphocytes induced acquisition of effector/regulatory phenotype by naïve NK cells. This cross-regulation

of lymphocytes disappeared in a trans-well system (no physical contact) indicating the necessity of cell-to-cell physical interaction during CD8⁺T—NK crosstalk. Notably, intercellular physical interaction led to cross-regulation of mitoCa²⁺ oscillations in both activated CD8⁺T and NK cells. Inhibition of mitochondrial Ca²⁺ uptake or Na⁺/Ca²⁺ exchanger with Ru360 and CGP37157, respectively, mimicked observed alterations in both lymphocytes. Further, NK cells displayed increased oxidative signaling, Tyk2, Jak 1 and 3, Stat2 and Stat6 phosphorylation while inhibiting TCR- and various cytokine receptor-mediated signaling. In turn, NK cells selectively restrained IL-2 signaling in CD8⁺T cells by dampening activation-induced up-regulation of CD25, Stat5 phosphorylation, IL-2 synthesis and elevation in IL-2 uptake.

Conclusions

These data suggest a model, where mitochondrial Ca²⁺ flux acts as a key biological controller to guide cellular crosstalk allowing acquisition of NK cell effector/regulatory and T cell central-memory phenotypes upon their interaction. Further understanding of the characteristics and regulatory factors involved in this NK—CD8⁺T cell physical play in the tumor microenvironment will provide new insights on controlling immune escape variants of tumor. This could lead to novel strategies for effective cancer immunotherapies, with a potential of relapse-free survival in cancer patients.

P379  Abstract Travel Award Recipient

Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade

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Background

Immune checkpoint blockade is able to achieve durable responses in a subset of patients, however despite such significant clinical progress we still lack a fundamental understanding of the mechanisms of anti-CTLA-4 and anti-PD-1 induced tumor immune rejection. CTLA-4 and PD-1 regulate T cell activation through different molecular and cellular mechanisms and act at different stages of T cell activation. As such, we hypothesized that anti-CTLA-4 and anti-PD-1 checkpoint blockade induce tumor rejection through distinct cellular mechanisms.

Methods

To address this hypothesis, we utilized mass cytometry which enables characterization of more than 40 parameters at single cell resolution and unsupervised cellular classification. Using this approach, we comprehensively profiled the immune infiltrates of MC38 and B16BL6 murine tumors from mice treated with anti-CTLA-4, anti-PD-1, or control antibodies. We also performed similar analyses of surgically resected melanomas from patients being treated with checkpoint blockade therapy.

Results

In both tumor models, more than 13 distinct tumor infiltrating T cell populations were identified. Both anti-CTLA-4 and anti-PD-1 checkpoint blockade modulated the frequencies of only a subset of these tumor infiltrating T cell populations. Furthermore, of multiple exhausted-like CD8 T cell populations identified, the frequencies of only two subsets correlate with outcome, suggestive of functional heterogeneity within phenotypically exhausted T cells. Most notably, we find that anti-CTLA-4, but not anti-PD-1, modulates the CD4 effector compartment by inducing the expansion of Th1-like CD4 effector T cells. Observations from mass cytometry analyses of surgically resected melanoma tumors from patients being treated with anti-CTLA-4 or anti-PD-1 therapy were consistent with these preclinical findings.

Next we utilized similar methodologies to investigate the cellular mechanism of combination anti-CTLA-4 and anti-PD-1 therapy. Although combination therapy largely enhanced effects observed in monotherapies, combination therapy differentially modulated specific exhausted-like CD8 T cell populations. These data suggest that combination therapy modulates T cell function and mediates tumor rejection through mechanisms that are in part distinct from either monotherapy.

Conclusions

Our findings indicate that anti-CTLA-4 and anti-PD-1 modulate specific tumor infiltrating T cell populations and utilize distinct cellular mechanisms to induce tumor rejection. Anti-CTLA-4, but not anti-PD-1, induces expansion of CD4 effector T cells. Furthermore, these data suggest that combination therapy modulates T cell function differently than monotherapies. These findings have implications for the rational design of combinatorial therapeutic approaches and expand our understanding of the mechanisms that regulate T cell activity.

P380

The role of radiotherapy in driving anti-tumor CD8 T cell responses

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Background

Radiotherapy (RT) is one of the three main arms of traditional cancer therapy, and has been shown to synergize well with immunotherapies in preclinical models. Consistent with the findings of other investigators, in immunogenic murine models of radiation therapy and immunotherapy we have observed CD8+ T cell-dependent clearance of tumors given single, high-dose radiation (20Gy) and anti-PD1 checkpoint blockade. In this setting, RT has

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been proposed to generate tumor antigen-specific T cells following the release of antigens by dying cancer cells, and PD1 blockade allows T cell control of residual cancer cells in the suppressive tumor environment. We hypothesized that the role of radiation is to boost the number of existing tumor antigen-specific CD8 T cells and drive an influx of tumor-antigen reactive cytotoxic T cells into the tumor. If this hypothesis is true, then providing T cells through other means should be sufficient to replace radiation therapy in this therapeutic combination.

Methods

In order to deliver targeted beams of radiation, we use the Xstrahl Small Animal Radiation Research Platform (SARRP). Tumor-reactive CD8 T cell numbers were boosted using live-attenuated *Listeria monocytogenes* vaccines against potent tumor-specific CD8 model antigens. Nur77-GFP transgenic mice were used to measure antigen recognition by T cells *in vitro* and *in vivo*.

Results

We demonstrate that RT generates marginal increases in tumor-antigen specific T cells in the peripheral circulation. In order to determine whether higher numbers of tumor-responsive CD8s were driving RT plus checkpoint blockade efficacy, we vaccinated mice with attenuated *Listeria* expressing tumor-specific antigens. Using immunohistology and flow cytometry we demonstrate that these antigen-specific cells were present at high levels in the tumor. We demonstrate that antigen-specific T cells generated by vaccination kill antigen-pulsed targets in *in vivo* cytotoxicity assays, recognize cancer cells *ex vivo*, and respond to tumor-associated antigen within the tumor. However, mice treated with *Listeria* vaccination and PD1 blockade showed no tumor growth control advantage when compared to controls, despite the high level of antigen-specific T cells in the tumor.

Conclusions

These data demonstrate that while radiotherapy generates marginal increases in the number of antigen-specific T cells, which are log-fold fewer than generated by *Listeria* vaccination, RT is a superior partner for combination with checkpoint blockade. We find that generating large numbers of tumor antigen-specific T cells cannot substitute for the efficacy of radiotherapy in combination with anti-PD1 checkpoint blockade, and question whether the main driver of radiotherapeutic efficacy is augmenting CD8 T cell numbers.

Mechanisms of Resistance to Immunotherapy

P381

The BET bromodomain inhibitor ZEN-3694 modulates the expression of checkpoint receptors and immune suppressive factors in the blood of mCRPC patients

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Background

Epigenetic regulation of the immune system plays a significant role in the response to immunotherapies. The potential for epigenetic modulators to prime the immune system and increase the duration and frequency of response to checkpoint inhibitors has been supported by both pre-clinical and clinical evidence. ZEN-3694 is an orally available inhibitor of the bromodomain and extra-terminal (BET) domain family of proteins currently in phase I clinical trials in metastatic castration-resistant prostate cancer (mCRPC) (NCT02705469 and NCT02711956). Previously, we have shown that ZEN-3694 modulates multiple checkpoint receptors, immune suppressive factors and cytokines *in vitro*, and acts synergistically with a PD-1 mAb to inhibit tumor

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growth in a MC-38 syngeneic mouse model. To follow up with these findings, we examined the ability of ZEN-3694 to modulate the expression of Immuno-Oncology target genes in mCRPC patients at multiple doses in our current phase 1 clinical trial.

Methods

The Nanostring nCounter® PanCancer Immune Profiling Panel was used to measure immune marker expression in patient whole blood RNA taken at 0, 4 and 24 h post dosing with ZEN-3694.

Results

ZEN-3694 modulates multiple checkpoints, suppressive factors and cytokines in peripheral immune cells 4 hours after a single dose. Most of these changes return to baseline at 24 hours, following clearance of the drug. Significant effects were detected at all doses tested, including at well-tolerated doses below the maximum tolerated dose. Multiple checkpoint receptors, including TIM3 and PD-L1, chemokines CCL2/CCR2 and IL-8, and the suppressive factors IDO1 and ARG1, were significantly inhibited 4 h post-dose across 16 patients. These results were also confirmed by real-time PCR for several of these markers. Furthermore, mRNA levels of multiple co-stimulatory markers, including ICOSLG and CD28, were maintained or induced. Several of these markers show a strong dose/exposure response, and Ingenuity® Pathway Analysis suggests that lower doses may be superior to higher doses for cancer immune response modulation.

Conclusions

Taken together, these data suggest that ZEN-3694 has the potential to modulate multiple factors involved in adaptive resistance to therapeutic PD-1 blockade, and therefore may improve response rate and duration in combination with a checkpoint inhibitor. This is the first presented clinical evidence that a BET bromodomain inhibitor can modulate PD-L1 and other relevant immuno-oncology targets in patients at therapeutically well-tolerated doses. Follow up studies to correlate these changes with

protein expression, immune cell activation, and tumor-specific targets in patients are underway.

P382

ATR inhibition sequenced with radiation therapy abrogates immune exhaustion

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Background

Immunotherapy that targets the immunosuppressive interaction between the T cell activation-induced programmed cell death receptor 1 (PD-1) and its ligand PD-L1 and radiation therapy are used in the management of a majority of metastatic non-small cell lung cancer (NSCLC) patients. ATR is a DNA damage signaling kinase activated at damaged replication forks and ATR kinase inhibitors potentiate the cytotoxicity of DNA damaging chemotherapies and radiation

Methods

Here we show that ATR kinase inhibitors potentiate radiation-induced tumor immune responses in genetically engineered and syngeneic mouse models of Kras-mutant lung adenocarcinoma and colorectal carcinoma, respectively.

Results

ATR kinase inhibitors attenuate radiation-induced PD-L1 upregulation on tumor cells and dramatically decrease the number of tumor infiltrating Tregs. ATR kinase inhibitors further attenuate radiation-induced T cell exhaustion and increase T cell activity in the tumor microenvironment.

Conclusions

Our work raises the exciting possibility that a single pharmacologic agent may enhance the cytotoxic effects of DNA damaging chemotherapy and

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radiation while concurrently potentiating radiation-induced tumor immunity.

P383

Molecular and immune characterization of melanoma metastases with heterogeneous PTEN expression

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Background

There is growing evidence that oncogenic pathways in tumor cells can impact the anti-tumor immune response and the efficacy of immunotherapy. Previously we showed that loss of PTEN inhibits immune infiltration and promotes resistance to immune-mediated tumor killing in melanoma [1]. Although PTEN expression is generally uniform in melanoma, we identified a cohort of melanoma metastases with distinct areas with (+) and without (-) PTEN protein expression. Comparative molecular and immune analyses were performed on the PTEN (+) and (-) regions of these tumors to improve our understanding of the mechanisms, pathogenesis, and significance of PTEN loss.

Methods

Core biopsies were performed on formalin-fixed paraffin-embedded FFPE blocks to isolate analytes from tumor-matched regions with (+) and (-) PTEN expression by immunohistochemistry (IHC). DNA was analyzed for somatic mutations and copy number variations (CNVs) in 200 cancer-related

genes by next generation sequencing (NGS), and globally for DNA methylation patterns by Illumina Infinium Human Methylation450 arrays. Expression of proteins involved in signaling pathways (n=42) and immune populations/regulators (n=40) in distinct PTEN (+) and (-) regions within the tumors was quantified by Nanostring Digital Spatial Profiling.

Results

NGS showed that PTEN (+) and (-) regions did not differ in overall mutation patterns. PTEN (-) regions had increased rates of chromosomal loss affecting *PTEN*, *EMB* and *ZNF567*; gain of *LETM1*, *WDR17* and *SPATA4* was detected in PTEN (+) regions. Hierarchical clustering of DNA methylation patterns demonstrated overall clustering of samples by tumor. Loss of PTEN expression did not correlate with increased methylation of the *Pten* locus, but differential methylation of several potential regulatory transcription factors (*Pax3*, *Gata2*, *Ikzf1*, *Pax5*, and *Gata5*) was detected. Proteomic analysis identified multiple signaling and immune related proteins with significantly different expression between PTEN (+) and (-) regions. Loss of PTEN correlated with significantly increased P-AKT and P-P70S6K, consistent with increased activation of PI3K-AKT-mTOR signaling, and significantly decreased VISTA, CD8A, and CD3. PD-L1 expression did not correspond with PTEN status.

Conclusions

Intratumoral loss of PTEN in melanoma metastases corresponded with genetic alterations in the *Pten* gene but not its methylation status. Proteomic analysis supports that PTEN status corresponds locally with PI3K-AKT pathway activation and with heterogeneity of components of the anti-tumor immune response. Together the findings further support the biological significance of PTEN loss in melanoma.

Consent to publish

All patients provided informed consent.

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References

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P384

Mechanisms of efficacy during TGFbR1 inhibition/cytotoxic combination therapy for rectal cancer

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Background

Neoadjuvant chemoradiation is the standard of care for patients with locally advanced rectal cancer with the intention of downstaging and sphincter preservation prior to surgical intervention. Responses may be limited, however, in part due to TGFb1 regulated resistance to these cytotoxic modalities. We have shown previously that blocking ALK5 signaling through use of a small molecule inhibitor sensitizes CT26 tumor-bearing mice to the effects of radiation in part by boosting CD8⁺ T cell mediated cytotoxicity. The mechanism for this efficacy is currently unknown.

Methods

By using LY2157299 prior to 5-FU and hypofractionated radiation, tumor-bearing animals elicit suboptimal T cell responses capable of controlling tumor growth in a significant percentage of animals but fail to cure.

Results

Early results from a clinical trial of neoadjuvant TGFbR1 inhibition with chemoradiation in 3 rectal cancer patients demonstrate objective clinical responses and markers of enhanced anti-tumor immunity but indicators of compensatory immunosuppression are also observed in surgically

excised tissue. One visually prominent aspect in a proportion of these patients is the infiltration of plasma cells into tumor parenchyma. Typically these fully differentiated B cells would be a hallmark of immune mediated rejection through secretion of high affinity immunoglobulin, but previous reports suggest they may also function to suppress cytotoxic T cells through TGFb1, IL-10 and PDL1. Correspondingly, we also observe increases in B regulatory populations and IL-10 expression in CT26 tumor draining lymph nodes from treated animals.

Conclusions

As B cell subsets express apoptotic cellular receptors similar to macrophages, we propose B cells may be one mechanism of resistance to this combination therapy and the direct blockade of their activity may optimize T cell effectiveness for patients with rectal cancer.

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A HSP-TLR-Wnt5a paracrine signaling axis drives CXCR2 ligand recruitment of myeloid-derived suppressor cells and represents a novel adaptive resistance mechanism to anti-PD-1 antibody therapy

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Background

Despite the impressive impact generated by the anti-PD-1/PD-L1 antibody (ab) therapies in oncology, a significant percentage of patients

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exhibit either primary or secondary resistance to these treatment modalities. The evolution of these resistance mechanisms to cancer immunotherapy remains poorly understood. Myeloid-derived suppressor cells (MDSCs) have been demonstrated to suppress anti-tumor immunity and previous work has shown the granulocytic subset of MDSCs (Gr-MDSCs) to migrate in response to CXCR2 chemokine gradients.

Methods

We utilized RNAseq differential gene expression analysis, serial tissue biopsy qRT-PCR, and SILAC-AHA metabolic labeling mass spectrometry-based differential secretome analysis to identify those genes and secreted proteins upregulated in melanomas escaping anti-PD-1 ab therapy in an autochthonous BRAF^{V600E}PTEN^{-/-} melanoma model. Multi-parameter flow cytometry and immunohistochemistry were utilized to investigate MDSC recruitment to tumor tissues. Lentiviral shRNA gene silencing and *in vivo* CD8⁺ T cell ablation assays were conducted to define the underlying signaling mechanisms driving MDSC recruitment to anti-PD-1 ab-treated melanomas. Circulating melanoma cells (CMCs) were derived from patients undergoing active anti-PD-1/PD-L1 ab therapy using a photoacoustic cytometry technique for single cell qRT-PCR validation of pre-clinical findings.

Results

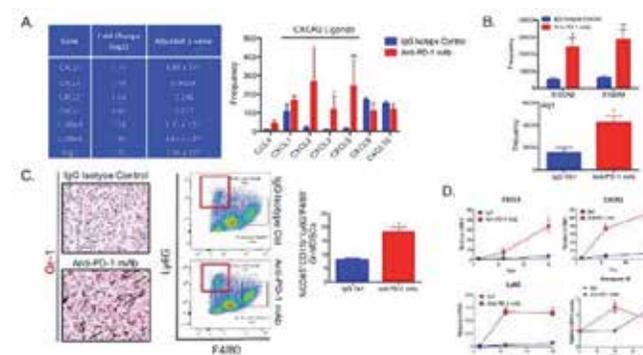
Autochthonous BRAF^{V600E}PTEN^{-/-} melanomas upregulate the expression of several CXCR2 ligands, including CXCL5, following escape from anti-PD-1 ab therapy (Figure 1A-B). This is associated with the rapid recruitment of Gr-MDSCs and an inhibition of local CD8⁺ T cell responses (Figure 1C-D). We determined that melanoma CXCL5 expression is induced by a Wnt5a-YAP pathway and that Wnt5a is, in turn, regulated by local CD8⁺ T cell killing and paracrine heat shock protein (HSP)-TLR2 signaling (Figure 2). The individual genetic silencing of Wnt5a and CXCL5 reverses Gr-MDSC recruitment in response to anti-PD-1 ab therapy and sensitizes these BRAF^{V600E}PTEN^{-/-} melanomas to checkpoint inhibition (Figure 3). Indeed, blockade of Wnt-

mediated signaling with a OMP-54F28 'Wnt trap' diminishes Gr-MDSC recruitment and augments the efficacy of anti-PD-1 ab therapy in this autochthonous melanoma model (Figure 4). Plasma levels of CXCL5 correlate with progression and anti-PD-1 ab escape in the transgenic melanoma model while patient-derived CMCs also demonstrate CXCR2 ligand upregulation following progression on anti-PD-1/PD-L1 ab therapy (Figure 5).

Conclusions

A HSP-TLR-Wnt5a paracrine signaling axis mediates the recruitment of Gr-MDSCs in response to anti-PD-1 ab therapy-mediated CD8⁺ T cell killing and represents a novel adaptive resistance mechanism to cancer immunotherapy. Additional studies are ongoing to exploit this pathway as a strategy for augmenting the therapeutic efficacy of anti-PD-1 ab therapy and for monitoring the development of immunotherapy resistance in advanced melanoma patients.

Figure 1. Escape from anti-PD-1 Ab Immunotherapy in a Transgenic Melanoma Model Involves the Recruitment of Gr-MDSCs

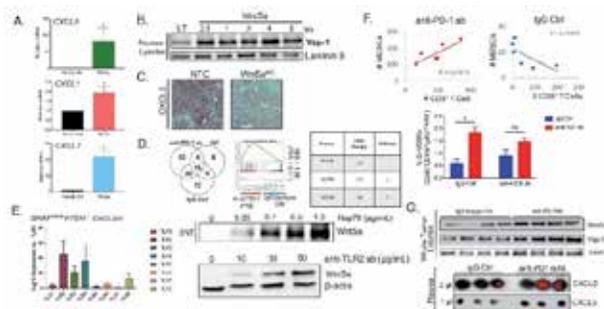


A. RNAseq differential gene expression analysis showing an upregulation of several CXCR2 ligands following anti-PD-1 ab escape in a transgenic BRAF(V600E)-PTEN^{-/-} melanoma model. B. RNAseq differential gene expression analysis demonstrating increased MDSC markers in BRAF(V600E)-PTEN^{-/-} melanomas following escape from anti-PD-1 ab therapy. C. Immunohistochemistry and flow cytometry analysis demonstrating increased levels

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of Gr-MDSCs in anti-PD-1 ab-treated versus IgG isotype control-treated BRAF(V600E)-PTEN^{-/-} melanomas. Representative IHC images and flow cytometry dot plots are provided. D. Serial tissue biopsy and qrt-PCR analysis of anti-PD-1 ab-treated versus IgG isotype control-treated BRAF(V600E)-PTEN^{-/-} melanomas. All data is mean +/- SEM. P<0.05.

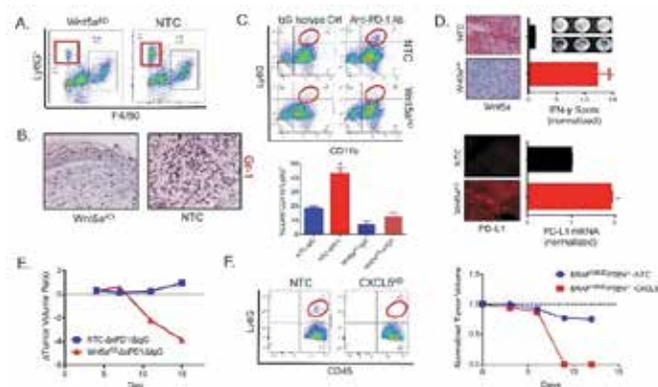
Figure 2. Induction of CXCL5 in Melanomas Undergoing Treatment with Anti-PD-1 Ab Therapy is Dependent Upon a Paracrine HSP-TLR-Wnt5a Signaling Pathway



A. Qrt-PCR analysis shows that Wnt5a stimulates the expression of several CXCR2 ligands in a BRAF(V600E)-PTEN^{-/-} melanoma cell line. B. Western blot analysis of nuclear YAP1 levels in response to Wnt5a stimulation in a BRAF(V600E)-PTEN^{-/-} melanoma cell line. C. Immunohistochemistry studies show that CXCL5 expression is significantly suppressed in Wnt5a-silenced BRAF(V600E)-PTEN^{-/-} melanomas. D. SILAC-AHA metabolic labeling mass spectrometry-based differential secretome analysis and RNAseq differential gene expression analysis of BRAF(V600E)-PTEN^{-/-} melanomas following treatment with anti-PD-1 ab vs IgG isotype control ab. Several cellular stress pathways identified as being upregulated in those melanomas undergoing anti-PD-1 ab therapy. Several heat shock proteins including HSP70 noted to be induced in anti-PD-1 ab-treated melanomas. E. BRAF(V600E)-PTEN^{-/-} melanomas express elevated levels of TLR2 and TLR4 based on qrt-PCR. Western blot demonstrates that HSP70 and agonistic anti-TLR2 ab stimulates

Wnt5a expression in the BRAF(V600E)-PTEN^{-/-} melanoma cell line. F. top, CD8+ T cell levels and Gr-MDSC levels correlate significantly in BRAF(V600E)-PTEN^{-/-} melanomas only when treated with anti-PD-1 ab therapy (p = 0.044). bottom, Ablation of CD8+ T cells diminishes the recruitment of Gr-MDSCs in response to anti-PD-1 ab therapy. G. top, Whole tissue Western blot analysis shows that anti-PD-1 ab therapy induces the upregulation of Wnt5a and YAP1 in BRAF(V600E)-PTEN^{-/-} melanomas. bottom, Dot blot analysis shows that CXCL5 expression is upregulated in the serum of transgenic BRAF(V600E)-PTEN^{-/-} mice undergoing treatment with anti-PD-1 ab therapy (red, blot density analysis). Representative blots shown. All data is mean +/- SEM. P<0.05.

Figure 3. The Wnt5a-CXCL5 Pathway Drives Gr-MDSC Recruitment in Response to anti-PD-1 Ab Therapy and Promotes the Development of anti-PD-1 Ab Adaptive Resistance

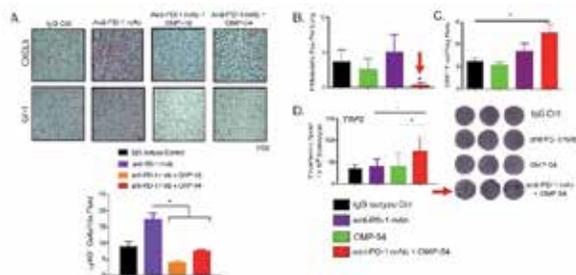


A. Flow cytometry analysis of Gr-MDSCs in Wnt5a-silenced BRAF(V600E)-PTEN^{-/-} melanomas. B. Immunohistochemistry of Gr-MDSCs in Wnt5a-silenced BRAF(V600E)-PTEN^{-/-} melanomas. C. Flow cytometry analysis demonstrates that Gr-MDSC recruitment in response to anti-PD-1 ab therapy is ablated in Wnt5a-silenced BRAF(V600E)-PTEN^{-/-} melanomas. Representative flow cytometry dot plots provided. D. IFN γ ELISPOT and tissue immunofluorescence show that melanoma Wnt5a silencing enhances the activation of TRP2-specific CD8+ T cells and promotes the expression of PD-L1.

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Representative images provided on the left. E. Wnt5a silencing sensitizes BRAF(V600E)-PTEN^{-/-} melanomas to anti-PD-1 ab therapy. F. CXCL5 silencing suppresses Gr-MDSC recruitment and sensitizes BRAF(V600E)-PTEN^{-/-} melanomas to anti-PD-1 ab therapy. Representative flow cytometry dot plots shown. All data is mean \pm SEM. $P < 0.05$.

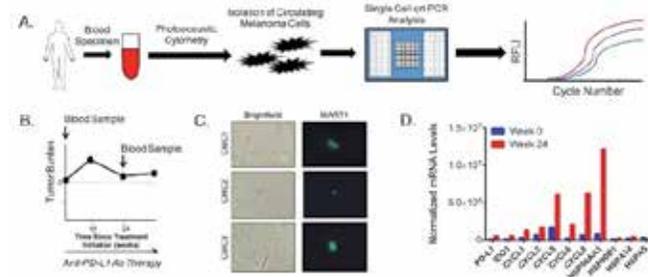
Figure 4. Inhibition of Wnt Signaling Suppresses Gr-MDSC Recruitment in Response to anti-PD-1 Ab Therapy and Augments the Efficacy of this Checkpoint Inhibitor in a Transgenic BRAF(V600E)-PTEN^{-/-} Melanoma Model



A. Immunohistochemistry of CXCL5 and Gr-1 in BRAF(V600E)-PTEN^{-/-} melanoma tissue undergoing treatment with anti-PD-1 ab therapy in the absence and presence of OMP-18R5 (vantictumab, anti-Fzd7 ab) or OMP-54F28 (ipafricept, Fzd8-Fc). B. Histologic analysis of resected lung tissues to enumerate lung metastases in the transgenic BRAF(V600E)-PTEN^{-/-} melanoma model following the indicated treatment regimens. C. Flow cytometry analysis of BRAF(V600E)-PTEN^{-/-} melanoma tissue following the indicated treatment regimens. CD45⁺CD3⁺CD8⁺ T cells and CD45⁺CD4⁺FoxP3⁺ regulatory T cell (Treg) populations quantitated and expressed as a CD8⁺ T cell : Treg ratio. D. TRP2 IFN γ ELISPOT analysis performed using harvested splenocytes from transgenic BRAF(V600E)-PTEN^{-/-} mice after undergoing the indicated therapy. Representative wells shown on the right. All data is mean \pm SEM. $P < 0.05$.

Figure 5. Circulating Melanoma Cell (CMC) Qrt-PCR Analysis Demonstrates Enhanced CXCR2 Ligand

Expression in Progressing Melanoma Patients Following anti-PD-1/anti-PD-L1 Ab Therapy



A. Schematic showing clinical specimen acquisition and analysis work flow. Samples are harvested from patients before and during checkpoint inhibitor immunotherapy. CMCs are enriched through centrifugation, analyzed by the photoacoustic flow cytometer, and subjected to integrated fluidic chip single cell qrt-PCR analysis of select target genes. Samples positive for CD45 or negative for both MART1 and S100 are excluded from analysis. Target gene expression normalized to Ubb expression levels. 6 CMCs analyzed per patient time point. B. Spider plot of target lesion tumor volume versus time for an advanced melanoma patient undergoing anti-PD-L1 ab therapy. Blood samples taken at week 0 prior to therapy and week 24. C. MART1 immunofluorescence analysis of captured CMCs. Brightfield images shown on left. D. CMC single-cell qrt-PCR analysis and quantitation of CXCR2 ligands and other target genes of interest in the same patient described in B. Data has been normalized to week 0 expression levels of respective target genes.

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Tracking the dynamic response to vaccine prime/oncolytic boost immunotherapy identifies key mechanisms of immune resistance in metastatic ovarian cancer

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Background

While spectacular responses to cancer immunotherapy have been observed, most responses are short-lived with ultimate tumor relapse. Using a vaccine prime/oncolytic boost immunotherapy, we sought to define robust biomarkers indicative of therapeutic response, response durability, as well as changes leading to tumor relapse in a pre-clinical mouse model of intraperitoneal metastatic ovarian cancer.

Methods

We utilized a heterologous prime/boost vaccine to treat ovarian cancer, combining an adjuvant-based vaccine with a novel oncolytic Maraba viral vector to generate robust anti-tumor T cell responses. To investigate treatment impact on both anti-tumor immunity and local events within the tumor, we combined analyses of immune cells within the tumor microenvironment with transcriptional profiling of solid tumors, and pre-clinical MRI to directly monitor changes in intraperitoneal tumors in response to therapy. This approach led to the identification/testing of candidate combination treatments with the potential to improve prime/boost vaccination.

Results

Heterologous prime/boost vaccination elicited robust tumor-specific CD8+ T cell responses, with high numbers of therapy-induced CD8+ T cells effectively trafficking to the tumor microenvironment. This approach greatly improved tumor control and long-term survival compared to vaccine priming alone, however the combination therapy was not curative and cellular analysis suggested that T cells within the tumor microenvironment were functionally suppressed. Tumor transcriptional profiling revealed that prime/boost vaccination led to induction of

numerous inflammatory processes, with gene signatures consistent with CD8+ T cell infiltration, upregulation of multiple co-stimulatory/ immune checkpoint receptors, and induction of chemokine networks associated with lymphoid and myeloid cell trafficking. Based on these signatures, we tested candidate combination therapies to further enhance the impact of prime/boost vaccination, of which checkpoint blockade using α PD-1 produced the greatest benefit. Analysis of the response to prime/boost vaccination + α PD-1 by MRI revealed significant intratumoral inflammation after Maraba boosting followed by dramatic tumor regression, consistent with pseudo-progression. Tumors were effectively controlled, but ultimately relapsed. Using MRI guided assessment, treated tumors were subsequently isolated during all phases of this response and subjected to RNAseq analysis. The findings from these studies will be presented.

Conclusions

Our data suggest that interrogating the tumor microenvironment during the course of vaccine prime/oncolytic boost immunotherapy can identify candidate therapeutic targets, as well as key biomarkers of therapeutic response and/or intratumoral changes leading to tumor progression.

P387  **Abstract Travel Award Recipient**

IFN γ signaling in T_{regs} acts as a barrier to immunotherapeutic response.

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Background

Checkpoint blockade (anti-PD1/PDL1) has displayed striking, durable responses in cancer patients; however, many still succumb to disease. The underlying reasons for this lack of response remain obscure. Therefore, identifying resistance mechanisms to current immunotherapies is critical. While regulatory T cells (T_{regs}) are required to maintain immune homeostasis, they are also a major barrier to the anti-tumor immune response, and as such, are an attractive therapeutic target. We have previously shown that intratumoral T_{reg} stability is reliant on the Neuropilin-1 (Nrp1):Semaphorin-4a axis. In its absence, T_{regs} become fragile and secrete IFN γ while maintaining *Foxp3* expression [1,2]. However, 1) whether T_{reg} fragility is required for response to immunotherapy, and 2) the underlying T_{reg} -mediated mechanisms of immunotherapeutic resistance in mice and patients remain unclear.

Methods

We utilized syngeneic tumor models, including MC38 (anti-PD-1 sensitive) and B16.F10 (anti-PD-1 resistant) in both *Foxp3*^{Cre-YFP} and *Ifngr1*^{L/L}*Foxp3*^{Cre-YFP} mice. Mice were treated with various immunotherapies to assess the role of IFN γ signaling by T_{regs} in mediating resistance. In addition, we are assessing the direct effect of IFN γ *in vivo* through the use of novel delivery mechanisms. Lastly, we are determining the impact of T_{reg} fragility on patient response rate after PD-1 blockade in metastatic melanoma and head and neck squamous cell carcinoma.

Results

Using *Foxp3*^{Cre-YFP} and *Ifngr1*^{L/L}*Foxp3*^{Cre-YFP} mice, we found that response to PD-1 blockade required IFN γ -induced T_{reg} fragility, as *Ifngr1*^{L/L}*Foxp3*^{Cre-YFP} mice treated with anti-PD1 were completely resistant to therapy. In addition, PD-1 blockade led to increased IFN γ secretion by T_{regs} , an effect that was absent in *Ifngr1*^{L/L}*Foxp3*^{Cre-YFP} mice. Furthermore, pre-treatment of tumor-derived T_{regs} with IFN γ led to significantly reduced suppressive function in both mouse and human T_{regs} . We are

currently extending our studies to patients treated with anti-PD1 in order to determine whether T_{reg} fragility is associated with immunotherapeutic response.

Conclusions

Overall, we have shown that IFN γ -induced T_{reg} fragility is required for anti-PD1 response and that IFN γ leads to reduced T_{reg} suppressive capacity while maintaining *Foxp3* expression. These studies uncover a novel potential resistance mechanism to immunotherapy, and provides an avenue to target T_{regs} selectively in the tumor microenvironment to bolster current immunotherapies.

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Neutrophil-rich inflammation and PD1/PD-L1 inhibitor resistance in advanced non-small cell lung cancer patients

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Background

The pro-tumoral role of neutrophils in chronic inflammation in carcinogenesis and metastasis has been well-reported in the animal models in the literature.[1] However, the role of neutrophil-to-lymphocyte ratio (NLR) and the acquired resistance to PD1/PD-L1 inhibitors (PD1/PD-L1i) in advanced

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non-small cell lung cancer patients (NSCLC) has not been described.

Methods

We retrospectively evaluated 115 advanced NSCLC patients who received PD-1 and PD-L1 inhibitors as their second-line therapy after platinum-based chemotherapy. Two groups were defined: Progressors (P) as patients who had radiographic and clinical progression after at least 2 months of treatment and Clinical Durable Responders (CDR) as patients with at least treatment duration of 6 months and ongoing response. Patients with baseline NLR > 10 were excluded. We performed paired-samples T test to analyze the difference of each patient's NLR at baseline and at progression in P group. In CDR group, the difference of each patient's NLR at baseline and at censored time while receiving ongoing PD-1/PD-L1i above 6 months were compared. Mann-Whitney test was performed to compare individual patient's NLR at baseline and at progression with the presence of bone metastasis.

Results

There were 72 patients in P group and 43 patients in the CDR group. Total 82 patients received nivolumab, 25 patients received pembrolizumab, and 8 patients received atezolizumab. The duration of clinical response in P ranged from 2 to 17.4 months and 6.5 to 23.8 months in CDR group. The mean NLR at baseline in P group was 4.89 and 6.59 at progression. Patients in P group had statistically significant increases of NLR at progression with mean increase of 1.69. The 95% confidence interval was 3.04 to 0.35 (p-value = 0.01). However, there was no statistical difference between the NLR at baseline and censored time while receiving ongoing treatment in CDR group (95% C.I.: -1.90 - 0.822, p-value=0.428). Bone metastasis did not correlate with the degree of NLR. (p=0.56) Patients who received corticosteroid at the time of progression were not included in the study.

Conclusions

Persistent increase of NLR may represent unfavorable pro-tumoral inflammation and can represent unfavorable peripheral balance of PD-1 and PD-L1 interplay in myeloid-effector T cell immune synapse. This may serve as acquired resistance mechanism of PD-1/PD-L1i and warrants further investigation.

References

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Aurora kinase inhibition enhances the efficacy of immunotherapy

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Background

Immunotherapy has improved clinical outcomes for a subpopulation of melanoma patients, but over

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half of treated patients develop primary or secondary resistance. Current efforts are thus focused on developing combinatorial strategies involving immunotherapy and other treatment modalities, such as targeted therapy, to improve clinical outcomes. We aimed to identify factors that regulate the tumor cell response to T cell mediated cytotoxicity, with the goal to develop more efficacious treatment combinations.

Methods

A high-throughput screen of 850 bioactive compounds was used to identify proteins/pathways that confer resistance to T cell mediated cytotoxicity (Figure 1A). A comboscore was calculated for apoptosis (cleaved caspase-3) induced by combined compound and T cell treatment compared to either treatment alone (Figure 1B). A library of 576 ORF was subsequently used to identify proteins that induce resistance to T cell mediated cytotoxicity. The genes with the lowest comboscore in the ORF screen were analyzed using Nanostring on 23 samples from adoptive cell therapy (ACT) treated melanoma patients. One of the hits was further validated using *in vitro* and *in vivo* assays.

Results

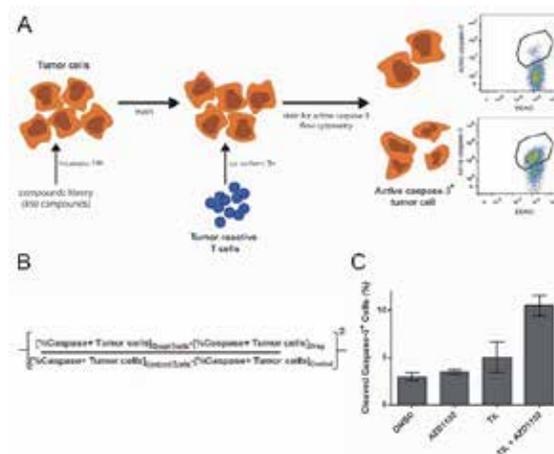
After challenging melanoma cell lines with the compounds and autologous TILs, Aurora kinase inhibitors (AURKi) obtained a high comboscore. Two Aurora kinase inhibitors were validated to synergize with T cell mediated cytotoxicity in multiple melanoma cell lines (example of enhanced cytotoxicity in one cell line shown in Figure 1C). Complementary to this finding, Aurora kinase A (AURKA) and AURKB overexpression induced resistance to T cell cytotoxicity. Additionally, patients who did not respond to ACT expressed higher levels of AURKA compared to responding patients ($p=0.04$), further demonstrating the relevance of Aurora kinase in immune suppression. The combination of AURKi AZD1152 and anti-CTLA4 significantly reduced tumor growth ($p<0.05$) and enhanced survival ($p<0.01$) as compared to either therapy alone in an MC38/gp100 *in vivo* model. Ongoing studies are now aimed at identifying the

molecular mechanisms by which AURKi enhance T cell mediated cytotoxicity.

Conclusions

Aurora kinase inhibition may not be cytotoxic per se, but we showed that AURKi treatment sensitizes tumor cells to T cell mediated cytotoxicity and enhances the efficacy of immunotherapy. The combination of AURKi treatment and immunotherapy may thus induce an effective and durable response in melanoma patients, making Aurora kinase inhibitors a promising combination strategy with immunotherapy, especially for patients who do not respond to immunotherapy alone.

Figure 1. Aurora kinase inhibitors were identified to enhance T cell mediated cytotoxicity



(A) Patient derived melanoma tumor cells were incubated with one of 850 different compounds per well in triplicate in a high-throughput screen. After 24 hours, the compound was washed off and the tumor cells were co-cultured with autologous TILs for three hours, followed by intracellular staining for cleaved caspase-3. The percentage caspase-3 positive tumor cells is used as a read-out for the amount of apoptosis among DDAO stained tumor cells. (B) The comboscore is calculated to assess the enhanced cytotoxicity of combined compound and TIL treatment over either treatment alone. (C) Mel2549 cells were treated with 2uM AZD1152 or

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DMSO, TIL at a 3:1 E:T ratio or the combination of AZD1152 and TIL. Four other patient derived melanoma cell lines showed similar enhanced cytotoxicity for the combination treatment.

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Combination radiotherapy and α OX40/ α CTLA-4 immunotherapy reverses anergy and prevents development of functional exhaustion within tumor-specific CD8 T cells

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Background

The immunosuppressive tumor microenvironment and chronic T cell stimulation that occurs in the presence of cancer results in CD8 T cell dysfunction that has been difficult to reverse with immunotherapy (IT) alone. This dysfunction can be separated into two categories of anergy and exhaustion. Anergy being a poor proliferative response to stimulation after initial priming with inadequate costimulation or suboptimal T cell receptor ligation, and functional exhaustion being the inability of T cells to kill or produce effector cytokines in response to stimulation. We hypothesized the addition of ionizing radiotherapy (RT) to IT with agonist α OX40 & blocking α CTLA-4 antibodies would reverse tumor associated CD8 T cell anergy and exhaustion.

Methods

We utilized a model of CD8 T cell anergy in which transgenic, OVA-specific, OT-I CD8 T cells are adoptively transferred into centrally tolerant POET-1 mice bearing OVA expressing tumors. After development of anergy/exhaustion, 21 days after transfer, animals were treated with a single 20 Gy fraction of RT, α OX40/ α CTLA-4, or combined IT/RT.

To assess anergy/exhaustion in endogenous CD8 T cell responses, CT26 colon carcinoma bearing BALB/c mice were treated with IT, RT, or combined IT/RT either 10 or 17 days after tumor implantation. The frequency/function of tumor-specific (OT-I or AH1) CD8 T cells was monitored in blood, lymph node (LN), and tumor infiltrating lymphocytes (TIL). Reporter Nur77-GFP-OT-I CD8 T cells expressing green fluorescent protein in response to TCR ligation were used to measure TCR signaling following IT/RT.

Results

We demonstrate OT-I cells become anergic/exhausted and produce few effector cytokines 21 days after adoptive transfer. Combination therapy with IT/RT resulted in increased TCR stimulation (measured by GFP expression) and reversed anergy (measured by proliferation) within OT-I T cells in blood, LN, and TIL compared to IT or RT alone. Combination therapy was able to reverse functional exhaustion (measured by cytokine production) within LN but not TIL in this model. In CT26 tumor bearing mice combination IT/RT significantly increased (up to 4-fold) the frequency of tumor-specific endogenous AH1 CD8 TIL and prevented development of functional exhaustion within TIL compared to IT or RT alone.

Conclusions

Combined ablative RT and IT with α OX40/ α CTLA-4 results in increased CD8 TCR signaling, reversal of T cell anergy, and can prevent or reverse functional exhaustion in some cases. These novel results suggest the addition of RT to IT can effectively reverse tumor-associated T cell dysfunction where IT alone is insufficient and provide rationale for early phase clinical trial design.

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Non-conventional inhibitory CD4⁺Foxp3⁻PD-1^{hi} T cells as a biomarker of immune checkpoint blockade activity

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Background

A significant proportion of cancer patients do not respond to immune checkpoint blockade therapy. To deepen our understanding of the mechanisms of action of this strategy, we explored the role of a subset of CD4⁺Foxp3⁻ T cells expressing PD-1 (4PD1hi), which we found to be up-regulated after CTLA-4 blockade in association with limited response to treatment.

Methods

4PD1hi frequency was monitored by flow cytometry. Their function was tested in standard in vitro suppression assays and 3D collagen-fibrin gel killing assays. RNAseq gene expression analyses were performed on a Proton sequencing system at the MSK Genomics Core Facility.

Results

We observed that 4PD1hi accumulate intratumorally as a function of tumor burden in untreated tumor-bearing hosts. In addition, mouse and human circulating and intra-tumor 4PD1hi inhibit T-cell functions in a PD-1/PD-L1 dependent fashion. Interestingly, CTLA-4 blockade promotes intratumoral and peripheral 4PD1hi increases in a dose-dependent manner, while combination with PD-1 blockade mitigates this effect and significantly improves anti-tumor activity. In keeping with these observations, we found that patients have a significantly higher risk of death if high 4PD1hi levels persist after PD-1 blockade. Mechanistically, we

provide evidence that mouse and human 4PD1hi resemble follicular-helper-T-cell(TFH)-like cells and that CTLA-4 blockade increases B-cell co-stimulatory potential in vivo and in vitro, pointing to 4PD1hi as a possible downstream effect of enhanced T-cell priming during CTLA-4 blockade.

Conclusions

These findings broaden our understanding of the incremental activity of combination checkpoint blockade. They also provide a new pharmacodynamic and prognostic biomarker that may inform the design of optimal combination schedules and checkpoint blockade dosage.

Microbiome

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Microbiome analyses in patients with previously treated, deficient DNA mismatch repair/microsatellite instability-high metastatic colorectal cancer treated with nivolumab ± ipilimumab: CheckMate 142

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Background

Microbiota may have a role in the carcinogenesis of colorectal cancer (CRC) through chronic low-grade

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inflammation and the influence of microbiota-derived metabolites on host metabolism and immune function.[1,2] Microbiome diversity was significantly different between patients with melanoma who responded to anti-PD-1 therapy and those who did not respond.[3] Here, we explore the relationship between microbiota and response to nivolumab ± ipilimumab in patients with deficient DNA mismatch repair/microsatellite instability-high (dMMR/MSI-H) CRC enrolled in CheckMate 142 (NCT02060188).

Methods

Patients received nivolumab 3 mg/kg Q2W or nivolumab 3 mg/kg + ipilimumab 1 mg/kg Q3W × 4 doses followed by nivolumab 3 mg/kg Q2W until discontinuation. 16S ribosomal RNA sequencing was performed on baseline stool samples. Alpha diversity and differential measures of operational taxonomic units were evaluated at each taxa level. Microbiota differences were compared between patients with an investigator-assessed best overall response (BOR) of partial response (PR) vs those with a BOR of progressive disease (PD). Due to small patient numbers, those treated with nivolumab and nivolumab + ipilimumab were combined for the analysis.

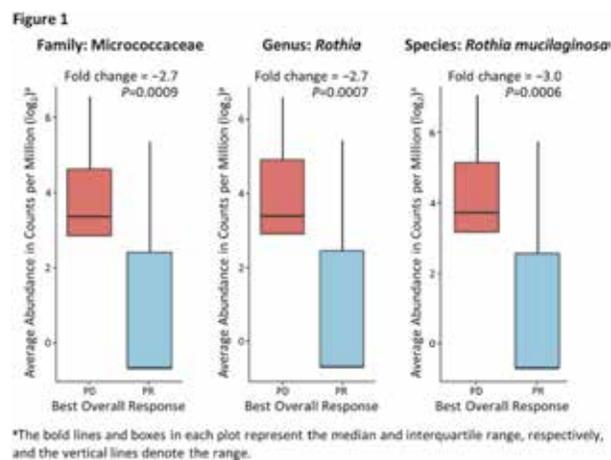
Results

Baseline stool samples were collected from 72 patients. Among these patients, BORs of PR were achieved in 25, stable disease in 31, and PD in 14; 2 were not evaluable. Alpha diversity was not statistically different between patients with a BOR of PR vs those with PD. The abundance of Actinobacteria Micrococcaceae was 2.7-fold lower in patients with a BOR of PR compared with patients with a BOR of PD ($P=0.0009$; Figure 1); significant differences were also observed in the genus *Rothia* (2.7-fold lower in patients with a PR; $P=0.0007$). These results remained significantly different ($P=0.04$) after corrections for false discovery rate. Analyses on additional microbiota will be presented.

Conclusions

Patients with dMMR/MSI-H CRC who achieved a BOR of PR with nivolumab ± ipilimumab treatment were found to have a lower abundance of Actinobacteria Micrococcaceae, and in particular, the genus *Rothia*, compared with those with a BOR of PD. These results suggest that there may be differences in the gut microbiota of patients with dMMR/MSI-H CRC who respond to treatment with checkpoint inhibitors vs those who progress. Additional analyses investigating correlations with tumor immune phenotype and microenvironment, and other clinical parameters, are warranted.

Figure 1.



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Association of microbiome to nivolumab response in metastatic renal cell carcinoma (mRCC)

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Regular Abstract Poster Presentations

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Background

Checkpoint inhibitors (CPI) have improved clinical outcomes in various cancer types, including mRCC [1]. Predictive factors of benefit from CPIs are not well defined. Relationship of the microbiome to benefit from CPIs has been suggested in preclinical studies [2]. Here we provide the first evidence of association between microbiome composition and response to nivolumab in mRCC patients (pts).

Methods

Stool samples were prospectively collected from pts with mRCC at 3 time points relative to nivolumab treatment (baseline, week 4 and week 12) and used to assess gut microbiota composition in two different groups: responders (R; including complete/partial response and stable disease) and non-responders (P; primary progression). In short, microbial DNA was extracted, 16s rRNA gene tags (v4) were generated by PCR amplification and sequenced using MiSeq (Illumina). Sequence reads were processed by Mothur software, assembled in operational taxonomic units (OTUs), taxonomically annotated to the level of genus and used to construct Bray-Curtis dissimilarity matrices. The similarity of samples was visualized by principal coordinates analysis (PCoA) and further confirmed by k-means clustering (k=2) and ANOSIM tests. Differentially abundant taxa were determined by METASTATS and compared between R and P.

Results

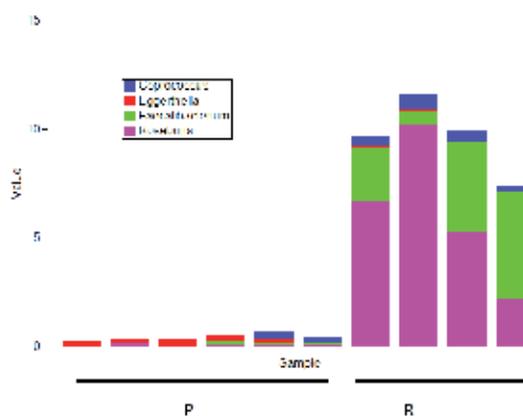
Of 11 pts, 9 were evaluable for response. In this study, 25,304 OTUs were attributed to 165 genera from 8 phyla. PCoA analysis reveals that the first two principal coordinates can explain 49.2% of data set variation. Subsequent k-means clustering shows an almost complete separation of microbiota in R and P groups. The produced clusters almost

perfectly aligned with R and P groups, although ANOSIM of this separation was not significant ($p=0.07$). The analysis of microbiota membership in P and R groups revealed 4 differentially abundant taxonomic units at the genus level (Figure 1), with 2 present above 1% abundance. Namely, *Roseburia* spp and *Faecalibacterium* spp were significantly elevated in R as compared to P ($p<0.05$). Notably, no significant differences were noted in *Bifidobacterium* spp or *Bacteriodes* spp in R and P ($P=0.15$ for both).

Conclusions

Our results are the first to associate specific microbial genera to nivolumab response in mRCC and warrant confirmation in larger series. Furthermore, manipulation of the stool microbiome as a means of modulating nivolumab response should be investigated.

Figure 1. Relative abundance of selected genera demonstrating significant differences between responders (R) and progressors (P).



P: Primary progression group. R: Responders group.

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P394  **Abstract Travel Award Recipient**

Commensal microbiota associated with anti-PD-1 efficacy in metastatic melanoma patients

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Background

Anti-PD-1-based immunotherapy is having a major impact on cancer outcomes, but only a subset of patients respond favorably. To predict response and ultimately improve therapeutic efficacy, it is of interest to identify variables that cause this inter-patient heterogeneity. Pre-clinically, commensal bacterial species have been demonstrated to modulate spontaneous anti-tumor immunity and the outcome of immunotherapy and chemotherapy in mice (1-3). Here, we examine the association between gut microbiota composition and response to anti-PD-1 immunotherapy in patients with melanoma.

Methods

Stool samples were collected from 42 metastatic melanoma patients prior to immunotherapy and microbial composition was analyzed through an integration of 16S rRNA gene sequencing, metagenomic shotgun sequencing, and quantitative PCR for selected bacteria. Clinical response was determined using Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1. Germ-free mice were colonized with stool from responding and non-responding patients, and tumor growth kinetics and anti-tumor immune responses were evaluated.

Results

There were 16 responders and 26 non-responders. There was a significant association between the

commensal microbiota composition and clinical response. Sixty-two operational taxonomic units (OTUs) assigned from the 16S rRNA gene sequencing dataset were differentially abundant in responders relative to non-responders. Forty-three of these OTUs were assigned potential species-level identities, based on metagenomics sequencing of the same stool samples. Selected responder-associated species, including *Bifidobacterium longum*, *Akkermansia muciniphila*, *Collinsella aerofaciens*, and *Enterococcus faecium* were also confirmed by quantitative PCR. Reconstitution of germ-free mice with fecal material from a responding patient led to improved tumor control, augmented T cell responses, and a distinct microbiota compared to a non-responding patient.

Conclusions

Our results indicate a correlation between the abundance of certain gut bacteria and clinical response to anti-PD-1 immunotherapy in cancer patients, and suggest a mechanistic impact of the commensal microbiota on anti-tumor immunity

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DNA repair genetic aberrations and tumor mutation burden in biliary tract cancers

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Regular Abstract Poster Presentations

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Background

Immune checkpoint inhibitors (ICPI) has been approved for several cancers. The association between tumor mutational burden (TMB) and ICPI has been previously studied and high TMB is associated with prolonged progression free survival (PFS) after ICPI. DNA repair gene aberrations (GAs) including those leading to microsatellite instability (MSI) is associated with response to ICPI. Data regarding this in biliary tract cancers (BTC) are limited.[1]

Methods

A comprehensive genomic profiling of 422 formalin-fixed, paraffin-embedded tumor tissue from BTC patients, including 92 gall bladder cancer and 330 cholangiocarcinoma, was performed using next generation sequencing. We included 20 DNA repair genes that were previously described.[1] These included direct DNA repair genes (*ATM*, *ATR*, *BRCA1*, *BRCA2*, *FANCA*, *FANCD2*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *POLD1*, *POLE*, *PRKDC*, *RAD50*, *SLX4*) and caretaker genes that induce genomic instability (*BAP1*, *CDK12*, *MLL3*, *TP53*, *BLM*). TMB was calculated in 205 samples by counting the number of mutations across a 1.25Mb region and classified into three groups; low (< 6 mutation/Mb), intermediate (6 – 19 mutation/Mb), and high (≥20 mutation/Mb). We assessed MSI status by a computational algorithm examining 114 intronic homopolymer loci.

Results

Direct DNA repair genes were identified in 61 BTC tumors (14.5%) including GAs in *ATM* (5.5%) or *BRCA2* (2.1%). Majority of the tumors had GAs in caretaker genes (42.9%), predominately in *TP53* (33.4%), and *BAP1* (9.5%). However, *FANCD2*,

POLD1, *POLE*, *PRKDC*, *RAD50*, *SLX4*, *BLM* mutations were not identified in our cohort. TMB was evaluated in 205 patients. Among patients with direct DNA repair GAs, 20 out of 35 (57.2%) had intermediate or high TMB as compared with 47 out of 170 (27.7%) patients without direct DNA repair GAs ($P < .0001$). Patients with caretaker GAs also had significantly higher TMB [intermediate and high (48.4%)] than patients without these mutations ($P < .0001$). MSI was determined in 88 patients and classified as stable, ambiguous, and high. All patients with MSI high had direct DNA repair GAs ($P < .0001$).

Conclusions

DNA repair GAs occur at a relatively high frequency in BTC and are associated with higher TMB and MSI. Future clinical trials targeting this subgroup with ICPI are warranted.

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P396

Evolving immune response in lung squamous carcinogenesis

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Background

Molecular characterization of pre-invasive and invasive bronchial lesions can provide insights into the mechanisms behind genesis and development of squamous cell carcinoma, revealing potentially promising new biomarkers for early detection and treatment of lung cancer.

Methods

At 8 different morphological stages of lung squamous carcinogenesis, from normal, to low grades dysplasia, high grades dysplasia, to carcinoma, fresh frozen human bronchial biopsies (n=122) were subjected to gene expression profiling (Agilent microarrays) from 77 patients. A linear mixed-effects model was applied to infer modules of gene co-expression, adjusted for smoking status, gender, and cancer history as fixed effects, and patient as a random effect. Immune cell-specific gene signatures were used to characterize the immune response through the different development stages.

Results

The gene expression alterations could distinguish four groups of successive stages of development. There were eight modules of co-expressed genes with different expression patterns across the stages: up- or down-regulation among the early or late steps, linear increase or decrease in expression, and a biphasic pattern. Transitioning to early grade, there was a depletion of immune gene expression. However, at the transition to high-grade dysplasia, there was a significant up-regulation of immune/inflammatory response genes, representing T helper 1, myeloid cells, as well as co-inhibitory and co-stimulatory molecules.

Conclusions

At a critical stage of carcinogenesis there is a significant modification of the immune response. The up-regulation of immune genes in high-grade suggests a major role of the surrounding microenvironment thorough adaptive and innate immune response, but also a tumor versus host immunosuppressive response before tumor invasion across the basal membrane. This analysis may identify promising new immune signatures of carcinogenesis, and may provide novel therapeutic targets for lung cancer.

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Withdrawn

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Tumor mutation load assessment of FFPE samples using an NGS based assay

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Background

Background – Tumors with high mutation load respond to therapy with immune checkpoint inhibitors. Hence high mutation load of a tumor can act as a predictive biomarker to immuno-therapy. We developed an Ion S5 Sequencing System assay to determine the mutation status of solid tumors.

Methods

Methods: Our assay uses an FFPE sample, utilizing an Ampliseq based target panel that covers 1.7 Mb of genomic DNA including 409 genes. The assay does not require matched normal sample to estimate the mutation status of a tumor.

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Results

Results: Our panel exhibits >90% sensitivity and >95% specificity in distinguishing between high and low mutation burden samples. Using a variety of public annotation sources, we have successfully eliminated approximately 98% of germline variants. Matched tumor-normal analyses on 9 lung and colorectal samples suggested that our single sample analysis on tumor samples detects mutation load with strong correlation ($r=0.9358$) with tumor-normal analysis. On engineered control from Acrometix, mutation load count consistently comprise true mutations with positive predictive value of 89%. To assess reproducibility, we compared the mutation load in library replicates for a cohort of 10 samples (FFPE and fresh frozen tumors, Acrometrix control, and NIST cell-lines) and observed high correlation ($r=0.9948$).

Conclusions

We have developed a single tumor-only assay for accurate estimation of tumor mutation burden, useful for cancer immuno-therapy research and development

P399

Rationally improving T cell-mediated cancer immunotherapy using *Sleeping Beauty* mutagenesis to identify novel drug targets

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Background

T cells have amazing potential to eliminate tumor cells throughout the body, evidenced by the clinical success of immunotherapies designed to enhance T cell function like immune checkpoint blockade and chimeric antigen receptor T cell (CAR-T) therapies. Expanding success to a broader number of patients is a top priority in the field. T cell infiltration into tumors appears to be an important prerequisite for the success of both immune checkpoint blockade and CAR-T therapies. Thus, increasing T cell

infiltration into tumors could have beneficial therapeutic impact.

Methods

We have designed a forward genetic screen to identify T cell genes that contribute to intratumoral T cell accumulation using *Sleeping Beauty* (SB) transposon insertional mutagenesis in T cells. Our systematic screen approach allows us to evaluate both loss- and gain-of-function mutations across the entire genome in immunocompetent melanoma and lymphoma models that preserve the complexity of the tumor microenvironment. Importantly, the vast majority of genes we identified were not previously known to be involved in tumor-associated T cell biology, and likely would not have been considered as drug targets to enhance immunotherapy. These genes have the potential to modify important T cell functions including trafficking to the tumor, clonal expansion, and sustained viability once inside the tumor.

Results

We identified 312 tumor-enriched genes that were mutated in tumors, but not the spleen, of individual mice. Twenty of these were detected in more than one mouse, representing strong gene candidates for validation. We demonstrate that one gene candidate is functionally associated with T cell response to activation signals. Specifically, CRISPR-mediated knockout of this gene in a murine T cell line resulted in cytokine production defects after T cell receptor stimulation. Additional screening has begun using other tumor models and anti-PD-1 therapy. Preliminary data from these indicate that few genes are conserved across tumor models and treatment groups.

Conclusions

We are currently investigating the role of candidate genes in intratumoral T cell accumulation and immunotherapy enhancement using an *in vivo* approach. Together, these experiments have the potential to expand our understanding of T cell infiltration into tumors and may provide previously

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unexplored strategies to rationally enhance immunotherapy efficacy.

P400

Evaluation of tumor genetics and microenvironment through next-generation sequencing (NGS)

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Background

The effectiveness of cancer immunotherapy is determined by the genetic basis of the patient's tumor as well as the state of the tumor microenvironment. For instance, tumor mutation burden (TMB) and microsatellite instability (MSI) status correlate with patient response to checkpoint inhibitors [1]. Also, the presence of immune cells in the tumor microenvironment correlates with immunotherapy responsiveness. Thus, this project aims to develop a next-generation sequencing (NGS) workflow that reports various biomarkers that can be used to guide cancer immunotherapy selection. Here we present our data on using whole exome sequencing (WES) and whole transcriptome sequencing (WTS) to report human leukocyte antigen (HLA) sequence, TMB load, prioritized neoantigens, and presence of immune cells.

Methods

WES and WTS libraries were generated using Illumina's TruSeq[®] Exome and RNA Access library preparation kits, respectively. The samples were paired-end sequenced (2x75bp) using HiSeq[®] 2000 and 2500 instruments.

Results

Here we demonstrate a workflow using tumor/normal WES and tumor-only WTS to determine MSI status and expressed TMB load. We also show data that WES can be used to haplotype

HLA Class I genes with 97% accuracy. Using the HLA haplotype sequence, we computationally predicted putative neoantigens and experimentally validated a subset, as true binders to their respective major histocompatibility complex. In addition, we applied FRICTION, a novel algorithm for cell deconvolution, to WTS data extracted from bulk tumor to quantify the fraction of immune cells present in the tumor microenvironment. Our titration experiments demonstrated our method's linearity in quantifying CD4⁺ T cells, CD8⁺ T cells, and CD19⁺ B cells in a variety of tissue backgrounds (median R² > 0.97). Finally, we applied FRICTION to WTS data from melanoma samples extracted from different subjects and showed that our workflow could also quantify the fraction of immune cells in these samples when compared to quantitation by flow cytometry.

Conclusions

In summary, we demonstrate the utility of a NGS workflow that uses WES and WTS to report biomarkers relevant to immunotherapy and characterize the inflammation status of the tumor microenvironment.

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Oncolytic Viruses and Intratumoral Therapies

P401

Intratumoral expression of IL12 using the ZVex[®] dendritic cell-targeting lentiviral vector exerts potent anti-tumor effects via induction of multiple immune effectors, including CD8 T cell responses

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Regular Abstract Poster Presentations

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Background

Interleukin 12 (IL12), produced by antigen-presenting cells, plays a pivotal role in the interplay between the innate and adaptive arms of the immune system. IL12 treatment has been shown to augment cytotoxic T lymphocyte and T_H 1 responses and anti-tumor effects. However, its use as a systemic therapeutic agent is limited due to toxicity. Intratumoral administration of IL12 is therefore being explored as an alternative route. We have previously reported on the strong anti-tumor effects of IL12 delivered intratumorally using Immune Design's dendritic cell-targeting lentiviral platform ZVex in various murine tumor models. Here, we evaluated the mechanism of action mediating the local and systemic immune responses induced by intratumoral ZVex/IL12 in different animal models.

Methods

Tumor cell lines (B16 melanoma, CT26 colon carcinoma, 4T1 breast cancer, A20 lymphoma, P815 mastocytoma, GL261 glioma) were implanted subcutaneously into mice. ZVex was engineered to express murine IL12 and administered as a single intratumoral injection into palpable tumors (50-100 mm³), typically at 7 days post-tumor inoculation. Mice were monitored twice weekly for tumor growth and survival. In lymphocyte depletion studies, anti-CD8, anti-CD4 or anti-NK1.1 antibodies were administered twice weekly, and their effect on immediate and long-term anti-tumor response was studied.

Results

A single intratumoral injection of ZVex/mIL12 induced complete tumor regression in B16, CT26, A20, GL261, and P815 models, delayed tumor growth in the 4T1 model. In mice depleted of either CD8 T, CD4 T, or NK cells, complete tumor regression was still observed in 13-40% of mice. However, only depletion of CD8 T cells together with CD4 T and/or NK cells completely abrogated the anti-tumor response, suggesting that CD8 T cells are required but not sufficient effectors in

ZVex/mIL12-mediated anti-tumor control. Furthermore, depletion of CD4 T cells resulted in very pronounced vitiligo, demonstrating that intratumoral ZVex/mIL12 profoundly breaks self-tolerance, likely in the absence of regulatory T cells.

Conclusions

Taken together, the results demonstrate that intratumoral administration of IL12 induces complex protective cellular immune responses, requiring CD8 effector T cells for maximum efficacy.

P402

Chemotherapy enhances oncolytic recombinant poliovirus efficacy

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Background

Oncolytic viruses are capable of direct tumor lysis along with the ability to activate pro-inflammatory programs that may contribute to the recruitment of adaptive immune effector responses. We use a recombinant poliovirus:rhinovirus hybrid, PVSRIPO, which has tropism for neoplastic cells, due to the ectopic expression of the poliovirus receptor, CD155, and a reliance on oncogenic signaling to initiate viral protein translation. PVSRIPO is currently in Phase-I/II clinical trials against recurrent glioblastoma multiforme (GBM). The first patients treated with PVSRIPO are radiographically tumor-free and clinically normal >52 months after single intratumoral infusion of PVSRIPO. On May 10th, 2016, the FDA/CBER granted Breakthrough Therapy Designation to PVSRIPO. A co-incidental finding emerging from our ongoing trial is remarkable durable, complete responses in patients upon treatment with chemotherapy following tumor progression after PVSRIPO administration.

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Methods

This study used syngeneic mouse tumor models (TRAMPC2 and CT2A) to test the same chemotherapy combination used in the clinic. Both the clinical trial and mouse study use chemotherapy doses that induce severe lymphotoxic side effects which have been suggested to augment antitumor immunity when combined with an immunotherapy regimen. To mirror what is done in the clinic the established mouse tumors receive a single injection of PVSRIPO followed a week later by a single round of the alkylating chemotherapeutic drug Temozolomide (TMZ). Tumor growth and end point immune cells populations were monitored.

Results

This study demonstrates that this PVSRIPO TMZ combination can be tested in syngeneic mouse models resulting in both an increase in survival and a delay in tumor growth over both PVSRIPO and TMZ treatment alone. Our data also suggests that this combination induces an increase in memory and infiltrating Treg cell populations.

Conclusions

Ongoing work is being done to further validate these findings in multiple mouse tumor models including intra-cerebral brain tumors models and to determine the mechanisms contributing to the synergy seen resulting from the PVSRIPO and chemotherapy combination. These intriguing results suggest that the memory T cells seen after PVSRIPO chemotherapy combination may be responsible for the new anti-tumor immune response. However, chemotherapy also induced an increased tumor infiltrating inhibitory Treg cells. Future work will look at ways to enhance this positive memory T cell population while preventing intratumoral Treg cell accumulation.

P403

Intratumoral administration of a multigene construct by electroporation can effectively modulate anti-tumor response in a murine B16.F10 model

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Background

Immunomodulatory cytokines, such as interleukin-12 (IL-12) are attractive candidates for cancer immunotherapy. IL-12 is a pro-inflammatory cytokine with potent anti-tumor effects; however, systemic administration of IL-12 shows limited clinical efficacy and dose-associated toxicity. In preclinical and clinical studies, intratumoral (IT) delivery of IL-12 plasmid DNA by electroporation (EP) can provide a safe and effective alternative for efficacious dosing. To augment the effects of IL-12, we developed a DNA plasmid platform that allows for delivery of agents that modulate multiple immune pathways as well as tumor- or patient-specific neoantigens. Polycistronic IL-12 Immune Modulator plasmid (PIIM) is a single plasmid encoding IL-12, and a fusion of Flt3L to an antigen. Flt3L is a ligand that stimulates dendritic cell (DC) maturation and enhances antigen processing and presentation. The encoded antigen can be a viral or shared antigen, or a patient-specific neoantigen, which enables customization to patient populations, as well as providing an aid to monitoring antigen-specific immune response(s) that can be correlated to patient outcomes. Here we demonstrate the first functional characterization of PIIM.

Methods

Two PIIM constructs were created for functional characterization: PIIM-OVA (IL12~Flt3L-OVA) for

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mouse experiments, and PIIM-NYESO1 (IL12~Flt3L-NYESO1) for testing in human cells. The expression and functional activity of PIIM components were determined. Treated tumors and spleens were assessed for transcriptional changes by NanoString® and phenotypic changes by flow cytometry. Systemic effects of PIIM were assessed using a syngeneic two-tumor model of B16.F10 in which only one tumor received IT-PIIM-EP while the other contralateral lesion remained untreated.

Results

PIIM-OVA and PIIM-NYESO1 secrete functional IL-12p70, Flt3L-OVA and Flt3L-NYESO1 fusion proteins as assessed by ELISA, flow and cell-based assays. PIIM promotes DC maturation and antigen-specific T cell proliferation both *ex vivo* and *in vivo*. Hydrodynamic-based gene delivery of PIIM-OVA lead to splenomegaly and significantly increased splenic CD11c+ DCs. Furthermore, IT-PIIM-EP lead to generation of splenic OVA-specific CD8s and increased APM gene expression. When introduced intratumorally in a mouse two-tumor model, IT-PIIM-EP delays B16.F10 tumor growth in both treated and contralateral tumors compared to untreated controls resulting in increased overall survival.

Conclusions

PIIM represents a novel approach to cancer immunotherapy. A combination of functional immune modulators can be expressed locally in the tumor microenvironment that increase inflammatory infiltrate, enhances antigen presentation and produces a systemic T cell response specific to the antigen encoded on the plasmid. This customizable approach has the potential to improve therapeutic outcome by enhancing adaptive-immunity and addressing patient-specific neoantigens needs.

P404

Targeting T-cells to human cancer associated fibroblasts using an oncolytic virus expressing a FAP-specific T-cell engager

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Background

Enadenotucirev (EnAd) is a chimeric oncolytic group B adenovirus with potent and selective anti-tumor activity against a range of epithelial cancer cells, with a blood stability profile that enables systemic dosing and has been administered intravenously to over 100 cancer patients. As an approach to immunogene therapy targeting stromal rich tumors, we have created transgene-modified variants of EnAd expressing a bi-specific T-cell engager (BiTE) molecule recognizing human fibroblast activating protein (FAP) on cancer associated fibroblasts (CAFs) and CD3 on T-cells. CAFs play a pivotal role in the development of solid carcinomas by facilitating invasion, coordinating angiogenesis and establishing and maintaining an immune suppressive microenvironment in the tumor stromal tissue. We hypothesize that production of BiTE proteins by virus infected tumor cells could be an effective way of modifying the stromal microenvironment to drive effective anti-tumor immunity and would bypass delivery and safety issues related to systemic dosing of BiTE proteins.

Methods

Bi-specific T-cell engager constructs comprising linked ScFv antibodies specific for human FAP and CD3 were designed and used to generate EnAd viruses expressing the BiTE such that transgene expression was under the control of either a CMV promoter or the virus major late promoter to allow

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broad or tumor-selective expression, respectively. Tumor and fibroblast cell lines, together with control BiTE proteins and BiTE-encoding viruses, were used to evaluate initial activities of the FAP-BiTE viruses prior to testing with freshly isolated malignant peritoneal ascites from patients with advanced cancer.

Results

We have shown that the viruses selectively express and secrete functional FAP-specific BiTEs following infection of tumor cell lines. In co-culture with FAP+ fibroblasts and PBMC-derived T cells this leads to T cell-activation and cytotoxicity towards the fibroblasts. Similarly, infection of primary malignant cancer ascites with FAP-BiTE-encoding viruses led to polyclonal activation of endogenous T-cells and depletion of FAP+ cells from the cultures. Unlike activation of T-cells with anti-CD3/CD28, FAP-BiTE activation was also shown to be effective even in the presence of immunosuppressive (cell-free) ascitic fluid samples added to the cultures.

Conclusions

In this study, we have shown that CAFs can be effectively targeted for T-cell mediated destruction by a FAP-BiTE transgene-bearing oncolytic virus, with an associated strong activation of endogenous T-cells to kill endogenous CAFs even in the presence of an immunosuppressive microenvironment. Systemic dosing of such a virus to patients with stromal rich tumors may provide an effective approach for driving effective anti-tumor immunity.

P405

Harnessing pre-existing antiviral immunity to treat solid tumors

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Background

Cancer immunotherapy rely on adaptive immune responses against tumor-associated antigens. The diversity of tumor antigens makes the development of cancer vaccines a highly personalized endeavor. Epitope spreading results from the induction of de novo responses against tumor antigens not initially included in vaccines [[1]. Intratumoral therapies also require to induce de novo responses against tumor antigen for abscopal therapeutic effect.

Human cytomegalovirus is highly prevalent in humans with polyfunctional T cell responses expanding with age [2]. We questioned whether redirecting pre-existing anti-cytomegalovirus T cells into solid tumor could arrest tumor growth, induce epitope spreading, and confer long-term anti-tumor immunity?

Methods

Persistently infected mice with mouse cytomegalovirus (MCMV) were challenged with TC-1 tumor cells expressing human papillomavirus (HPV) E6 and E7 oncogenes. We generated HPV pseudovirions expressing MCMV antigens or peptides derived from minimal MCMV epitopes. These reagents were injected intratumorally together with the poly(I:C), as adjuvant. We monitored tumor growth, survival and measured anti-tumor CD8⁺ T cell responses against an E7 immunodominant epitope using MHC-I tetramer and intracellular cytokine stainings. In rechallenge experiments, we evaluated the induction of long-term anti-tumor immunity. Using PanCancerImmune panel (Nanostring), we analyzed the tumor immune microenvironment after treatment.

Results

Transduction of TC-1 tumors with HPV pseudovirions expressing MCMV antigens or peptidic MCMV epitopes caused the expansion of MCMV-specific CD4⁺ and CD8⁺ T cells. These treatments also caused broad modifications of the

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tumor immune environment. Intratumoral injection of MCMV CD8 epitopes provoked the arrest of tumor growth. Intratumoral injection of MCMV CD4 epitopes with poly(I:C) promoted induction of E7-specific CD8⁺ T cells. Sequential administration of CD4 and CD8 MCMV epitopes together with poly(I:C) was the best protocol to eradicate pre-existing tumor of 5 to 10mm³, and rechallenge experiments showed anti-tumor immunity up to 4 months after the last treatment.

Conclusions

Our results provide a proof of concept to design “antigen-agnostic” intratumoral therapies based on pre-existing antiviral T cells. Such approach change the tumor immune microenvironment, induce epitope spreading, and confer long term anti-tumor immunity. These findings prompt further evaluation in other spontaneous tumor models and provide a model to decipher the mechanisms of epitope spreading and the role of CD4 help.

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P406

Dissecting the mechanisms underlying antitumor effects of live oncolytic vaccinia and heat-inactivated vaccinia.

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Background

Preclinical and clinical studies have shown that viral-based immunotherapy has the potential to overcome resistance to immune checkpoint blockade and to fill the unmet needs of many cancer patients. Oncolytic viruses are defined as engineered or naturally occurring viruses that selectively replicate in and kill cancer cells. Oncolytic viruses also induce antitumor immunity. We recently showed that intratumoral (IT) delivery of inactivated modified vaccinia virus Ankara (iMVA) induces antitumor systemic immunity via the STING-mediated cytosolic DNA-sensing pathway and Batf3-dependent CD103⁺/CD8α⁺ dendritic cells (DCs). The combination of IT iMVA and systemic delivery of immune checkpoint blockade is highly effective in treating large established tumors and distant metastasis [1]. MVA is non-replicative in tumor cells, and therefore whether or not viral replication is necessary for antitumor effects of IT oncolytic DNA virus is unknown.

Methods

In the study, we engineered a replication-competent, attenuated vaccinia virus (VC) by removing the Z-DNA-binding domain of vaccinia virulence gene E3 and inserting murine GM-CSF gene into the thymidine kinase (TK) locus (E3Δ83N-TK⁻mGM-CSF). We compared antitumor effects of live VC and iVC in murine tumor models.

Results

We found that IT Heat-inactivated VC (iVC; by heating the live virus at 55°C for 1 h) is more effective in generating systemic antitumor immunity than live VC murine tumor models. The antitumor effects of live VC also requires Batf3-dependent DCs. IT Heat-iVC induces higher numbers of infiltrating activated CD8⁺ and CD4⁺ T cells in the non-injected tumors than live VC and is more potent in depleting tumor-associated macrophages than live VC in both injected and non-injected distant tumors. The surviving mice treated with Heat-iVC are more effective in rejecting tumor rechallenge than those treated with live-VC, indicating that IT

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Heat-iVC generates stronger antitumor memory T cell responses than live VC.

Conclusions

In conclusion, our results support the use the IT delivery of inactivated vaccinia virus as a safe and effective cancer immunotherapy either alone or in combination with immune checkpoint blockade antibodies.

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P407

In situ vaccination for cancer immunotherapy: treat locally, respond systemically

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Background

Immunotherapy for cancer is making impressive impacts in the clinic. One strategy dating back to William Coley in 1900 is *in situ* vaccination. This approach puts immunostimulatory reagents into an identified tumor to break the local immunosuppression, stimulate a local anti-tumor response and most importantly stimulate systemic antitumor immune responses to eliminate metastatic disease. This is essentially an antitumor therapeutic vaccination, because the tumor

provides the antigens and the adjuvants are the immunostimulatory reagents, thus “*in situ* vaccination”. The approach has been part of standard of care for superficial bladder cancer using BCG bacteria. There are many immunostimulatory reagents that can be used and each has different capabilities. We have done previous studies with attenuated microorganisms including *Toxoplasma gondii*, and *Listeria monocytogenes*.

Methods

Our recent focus has been on a plant virus that cannot infect animals, compea mosaic virus (CPMV). Complete CPMV or CPMV lacking nucleic acids (eCPMV) are grown in plants and biochemically purified.

Results

CPMV nanoparticles were used in mouse cancer models and community dogs with oral melanoma and other tumors. These particles are composed of assembled viral capsid proteins. eCPMV has no nucleic acids and no recognized immunostimulatory reagents. However, eCPMV is strongly immunostimulatory through unknown pathways and causes changes in the tumor microenvironment that lead to primary tumor reduction or elimination and potent resistance to metastatic tumors (1). The treatment is immune-mediated but response in the lungs requires different immune components than response in flank tumors of the same B16F10 melanoma cell line. Tumor reduction or elimination occurs in many anatomic locations with multiple tumor types and in multiple strains of inbred mice. Treatment of primary tumors by direct intratumoral injection mediates robust rejection of a rechallenge with the same tumor. Response in community dogs was highly effective at both eliminating the local tumor and resisting metastatic disease. The mechanisms and pathways of immunostimulation are under investigation.

Conclusions

In situ vaccination has considerable potential for clinical use. In addition to the inherent immunostimulatory adjuvant properties of CPMV,

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they are a versatile platform to which other reagents for immune modulation can be attached. This demonstration of the value of select viral-like nanoparticles for treatment of cancer opens a new avenue of cancer immunotherapy.

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P408

Tumor cell-intrinsic STING signaling and regulation of IFN- β gene expression

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Background

Our laboratory has previously shown that immunogenic tumors spontaneously activate the innate immune system through the STING pathway. The STING pathway senses cytosolic DNA, which activates a signal transduction pathway culminating in phosphorylated IRF3 that translocates to the nucleus where it acts as a transcription factor to induce several genes including IFN- β . STING signaling and IFN- β receptor signaling in tumor-infiltrating immune cells, in turn, are required for optimal priming of CD8+ T cells against tumor antigens. Based on this notion, STING agonists were developed and tested as a pharmacologic approach to activate the pathway.

Methods

We stimulated various cell populations present in the tumor microenvironment as well as several tumor cell lines with STING agonists to test their ability produce IFN- β and analyze each step in STING pathway signaling.

Results

We observe that tumor cells themselves frequently fail to produce IFN- β in response to STING agonists or cytoplasmic DNA, arguing that loss of activation of this pathway might occur regularly as a component of oncogenesis. Surprisingly, we find that tumor cells retain expression of each gene in the STING pathway and STING signal transduction is intact up to and including nuclear translocation of IRF3. CHIP assays demonstrate IRF3 is unable to bind the IFN- β promoter but can still bind the promoters of other genes.

Conclusions

These data indicate tumor cells fail to express IFN- β following STING pathway activation due to a defect in transcription factor binding to the IFN locus. Based on ChIP experiments the defect in IRF3 DNA binding may be specific to the IFN- β locus. Sequencing of the locus shows no mutations in or around the binding site so we are currently investigating epigenetic mechanisms regulating IFN- β gene expression.

P409

Efficacious anti-melanoma immunity induced by OX40 ligand-expressing oncolytic adenovirus Delta-24-RGDOX

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Background

Oncolytic adenoviruses are highly immunogenic and cancer-selective but show limited efficacy as a single agent. Immune checkpoint modulation has shown efficacy in a variety of cancers but is associated with nonspecific T-cell activation, and has had a limited effect in tumors with a nonimmunogenic

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microenvironment. Thus, combining these two strategies likely resulted in both efficacious and specific cancer therapy. To this end, we constructed the oncolytic adenovirus Delta-24-RGDOX which expressed the immune co-stimulator OX40L. This new virus induces a superior immunotherapeutic effect in immunocompetent mouse glioma models than its predecessor Delta-24-RGDOX.

Methods

In a s.c./s.c. melanoma mouse model with B16F10-Red-FLuc cells, Delta-24-RGDOX and/or anti-CD47 antibody were injected into the first implanted tumor. The tumor growth of the first and second implanted tumors was monitored with bioluminescence. The survival curves were plotted according to the Kaplan–Meier method. Survival rates in the different treatment groups were compared using the log-rank test. The immune cells in the tumor were isolated and analyzed with flow cytometry.

Results

Delta-24-RGDOX expressed OX40L effectively in mouse melanoma cells. Compared to treatment with PBS, four doses of intratumoral injection of the virus significantly inhibited the growth of both the injected tumors and the untreated distant tumors, resulting in prolonged survival of the mice with 40% long-term survival ($P = 0.01$). The surviving mice is resistant to rechallenging with the same tumor cells but is susceptible to lung cancer cells, suggesting the development of immune memory specific to the virus-injected tumor type. Through flow cytometry analysis of the tumor-infiltrating lymphocytes, we found the virus injection increased the presence of CD3+ T lymphocytes, CD3+CD4+ helper T cells and CD3+CD8+ cytotoxic T cells in the tumor, causing a decreased ratio of CD4+/CD8+ cells. To further increase the efficacy of Delta-24-RGDOX, we combined the virus with intratumoral injection of an antibody against CD47, a “don’t eat me” signal overexpressed on tumor cell surface to protect them from phagocytosis. We found high expression levels of CD47 in cultured B16 cells and the tumor cells from the xenografts. Intratumoral injection of

Delta-24-RGDOX caused much more phagocytes presented at the tumor site than PBS treatment. Combination of anti-CD47 antibody with Delta-24-RGDOX mediated longer survival time than the virus alone (median survival: 56 days vs. 26 days).

Conclusions

Delta-24-RGDOX induced efficacious local and systemic anti-melanoma immunity in B16-C57BL/6 mouse model and combination of the virus with anti-CD47 antibody further increased the therapeutic efficacy.

P410

Generation of therapeutic RNAs to induce immunogenic cell death and interferon expression in cancer cells

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Background

Pattern-recognition receptors (PRRs) are immunological sensors that initiate the host defense response against infections. They are located at the cell surface, within endosomal compartments and in the cytoplasm, where they are poised to recognize different molecular signatures associated with invading pathogens. In addition to anti-infectious immunity, the activation of RNA-sensing PRRs can mediate programmed cell death of infected cells, which allows the host to efficiently block viral replication by sacrificing infected cells. Transfection with certain types of RNA ligands that stimulate RIG-I-like receptor (RLR) family can induce type I interferon (IFN) and immunogenic cell death (ICD) in cancer cells, which together orchestrate anti-tumor immune responses that can prevent tumor recurrence and metastasis. Currently RNA-sensing PRR agonists have demonstrated little or no overall benefit to patients with cancers because of, mostly but not exclusively, toxicity driven by non-specific induction of immune reactions. In this study, we

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generate and screen multiple nuclease-resistant RNA molecules that can differentially induce immunogenic cancer cell death with or without concomitant expression of IFN- β , pro-inflammatory cytokines and pro-coagulation mediators.

Methods

Therapeutic RNAs are designed using mFold RNA structure prediction program and generated by *in vitro* transcription. All RNAs contain 5'triphosphate and 2'fluoro pyrimidine that are recognized by RLRs and resistant to nucleases. Immunogenic cell death in human melanoma, pancreatic cancer and prostate cancer cells and PBMCs was analyzed by western blot, ELISA and Flow cytometry. *In vivo* anti-tumor effects of the RNAs were induced by intratumoral transfection with RNAs into immunocompromised mice bearing human melanoma.

Results

We generated two novel and distinct ssRNA molecules (Immunogenic Cell-killing RNA (ICR)2 and ICR4). ICR2 and ICR4 differentially stimulated cell death and PRR signaling pathways and induced different patterns of cytokine expression in cancer and innate immune cells. Interestingly, damage-associated molecular patterns (DAMPs) released from ICR2- and ICR4-treated cancer cells had distinct patterns of stimulation of innate immune receptors and coagulation. Finally, ICR2 and ICR4 inhibited *in vivo* tumor growth as effectively as polyI:C. ICR2 and ICR4 are potential therapeutic agents that differentially induce cell death, immune stimulation and coagulation when introduced into tumors.

Conclusions

ICR2 and ICR4 are potent PRR-stimulating cytotoxic agents against human cancers. ICR2 and ICR4 have distinctive immune stimulatory and coagulating activities. Combination of dual checkpoint inhibitors and ICR4 and/or ICR2 would be a potent and effective anti-cancer therapies against advanced cancers.

P411

Immunotherapy with BO-112, a novel double-stranded RNA-based agent, promotes tumor cell death and boosts T cell immunity in preclinical mouse models

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Background

Unresponsive or refractory tumors are the main research objective in cancer immunotherapy. Strategies that promote immunogenic tumor cell death in the context of type I interferon (IFN) signaling can be combined effectively with existing therapies that target checkpoint inhibitors to boost anti-tumor immunity. BO-112 is a double-stranded synthetic RNA currently undergoing phase I clinical trials for intratumor injection. To enhance local antitumor immunity, BO-112 bears poly I:C chains that trigger endosomal Toll-like receptor 3 and cytosolic helicases.

Methods

B16F10 and B16F10-OVA melanoma, MC38 colon carcinoma or 4T1 breast carcinoma cells (5×10^5) were implanted subcutaneously in the right flank of C57BL/6J and BALB/c mice; a second tumor was engrafted in the left flank to evaluate distant antitumor effects. Beginning 7-10 days post-implant, mice were treated intratumorally with BO-112 only in the primary lesion. Therapeutic efficacy was evaluated by monitoring tumor growth and survival, and cellular responses were analyzed by multiparametric flow cytometry. Antigen-specific CD8+ T cell *in vivo* priming was addressed by H-2K^b

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tetramer analysis in B16F10-OVA tumors. The role of CD4+ or CD8+ T cells was studied by depletion and depletion/rechallenge experiments. Combined BO-112 and anti-PD-L1 (programmed death-ligand 1) antibody therapy was evaluated in established B16F10 melanomas.

Results

In vitro and *in vivo* studies showed that BO-112 triggered cell death in tumor cells of distinct tissue origins (melanoma, colon or breast carcinoma). The cytotoxic effect was also detected in engineered B16 cells that do not respond to type I IFN. Intratumor BO-112 administration in all three mouse tumor models showed therapeutic effects, with complete tumor regression in up to 30% of mice. FACS analyses of tumor-derived cell suspensions showed that BO-112 increased the tumor immune infiltrate and CD8+ T cell frequency. Moreover, the receptors PD1 and CD137, which denote activation, were upregulated on CD8+ T cells. BO-112 treatment enhanced intratumor OVA-specific CD8+ T cells, and depletion and rechallenge experiments confirmed CD8+ T cell involvement in its immunotherapeutic effects. A combination of BO-112 and anti-PD-L1 antibody significantly improved the poor response to PD-L1 blockade in B16F10 and B16F10-OVA tumor-bearing mice. Responses were observed in treated and in untreated distant tumors, which provides a rationale for clinical testing of this combination.

Conclusions

These data identify local BO-112 release as a new immunotherapy agent beneficial in the treatment of refractory and recurrent tumors, especially when combined with checkpoint inhibitors. A phase Ib clinical trial is being designed to address this question.

Trial Registration

<http://ClinicalTrials.gov> identifier, NCT02828098

P412

Antitumor immunity in patients with locally advanced soft tissue sarcoma treated with hafnium oxide nanoparticles and radiation therapy

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Background

Soft tissue sarcoma (STS) is a large and heterogeneous group of malignant mesenchymal neoplasms characterized by a strong tendency toward local recurrence and metastatic spreading. Consistently, the immune microenvironment in sarcomas is highly variable. A new class of material with high electron density, hafnium oxide, was designed at the nanoscale to efficiently absorb ionizing radiation from within the tumor cells and augment the dose deposited to a tumor. These nanoparticles (HfO₂-NP) administered in a single intratumor injection and activated by fractionated radiotherapy are evaluated in a phase II/III trial in patients with locally advanced STS as neoadjuvant treatment. Besides, beyond the broadly cytotoxic

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effect of radiation therapy (RT), RT may promote the release of tumor neoantigens during cancer cell death and stimulate local immunological effects.

Here, we explore the effects of nanosized hafnium oxide exposed to RT in terms of tumor immune profile changes in patients with STS when compared to RT alone.

Methods

Tumor tissues pre- (biopsy) and post-treatment (resection) are collected from patients with locally advanced STS (NCT02379845), who received either HfO₂-NP activated by RT or RT alone.

Immunohistochemistry and Digital Pathology for immune biomarkers and Pan-Immune gene expression profiling are analyzed.

Results

A significant increase of CD8+ T cells and a marked increase of CD3+ and PD-1 T cells and CD103+ immune cell infiltration post- vs pre-treatment are observed for HfO₂-NP + RT while no differences are seen for RT alone (more than 10 patients analyzed in each arm). Functional analysis of genes expression up-regulated in HfO₂-NP + RT post- vs pre-treatment shows an enrichment of cytokine activity (IL7, IFNA, IL11, IFNG), adaptive immunity (RAG1, TAP1, TAP2, TBX21, IFNG, LTK, CD37, CD22) and T cell receptor signaling pathway (CD28, CTLA4, CD274, BTLA, TIGIT, CD5, ZAP70) when compared to RT.

Conclusions

Promising results are observed in patients who received HfO₂-NP + RT in terms of immune cells infiltration post- vs pre-treatment when compared to RT. Moreover, HfO₂-NP + RT induces a specific adaptive immune pattern. So far, nanosized hafnium oxide exposed to RT bring substantial changes to the tumor immune profile in patients with STS when compared to RT. As such, it may convert immunologically “cold” tumor into “hot” tumor and be effectively combined with immunotherapeutic agents across oncology. More

tissue samples are under evaluation to reinforce these findings.

P413

Transforming immunologically “cold” tumor into “hot” tumor with hafnium oxide nanoparticles and radiation therapy

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Background

Hafnium oxide, an electron-dense material, was designed at the nanoscale to increase the radiation dose deposited from within the cancer cells: “Hot spot” of energy deposit where the nanoparticles are when exposed to radiation therapy (RT). Preclinical studies have demonstrated increase of cancer cells killing *in vitro* and marked antitumor efficacy *in vivo* with presence of these nanoparticles (HfO₂-NP) exposed to RT, when compared to RT alone. HfO₂-NP is intended for a single intratumor injection and is currently evaluated in clinical trials including soft tissue sarcoma, head and neck, prostate, liver and rectum cancers.

Here, we explore the ability of nanosized hafnium oxide exposed to RT to bring substantial immune cells infiltrations in the tumors and convert immunologically “cold” tumor into “hot” tumor.

Methods

CT26 (murine colorectal cancer cells) were subcutaneously injected in the flank of BALB/c mice. Once the mean tumors volume reached 115±30 mm³, tumors were intratumor injected with HfO₂-NP and irradiated with 2Gyx3 or 4Gyx3, or irradiated only. Tumors were collected 5 days after the last RT fraction and analyzed for immune cell infiltrates by immunohistochemistry (2Gyx3 and 4Gyx3) and cytokines content by flow cytometry (2Gyx3).

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A second study evaluated HfO₂-NP exposed to RT vs RT alone using the 4T1 murine breast cancer model. Cells treated or not with HfO₂-NP were exposed to irradiation (40Gy). Irradiated cells (1.10⁶) (or phosphate-buffered saline as control) were inoculated subcutaneously into the flank of BALB/c mice (vaccination phase). Seven days after, mice were challenged with untreated 4T1 cells (1.10⁶) (challenge phase). Grown tumors (challenge site) were collected 19 days after the challenge phase and analyzed for immune cell infiltrates by immunohistochemistry.

Results

In mice bearing CT26 tumors, a marked increase of cytokines content and immune cell infiltrates was observed with HfO₂-NP + 2Gyx3 when compared to RT alone. The tumor immune cell infiltrates were further enhanced with HfO₂-NP + 4Gyx3.

In mice inoculated with 4T1 cells treated with HfO₂-NP + 40Gy, a marked increase of immune cell infiltrate (CD8+) was observed in tumors when compared to tumors in mice inoculated with 4T1 cells treated with 40Gy and control.

Conclusions

These *in vivo* data generated from CT26 and 4T1 tumor models suggest that HfO₂-NP + RT triggers immunogenic conversion of the tumor microenvironment when compared to RT alone. HfO₂-NP treatment may represent a therapeutical approach for broad applications since it does not rely on any molecular characteristics of the tumor.

P414

PVSRIPPO is an interferon-resistant, immunotherapeutic oncolytic virus

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Background

Oncolytic viruses are attractive cancer therapies because they selectively lyse cancer cells, but show attenuation in normal cells. Many oncolytic virus are attenuated through susceptibility to innate antiviral immune responses, like Type I Interferon (IFN) responses. Until recently it was believed that cancer cells lack these responses. However, our lab and others have shown cancer cells can have intact IFN responses which inhibit IFN-susceptible viruses, but may activate potent anti-cancer immunity. Innate immunity in cancer is thus an area of extreme interest.

Our lab developed an oncolytic human rhinovirus:poliovirus chimera, PVSRIPPO, which is in clinical trial against glioblastoma multiforme, an aggressive form for brain cancer. Using PVSRIPPO, and the related picornavirus encephalomyocarditis virus (EMCV), we investigated MDA5 and IFNs in infection of cancer cells. MDA5 is a pattern recognition receptor, targeted to viral double-stranded RNA produced by picornaviruses. We also investigated PVSRIPPO's role in anti-tumor immune responses after treatment of cancer cells.

Methods

DM440, a human melanoma cell line, was engineered with stable MDA5 knock-down by lentivirus transduction containing MDA5-targeting small-hairpin RNA. PVSRIPPO or EMCV was added at a multiplicity of infection (MOI) of 0.1 or 0.01 and samples collected for immunoblot, ELISA, or viral titer. Cells were also incubated with IFN- α 2 for 24 hours, infected, and assayed as above. Human dendritic cells (DCs) were infected at 1, 10, 50, or 100 MOI and collected as above. DCs were also treated with oncolysate from PVSRIPPO-infected SUM149 human breast cancer cells, non-infectious lysate, or virus-filtered lysate. DCs were analyzed by flow cytometry or used for a cytotoxic HLA-matched T-cell assay using europium-labeled target cells, including cancer cells, tumor antigen transfected DCs, and control antigen expressing DCs.

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Results

DM440 cells produce and respond to IFNs in an MDA5-dependent manner after PVSRIPO and EMCV infection. MDA5 activation and IFN treatment inhibits EMCV replication significantly more than PVSRIPO replication. Human DCs can be productively infected and activated with PVSRIPO without toxicity. Oncolysate activates human DCs in a virus dependent manner, and DCs activated in this way promote cytotoxic T-cell activation against tumor-specific antigen.

Conclusions

PVSRIPO is an IFN-resistant oncolytic virus that provokes MDA5-dependent IFN responses in cancer cells, without significant viral inhibition. Further, PVSRIPO infects DCs, activating them without significant cytotoxicity, which can lead to tumor-specific cytotoxic T-cell responses, and may lead to antigen spreading. It is possible this potent DC activation phenotype is due to PVSRIPO's resistance to IFNs.

P415

Upregulation of PD-L1 in tumor microenvironment is a resistance mechanism for oncolytic virus immunotherapy

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Background

Intralesional therapy with oncolytic viruses (OV) leads to activation of multiple immune pathways such as type I IFN. Both OV and OV-activated type I IFN pathway can exert a variety of pleiotropic effects on both immune and non-immune cells, activating resistance to the immune system on both local and abscopal level. Identification of such pathways could provide insights into the mechanisms of OV-mediated T cell activation as well as generate rationale for combinatorial strategies.

Methods

Using Newcastle Disease Virus (NDV) as a model OV, we explored the effect of intratumoral OV therapy on the microenvironment of the treated and distant tumors in syngeneic bilateral flank tumor models, and explored combination therapies using intratumoral NDV with systemic immune checkpoint blockade.

Results

Intratumoral therapy with NDV led to upregulation of a range of activating and inhibitory immune targets in the treated and distant tumors. While NDV therapy shifted the balance from exhausted to effector T cell phenotype in distant tumors, it was not sufficient for complete tumor rejection. We further demonstrate that infection with NDV leads to upregulation of PD-L1, an effect that is mediated early through paracrine action of type I IFN. Consistent with these findings, intratumoral therapy with NDV or IFN α results in upregulation of PD-L1 on both tumor cells and tumor-infiltrating lymphocytes in the treated tumors. In contrast to NDV, intratumoral IFN α therapy had no effect on late PD-L1 expression, immune cell infiltration, or growth of distant tumors, highlighting that late PD-L1 upregulation is likely a reflection of adaptive immune resistance to the increased tumor infiltrating lymphocytes. Systemic therapeutic targeting of PD-1 or PD-L1 in combination with intratumoral NDV resulted in rejection of both treated and distant tumors. Anti-tumor efficacy was fully dependent on CD8 cells and on early presence of NK cells.

Conclusions

These findings provide implications for timing of PD-1/PD-L1 blockade in conjunction with OV therapy and highlight that understanding of adaptive mechanisms of immune resistance to specific OV will be important for rational design of appropriate combinatorial approaches with these agents.

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Other

P416

Anti-tumor effects and immunological response following immune stimulating interstitial laser thermotherapy

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Background

Immune stimulating interstitial laser thermotherapy (imILT) is a local ablation modality designed for local destruction of tumor tissue as well as to optimize systemic immunologically mediated anti-cancer effects. Safety and feasibility in the clinic has been studied and previous rodent data show abscopal effects. This study expands on the mechanisms behind these immunological effects.

Methods

An imILT treatment protocol was developed and a syngenic mouse tumor model was established. Mice were inoculated subcutaneously with tumor cells and tumor growth was monitored. When tumors reached adequate size imILT treatment was performed and subsequently another tumor was inoculated and tumor progression was measured. In another set of experiments, two tumors were inoculated simultaneously (twin tumor model) and one of them was treated at appropriate size. Tumor progression of both tumors were monitored. Immune infiltrate and morphology of all tumors were studied.

Results

Preliminary data show both abscopal effects in the twin tumor model as indicated as reduced growth of the non-treated tumor of the imILT treated mice as compared to the control mice. Memory effects can also be demonstrated in the first set of experiment as seen as reduced growth and take of tumor

inoculation in mice which have previously been receiving imILT as compared to control mice.

Conclusions

Taken together these data suggest immunologically mediated effects are evoked following imILT treatment. This new information will be useful for deducing the mechanisms of action involved in these effects.

P417

Post-marketing safety of checkpoint inhibitors: analysis of the FDA adverse event reporting system

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Background

Information about the toxicity profile of immune checkpoint Inhibitors (ICIs) is limited. Available data primarily comes from clinical trials. However, subjects enrolled in clinical trials are usually fitter than patients typically seen in clinical practice and therefore may have a different toxicity profile.

Methods

We reviewed data from the FDA Adverse Event Reporting System (FAERS) summarizing adverse events (AEs) associated with the PD-1 inhibitors (nivolumab and pembrolizumab); PD-L1 inhibitor (atezolizumab); and CTLA-4 inhibitor (ipilimumab). We restricted our analysis to reports that included only an ICI as suspect agent. For each agent, we performed a descriptive analysis of hospitalization (HO) and death (DE) outcomes, as well as AEs of special interest (AESI). We compared the distribution of each outcome within age groups (<65 years; 67-75; >75) using Mantel-Haenszel chi-square test for trend.

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Results

Our analysis included a total of 21,588 safety reports. 415 for atezolizumab, 10,026 for nivolumab, 4,808 for pembrolizumab, and 6,339 for ipilimumab (Table 1). There was a statistically significant trend of more hospitalization with increasing age for all drugs. Prevalence of any of the AESI was higher as age increased for all drugs ($p < 0.0001$) except for atezolizumab ($p 0.12$). However, no statistically significant trend by age was found for any individual AESI (data not shown). Proportion of older patients who experienced death was higher for pembrolizumab ($p < 0.001$) and ipilimumab ($p 0.002$).

Conclusions

Our analysis suggests that older patients receiving pembrolizumab, Nivolumab or ipilimumab are more likely to develop immune related AEs, and to be hospitalized. This resulted in higher rate of deaths only in pembrolizumab and ipilimumab. The smaller sample size of the patients receiving atezolizumab may have contributed to the lack of statistical significance. Older patients treated with ICIs should be monitored carefully for treatment-related AEs. Toxicity of ICIs should be further evaluated prospectively in the setting of trials in older adults that would take in consideration impact of physiologic and immune aging.

Table 1.

	Atezolizumab				
	Overall	< 65 y	65-75 y	> 75 y	P value
	N=415	N=273	N=101	N=41	
Hospitalization, N (%)	231 (56)	124 (45)	80 (79)	27 (66)	<0.0001
Death, N (%)	85 (20)	56 (21)	18 (18)	11 (27)	0.64
Any AE, N (%)	87 (21)	52 (19)	23 (23)	12 (29)	0.12
	Nivolumab				
	Overall	< 65 y	65-75 y	> 75 y	p value
	N=10026	N=7342	N=1896	N=788	
Hospitalization, N (%)	3028 (30)	1677 (23)	970 (51)	381 (48)	<0.0001
Death, N (%)	3837 (38)	2905 (40)	670 (35)	262 (33)	<0.0001
Any AE, N (%)	1888 (19)	1187 (16)	456 (24)	245 (31)	<0.0001
	Pembrolizumab				
	Overall	< 65 y	65-75 y	> 75 y	p value
	N=4808	N=3319	N=947	N=542	
Hospitalization, N (%)	1482 (31)	855 (26)	386 (41)	241 (44)	<0.0001
Death, N (%)	1518 (32)	970 (29)	359 (38)	289 (35)	<0.0001
Any AE, N (%)	740 (15)	445 (13)	174 (18)	121 (22)	<0.001
	Combined PD-1 & PD-L1 inhibitors				
	Overall	< 65 y	65-75 y	> 75 y	p value
	N=15249	N=11004	N=2890	N=1355	
Hospitalization, N (%)	4621 (30)	2589 (24)	1393 (48)	639 (47)	<0.0001
Death, N (%)	5403 (35)	3906 (36)	1039 (36)	458 (34)	0.44
Any AE, N (%)	2674 (18)	1656 (15)	644 (22)	374 (27)	<0.0001
	Ipilimumab				
	Overall n (%)	< 65	65-75	> 75	p value
	N=6339	N=4697	N=1120	N=522	
Hospitalization, N (%)	2401 (38)	1477 (31)	645 (58)	279 (53)	<0.0001
Death, N (%)	1421 (22)	1021 (22)	250 (22)	150 (29)	0.002
Any AE, N (%)	1844 (29)	1258 (27)	407 (36)	179 (34)	<0.0001

P418  Abstract Travel Award Recipient

Bromodomain and extraterminal region inhibitors slow melanoma tumor growth by altering the tumor microenvironment.

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Background

Melanoma is the deadliest form of skin cancer, yielding a 2-16% survival rate for metastatic patients. Despite the onset of new successful therapies, i.e. targeted inhibitors and immunotherapy, there is a large need for drugs that

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alter the entire tumor microenvironment. To this end, we aim to study drugs that have widespread tumor microenvironment effects, such as epigenetic modifiers, i.e. bromodomain and extra terminal domain inhibitors (BETi), which slow tumor growth in a variety of cancers and effect inflammatory responses. Thus, BETis potentially represent a novel treatment for metastatic melanoma, as they can target tumor cell growth and anti-tumor immune mechanisms.

Methods

Several mouse and human BRAF^{V600E} melanoma cell lines, immune competent mouse models, and a patient-derived xenograft model were used to uncover the effects of BETi treatment on melanoma.

Results

Here we show that the BETi PLX51107 was able to differentially impact BRAF^{V600E} melanoma tumor growth in a number of mouse and human melanoma cell lines in vitro and in vivo. PLX51107 induced cell cycle arrest and cell death of melanoma cell lines by increasing Bim and decreasing Cyclin B1 in mouse and human lines. Using immune competent mouse models, BETi treatment altered the immunogenicity of the microenvironment of responsive melanoma tumors by lowering the expression of anti-inflammatory PD-L1 and FasL, while increasing pro-inflammatory MHC-I. This was also seen in mouse and human melanoma cell lines and cancer-associated fibroblasts. PLX51107 treatment differentially altered tumor infiltrating lymphocyte (TIL) populations in mouse melanoma models, increasing activated, proliferating, and functional intratumoral CD8+ T cells and leading to a CD8+ T cell mediated tumor growth delay in highly responsive tumors. However, in moderately or poorly responsive tumors, these CD8+ T cell populations were unchanged or decreased after BETi treatment. As BETi treatment was a successful primary therapy, it was prudent to determine its efficacy as a second line therapy. In this vein, an immune competent mouse model and patient-derived xenograft model were used to demonstrate

BETi efficacy on anti-PD-1 resistant tumors. BETi treatment was able to delay the growth of anti-PD-1 resistant tumors, by increasing intratumoral CD8+ T cells and pushing tumors to a more responsive IPRES signature.

Conclusions

As hypothesized, BETi treatment had wide-ranging effects on melanoma tumors by altering the tumor cells themselves, the microenvironment, and anti-tumor immunity to induce robust tumor growth delay. From these findings, BETis represent a potential primary or secondary treatment for metastatic melanoma patients.

P419

Tobacco use, awareness and cessation among Malayali tribes, Yelagiri Hills, Tamil nadu, India.

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Background

Health is a state of complete wellbeing free from any discomfort and pain. Despite remarkable worldwide progress in the field of diagnostic, curative and preventive medicine, still there are large populations of people living in isolation in natural and unpolluted surroundings far away from civilisation, maintaining their traditional values, customs, beliefs and myths. India has the second largest tribal population of the world next to the African countries. About half of the world's autochthonous people live in India, thus making India home to many tribes which have an interesting and varied history of origins, customs and social practices. The present study was conducted to assess the tobacco use, awareness and its effect on health among Malayali tribes, Yelagiri Hills, Tamil nadu, India.

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Methods

The inhabitants of the 14 villages of the Yelagiri hills, who have completed 18 years and residing for more than 15 years present on the day of examination and who were willing to participate in the study were included.

Data was collected from a cross-sectional survey, using a Survey Proforma, clinical examination and a pre-tested questionnaire which included Demographic data, tobacco habits. An intra-oral examination was carried out by a single examiner to assess the Oral Health Status using WHO Oral Health Surveys – Basic Methods Proforma (1997). SPSS version 15 was used for statistical analysis.

Results

Results showed that among 660 study population, 381 (57.7%) had no formal education. Among the study population 75% had the habit of alcohol consumption. Of those who had the habit of smoking, 26% smoked beedi, 10.9% smoked cigarette, 65% chewed raw tobacco, 18% chewed Hans and 28% had a combination of smoking and smokeless tobacco usage. The reason for practicing these habits were as a measure to combat the cold, relieving stress and body pain after work, and the lack of awareness of the hazards of the materials used. Prevalence of oral mucosal lesions in the study population was due to tobacco usage and alcohol consumption and lack of awareness regarding the deleterious effects of the products used.

Conclusions

From the results of this study it may be concluded that the Malayali tribes were characterized by a lack of awareness about oral health, deep rooted dental beliefs, high prevalence of tobacco use and limited access to health services.

P420

Selection of first-in-human starting dose of anti-OX40 agonist monoclonal antibody BMS-986178 using a pharmacokinetic/pharmacodynamic-based approach

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Background

Following the clinical success of checkpoint blockade, the field of cancer immunotherapy rapidly expanded. Co-stimulatory molecules from the TNF receptor superfamily, including OX40, may be a promising approach to enhance the benefits of immunotherapy. BMS-986178 is a fully human agonist antibody of the immunoglobulin G1 isotype that binds with high affinity to the human OX40 receptor, currently being developed for the treatment of advanced solid tumors.

Methods

BMS-986178 does not bind to mouse OX40, necessitating antibody surrogates to assess *in vivo* activity of OX40 agonism. Two hamster anti-mouse OX40 agonist mAbs (reformatted as mouse antibodies: mIgG1 and mIgG2a) were studied in the mouse MC38 colon adenocarcinoma model. A pharmacokinetic/pharmacodynamic (PK/PD)-based approach was employed to integrate the preclinical data and guide the first-in-human (FIH) starting dose selection.

Results

In vitro, binding EC₅₀ of mIgG1 and mIgG2a mAbs in activated mouse T-cells was similar to *in vitro* binding EC₅₀ of BMS-986178 in activated human T-

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cells. PK/PD analysis of the anti-tumor efficacy data showed maximum efficacy at an AUC of 100 $\mu\text{gh/mL}$ with a C_{max} of 25 $\mu\text{g/mL}$. Targeting the same AUC and C_{max} in mice into a human PK/PD prediction model, the human efficacious dose was projected to be 1 mg/kg.

The proposed FIH starting dose was then selected to be 0.25 mg/kg, 4-fold below the projected efficacious dose. First, using preclinical GLP safety assessments, the proposed starting dose was 68- to 80-fold lower than 1/6th of the highest non-severely toxic dose (120 mg/kg/week) in monkeys. Second, the C_{max} (6.25 $\mu\text{g/mL}$) at the starting dose was 5-fold below the highest no-effect concentration (33 $\mu\text{g/mL}$) evaluated in the *in vitro* cytokine release assay. Third, there was a >10-fold exposure margin between the minimal pharmacologic effect (antigen-specific T-cell or antibody responses) observed in monkeys at doses of 2-4 mg/kg and the FIH starting dose selected.

The FIH trial was successfully initiated at 20 mg (0.25 mg/kg with a BW of 80 kg). BMS-986178 was well tolerated without any adverse event at this dose. OX40 receptor occupancy observed in peripheral blood at the starting dose maintained ~80% in line with the prediction made from *in vitro* cellular binding affinity and clinical information observed from other anti-OX40 antibodies.

Conclusions

This PK/PD-based approach was successfully applied to select the FIH starting dose of BMS-986178. This strategy appropriately balanced the selection of a safe dose, while minimizing the number of cancer patients receiving sub-therapeutic doses.

P421

Co-administration of dexamethasone with checkpoint blockade therapy increases survival in brain tumor model

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Background

The use of corticosteroids for therapeutic benefit must be weighed against risks of adverse consequences associated with these drugs. Brain tumor patients are routinely prescribed dexamethasone to reduce tumor-associated edema. Checkpoint blockade, a type of immune therapy, is currently being investigated as a potential treatment for brain tumors. Although glucocorticoid signaling has been shown to attenuate the immune response, the effect of glucocorticoids on the anti-tumor immune response during checkpoint blockade remains unclear. Here, we propose that dexamethasone's ability to upregulate T-cell checkpoint molecules such as CTLA-4 might be an immunosuppressive mechanism, but can be countered in unison with checkpoint blockade therapy.

Methods

Healthy donor T cells were tested for response to dexamethasone. T cell proliferation, cell cycle analysis, apoptosis, glucose uptake, and protein expression were assessed with flow cytometry and Western blots. Transcriptional changes were assessed with qPCR. CTLA-4 was blocked using monoclonal antibodies *in vitro* on human PBMCs and *in vivo* with the GL261 syngeneic glioblastoma model.

Results

Checkpoint molecule CTLA-4 was increased by dexamethasone treatment upon stimulation. Unexpectedly, dexamethasone did not elicit a direct lymphotoxic effect on T cells but significantly reduced T cell entry into cell cycle. Dexamethasone also abated CD28 signaling as shown by reduced AKT phosphorylation and reduced glucose uptake. Blockade of CTLA-4 resulted in a substantial reversal of these effects. In addition, we discovered that antigen-experienced memory T cells were invulnerable to dexamethasone treatment. *In vivo*,

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dexamethasone and CTLA-4 blockade provided a survival benefit to tumor bearing mice.

Conclusions

These results suggest that mature T cells, which predominate in the tumor microenvironment, are resilient to the negative effects of corticosteroids. Thus, corticosteroids are unlikely to destroy anti-tumor T cells. For patients who have not responded to immune therapy, CTLA-4 blockade may provide protection from corticosteroids to the naïve T cell pool.

P422

Overall survival and treatment patterns among real-world patients with metastatic non-small cell lung cancer not previously treated with systemic therapy for advanced cancer

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Background

The majority of patients with non-small cell lung cancer (NSCLC) are diagnosed at an advanced stage of disease, typically with a poor prognosis. While treatment of metastatic NSCLC is focused on extending survival, real-world data on the effectiveness of existing therapies and patterns of treatment is limited.

Methods

Patients with metastatic NSCLC, that are EGFR and ALK WT and no prior systemic therapy for advanced disease were identified from the Flatiron Oncology electronic medical record database from 2013–2016. Treatment patterns, including regimens administered and time to treatment, were summarized. The median overall survival (OS) from the initial diagnosis of metastatic NSCLC was

calculated from Kaplan-Meier curves, with associated 95% confidence intervals (CI).

Results

A total of 10,123 patients were eligible for analysis. The mean age of patients was 68.0 ± 10.0 years, and 54.0% were male. The most commonly observed regimens after the diagnosis of metastatic disease were nivolumab (17.4%), carboplatin + pemetrexed (16.0%), and carboplatin + paclitaxel (15.0%), while 25.4% did not receive treatment. The median time from diagnosis of metastatic disease to start of first-line therapy was 36 days (range: 1–1,770) and the median number of therapy lines was 1 (range: 1–8). The median OS was 11.2 months (95% CI: 10.9–11.6) from the initial diagnosis of metastatic disease (Figure 1).

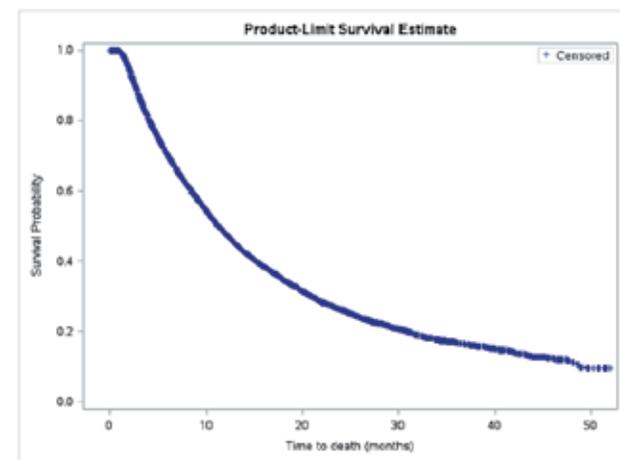
Conclusions

In this real-world study, overall survival of metastatic patients was less than 12 months, and one quarter of patients did not receive treatment after diagnosis. This evidence indicates a potential unmet need for additional treatment options, as more patient information such as biomarkers become available to physicians at initial diagnosis.

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Figure 1. Overall Survival for Patients with Stage IV NSCLC, From Initial Diagnosis of Advanced or Metastatic NSCLC

Figure 1. Overall Survival for Patients with Stage IV NSCLC, From Initial Diagnosis of Advanced or Metastatic NSCLC



Median	95% CI
11.2109	10.9151, 11.6383

P423

Overall survival and treatment patterns among real-world patients with stage IIIB non-small cell lung cancer treated with platinum-based chemotherapy

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Background

Platinum-based chemotherapy has been the standard of care in the management of patients with advanced non-small cell lung cancer (NSCLC), but evidence of effectiveness and patterns of treatment in a real-world setting is needed.

Methods

Electronic medical record data of patients from the Flatiron Oncology database with Stage IIIB NSCLC treated with at least two cycles of platinum-based chemotherapy during 2013–2016 were identified; patients with prior evidence of metastatic disease were excluded. Treatment patterns were examined, including the duration and number of lines received. The median overall survival (OS) from the start date of the third cycle of chemotherapy was calculated from Kaplan-Meier curves, with 95% confidence intervals (CI).

Results

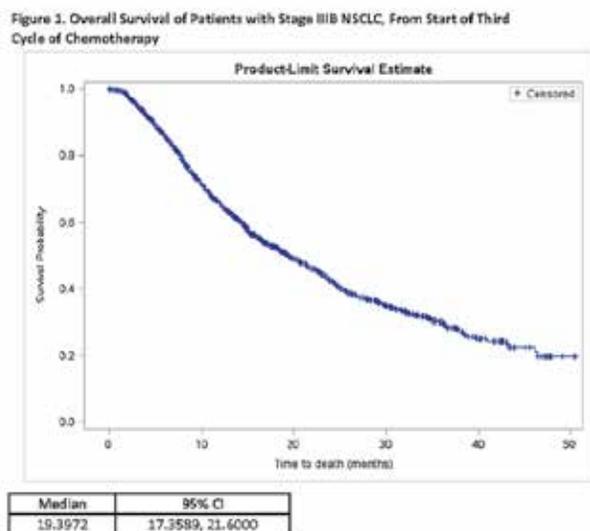
A total of 1,226 patients met all criteria for study inclusion. The mean age of patients was 68.5 ± 9.0 years, and 51.9% were male. The most common regimens observed at any time during follow-up were carboplatin + paclitaxel (43.9%), nivolumab (23.7%), and carboplatin + pemetrexed (21.0%); patients received a median of 2 lines of therapy (range: 1–7). The median OS was 19.4 months (95% CI: 17.4–21.6) from the start of the third cycle of chemotherapy (Figure 1).

Conclusions

Overall survival among Stage IIIB NSCLC patients who were treated with platinum-based chemotherapy in a real-world setting was less than 20 months. Previous literature indicates survival of 7–13 months among advanced NSCLC patients treated with chemotherapy. However, the present study excludes patients with metastatic disease. The difference in OS may also be related to the use of newer, more effective treatments that were not available in earlier studies.

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Figure 1. Overall Survival of Patients with Stage IIIB NSCLC, From Start of Third Cycle of Chemotherapy



P424

Efficacy of pembrolizumab (MK-3475) in patients with adrenocortical carcinoma

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Background

Adrenocortical carcinoma (ACC) is an orphan endocrine malignancy with poor prognosis and limited response to chemotherapy. ACC can express immune markers which could make it susceptible to immunotherapy. So far, there is no published clinical trial about using immunotherapy in ACC.

Methods

This is a phase II study of pembrolizumab in patients with rare tumors including a pre-specified ACC

cohort (<http://ClinicalTrials.gov> identifier, NCT02721732). Patients received pembrolizumab 200 mg intravenously once every 3 weeks. Response was assessed every 9 weeks using RECIST1.1. The primary end point was progression-free survival at 27 weeks (PFS27wk). Mandatory biopsies are taken at baseline, on cycle 1 day 15 -21, and at the time of progression.

Results

A total of 11 patients were enrolled and treated. At the time of analysis, patients received a median of 6 treatment cycles (range 2-17). A PFS of greater than 27 weeks was seen in 3/11 patients (27%). The first patient reached 37% tumor reduction (partial response) while the second patient had a response of 41% tumor reduction (partial response) and the third patient had stable disease. The remaining 8 patients had evidence of disease progression within 27 weeks of starting the study (median time 18 weeks, range 6-27 weeks). Progression was seen in most patients with hormonally active ACC (6/7) and only in 2/4 patients without evidence of hormonal excess at the time of their initial diagnosis. The safety profile of pembrolizumab was very favorable and none of these patients had severe grade 3 or grade 4 adverse effects.

Conclusions

Single agent pembrolizumab may be an effective option in subset of ACCs without hormonal overproduction while it is associated with high failure rate among patients with hormonally active ACCs. Expanding the study to hormonally silent ACCs is needed to verify the initial observation from limited number of patients treated in this study. Translational data will be presented at this meeting.

Trial Registration

<http://ClinicalTrials.gov> identifier, NCT02721732

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P425

Efficacy of pembrolizumab in patients with cutaneous squamous cell carcinoma

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Background

Cutaneous squamous cell carcinoma (cSCC) is the 2nd most common malignancy in the US. While the majority of patients are cured with surgery, approximately 6,400 die annually as a consequence of disease. There is limited data on the role of chemotherapy in the treatment of metastatic cSCC. The high-mutational burden, the presence of tumor-infiltrating lymphocytes, immunosuppression as a risk factor, and evidence of direct immunosuppressive effects of UV radiation suggest that cSCC is appropriate for the clinical study of checkpoint inhibitors.

Methods

This is a phase II study of pembrolizumab in patients with rare tumors including a pre-specified cutaneous squamous cell carcinoma cohort (<http://ClinicalTrials.gov> identifier, NCT02721732). Patients received pembrolizumab 200 mg intravenously once every three weeks. The response was assessed every nine weeks using RECIST1.1. The primary end point was progression-free survival at 27 weeks (PFS27wk). Mandatory biopsies are taken at baseline, on cycle 1 day 15 -21, and at the time of progression.

Results

A total of 11 patients were enrolled and treated. At the date of analysis, patients received a median of

5.5 treatment cycles (range 1-15). Three out of eleven patients have been treated beyond 27 weeks. Four patients had a partial response and the tumor reduction from the baseline have reached 33%, 55%, 63%, and 70%. The fifth patient had stable disease while the other five patients had a progressive disease within 27 weeks of starting the study. The last patient has not had his first restaging scans yet. The safety profile of pembrolizumab was very favorable.

Conclusions

Single agent pembrolizumab may be effective in a patient with advanced cutaneous squamous cell carcinoma. Expanding the study is needed to verify the initial observation. Translational data will be presented at this meeting.

Trial Registration

<http://ClinicalTrials.gov> identifier, NCT02721732

P426

Antibody-drug conjugate induced cytotoxicity of tumor cell lines by targeting the SAS1B N-terminus

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Background

SAS1B (ASTL, Ovastacin) is a zinc metalloproteinase found normally in growing oocytes that has been detected in uterine cancer cells, and its expression is restricted to oocytes, among adult tissues. Thus, it is classified as a cancer-oocyte neoantigen [1]. Treatment of uterine tumor cells with anti-SAS1B polyclonal antibodies and complement arrested growth, and a saporin-immunotoxin directed to

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SAS1B caused cell death. The goal of the present study was to develop immunotherapeutic monoclonal antibodies against the human SAS1B target, to reduce risks of off-target effects during therapy.

Methods

Monoclonal antibodies (mAbs) were generated to recombinant (r) human (h) SAS1B immunogen without its signal peptide.

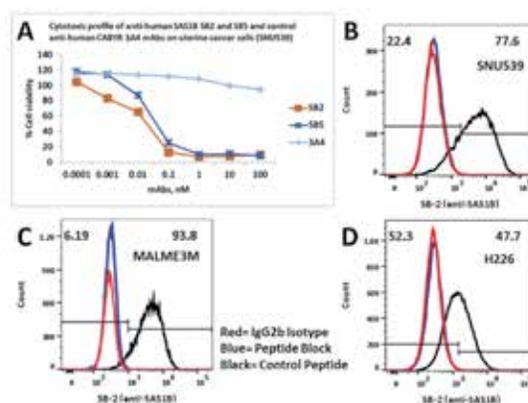
Results

SAS1B transcript in human uterine and lung cancer cell lines was confirmed by PCR and sequencing. SB2 and SB5 mAbs demonstrated Western immunoreactivity to rhSAS1B expressed in *E. coli* or HEK293T cells, and recognition of SAS1B was confirmed by mass spectrometry on immunoprecipitated antigen. SAS1B deletion constructs mapped the epitopes recognized by the SB2 and SB5 mAbs to the N-terminal domain between residues 32-40. Surface expression of SAS1B in live uterine, lung and pancreatic tumor cell lines was detected by both mAbs. The expression of SAS1B N-terminus on the surface of live tumor cells and its cytoplasmic expression from uterus (SNU539, Figure 1B), ovary, breast, melanoma (Malme3M, Figure 1C) lung (H226, Figure 1D), and were confirmed by flow cytometry and SAS1B peptide blocking. SAS1B expression was not evident on the surface of normal peripheral blood mononuclear cells. Monoclonal antibodies SB2 and SB5, complexed with a Fab-Duocarmycin DM, that incorporated a cathepsin-cleavable linker arm, were cytotoxic to SAS1B positive uterine (SNU539, Figure 1A), pancreatic and lung tumor cells at 10 - 100 picomolar concentrations.

Conclusions

The SB2 and SB5 mAbs offer promise as candidate immunotherapeutic entities for targeting SAS1B positive cancer cells with defined epitopes located in the SAS1B N-terminal domain suitable for immunotherapeutic targeting.

Figure 1. Profile of SAS1B expression on tumor cell lines by ADC and flow cytometry



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P427

Democratizing analysis of cancer data using the cancer genomics cloud

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Background

The advent of next generation sequencing has resulted in the generation of petabytes of multi-dimensional information, but the access and analyses of this data remains challenging. This difficulty is exemplified when we consider data generated by the efforts of The Cancer Genomics Atlas (TCGA) network. Historically, the only means for gaining insight from the TCGA data was to download the complete TCGA repository, which can require several weeks with a highly optimized network connection, followed by analyses in a very expensive high performance computational cluster.

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Methods

The Cancer Genomics Cloud Pilots project seeks to democratize cancer data analysis by co-localizing data with the computational resources. Funded by the National Cancer Institute, the Cancer Genomics Cloud Pilot (www.cancer-genomics-cloud.org) project enables researchers to leverage the power of cloud computing to gain actionable insights on cancer biology and human genetics from massive public datasets including TCGA.

Results

The CGC removes the need to download data, enables easy querying, and much more.

Conclusions

We will highlight our approach to optimized computation, data mining, and visualization solutions that address the challenges associated with analyses of petabyte-scale datasets and beyond.

P428

PD-1 blockade activates CD4 T cells and the innate immune response for glioblastoma eradication

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Background

Cancer immunotherapy has come of age as a result of substantive advances in basic and translational tumor immunology [1]. In fact, multiple advanced clinical trials with human anti-PD-1 antibodies (i.e. nivolumab and pembrolizumab) have convincingly established the ability of anti-PD-1 antibodies to trigger clinically significant tumor destruction in multiple tumor types, reinforced by dramatic anti-cancer responses in animal models and encouraging durability in early clinical trials [2].

Methods

Through flow cytometry and *in vivo* intracranial injection of mouse glioma tumor cells we recently demonstrated that PD-1 blockade elicits an anti-tumor immune response resulting in tumor rejection and long-term survival in approximately 50% of mice, despite the absence of accumulating CD8+ cytotoxic T cells in the tumor or draining lymph nodes [3].

Results

In this investigation, we provide evidence for the role of conventional CD4+ T cells and the innate immune response in PD-1 mediated anti-glioma immunity in this model. In response to anti-PD-1 monotherapy, intratumoral CD4+ T cells, but not CD8+ T cells, expressed significantly elevated levels of IFN- γ and TNF- α pro-inflammatory cytokines and the cytotoxic enzyme, granzyme B. Tbet, GATA3, and EOMES, transcription factors required for T cell proliferation, activation, and effector function, were also up-regulated in CD4+, but not CD8+ T cells in the brains of mice treated with PD-1 mAbs when compared to controls. We demonstrated that depletion of CD4+ or CD8+ T cells, but not NK, was sufficient to completely ablate anti-PD-1-mediated tumor eradication and long-term survival. CD4+ T cell activation was accompanied by the classical activation and M1 polarization of resident microglia and tumor-infiltrating macrophages. Moreover, CSF-1 inhibition with PLX3397 to deplete microglia and macrophages did not affect tumor growth, but did lead to a significant survival advantage when combined with anti-PD-1 mAb.

Conclusions

Together, these studies demonstrate for the first time a role for CD4+ T cells and the innate immune response in the eradication of glioblastoma by PD-1 blockade.

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Personalized Vaccines and Technologies/Personalized Medicine

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Nanoparticle based enrichment and expansion of self and neo-epitope specific CD8⁺ T cells in murine melanoma

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Background

T cell responses against neo-antigens has created a new avenue to target and kill patients' tumors while decreasing autoimmune side effects. Neo-antigen-specific CD8⁺ T cells have the potential benefit to be a source of T cells that are not limited by peripheral tolerance or deleted from the T cell repertoire by central tolerance. To understand the differences between T cell responses against neo- and self-antigens, these two types of T cell populations must be compared side-by-side for anti-tumor responses and phenotypic differences.

Methods

Following another group that identified mutated proteins in B16 murine melanoma [1], our current work has identified the short peptide epitopes that can stimulate neo-antigen T cell responses. Artificial antigen presenting cells (aAPCs) were used to access neo- and self-antigen tumor specific responses in the endogenous T cell population of naïve and B16

tumor bearing animals. In a B16-SIY melanoma model, neo-epitope Kb-SIY serves as a model to collect T cells for self and neo-epitopes and analyze the T cell receptor (TCR) repertoire of the separate populations.

Results

After exposure to tumor, neo-epitope T cell responses showed larger fold expansion, while T cell expansions for self-antigen Kb-TRP2 expanded similar in the naïve animals. TCR repertoire analysis of neo- and self-antigen reactive T cells showed a highly conserved V beta gene usage in the naïve Kb-SIY TCR repertoire, while the naïve Kb-TRP2 TCR repertoire had a more broad V beta selection. The tumor did not alter the V beta usage for Kb-SIY repertoire, however the Kb-TRP2 repertoire changed to different V beta genes after tumor exposure.

Conclusions

The increased expansion ability of neo-antigens after tumor exposure demonstrates the potential anti-tumor ability of neo-epitope responses compared to self-epitope T cell responses. TCR repertoire analysis of neo- and self-antigen reactive T cells shows that the tumor shifts the TCR clonal diversity of self-antigen specific populations to but leaves the neo-epitope repertoire intact and similar to the naïve repertoire. From this work, we show proof of concept of a streamlined method to screen candidate neo-epitopes, identify rare endogenous T cell populations, and assess the benefit of T cell directed immunotherapies on different T cell populations.

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Neoantigen identification using ATLAS™ across multiple tumor types highlights limitations of prediction algorithms

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Background

Neoantigens arise from tumor-specific, somatic mutations and have the potential to be recognized by T cells that are associated with anti-tumor immune responses. Since they are non-self, they are hypothesized to provide an attractive therapeutic modality since T cells that can respond to those sequences have not undergone thymic selection. The ATLAS™ platform enables identification of biologically relevant CD4⁺ and CD8⁺ T cell neoantigens in any subject in an unbiased manner.

Methods

Multiple patients with solid tumors were analyzed. CD14⁺ monocytes and T cell subsets were isolated from patient peripheral blood mononuclear cells. Monocytes were differentiated into dendritic cells (MDDC), and T cells were non-specifically expanded. Whole exome sequencing was performed on tumor biopsies and matched normal genomic DNA, and tumor specific changes (single nucleotide variants and insertion/deletions) were identified and cloned into *E. coli* expression vectors with and without co-expressed listeriolysin O to enable presentation via MHC class I or class II, respectively. For each patient, their unique clones were co-cultured with autologous MDDCs in an ordered array, then their CD4⁺ or CD8⁺ T cells were added and incubated overnight. T cell activation was determined by measurement of TNF- α and IFN- γ in the supernatants by meso-scale discovery. Cytokine concentrations were normalized against responses

to negative control bacteria and neoantigens were defined as clones that elicited responses >3 median absolute deviations of the median of negative controls.

Results

ATLAS™ identified CD4⁺ and CD8⁺ T cells responses to up to 15% of mutant polypeptide sequences, across a broad cohort of patients with different tumor types, including tumors with either low or high mutational burden. “Inhibitory” neoantigens, which shut off all cytokine production, were also identified in each subject. Many neoantigens were not predicted by algorithms. When exploring neoantigens by tumor type, no patterns in overall mutational burden, RNA expression level, or DNA mutant allele frequency have yet been identified.

Conclusions

The ATLAS™ platform empirically defines which potential neoantigens created by somatic mutations elicit immune responses in individual patients independently of a patient's HLA type and T cell receptor repertoire. The identification of activating and inhibitory neoantigens for CD8⁺ T cells, as well as for CD4⁺ T cells for which the algorithmic approaches do not perform nearly as well, provides the opportunity to identify better targets to include in a vaccine formulation. Genocea is currently developing personalized cancer vaccines with neoantigens prioritized by ATLAS™ which we hypothesize will provide better therapeutic impact than algorithmic approaches.

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Identification of immunogenic breast cancer neoantigens exposed by radiation therapy

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Background

Recent studies have highlighted the key role of mutation-generated neoantigens in tumor response to immunotherapy [1]. We have previously shown in the 4T1 mouse model (syngeneic with BALB/c mice) of immune-checkpoint blockade-resistant metastatic breast cancer that local radiation therapy (RT) combined with CTLA-4 blockade induces the CD8⁺ T cell-mediated regression of irradiated tumors and limits metastatic lung colonization [2]. Preliminary analysis of the T-cell receptor repertoire indicated that unique clonotypes are expanded in treated tumors, suggesting that tumor rejection involves T cells reactive to a set of tumor antigens that are made available to the immune system by RT. Therefore, we hypothesize that RT, by changing the transcriptional profile of cancer cells, may expose antigenic mutations not transcribed at sufficient levels in untreated tumors and hence promote priming of T cells to these unique mutated antigens.

Methods

We performed whole-exome sequencing and RNA sequencing (RNA-seq) of 4T1 cells irradiated (8GyX3) or not (0Gy) *in vitro* to identify tumor-specific mutations. We used several algorithms to predict MHC-I (H2^d)-binding epitopes from these mutated genes, and we selected those with a predicted affinity <500 nM. Based on the RNA-seq data, we prioritized variants that were upregulated by radiation. These candidate peptides were synthesized and tested *in vitro* for binding to H2-L^d or H2-K^d in a MHC stabilization assay using RMA-S cells. Finally, the mutated peptides with the highest affinity were used to vaccinate BALB/c mice, followed by challenge with 4T1 cells to test for the induction of protective anti-tumor immunity.

Results

Out of 309 total mutations initially identified in the 4T1 cancer cells, we selected and tested *in vitro* 17 candidate neo-epitopes. Two of them bound H2-L^d with a high affinity and were used alone or in combination to vaccinate mice. Our preliminary data indicate that the combination of these two

neoepitopes is immunogenic and delays the tumor growth after challenge with 4T1 cells.

Conclusions

In conclusion, these data provide initial proof-of-principle evidence that RT can expose existing neoantigens to the immune system.

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Computational pipeline for the PGV-001 (personalized genomic vaccine) clinical trial

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Background

PGV-001 (NCT02721043) is a phase I clinical trial at Mount Sinai Hospital, studying the safety and immunogenicity of a multi-peptide personalized genomic vaccine for treatment of several different malignancies. Patients are treated with a TLR3 agonist (Poly-ICLC) and ten synthetic long peptides containing tumor mutations that are predicted to be abundantly expressed and to form mutated MHC

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ligands. The personalized vaccine is administered as an intramuscular injection and is given to each patient 10 times over a span of 6 months. Thus far, two patients (of an eventual twenty) have enrolled in the trial and one has been treated. The vaccine is administered in the adjuvant setting for patients who undergo a complete resection and have no evidence of residual disease.

Methods

Tumor DNA and RNA are extracted from fresh frozen tissue immediately following surgery. Normal DNA is extracted from a patient blood sample. Both DNA samples are enriched for coding regions using an exon capture kit. Normal DNA is sequenced to mean on-target coverage of 150x, whereas tumor DNA is sequenced to 300x coverage. RNA is enriched for mRNA using poly-A capture and then >100M paired-end reads are sequenced. Somatic variants are detected from the tumor and normal DNA sequencing data. The RNA sequencing data is then used to assemble the most abundant coding sequence for each variant, which naturally phases adjacent variants and allows for an allele-specific estimate of expression. Each coding sequence is then translated to a mutated protein fragment, which is ranked according to (1) expression and (2) predicted binding affinity to patient MHC I molecules. The window of wildtype amino acids around a mutation in each peptide is optimized to improve the odds of successful solid phase synthesis.

Results

Though only two patients have enrolled in PGV-001 so far, we have evaluated the pipeline on a variety of patient (and mouse) samples across different kinds of cancers. We demonstrate that even cancers with intermediate mutational burden still yield sufficient predicted neoantigens to potentially benefit from vaccination. Furthermore, even with our existing attempt to optimize peptide sequences for manufacturability, we have still found many peptide sequences cannot be synthesized or purified. Analysis of these peptide sequences hint at

additional rules which may improve synthetic yield for peptide-based neoantigen vaccines.

Conclusions

We hope that discussion of the design and rationale for PGV-001's computational pipeline will help other groups start their own personalized vaccine programs.

Trial Registration

NCT02721043

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Great Apes Adenoviral vaccine encoding neoantigens synergizes with immunomodulators to cure established tumors in mice.

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Background

Current immunotherapies based on checkpoint inhibitors (CPI) revealed the importance of neoantigens-specific T cells for tumor control. Here, we developed a novel neoantigens vaccine approach based on the use of a viral vector, Great Apes Adenovirus (GAd), encoding multiple cancer neoantigens in tandem. NKTR-214 is a CD122-biased cytokine agonist currently in clinical trials. NKTR-214 is designed to expand a specific population of cancer-killing T cells, known as tumor-infiltrating lymphocytes. Synergy between the two drugs was investigated.

Methods

Balb/c mice were implanted subcutaneously with CT26 colon carcinoma cells. Once tumors were

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established, mice were randomized and vaccinated with a single intramuscular injection of GAd encoding up to 20 different CT-26 neoantigens identified by NGS and selected with a NousCom proprietary pipeline. NKTR-214 was administered i.v. on q9dx3 schedule. Tumor growth in treated animals was monitored and neoantigen-specific T cells were measured both in the periphery and in tumors.

Results

Combination treatment of a GAd-CT26 neoantigens vaccine with NKTR-214 results in tumor regression in 90% of mice. Single agent NKTR-214 treatment led to tumor regression in < 40% of mice. Interestingly, all animals cured by the combo were resistant to a second tumor challenge. NKTR-214 treatment rescued CD4 T cell reactivity against at least one of the predicted neoantigens, likely induced by the tumor itself. GAd vaccination in the presence of NKTR-214 induced a greater breadth and depth of immune response. When compared to Gad vaccine alone, the Gad + NKTR-214 combination had immune reactivity detected against a larger number of vaccine-encoded neoantigens and there was a greater proportion of IFN-gamma+ CD4 and CD8 T cells against each vaccine-encoded neoantigen. Tumors in regression of mice treated by the combo were highly enriched in T cells reactive to vaccine-encoded neoantigens.

Conclusions

Vaccination with GAd encoding neoantigens is a very effective and safe approach to strengthen and broaden tumor-killing T cells. Therapeutic cancer vaccines have been unsuccessful in the past likely because T cell growing capacity in the tumor is suppressed by the tumor itself. Vigorous growth of vaccine-induced T cells in the tumour can be successfully achieved by the cytokine NKTR-214, overcoming the tumor inhibitory activity. NKTR-214 was shown to be safe and well tolerated in cancer patients and GAd vaccines have been safely used in many healthy volunteers, opening up the opportunity for a fast evaluation of a combo treatment in the clinic.

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Immunogenomics for the development of personalized T cell therapy for ovarian cancer

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Background

Ovarian cancer (OC) is the fifth leading cause of cancer death in the United States with approximately 20,000 women being diagnosed every year. Since OC development is mainly asymptomatic, patients are often diagnosed at late stage and present local and distal metastases. This offers a clinical challenge, as roughly 70% of the patients develop chemoresistant disease. With the advent of cancer immunogenomics it is now possible to identify tumor specific mutations, or neoantigens/neoepitopes, that can be exploited for the development of personalized T cell therapies via DC vaccines or adoptive cell therapy (ACT). We therefore hypothesize that tumor cells isolated from patients with ovarian cancer offer a unique opportunity to identify clinically actionable tumor neoantigens.

Methods

Somatic mutations were identified by whole exome sequencing (WES) and RNASeq analysis in tumor samples, using PBMCs as germline control. In parallel ovarian cancer PDXs were established in NSG mice by injecting 2x10⁶ patient tumor cells/mouse subcutaneously (SQ, n=20). Tumor growth was monitored weekly via caliper measurement. Mutational analysis and immunogenicity prediction for the mutated peptides was done using a combination of home-developed bioinformatic approaches and NetMHC. Immunogenicity predictions were validated *in-vitro* by pulsing wild type and mutated peptides into CD8+ depleted PBMCs and co-cultured with CD8+ T cells for up to 20 days. T cell activation was quantitated via ELISA measuring IFN-g release and

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via flow cytometry by staining for CD137 expression. Lastly, the therapeutic potential of ACT targeting neoantigens was tested *in-vivo* in ovarian cancer PDXs and tumor size is being monitored weekly.

Results

We have established patient-derived xenografts (PDX) mice and interrogated the tumor mutational landscape. Targeted mutations were confirmed in the following PDX passages by NGS and Sanger sequencing. Out of 168 mutated peptides predicted from the patient tumor, 13 were found to have high affinity for different HLAs compared to the wild type peptide. In vitro screening of high affinity peptides showed that few of the mutated peptides can activate the T cells from patient PBMCs and tumor infiltrating lymphocytes thereby confirming the presence of neoantigen reactive T cells.

Conclusions

In this study, we demonstrate that the patient's own T lymphocytes can recognize tumor neoantigens and could be used as personalized therapy in ovarian cancer patients

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***In vivo* self-assembled albumin/vaccine nanocomplexes for lymph-node-targeted vaccine delivery in combination cancer immunotherapy**

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Background

Cancer immunotherapy aims to normalize the immune system in patients to harness the power of the immune system to systemically treat cancer. Molecular vaccines hold tremendous potential for tumor immunotherapy, but its clinic outcome has yet been optimal, largely due to inefficient co-delivery of heterogeneous peptide antigens and adjuvants to secondary lymphoid organs such as

lymph nodes (LNs). Nanovaccines were attempted to improve vaccine delivery, however, clinical translation of most of them have been impeded by complicated large-scale manufacturing and safety concerns.

Methods

We developed an albumin-binding Evans blue derivative as LN-homing moieties and cell uptake mediators for cancer vaccine delivery. The safety of this derivative has been validated in 2 ongoing clinical trials involving more than 200 patients and healthy volunteers. By conjugating this derivative with molecular vaccines (CpG adjuvant, B16F10 melanoma-associated antigen Trp2, and MC38 colon adenocarcinoma-specific neoantigen Adpgk), we developed albumin-binding vaccine (AlbiVax) (Figure 1A) to efficiently co-deliver molecular adjuvants and antigens to LNs. By PET imaging of radiolabeled AlbiVax in mice, we systematically optimized AlbiVax for LN-targeted delivery, and light-sheet fluorescence imaging and super-resolution confocal microscopy were employed to study vaccine behaviors in transparent LNs and single antigen-presenting cells (APCs). In three tumor models, we studied the antigen-specific immunogenicity and tumor therapy efficacy of AlbiVax, either alone or in combination with anti-PD-1 or Abraxane.

Results

Quantitative PET imaging revealed that AlbiVax was delivered to LNs more efficiently (20-fold for CpG, up to 91-fold for antigens) than unconjugated vaccines or vaccines emulsified in Incomplete Freund's Adjuvant (IFA) (Figure 1B, Figure 1F). Light-sheet fluorescence imaging elucidated the distribution of AlbiVax in transparent draining LNs (Figure 1, Figure 1D); and super-resolution imaging revealed efficient intracellular co-delivery of AlbiVax *via* albumin into endolysosome of APCs (Figure 1E). AlbiVax elicited >21-fold higher frequency of Antigen-specific CD8⁺ cytotoxic T lymphocytes (CTLs) than IFA-emulsified vaccines and induced immune memory for > 5 months (Fig. g). AlbiVax dramatically regressed or inhibited the progression

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of established primary or lung metastatic EG7.OVA (Figure 1G), B16F10 (Figure 1H), and MC38 tumors (Figure 1I). Remarkably, combining AlbiVax with immune checkpoint inhibitor anti-PD-1 further improved the therapeutic efficacy, and led to the complete regression of 60% MC38 tumors for at least 5 months, and triple combination of AlbiVax, anti-PD-1, and Abraxane exhibited synergistic therapeutic efficacy (Figure 1H, Figure 1I). AlbiVax showed excellent safety profile throughout the study.

Conclusions

AlbiVax represents a promising safe, widely applicable, and robust T cell vaccine for combination cancer immunotherapy.

Figure 1. In vivo self-assembled albumin/vaccine nanocomplexes for lymph-node-targeted vaccine delivery in combination cancer immunotherapy

(a) Schematics of albumin-binding vaccine (AlbiVax) for LN-targeted vaccine delivery for cancer immunotherapy. (b, f) by PET imaging of radiolabeled AlbiVax in small animals, AlbiVax was systematically optimized for optimal LN-targeted delivery. (c, d) By light-sheet fluorescence imaging, we elucidated the distribution of AlbiVax in draining LNs that were cleared to be transparent. (e) By super-resolution imaging, we discovered efficient intracellular co-delivery of AlbiVax via mouse serum albumin (MSA) into endolysosome of bone marrow derived dendritic cells (BMDCs). (g) AlbiVax elicited 21-fold higher frequency of Antigen-specific CD8+ cytotoxic T lymphocytes (CTLs) than IFA-emulsified vaccine. (h-j) AlbiVax dramatically regressed or inhibited the progression of EG7.OVA (g), B16F10 (h), and MC38 tumors (i).

PHYSICIAN/NURSE/PHARM: Best Practices for Improving Cancer Immunotherapy

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Mapping the treatment pathway for metastatic uveal melanoma (mUM) patients in England: A qualitative pilot study.

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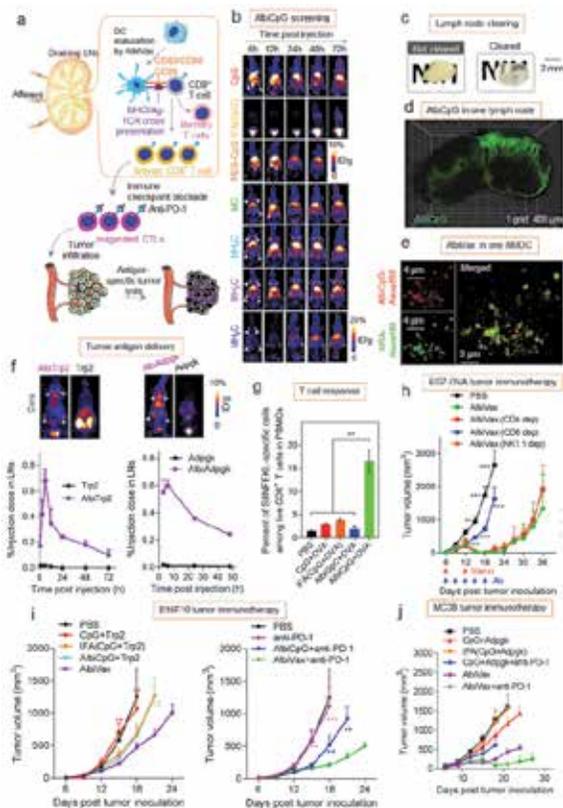
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Background

mUM is a rare disease (annual UK incidence <150 patients), with a reported median overall survival of 7-12 months. In 2015, the UK published their first national uveal melanoma guidelines [1]. However, with few therapeutically effective options available for metastatic patients, it is unclear what patients actually receive as standard of care (SoC). This pilot study aimed to map the real-world pathway for recently diagnosed mUM patients, through qualitatively evaluating their clinical management and treatments received within SoC.

Methods

Based on treatment recommendations within national guidelines, an outline decision-tree for mUM patients was developed. On presenting the decision-tree to 5 specialist centres across England (January-June, 2017), 6 senior clinicians were interviewed about their typical: clinical management; medical resource use; treatment decision-making and patient flow(s) between local and regional facilities, when treating mUM patients. The interview data were analysed to populate centre-level decision-trees, and consolidated to inform a consensus SoC pathway.

Results

Interviews revealed considerable variation in first-line treatments offered at specialist centres:

- hepatic resection for liver metastases [1-25%];
- loco-regional therapies for liver metastases (including percutaneous hepatic perfusion and radiofrequency ablation) [0-20%];
- new treatments in clinical trials (TRAP, SelPac or IMCgp100) [10-80%];
- immunotherapies (Ipilimumab, Pembrolizumab or Nivolumab) [0-54%]; and
- chemotherapies (Dacarbazine or Temozolomide) [0-1%].

There was, however, consensus on treatment priorities - if liver metastases were operable then surgical options were considered first. If non-

operable, clinical trials were subsequently considered, before other therapeutic options. Whilst centres agreed that treatment should be initiated at a specialist (supra-regional) mUM centre, there were mixed attitudes on “if” and “how” patients could receive ongoing treatments at more local centres.

Conclusions

The main reason suggested for treatment pathway variations amongst centres, was the absence of effective treatments. Introducing an effective therapeutic option at a defined optimum point in the pathway would be considered a “catalyst to transform the management of care”. This in turn would lend itself to greater consistency of practice, harmonise care pathways and improve overall outcomes for mUM patients.

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Continued challenges and opportunities in oncologists' COMPREHension of clinical immunology and its relationship with cancer immunotherapy

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Background

Cancer immunotherapy (CIT) is revolutionizing the treatment of cancer. As part of an ongoing educational curriculum, assessment of oncologists'

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knowledge, competence, and performance in the use of CIT identified multiple challenges and barriers. This study's objective was to assess and compare practicing oncologists' comprehension of, and confidence in, the interplay between the immune system and cancer tumor development and metastasis.

Methods

An expert panel was convened to identify knowledge, competence, and performance gaps in the area of CIT. Educational curricula consisting of 8 activities that focused on foundational education were developed in 2014-2016, and posted online. Intra-activity questions embedded in each activity allowed learners to self-report their confidence with CIT concepts, while matching participants' responses to knowledge-based questions presented before and after education were collected to measure reinforced learning and gains in comprehension. Confidentiality of respondents was maintained. Responses were collected from 5/2014-10/2016.

Oncologists (N=4,684) who participated in the activities demonstrated on average a 20% improvement from initial baseline between years 1 and 2. Additional education in year 3 was provided to help prevent decay in oncologists' comprehension of immune system functions. However, comparison of oncologists' knowledge of the role of immune checkpoints indicates that while gains observed in year 1 were maintained, a drop off was observed between years 2 and 3. This is likely a result of more data becoming available on alternative CITs while simultaneously less foundational education was available.

Results

Oncologists continued to report a lack of confidence with the concepts of clinical immunology. Only 2%-11% of oncologists were very confident in their comprehension of the relationship between the immune system and tumor development. In addition, fewer than 50% of participants post-activity could recognize common T cell co-receptors

that could be targeted as part of CIT. One quarter did not realize that tumors interfere with signal 1 by blocking MHC antigen processing.

Conclusions

This study demonstrates the value of and continued need for educational interventions on CIT. Retention of CIT concepts and improvements in knowledge of clinical immunology should facilitate competence among oncologists, which has valuable implications for use of CIT and for patient outcomes.

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PDL-1 status and value for Extrapulmonary small cell carcinomas

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Background

Extrapulmonary small cell carcinomas (ESCCs) are rare but aggressive tumors with an overall 5-year survival rate of less than 15%. Relapse is common despite rigorous chemotherapy and radiotherapy. Cancer immunotherapy targeting PD-1/PD-L1 pathway emerges as an increasingly appealing strategy [1, 2, 3].

Methods

We investigated PD-L1 expression by immunochemistry (IHC) in ESCCs diagnosed at our institution from 1999-2016. 34 cases with sufficient material were selected for PD-L1 IHC analysis using clone E1L3N. Sites of origin include bladder (9), cervix (3), prostate (2), ovary (2), gallbladder (2), duodenum (1), esophagus (1), biliary tract (1), vagina (1), palate (1), ureter (1), and unknown primary (10).

Results

2 cases showed diffuse PD-L1 expression in both tumor and stromal cells, one from ureter and one

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from a gallbladder in origin. 10 cases (29%) showed at least patchy PD-L1 expression in stromal cells. 22 cases (65%) were completely negative for PD-L1 expression. Patients of both groups who was treated, did received chemotherapy +/- surgery or radiation. The overall response rate for PDL positive group was 80% while it was 67% for PDL-1 negative group (Table 1). The median overall survival for PD-L1 positive staining group, regardless of stage was 11.5 months' vs 7 months for PDL-1 negative group (Figure 1). Patients with limited stage disease with positive PDL-1 showed an impressive 53 months' survival compared to PDL-1 negative limited disease with 15 months.

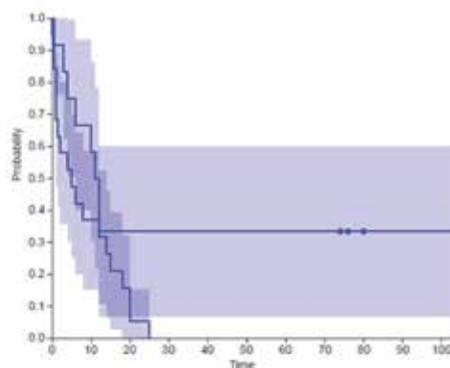
Conclusions

This study suggests that PD-L1 staining in extrapulmonary small cell carcinoma may be of prognostic relevance and further studies are required to understand the implications of immune dysregulation in these aggressive tumors. PD-1 inhibitors should be investigated in this group of patients.

Table 1.

Trial	Positive PDL-1 (n=12)	Negative PDL-1 (n=22)
Received Treatment	10 (83%)	16 (73%)
Response rate to treatments	8 (80%)	11 (67%)
Median Overall Survival	11.5 months	7 months

Figure 1.



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PHYSICIAN/NURSE/PHARM: Case Studies demonstrating exceptional responses

P440

Durable complete response with PANVAC and Trastuzumab in metastatic triple positive breast cancer (mTPBC)

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Background

First line treatment of metastatic HER2+ breast cancer with Docetaxel+Trastuzumab (TH) carries a median PFS of 12 months and OS of 40 months.¹ The addition of Trastuzumab+/-Pertuzumab to chemotherapy prolongs survival in the metastatic setting¹ but no therapies are curative. Trastuzumab has immune-related effects including antibody-dependent cell-mediated cytotoxicity and cross-presentation of tumor antigens. PANVAC is a recombinant poxviral vaccine that contains transgenes for MUC-1, CEA and T-cell costimulatory molecules (B7.1, ICAM-1 and LFA-3). For optimal response, PANVAC employs a prime-boost regimen, using vaccinia (PANVAC-V) as the primer and fowlpox (PANVAC-F) as the boost. We report an exceptional response of mTPBC to PANVAC+Trastuzumab after progression on Letrozole+Trastuzumab.

Methods

A pilot study of PANVAC+Sargramostim (NCT00088413) demonstrated minimal side effects and suggested clinical benefit in low disease burden.² On Day 1 (D1), patients received PANVAC-V 2x10⁸ pfu SC followed by PANVAC-F 1x10⁹ pfu SC every 2 weeks x 3, then monthly x 12 followed by maintenance every 3 months until progression. Sargramostim 100 µg SC was given on D1-D4 of each PANVAC injection. Scans were performed every 2-3 months.

Results

A 32-year-old female, BRCA wildtype, with poorly differentiated, invasive ductal carcinoma, ER60%, PR70%, HER2+(IHC3+) had biopsy-confirmed metastasis to an axillary node and liver shortly after diagnosis. After bilateral oophorectomy, she received THx6 cycles followed by a partial hepatectomy, which demonstrated a pathologic complete response. She received radiation to supraclavicular lymph nodes with a tumor bed

boost. After 12 months on Letrozole+Trastuzumab she recurred. While on Letrozole she completed 6 injections for a HER2/neu peptide vaccine trial (NCT00194714). Due to progression in bilateral hilar and subcarinal (2.8 cm) lymph nodes determined by PET, she enrolled on trial at NCI in January 2008. Per protocol above, she received PANVAC-V followed by PANVAC-F. Restaging at 10 months showed a partial response (0.8 cm) and at 18 months showed a complete response (CR). She has remained on-study and receives PANVAC-F+Sargramostim every 90 days and Trastuzumab every 3 weeks. Restaging in May 2017 shows a continued CR at 113 months.

Conclusions

This case report highlights the potential of immunotherapy and possibly multiple immunotherapies in a patient with breast cancer. Further study is required to understand the mechanism of response in this patient and if it can be applied on a broader scale.

Trial Registration

NCT00088413

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P441

Activity of Nivolumab (Opdivo®) in relapsed progressive peripheral T-cell Lymphoma-NOS (PTCL-NOS)

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Background

Nivolumab is a member of the family of check-point inhibitors. It is a PD-L1 inhibitor that has been approved by the FDA as treatment for several solid tumors including non-small cell lung cancer. It's also approved to treat Hodgkin's Lymphoma after failure of several lines of therapy. PTCL is often aggressive and incurable. Compared to B-cell lymphomas, they tend to present at more advanced stages and respond less frequently and with shorter duration to the same chemotherapy regimens. Nevertheless, PCTL is still approached with suboptimal B-cell therapeutic strategies. As to the recently approved PTCL specific therapies, they are palliative in nature. An urgent need for better therapeutic agents in this desperate lymphoma continues to exist.

Methods

We report on the activity of Nivolumab as a 4th line treatment in a case of relapsed and rapidly progressive PTCL-NOS.

Results

Our patient was a 62 y.o. white man who was diagnosed with poor risk stage IVA PTCL and Stage IA lung squamous cell carcinoma in 2014. Due to his borderline functional performance status and decreased left ventricular ejection fraction, he was treated with Pralatrexate that is approved for PTCL and has activity in lung cancer. He received 2 cycles of Pralatrexate which stabilized both malignancies for 3 months after which they progressed. Subsequently, he was given Carboplatin and Gemcitabine combination that has activity in both malignancies. This combination seemed to stabilize his lung cancer and PTCL for 4 months. After the 5th cycle, the patient's lymphoma progressed. At this time, the patient was started on Belinostat to address his PTCL. Initially, PTCL responded to Belinostat and then stabilized for 5 months. After the fifth cycle, his lung cancer started progressing requiring lung cancer directed therapy. He was started on Nivolumab which stabilized his lung cancer. After the 4th dose of Nivolumab further

unexpected PTCL response was noted clinically and on follow up imaging. This response was maintained for a period of 15 months spanning 27 cycles of Nivolumab without any significant toxicity. Unfortunately, by the 27th cycles the patient's lung cancer progressed and he decided to stop all treatment and enroll in hospice where he eventually expired due to his progressive lung cancer.

Conclusions

This case demonstrates for the first time the notable activity and clinical benefit of Nivolumab as a single agent in PTCL-NOS. Further evaluation of Nivolumab's activity in PTCL as well its incorporation into PTCL treatment approaches need additional study and validation.

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Pseudoprogession manifesting as recurrent ascities with anti-PD-1 therapy

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Background

Pseudoprogession occurs when patients treated with immunotherapy have an initial increase in apparent tumor size prior to regression. This phenomenon results from an influx of activated immune cells, occurring in up to 7% of patients [1]. Ascites is also a common manifestation of progressive malignancy from peritoneal metastases. However, ascites has not been previously reported as a manifestation of pseudoprogession. We describe a metastatic urothelial cancer patient with progressive ascites and concurrent reduction in tumor burden on anti-PD-1 therapy.

Methods

Pembrolizumab was given 200 mg IV every 3 weeks. Computed tomography scans were obtained and tumor burden assessed by RECIST v1.1. Peritoneal

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fluid was analyzed for cytology and fluorescence-activated cell sorting (FACS) using FlowJo10.1r5. Multiplex cytokine assay was performed on peritoneal fluid. Normal donor PBMCs stimulated with phorbol myristate acetate/ionomycin was used as a positive control.

Results

A 62-year-old female with urothelial bladder cancer previously treated with chemotherapy and radiation presented with recurrent metastatic disease including two peritoneal nodules and an enlarged para-aortic lymph node. Pembrolizumab was started and she had a significant partial response with a 49% reduction in target lesions. During therapy, she developed large-volume ascites. Initial paracentesis showed a serum-ascites albumin gradient >1.5, total protein of 1.2 g/dL, and leukocyte count of 231/ μ L including 45% lymphocytes. Cytology from four separate paracenteses over 8 weeks showed reactive cellular changes/inflammation, but no malignant cells were identified.

FACS analysis on peritoneal fluid showed CD8⁺ lymphocytes comprised 53% of CD45⁺CD3⁺ cells. The majority of CD8⁺ lymphocytes were PD-1⁺ (78%). Class II-MHC (HLA-DR) and CD40 ligand (CD154) were expressed on 43% and 20% of CD8⁺ lymphocytes, respectively. All three markers were increased relative to normal donor PBMC controls, suggesting a locally high number of antigen-experienced, activated T cells. FoxP3⁺CD25⁺ Tregs were also detected in 17% of the CD45⁺CD3⁺CD4⁺ compartment. Multiplex cytokine assay for 38 analytes was performed. The most significantly elevated analytes were IL-6, IL-15, G-CSF and FGF-2 (each >2-fold above control).

Conclusions

Pseudoprogression in cancer patients treated with immunotherapy can manifest as recurrent ascites due to a rapid influx of activated T lymphocytes into the peritoneum. Since ascites can also be caused by disease progression, it is important to clinically

distinguish the two, so that treatment may be appropriately continued or stopped.

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P443

Malignant melanoma metastatic to the heart: exploring patient outcomes and cardiovascular complications in the era of immunotherapy

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Background

Cardiac metastasis (CM) from melanoma is being recognized more frequently, but often presents without signal symptoms. A rate of CM of 64% has been quoted in one autopsy series [1], though the clinical diagnosis rate is considerably lower [2]. We evaluated patients with known CM for cardiac complications and impact of therapy, and defined survival among these patients.

Methods

This retrospective review identified patients with CM seen in the University of Virginia Oncology clinic between 2010 and 2017. Eligible patients were at least 18 years old with metastatic melanoma (MM) and CM.

Our primary outcome metrics were 2 year survival from time of diagnosis of melanoma (M) and MM. We recorded cardiac complications, diagnostic procedures, patient outcomes, and treatment modality. Ten patients were identified with the demographics outlined below. Longest follow up was 21 months from diagnosis of CM (DCM).

Results

Two year survival from M diagnosis was 70%. Two year survival from DCM could not be calculated

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(two patients are still living at 9 and 21 months from DCM). Figure 1 shows Kaplan-Meier survival analysis. Of the eight patients who died, all died within one year of DCM. Responses to immunotherapy (IO) are shown in Table 2.

Conclusions

The rate of cardiac complications was low. Only one death was a direct result of CM (patient 10).

90% of patients received IO. At the time of data collection, two patients remain (2 and 3) alive nine and 21 months since DCM; both received >20 cycles of P. Patients 1, 2, 3, 8, 9, and 10 received IO after DCM, but only patient 3's CM responded to IO (IN induction, P maintenance). Patient 1 did not respond to I; patient 2 was lost to follow up on IL-2; patient 8's response cannot be determined due to death from septic shock; patient 9 progressed through P; and patient 10 did not respond after one cycle of I.

This study is limited by small patient numbers, but supports a poor prognosis for patients with CM. Responses can be seen with IO, specifically PD-L1 inhibitors, which may improve survival. Other treatments cannot be assessed. The only patient who survived one year after DCM has had an ongoing response to P.

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PHYSICIAN/NURSE/PHARM: irAE Management: Clinical Care and Best Practices

P444

Inflammatory arthritis induced by the use of checkpoint inhibitors for immunotherapy of cancer

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Background

Checkpoint inhibitors (CPI) have revolutionized cancer treatment with remarkable survival benefits in multiple cancer patients. Yet, their use can be hampered by frequent immune-related adverse events (irAEs), affecting any organ, with signs and symptoms usually seen in inflammatory and autoimmune diseases. There is paucity of data on CPI induced arthritis, primarily from case reports or small series.

Methods

Patients developed arthritis while using CPI were identified from the Rheumatology Clinic at UT MD Anderson Cancer Center. We diagnosed arthritis if patients developed joint swelling after receiving any FDA approved CPI, after ruling out other potential underlying causes or pathologies. Here we report 13 pts. Cancer types included melanoma (n=5), non-small cell lung cancer (n=4), renal cell carcinoma (n=2), acute myeloid leukemia, and cancer colon (1 each). CPI included nivolumab (n=8), pembrolizumab (n=3), and ipilimumab and atezolizumab (1 each).

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Results

Patients age ranged from 47-74 years and 62% were male. CPI induced arthritis was the sole irAE in eight pts (62%), and was associated with other irAEs including sicca symptoms (xerostomia, dry eyes), colitis, pancreatitis, or dermatitis in the remaining five (38%). Overall, median time to onset of arthritis after the initiation of CPI was 6 (range 1 to 15) months. When occurred with other irAEs, arthritis always manifested up to 4 months after the first occurring irAE despite sufficient immunosuppressant treatment (infliximab and/or high dose steroid) and symptoms control, with the exception of one patient with sicca symptoms. Initial symptoms varied widely between small and large joints of upper and lower extremities. Over time, symmetrical polyarthritis resembling rheumatoid arthritis, predominantly involving small joints of the hands and wrists, along with larger joints was reported in six patients. Two had oligoarthritis resembling seronegative spondyloarthropathy with axial pain and enthesopathy. Four had asymmetrical inflammatory polyarthritis, and one had oligoarthritis. Rheumatoid factor and/or anti-citrullinated peptide antibodies were positive in two patients, and antinuclear antibodies in two others. Eleven patients were treated with steroids, six were controlled, while five needed additional biological agents (4 tocilizumab, 1 infliximab) to achieve appropriate steroid taper (< 7.5 mg). One patient improved with intra-articular steroid injection, and one received tocilizumab as first line.

Conclusions

Our data suggest that arthritis is a late occurring irAE that may be due to a different immunopathology. A multi-institutional collaborative work including tissue analysis is needed to enhance our understanding of the pathogenesis and design optimal therapy without dampening antitumor immunity.

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Clinical features of immune checkpoint inhibitor-induced inflammatory arthritis differ in those treated with combination versus single agent therapy

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Background

Multi-system immune-related adverse events (irAE) can occur as a result of immune checkpoint inhibitors (ICIs) targeting PD-1, PD-L1, and CTLA-4, with variations depending on tumor type and agent used. Inflammatory arthritis (IA) has been increasingly recognized as an irAE that may occur as a result of anti-PD-1/PD-L1/CTLA-4 therapy used in the treatment of malignancy [1, 2]. We evaluated associations between ICI regimen and clinical presentation of IA in a single institution.

Methods

Patients referred to rheumatology for suspected ICI-induced IA, were identified January 2013- July 2017. Patients were included if they developed de novo IA during or after ICI therapy and had no history of pre-existing autoimmune disease. Patients were included for the single agent group if they were on a PD-1 or PD-L1 inhibitor without CTLA-4 inhibition. Those in the combination group were treated with PD-1 and CTLA-4 inhibition. Clinical, demographic and laboratory data were obtained from the medical records.

Results

Tumor types for included patients were melanoma, non-small cell lung cancer, small cell lung cancer, colon cancer, Hodgkin or cutaneous lymphoma, renal cell carcinoma, endometrial carcinoma, duodenal carcinoma, merkel cell carcinoma, basal cell carcinoma, or squamous cell carcinoma. The

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group treated with combination CTLA-4/PD-1 inhibition was significantly younger than the monotherapy group; those in the combination group were also more likely to be male and to have melanoma (Table 1). Combination therapy was associated with higher levels of C-reactive protein (4 mg/dl vs. 0.7 mg/dL) and a higher likelihood of having a large joint affected first in the arthritis course (table 1). All instances of reactive arthritis-like presentation (arthritis + sterile urethritis, conjunctivitis) were in the combination therapy group. Patients who received PD-1/PD-L1 monotherapy were more likely to have IA as their first iRAE. No patients had CCP antibodies; two patients were positive for rheumatoid factor.

Conclusions

This study illustrates the diversity in clinical phenotypes of patients with ICI-induced IA. Differences in presentation may be explained by the regimen used to treat a patient's malignancy. Understanding the diversity of clinical presentations will help oncologists identify and treat this increasingly important iRAE.

Table 1.

	Anti-PD-1 (n = 22)	PD-1/PD-L1 monotherapy (n = 18)	Combination CTLA-4/PD-1 therapy (n = 12)	p-value*
Age (median) (y)	64 (10-81)	62 (36-77)	62 (39-84)	0.00
Female sex, n (%)	12 (54.5%)	10 (55.6%)	5 (41.7%)	0.05
Tumor type, n (%)				0.05
Melanoma	11 (50.0%)	10 (55.6%)	10 (83.3%)	
Lung	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Colorectal	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Other	9 (40.9%)	6 (33.3%)	0 (0%)	
Time to first iRAE (mo)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Time to last iRAE (mo)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Concomitant use of other iRAE†				
Thyroid disease	5 (22.7%)	3 (16.7%)	3 (25.0%)	
Hypophysitis	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Rash	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Other	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Time to first iRAE (days)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Total n = 52	22 (42.3%)	18 (34.6%)	12 (23.1%)	0.00
Female sex, n (%)	12 (54.5%)	10 (55.6%)	5 (41.7%)	0.05
Time to first iRAE (days)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Time to last iRAE (days)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Concomitant use of other iRAE†				
Thyroid disease	5 (22.7%)	3 (16.7%)	3 (25.0%)	
Hypophysitis	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Rash	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Other	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Time to first iRAE (days)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Time to last iRAE (days)	1.2 (0.1-3.8)	1.1 (0.1-3.8)	1.1 (0.1-3.8)	0.00
Concomitant use of other iRAE†				
Thyroid disease	5 (22.7%)	3 (16.7%)	3 (25.0%)	
Hypophysitis	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Rash	1 (4.5%)	1 (5.6%)	1 (8.3%)	
Other	1 (4.5%)	1 (5.6%)	1 (8.3%)	

*p-values are based on Fisher's exact test. †Concomitant use of other iRAE includes thyroid disease, hypophysitis, rash, and other autoimmune diseases. ‡Concomitant use of other iRAE includes thyroid disease, hypophysitis, rash, and other autoimmune diseases. §Concomitant use of other iRAE includes thyroid disease, hypophysitis, rash, and other autoimmune diseases. ¶Concomitant use of other iRAE includes thyroid disease, hypophysitis, rash, and other autoimmune diseases. ††Concomitant use of other iRAE includes thyroid disease, hypophysitis, rash, and other autoimmune diseases. †††Concomitant use of other iRAE includes thyroid disease, hypophysitis, rash, and other autoimmune diseases.

Demographic and clinical variables at initial evaluation for inflammatory arthritis

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P446

Immunosuppression use and clinical outcomes in patients with immune checkpoint inhibitor - induced inflammatory arthritis

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Background

Inflammatory arthritis (IA) resulting from immune checkpoint inhibitors (ICIs) is an increasingly recognized immune-related adverse event. The requirement for immunosuppression to treat IA, including TNF-inhibitors (TNF-I), has been documented in previous case series [1, 2]. How often immunosuppression is required to treat ICI-induced IA is unclear. The consequences of long-term immunosuppression on tumor response from ICIs is also uncertain.

Methods

Patients treated at Johns Hopkins Rheumatology for IA due to ICIs were included if they received anti-CTLA-4, anti-PD-1, and/or anti-PD-L1 therapy and developed IA during or after therapy. Patients with pre-existing autoimmune disease were excluded. Patients were managed by rheumatology and oncology based on preliminary recommendations for ICI-induced IA treatment [3].

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Results

Included patients (n=32) had melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer, colon cancer, Hodgkin or cutaneous lymphoma, renal cell carcinoma, endometrial carcinoma, duodenal carcinoma, merkel cell carcinoma, basal cell carcinoma, or squamous cell carcinoma. Of these patients, 20 (63%) had a partial or complete tumor response to ICIs clinically or by RECIST criteria. Twenty six (81.3%) required corticosteroids for management of their IA. Eleven patients (34.3%) required additional immunosuppression. TNF-Is were used in 7 patients (tumor types: melanoma n=6, RCC n=1). Methotrexate or leflunomide was used in 4 patients (tumor types: NSCLC, small cell lung cancer, duodenal carcinoma, endometrial carcinoma). Duration of TNF-I use ranged from 2-16 months. Four patients treated with TNF-I, all with melanoma, had complete response by RECIST criteria or no evidence of disease at ICI start. None of these patients had tumor progression while on TNF-I (duration: 3-16 months). Of the patients treated with methotrexate or leflunomide, 3 had a complete or partial response to ICIs, and none had tumor progression during 2-12 months of IA management.

Conclusions

In a cohort of patients with ICI-induced IA, the majority of patients required corticosteroids to control IA symptoms. In this series, TNF-inhibitors and other forms of non-corticosteroid immunosuppression did not affect tumor response in those who had an initial response to ICIs for malignancy.

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P447

Infliximab associated with faster symptom resolution compared to corticosteroids alone for management of immune related enterocolitis

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Background

Immune-related enterocolitis (irEC) is the most common serious complication from checkpoint inhibitors (CPIs) that can lead to bowel perforation, sepsis, and death. The current front-line treatment for irEC is high-dose corticosteroids (CS). However, prolonged high-dose CS therapy has significant toxicities and side effects, and prolonged therapy may reduce CPI anti-tumor activity. Early addition of TNF-alpha inhibitors such as infliximab may expedite symptom resolution and shorten CS duration. The only prospective trial evaluating infliximab with CS versus CS alone for irEC (NCT02763761) has recently been terminated. Thus we conducted a retrospective study to evaluate symptom resolution in patients with irEC treated with infliximab + CS versus CS alone.

Methods

The medical records of patients with irEC between January 1, 2012 and June 30, 2017 were reviewed under an institution review board-approved

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protocol. Patient demographics, CPI regimen (aCTLA-4, aPD-1/L-1, alone or in combination), colitis grade, colitis treatment, treatment-related toxicities, time to symptom resolution, and CS duration were collected. The primary-endpoint was time to symptom resolution for irEC (times to diarrhea resolution and to initiation of steroid titration) for patients managed with versus without infliximab. Duration of CS and rate of CS-associated toxicities were secondary-endpoints. Categorical and continuous variables were assessed between groups by Fisher's exact test and Kruskal-Wallis test (or two-sample t-test).

Results

Among 75 patients with irEC, 39 (52%) received CS alone and 36 (48%) received infliximab within a median of 8 days after CS initiation. Patient characteristics (age, comorbidities, cancer type, immunotherapy regimen, and initial steroid dose), were similar between the two arms. The incidence of grade 3+ colitis was higher in the infliximab + CS arm (86.1% vs. 34.2%, $p < 0.001$). Despite this, times to diarrhea resolution (median 3 vs. 9 days, $p < 0.001$) and to steroid titration (median 4 vs. 13 days, $p < 0.001$) were shorter in patients treated with infliximab + CS versus CS alone. Rates of CS-associated toxicities (39% vs. 54%; $p = 0.249$) and total steroid duration (median 35 days vs. 51 days; $p = 0.241$) were numerically lower in the infliximab-treated patients (Table 1).

Conclusions

To our knowledge this is the first study to compare time to symptom resolution for irEC with infliximab + CS versus CS alone. Despite higher incidence of grade 3+ colitis, infliximab treatment was associated with a statistically significant shorter time to symptom resolution without additional toxicities. The data suggest that early introduction of infliximab should be considered for patients with irEC until definitive prospective clinical trials.

Table 1.

Variable	n	Overall	Infliximab	Steroid	P
Grade 3+ colitis, n (%)	75				< 0.001
Infliximab		8 (11.9)	0 (0.0)	8 (21.5)	
Steroid		22 (29.3)	5 (13.9)	17 (47.2)	
Total		44 (58.5)	5 (13.9)	39 (51.4)	
Symptom Resolution					
Time to diarrhea resolution	75	6	4	13	< 0.001
Time to steroid titration	75	4	3	13	< 0.001
Total duration of steroid, Median	67	46	35	51	0.241

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Incidence of thyroid test function abnormalities in patients receiving immune-checkpoint inhibitors for cancer treatment

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Background

With the advent of immune-checkpoint inhibitor (ICI) therapy (anti-CTLA-4, anti-PD-1), immune related adverse events (irAE) such as thyroid function test abnormalities (TFTA) are common with a reported incidence range of 2-15% depending upon the ICI utilized [1,2]. The aim of this study was to describe the incidence of TFTAs retrospectively in patients who received ICI therapy.

Methods

A total of 285 patients were reviewed (178 male, 107 female; ages 16-94) of which 218 had no baseline TFTA, 61 had baseline TFTAs, and 6 had history of thyroidectomy and were excluded. Patients received at least one dose of ipilimumab and/or nivolumab or pembrolizumab. Post-ICI therapy TFTA was classified according to definitions of thyroid abnormalities when possible [3].

Results

A total of 35% (76/218) patients had new onset TFTAs on ICI therapy. Of note, 70.5% (43/61) had baseline TFTA that were exacerbated by ICI therapy. Median time to new onset or exacerbated baseline TFTA were 46 & 33 days respectively. Of note, 65% (20/31) of patients on both ipilimumab and

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nivolumab had new onset TFTA, compared to 31.3% (15/48) with ipilimumab, 31.5% (28/89) nivolumab, 26% (13/50) pembrolizumab (Table 1).

Conclusions

The incidence of TFTAs with ICI therapy was higher than previously reported. Patients with baseline TFTA and/or receiving ipilimumab and nivolumab combination therapy had a higher incidence of TFTA than one agent ICI therapy. In conclusion, we recommend more frequent evaluation of TFT in the first two months, especially in those with baseline TFTA.

Table 1.

Thyroid function test abnormality	I&N, n=31	I, n=48	N, n=89	P, n=50	Total n=218
Primary hypothyroidism	6.5% (2)	4.2% (2)	10.1% (9)	14% (7)	20
Subclinical hypothyroidism	6.5% (2)	8.3% (4)	11.2% (10)	8% (4)	20
Central hypothyroidism	16.1% (5)	8.3% (4)	1.1% (1)	2% (1)	11
Primary overt hyperthyroidism	25.8% (8)	2.1% (1)	0% (0)	2% (1)	10
Subclinical hyperthyroidism	0% (0)	4.2% (2)	5.6% (5)	0% (0)	7
Thyroiditis (Hyperthyroidism/Hypothyroidism)	9.7% (3)	4.2% (2)	3.4% (3)	0% (0)	8
Total % (n/x)	64.5% (20/31)	31.3% (15/48)	31.5% (28/89)	26% (13/50)	35% (76/218)
Required thyroid replacement therapy	32.3% (10/31)	14.6% (7/48)	13.5% (12/89)	12% (6/50)	17.4% (35/218)
Required thionamide treatment for overt hyperthyroidism	22.6% (7/31)	0% (0)	0% (0)	2% (1/50)	3.7% (8/218)

Summary of new onset TFTA

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P449

Neurotoxicity in patients with metastatic solid tumors treated with immune checkpoint inhibitors: a single institution retrospective analysis

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Background

Immune checkpoint inhibitors (ICIs) have dramatically improved survival for patients with melanoma and are playing an increasingly important role in the management of other tumor types. Anti-CTLA4, anti-PD1, and anti-PDL1 antibodies have been approved for multiple tumor types based on their clinical benefit, however they can be associated with a unique set of toxicities termed immune related adverse events (irAEs). We aimed to identify the incidence and clinical manifestations of patients who developed neurologic irAEs with ICIs at our institution in order to inform investigation and management guidelines.

Methods

An IRB approved retrospective study involved review of charts and institutional databases at MSKCC. We identified patients who developed neurotoxicity while on at least one of the following ICIs: anti-CTLA4, anti-PD1 or anti-PD-L1 over a 6 year period (1/1/2010-11/16/2016).

Results

We identified a total of 3,804 patients who were treated with one or more ICI during the period of review. Neurotoxicity was observed in 81 patients (2.1%) and affected both central and peripheral nervous systems. 31 patients (38.2%) received more than one ICI. Median number of cycles prior to developing toxicity was 3 (1-29). Ten patients had more than one neurotoxicity. The various

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neurologic phenotypes observed in patients included: sensory neuropathy, encephalopathy, headache and aseptic meningitis, myasthenia gravis-like syndrome, myopathy, autonomic neuropathy, radiculopathy, brachial plexitis, mononeuritis multiplex, Guillain-Barre syndrome or chronic inflammatory demyelinating polyneuropathy, paraneoplastic neurologic disorder, and PRES. 43 patients required hospital admission and 2 required ICU level of care. A diagnosis of neurotoxicity was based upon the temporal association with ICIs and appropriate workup including but not limited to neurologic consultation, lumbar puncture, and neuroimaging. Patients were treated with drug holiday, observation, corticosteroids, plasma exchange and/or IVIG.

Conclusions

As ICIs are being used with increased frequency, oncologists and other health care providers should be aware of the varied manifestations of neurotoxicity in order to ensure appropriate diagnosis, work up and treatment. Prompt treatment may relay a benefit in regards to outcome.

PHYSICIAN/NURSE/PHARM: Patient Experience: Patient Education Regarding Immunotherapy and Side Effect Management

P450

Educating cancer patients about immunotherapy: gains from attending a national educational immunotherapy workshop

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Background

Immunotherapy continues to offer promising treatments for many tumor types. Unfortunately, patients potentially eligible for immunotherapy are often not aware of options. According to a 2014 Cancer Support Community (CSC) online survey, only 34.8% of cancer survivors knew the term “immuno-oncology” and 64.9% had heard of “immunotherapy.” Despite low awareness, most (84%) wanted to know more about these topics. Recognizing this need, the CSC developed a national psychoeducational immunotherapy workshop. The current analyses investigate the program’s informational and empowerment outcomes among attendees with cancer.

Methods

From 2014-16, 706 program attendees of the CSC’s *Frankly Speaking About Cancer: Your Immune System & Cancer Treatment* workshop completed a post-program evaluation assessing knowledge (on 5-point scale) and program outcomes, including program satisfaction. Individuals with cancer (n=478) comprised 68.0% of participants in the workshop. These attendees were White (81.7%), female (79.6%), and averaged 62.6 years old [1]. Workshop outcomes were ascertained by descriptive analyses and ANOVAs.

Results

Over half of workshop attendees with cancer (53.0%) reported generally being very involved in their overall treatment decision-making, yet 47.2% had been unsure whether immunotherapy was a treatment option for them[m1] . [AKZ2] About one-third (36.8%) had spoken with their doctor about immunotherapy and 26.2% inquired about potential side effects of immunotherapy; 44.1% had searched for information about immunotherapy on their own before the workshop. The majority (74.6%) reported low knowledge about immunotherapy before the workshop.

After the workshop, most attendees with cancer reported a high level of knowledge of immunotherapy (57.8%), representing a significant

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gain from attending the program ($F(4,416) = 39.2, p < .01$). Post-workshop, most (86.5%) felt confident speaking about immunotherapy with their doctors. Furthermore, a majority felt “more confident” discussing potential side effects (86.3%), asking about treatment options generally (90.3%), and making decisions with their doctors (84.8%) post-workshop. Nearly all (92.5%) strongly or moderately recommend attending the workshop.

Conclusions

Results from the attendee evaluations demonstrate significant informational and confidence gains from attending a two-hour psychoeducational workshop. Attendees reported increased awareness of options, knowledge, and efficacy in discussing potential options for them. Results suggest that improving access to comprehensive information about immunotherapy and promoting communication between the patient and the healthcare team about treatment options and decision-making via an educational workshop is viable and can be implemented nationally to reach many.

References

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Tumor Microenvironment (Mechanisms and Therapies)

P451

Alpha-TEA demonstrates antitumor activity against trastuzumab sensitive and resistant breast cancer

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Background

Alpha-tocopheryloxyacetic acid (α -TEA) is an orally administered, small molecule, multi-pathway cancer therapeutic agent that drives tumor cell apoptosis and autophagy to stimulate antigen cross-presentation. Anti-tumor activity of α -TEA in combination with trastuzumab was evaluated in vivo against established Her2/neu-expressing mouse tumors (MMC) and in vitro against HER2 positive, human trastuzumab-sensitive (BT-474) and trastuzumab-resistant (HR6) tumor cells.

Methods

In vivo studies: Alpha-TEA was formulated in mouse chow and administered to mice ad libitum. FVB/N-TgN (MMTVneu) on nutrient-matched control chow mice received s.c. implant of 1 million Her2/neu positive murine mammary cancer (MMC) cells. On day 25, after tumors were established, mice were transferred to α -TEA diet, and treatment with anti-Her2/neu monoclonal antibody (7.16.4) was initiated. Mice received 3 injections of 7.16.4 antibody 3 times a week for 1 month and tumor growth was monitored until day 60. Mice demonstrating complete tumor regression were challenged with viable tumor cells to assess the establishment of a memory response. Spleen cells were removed from treated mice and assessed for cytokine production by ELISPOT assay.

In vitro studies: Human trastuzumab-sensitive (BT-474) and a resistant sub-clone (HR-6) were treated continuously with α -TEA alone or in combination with trastuzumab, both at various doses and evaluated for clonogenic potential and activation of ERK and AKT signaling pathways.

Results

Treatment of mice harboring established MMC tumors with α -TEA plus trastuzumab caused complete tumor regression, and increased cytokine production by splenic lymphocytes. Individually, α -

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TEA and trastuzumab caused cell death and reduced colony formation of BT-474 tumor cells in vitro; however only α -TEA was able to kill and reduce the clonogenicity of the trastuzumab-resistant HR6 cell line. Furthermore, α -TEA-mediated cell death of HR6 tumor cells involved inhibition of AKT and ERK phosphorylation. Treatment with α -TEA did not affect the expression of HER2/neu by both cell lines.

Conclusions

- Alpha-TEA synergizes with trastuzumab to cause regression of established Her2/neu-expressing tumors.
- Anti tumor activity of α -TEA is due to apoptosis induction and suppression of AKT and ERK signaling pathways.
- Alpha-TEA represents a potential opportunity for combination treatment with Herceptin in HER2 positive breast cancer.

P452

Defining IL-15 expressing myeloid cells in the tumor microenvironment

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Background

Loss of IL-15 within colorectal tumors correlates with higher risk of relapse, decreased survival, lower T cell density and decreased T cell proliferation [1] suggesting IL-15 expression within the tumor is important for anti-tumor responses. Furthermore, we have found that soluble IL-15 (sIL-15) complexes are produced in early tumors of mice implanted with tumor cell lines. However, the cell types expressing IL-15 and the mechanisms regulating IL-15 responses in the tumor microenvironment are not known. The goal of this study was to identify myeloid cell populations expressing IL-15 in the

tumor microenvironment and investigate the mechanisms of expression.

Methods

Both IL-15 transcriptional and translational EGFP (enhanced green fluorescent protein) reporter mice were injected subcutaneously with 3×10^5 B16 melanoma, MCA-205 fibrosarcoma or MC-38 murine adenocarcinoma cell line. After two weeks tumors were stained for myeloid cell surface markers and analyzed via flow cytometry. Distribution of myeloid cell populations in tumors were also monitored via confocal laser-scanning microscopy of IL-15 reporter mice. CCR2⁺ IL-15⁺ double reporter mice were used to further characterize myeloid cells. To investigate the cellular source of sIL-15 complexes, we used mice with a conditional deletion of IL-15R α from DCs (CD11c-Cre x IL-15R α fl/fl) or macrophages (LysM-Cre x IL-15R α fl/fl); or mice were treated with anti-Ly6G depleting antibodies.

Results

In both IL-15 reporter lines, we found that the EGFP expressing cells in the tumor microenvironment are composed of three main populations: CD11b⁺Ly6C^{hi}Ly6G⁻, CD11b⁺Ly6C⁺Ly6G⁺ and CD11b⁺Ly6C^{lo}Ly6G⁻ cells with the CD11b⁺Ly6C^{hi}CCR2⁺ cells being the most abundant. Similar composition of EGFP⁺ myeloid populations was also seen in MCA-205 and MC-38 tumors. Imaging studies of MCA-205 tumors showed that the majority of the F4/80⁺ or CCR2⁺ cells were also EGFP⁺. In MCA-205 tumors, EGFP expression was decreased in CD11b⁺Ly6C⁺Ly6G⁺ cells compared to the spleen. Analyses with conditional knockout mice or depleting antibodies showed that the monocytic cells and macrophages are major sources of sIL-15 complexes in early B16 tumors.

Conclusions

In the tumor microenvironment, IL-15 is primarily produced by inflammatory monocytes and macrophages in early tumors and is down regulated with tumor growth.

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References

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P453

Targeted tumor stroma depletion in combination with a whole cell tumor vaccine enhances effector T cell infiltration in a mouse model of pancreatic cancer

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Background

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with dismal prognosis due to conventional therapy resistance. The tumor microenvironment (TME) of PDAC is characterized by a desmoplastic reaction that excludes effector immune cell infiltration, thus preventing optimal efficacy of immunotherapy. A human recombinant hyaluronidase (PEGPH20) has been clinically formulated to enzymatically deplete hyaluronan (HA) in the tumor extracellular matrix. We tested the hypothesis that combining the stromal targeting agent PEGPH20 with a GM-CSF secreting allogenic pancreatic tumor vaccine (GVAX) would increase immune cell infiltration and improve survival of PDAC bearing mice.

Methods

C57BL/6 mice were orthotopically transplanted with 2×10^6 murine KPC pancreatic tumor cells to form liver metastases by a hemisplenectomy technique on day 0. Following tumor transplantation, mice were treated subcutaneously with GVAX in combination with intravenous PEGPH20 or the appropriate control. PEGPH20 was given on day 6

and GVAX on post-operative day 7. An immune modulating dose of Cyclophosphamide (Cy) was given the day before GVAX in all groups. Tumor immune lymphocyte infiltration was assessed by immunohistochemistry and flow cytometry. RT-PCR was performed on tumor associated macrophages isolated with CD11b beads. Mice were sacrificed on day 16 for immune and gene expression analysis. Additional survival analysis followed mice until death.

Results

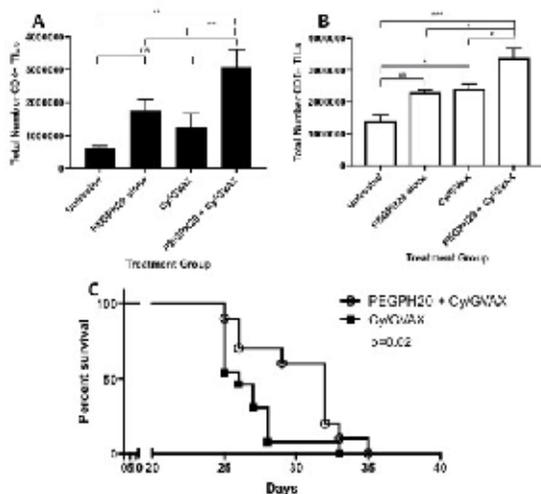
Combination therapy with PEGPH20 and GVAX yielded higher infiltration of effector CD8+ and CD4+ tumor infiltrating lymphocytes in the TME compared to untreated and monotherapy groups (all $p < 0.05$) (Figure 1). Murine survival was improved in the combination therapy group as compared to GVAX therapy alone (Hazard Ratio: 0.286 [0.10-0.82] $p = 0.02$). Additionally, a larger percent of tumor-infiltrating CD8+ cells were noted to express the lymphocyte activation surface marker CD137 in combination therapy compared to monotherapy controls. A trend towards lower CXCR4 expression was noted in the combination therapy group, suggesting a possible mechanism to CD8+CD137+ infiltration beyond decreased vascular impedance.

Conclusions

Our preclinical murine model of PDAC demonstrates enhanced efficacy of GVAX in combination with the stromal targeting agent PEGPH20 with increased immune cell infiltration and a survival advantage. This study provides rationale for further study of this combination for PDAC treatment.

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Figure 1. Combination therapy of PEGPH20 with Cy/GVAX improves immune cell infiltration and survival in a PDAC mouse model.



Total number of live (A) CD8+ and (B) CD4+ tumor infiltrating lymphocytes after KPC hemispleen surgery and indicated therapy. Data represent mean \pm SEM from one representative experiment with three mice per group that was repeated twice. (C) Kaplan-Meier survival curves of mice that were implanted with PDAC cells and treated with Cy/GVAX with and without combination of PEGPH20. = p

P454

Molecular targeted radiotherapy facilitates in situ vaccination in a syngeneic murine melanoma model

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Background

We reported that 12Gy external beam radiation (EBRT) and intratumoral (IT) administration of the hu14.18-IL2 immunocytokine (IC) [an anti-disialoganglioside (GD2) mAb fused to IL2] five days later elicits an *in situ* vaccination effect in GD2⁺ murine tumors. An established distant, untreated secondary tumor exerts an antagonistic effect, “concomitant immune tolerance” (CIT), which drastically reduces the efficacy of *in situ* vaccination. We have shown that CIT is mediated, in part, by tumor-infiltrating regulatory T cells (Tregs) and that CIT can be overcome by delivering 12Gy EBRT to both tumors. Clinically, it is not feasible to deliver this dose to all sites of metastatic disease. Systemically delivered molecular targeted radiotherapy (MTRT) with the tumor-selective alkyl phosphocholine analog ¹³¹I-NM404 could overcome this challenge, and potentially deplete immunosuppressive cells responsible for CIT in practically all sites of metastasis. We evaluated doses of EBRT to distant tumor sites needed to eliminate CIT, performed dosimetry to determine the administered activity of ¹³¹I-NM404 that approximates this EBRT absorbed dose, and tested whether systemic administration of MTRT may overcome CIT in a syngeneic murine melanoma model.

Methods

We monitored tumor volume, survival, and infiltrating lymphocyte populations in primary and secondary tumors treated with EBRT+IT-IC+/- intravenous MTRT in C57BL/6 mice bearing one or two ~250mm³ syngeneic GD2⁺ B78 melanoma flank tumors.

Results

We demonstrate that CIT can be substantially attenuated using low-dose (2Gy) EBRT to the secondary tumor. This lower dose is not adequate to generate an *in situ* vaccine response but appeared comparable to 12Gy in its capacity to overcome CIT. Using longitudinal ¹²⁴I-NM404 PET/CT imaging with Monte Carlo dosimetry, we estimated that 60 μ Ci of ¹³¹I-NM404, a dose which does not

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destroy B78 tumors, given IV provides approximately 2Gy to both tumors in animals bearing two B78 tumors. Following 60 μ Ci of ¹³¹I-NM404 to mice with two B78 tumors, we observed depletion of tumor-infiltrating Tregs by IHC staining over a 4-week period, without bone marrow suppression (thrombocytopenia/leukopenia/anemia). We observed that pretreatment with systemic ¹³¹I-NM404 one week prior to 12Gy EBRT+IT-IC to a single tumor site may substantially attenuate CIT and rescue a systemic response to *in situ* tumor vaccination.

Conclusions

We demonstrate that 2Gy EBRT is sufficient to attenuate CIT, and that this dose can be approximated using systemic MTRT. This work suggests that MTRT may enable conditioning of the tumor immune microenvironment to overcome intrinsic immune evasion mechanisms and restore efficacy of *in situ* tumor vaccination.

P455

Investigating the specificity and response of a native leukemia-reactive CD8⁺ T cell clone

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Background

We previously reported that high-affinity, antigen-specific CD8⁺ T cells were induced to undergo abortive proliferation and deletion in leukemia-bearing hosts, and have subsequently found that this deletional CD8⁺ T cell tolerance requires cross-presentation of the leukemia antigen by splenic Batf3-lineage dendritic cells.

Methods

Recently, we generated a T cell receptor (TCR) transgenic mouse strain (referred to as Tg101) that

expresses the TCR- α and - β chains of a CD8⁺ T cell clone specific for a native leukemia antigen.

Results

Having confirmed that Tg101 CD8⁺ T cells specifically recognize C1498 acute myeloid leukemia (AML) cells in the context of the MHC class I molecule, H-2D^b, we investigated the behavior and fate these cells in naïve and C1498 leukemia-bearing hosts. In naïve mice, Tg101 thymocytes predominantly developed along the CD8⁺ lineage, and existed as naïve CD8⁺ T cells in peripheral lymphoid organs, suggesting that the Tg101 antigen is likely leukemia-specific. In leukemia-bearing mice, antigen encounter by Tg101 T cells was delayed (day 10-12), and primarily occurred in the liver - a prominent site of leukemia progression. Subsequently, Tg101 cells expanded and upregulated expression of co-inhibitory receptors, including PD-1, LAG-3, TIM-3 and TIGIT, which correlated with a progressively dysfunctional phenotype. Surprisingly, antigen encounter by Tg101 T cells in leukemia-bearing mice occurred independently of Batf3-lineage DCs, suggesting that the Tg101 antigen was poorly cross-presented *in vivo*. Given this finding, we deleted H-2D^b in C1498 cells to determine the extent to which direct antigen presentation by leukemia cells is responsible for the acquisition of dysfunctional properties in Tg101 T cells *in vivo*. *In vitro* expansion was 2-fold decreased in Tg101 T cells compared to high-affinity 2C CD8⁺ T cells (which recognize the SIY peptide in the context of H-2K^b) upon culture with SIY-expressing C1498 cells (C1498.SIY), indicative of a lower avidity of Tg101 vs the 2C T cells for antigen. In contrast to Tg101 T cells, 2C T cells encountered leukemia antigen rapidly (day 3-4) in the context of splenic CD8a⁺ DCs, proliferated briefly, and were effectively deleted from the host by day 12.

Conclusions

Collectively, our data reveal that CD8⁺ T cell tolerance is effectively generated in the context of AML. However, the nature of the leukemia antigen, as well as the context in which it is presented, are

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critical determinants in the underlying mechanism of tolerance that ensues.

P456

CDK4 & 6 inhibitor Abemaciclib exerts intrinsic immunomodulatory effects on human T cells *in vitro*

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Background

Abemaciclib is an inhibitor of cyclin-dependent kinases 4 and 6 (CDK4 & 6) that suppresses DNA synthesis and cell proliferation as result of cell cycle arrest in G1 phase. Significant clinical activity of abemaciclib has been observed in hormone receptor-positive breast cancer patients highlighting the therapeutic potential of this compound in oncology, including combinations with other therapeutic modalities. In preclinical studies, pretreatment with abemaciclib followed by PD-L1 checkpoint blockade has demonstrated synergistic immune activation and antitumor efficacy. This has generated interest in combination studies of abemaciclib with immunotherapeutic agents. However, the mechanisms underlying immunomodulatory activity of abemaciclib have not been fully elucidated. Consequently, the objective of this study was to understand the direct effects of abemaciclib on primary human T cells.

Methods

Jurkat and primary human T cells were stimulated *in vitro* with anti-CD3/CD28 and treated with increasing concentrations of abemaciclib ranging between 0 uM to 1 uM for up to ten days. Cells were harvested at the end of the experiments and nuclear factor of activated T cells (NFAT) activation,

cell surface immune markers (costimulatory and coinhibitory receptors and ligands) and expression of immune-related genes were analyzed by signal reporter assay, flow cytometry and Quantigene Plex, respectively.

Results

Treatment of Jurkat T cells showed a dose dependent increase in NFAT reporter activation during abemaciclib treatment which was replicated in primary human T cells. Additionally, treatment of primary T cells with abemaciclib resulted in a distinct phenotype exemplified by up-regulation of CD137, GITR, TIM3, PD-1, PD-L1, and TIGIT on the surface of both CD4+ and CD8+ T cells correlating with gene expression changes associated with T cell activation phenotype (upregulated Granzyme B, CD137, OX40, TNF-alpha, IL2R, IP10, ICAM-1) with only a modest concentration-dependent decrease in T cell expansion. Furthermore, intermittent exposure of T cells to abemaciclib during various stages of expansion revealed that T cells were capable of recovering from abemaciclib-induced growth inhibition whereas expression of costimulatory/coinhibitory molecules was maintained once the drug was removed.

Conclusions

Taken together, these results demonstrate that abemaciclib can exert T cell-intrinsic effects at concentrations comparable to clinical exposure. This is exemplified by increased T cell activation phenotype with only a modest and reversible decrease in T cell expansion, providing further rationale for clinical exploration of combination studies with immunomodulatory agents such as PD-1 pathway inhibitors.

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P457

Pharmacodynamic activity of MEDI9197, a TLR7/8 agonist, administered intratumorally in subjects with solid subcutaneous or cutaneous tumors

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Background

MEDI9197 (formerly 3M-052), is a novel TLR7/8 dual agonist formulated for intratumoral (IT) injection and optimized for retention within the tumor after IT injection. IT administration aims to focus antigen presentation in the tumor by activation of both plasmacytoid (TLR7 expressing) and conventional dendritic (TLR8 expressing) cells, thus minimizing systemic inflammatory toxicities. We report the pharmacodynamic results of MEDI9197 from a phase I, first-in-human, dose-escalation study in patients with solid tumors (NCT02556463).

Methods

The dose-escalation trial of MEDI9197 (0.005 to 0.055 mg) has enrolled 24 patients with subcutaneous/cutaneous tumors. As reported at AACR 2017, the maximum tolerated dose is 0.037

mg and IT administration is feasible and safe. Here we present updated tumoral and peripheral pharmacodynamic effects in patients treated with MEDI9197 0.005, 0.012, and 0.037 mg.

Results

Local pharmacodynamic effects assessed by immunohistochemistry (IHC) in longitudinal biopsies demonstrated the majority of patients treated with 0.037 mg had an increase (≥ 2 fold) in CD8, CD40, CD56, or PD-L1 (tumor and immune cells) markers 3 weeks after a single dose of MEDI9197. RNAseq analysis of paired tumor biopsies showed an increase in TLR7/8-downstream regulated genes and innate and adaptive immune activation signatures such as Type 1 IFN, IFN γ and T effector signatures (≥ 1.5 fold) consistent with IHC.

Increases in peripheral levels of IFN γ , CXCL10, and CXCL11 were observed across all 3 doses (0.037, 0.012, and 0.005 mg). Within 24 hours of IT administration of MEDI9197 0.037 mg, median peak values of IFN γ , CXCL10, and CXCL11 observed were 236, 9286, and 558 pg/mL respectively. The fold change increase ranged from 4.5-43, 30-132, and 3.7-52 vs. baseline for IFN γ , CXCL10, and CXCL11 respectively. Peripheral levels of IFN γ showed a significant trend for a dose response with median peak values for those treated with MEDI9197 0.005 mg 4.1-fold less than those treated with 0.037 mg. Analysis of whole blood microarray data across all doses demonstrated increases (≥ 2 fold) in T_H1 and Type 1 IFN gene expression signatures with a transient decrease (≥ 1.5 fold) in *CD8A* transcript and NK cell signature expression, suggesting trafficking of T and NK cells.

Conclusions

IT administration of MEDI9197 induces Type 1 and 2 IFN as well as T_H1 responses, suggesting activation of both plasmacytoid and conventional dendritic cells. A combination trial of MEDI9197 with durvalumab (anti-PD-L1) and radiation in patients with advanced solid tumors is currently ongoing.

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Trial Registration

NCT02556463

P458 Abstract Travel Award Recipient

Nitric oxide synthase inhibition results in PDL-1 upregulation and ADAR1 inhibition, triggering immunogenic cell death: an in-vitro and in-vivo analysis

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Background

Response failure in checkpoint inhibitors, such as death ligand 1 (PD-L1) and its receptor programmed death 1 (PD-1), is associated with cancer-related immunosuppression. Inducible nitric oxide synthase (iNOS) product, nitric oxide, has been implicated in immunosuppressive environments. In triple negative breast cancer (TNBC), iNOS expression is associated with poor survival and increased tumor aggressiveness. We've shown ADAR1 (adenosine deaminase acting on RNA 1) is upregulated by iNOS activation in TNBC cell lines. ADAR1 RNA editing suppresses inflammation, thereby damping the immune response. This study aims to evaluate whether NOS inhibition plus anti-PD-1 is a feasible therapeutic combination in TNBC.

Methods

TNBC cell lines BT-549, SUM-159, SUM-157, HCC-70, MDA-MB-231 and MDA-MD-468 were treated with NOS inhibition therapy (L-NMMA, 4mM) + amlodipine (5µM). PDL-1, HMGB1, and ADAR1 expression was assessed via western blot. BALBc mice growing orthotopically injected 4t1 cells were treated weekly with: 1) vehicle (saline, oral/Rat-IgG2 i.p.); 2) NOS inhibition [L-NMMA (200mg/kg oral) + amlodipine (10mg/kg, i.p.), days 1-5]; 3) anti-PD-1 antibody (10 mg/kg i.p.; Bio X Cell Clone: RMP1-14, days 1, 3, 5); or 4) anti-PD-1 + NOS

inhibition. Humanized mice were developed by injecting hematopoietic stem cells (HSC, CD34+) into the tail vein of irradiated NOD-scid IL2Rynull mice. Mice were sorted and TNBC patient-derived xenografts (PDXs), representing 12 different patients, were implanted into the mammary fat pad (3 mice/PDX). Mice were sorted and treated weekly with: 1) vehicle; 2) pembrolizumab (anti-PD-1, 200µg, day 1, IV); or 3) pembrolizumab + NOS inhibitor.

Results

NOS inhibition increased PD-L1 in most cell lines. 4T1 syngeneic model helped assess the potential benefit of combined therapy. There were no significant differences in tumor growth between monotherapy and control group. However, anti-PD1 + NOS-inhibition resulted in a significant reduction in tumor growth. In immune-humanized TNBC PDXs, 40% responded to anti-PD1 monotherapy, 60% had improved response following combination therapy, and 33% responded only to combination therapy. In total, 66% of PDXs analyzed benefited from combination therapy. Furthermore, both *in-vitro* and *in-vivo* samples showed downregulation of ADAR1 levels when treated with NOS inhibition, with a subsequent increase in HMGB1, a marker of immunogenic cell death.

Conclusions

NOS inhibition upregulates PDL-1 expression in TNBC cell lines. Furthermore, combining NOS inhibition with anti-PD1 therapy improved antibody response by activating the immunogenic cell death pathway. This combination treatment may benefit cancer patients who do not respond to anti-PD-1 monotherapy.

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P459  Abstract Travel Award Recipient

Class IIa HDAC inhibition promotes an anti-tumor macrophage phenotype that induces breast tumor regression and inhibits metastasis

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Background

The breast tumor environment is complex and includes both neoplastic and immune cells. Among the most prevalent immune cell population within breast tumors is tumor associated macrophages (TAMs). Macrophages have the ability to polarize into either M1 cells, which have potent anti-tumor capabilities, or M2 macrophages which promote tumor progression by stimulating tumor vasculature, invasion, metastasis, and can enhance tumor resistance to chemotherapy. Generally TAMs in breast tumors are considered M2 macrophages and a high tumor density of TAMs clinically correlates to both overall worse prognosis and increased metastasis. Several therapeutic strategies exist to modulate TAMs clinically, focusing on depleting or inhibiting TAMs. However, macrophage are required for optimal tumor regression in response to both chemotherapy and immunotherapy. Their embedded location and their untapped potential provide impetus to the discovery of strategies to turn them against tumors and to harness for cancer therapy. Therefore here, we describe a novel method to polarize pro-tumor macrophages to an anti-tumor phenotype.

Methods

We recently reported that a first in class selective class IIa HDAC inhibitor (TMP195) influenced human monocyte responses to colony stimulating factors CSF-1 and CSF-2 *in vitro*. Here, we utilize a macrophage-dependent autochthonous mouse model of breast cancer to demonstrate that *in vivo* TMP195 treatment alters the tumor microenvironment and reduces tumor burden and pulmonary metastases through macrophage modulation.

Results

Here, we demonstrate that a first in class, class IIa histone deacetylase (HDAC) inhibitor, TMP195, can activate tumor macrophages *in vivo* to induce tumor regression and inhibit pulmonary metastases in a mouse model of breast cancer. We find that TMP195 induces macrophage recruitment and differentiation of highly phagocytic cells within the tumor, which increases tumor cell death while decreasing angiogenesis. Strikingly, we find that TMP195 enhances chemotherapy and immunotherapy to induce durable tumor reduction.

Conclusions

Our findings reveal an immunostimulatory effect of class IIa HDAC inhibition that contrasts with strategies of depleting or inhibiting TAMs for cancer therapy. Class IIa HDAC inhibition leverages the effector functions of macrophages, opening the door to clinically-relevant cooperation of checkpoint blockade, agonistic aCD40 or inhibitory aCD47 therapy, peptide vaccine therapy or the antibody-dependent cellular phagocytosis (ADCP) associated with mAb therapy. Indeed, the innate immune system is the natural complement to the adaptive immune system that surveys and fights tumors, and these studies demonstrate a novel approach to harness innate immune cells to cooperate with agents that stimulate an adaptive anti-tumor immune response in an otherwise resistant cancer.

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P460

Selenium, the element of the moon, improves immunotherapies on earth

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Background

Recent advances in immunotherapy have shown amazing results in cancer patients, albeit in small fraction. Attempts in developing immunotherapy to generate effective response against tumors are still faced with immune suppressive environment. The abundance of tumor-infiltrated T regulatory cells (Tregs) highly is one of the main mechanism by which tumors overcome immune responsiveness. Tregs also correlates with poor patient prognosis.

Selenium (Se), an essential trace element named after the goddess of the moon, is described to exhibit anti-carcinogenic potentials [1]. However, the underlying mechanisms of Se action are insufficiently understood. Since Se is also known to exhibit immune-modulatory properties [2] we hypothesized that Se in combination with vaccine-based immunotherapy might enhance anti-tumor responses.

Methods

TC-1 tumor-bearing C57BL/6 mice were treated either with Vaccine (E7, 100 µg/mouse; PADRE, 20 µg/mouse; every 7th day), Methylselenocysteine, as source of Se (MSC, 100 µg/mouse; daily) or with a combination of both. In addition, an untreated group served as the control. Spleens and tumor tissues were analyzed for T cell subsets via flow cytometry. ELISpot assays were performed to determine IFNγ production in E7/PADRE restimulated splenocytes. In vitro, CD4⁺ T cells

isolated from spleens of C57BL/6 mice were cultured under iTreg conditions, treated with Se and analyzed via Flow Cytometry and Immunoblotting.

Results

We found that combining Selenium with specific antigen vaccine led to significant enhancement of immune response leading to delay in tumor growth thereby prolonging the survival rate of mice. Importantly, Se significantly reduced the infiltration of Tregs into the tumor environment thereby enhancing the CD8⁺/Treg ratio leading to an increased vaccine-induced immune response. In vitro analysis of iTregs revealed that Se reduces Foxp3 expression and destabilizes Tregs.

Conclusions

Here we show for the first time that Se an essential trace element improves vaccine-based immunotherapies thereby opening a new window for the development of novel strategies to fight against cancer.

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P461

Listeria monocytogenes-based immunotherapies alter the suppressive phenotype of intratumoral regulatory T cells

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Background

Advaxis' *Listeria monocytogenes* (*Lm*)-based immunotherapies are live attenuated bacterial vectors that elicit tumor-reactive T cells through the delivery of tumor-associated antigens directly to dendritic cells. In addition, our *Lm*-based immunotherapies reduce the frequency and function of intratumoral regulatory T cells (Tregs), thereby counteracting the immunosuppressive tumor microenvironment (TME) and sustaining the newly generated antitumor immune response. The purpose of this study is to investigate further the mechanism by which Advaxis' *Lm*-based immunotherapies alter Treg function.

Methods

C57BL/6 mice were implanted with TC-1 tumor cells, which express the human papillomavirus (HPV)16 E6 and E7 proteins. On day 8, when tumors were palpable, mice were treated with either one dose of ADXS-HPV-Quad, an *Lm*-based vector targeting the E6 and E7 proteins from both HPV16 and HPV18, or PBS. Five days post-treatment, tumor infiltrating lymphocytes were phenotyped by flow cytometry and intact tumors were analyzed by immunofluorescence microscopy.

Results

We reasoned that *Lm*-based immunotherapies, to be effective at tumor control, must reprogram the immunosuppressive TME early during the course of treatment in order to induce and sustain infiltration of tumor-specific T cell effectors. Accordingly, we analyzed the TME 5 days after a single dose of ADXS-HPV-Quad. By flow cytometry, we compared the proliferative status and viability of Tregs in tumors of mice treated with either PBS or ADXS-HPV-Quad. The percentage of proliferating (Ki-67⁺) Tregs was comparable between the two treatment groups, but the percentage of Tregs undergoing apoptosis was 2-fold higher in ADXS-HPV-Quad-treated mice than in PBS-treated mice. Further phenotypic analysis revealed that the percentage of Tregs expressing CCR8, a chemokine receptor whose expression is essential for Treg survival and function, was almost 2-fold lower in ADXS-HPV-

Quad-treated mice than in PBS-treated mice. Expression levels of other Treg phenotypic markers, namely Foxp3 and CD39, were also reduced in the CCR8-negative Tregs. The phenotypic changes in intratumoral Tregs were associated with a loss in *in vivo* suppressive activity, as there was a dramatic increase in the infiltration of activated cytokine-producing T cell effectors into the tumor core of ADXS-HPV-Quad-treated mice but not into the tumor core of PBS-treated mice.

Conclusions

Within 5 days of administration, a single dose of ADXS-HPV-Quad alters the tumor microenvironment by impairing Treg survival and function and by promoting effector T cell recruitment and function. Changes in the suppressive Treg phenotype, specifically the downregulation of CCR8 expression, may be a key mechanism by which Advaxis' *Lm*-based immunotherapies impair Treg function.

P462

Ubiquitin-specific protease 6 (USP6) oncogene promotes immune cell recruitment in sarcoma

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Background

The de-ubiquitylating enzyme USP6 is a target of chromosomal translocation and the key etiologic agent in several benign bone and soft tissue tumors (BSTTs) [1]. We have shown that USP6 drives tumorigenesis by directly de-ubiquitylating the Jak1 kinase, leading to its stabilization and activation of STAT transcription factors [2]. We recently found that USP6 is also upregulated in sarcomas, malignancies of mesenchymal origin. Activation of the Jak-STAT pathway plays a key role in modulating

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the tumor microenvironment, however it is not known if or how USP6 affects immune cell infiltration and/or composition. We sought to determine USP6 effects on sarcoma tumor growth and immune cell migration/infiltration.

Methods

USP6 was expressed in a doxycycline-inducible manner in the patient-derived sarcoma cell lines A673 and RD-ES. USP6 expression levels were confirmed to approximate those in primary patient tumor samples.

Results

Overexpression of USP6 results in the production of several immunomodulatory cytokines, most notably *CXCL10*, *CCL5*, and *CCL20*. USP6 expression led to the hallmarks of a heightened immune response, such as enhanced migration, polarization, and activation of several classes of immune cells, such as CD4⁺ and CD8⁺ T cells, monocytes, and macrophages. In murine xenografts, sarcoma cells expressing USP6 had attenuated tumor growth and enhanced immune cell infiltration compared to parental lines. Furthermore, analysis of sarcoma patient datasets revealed that elevated USP6 expression correlated with an immune cell gene signature, and immunohistochemistry of USP6-translocated tumors revealed a high degree of CD4⁺, CD8⁺, and CD163⁺ infiltration.

Conclusions

USP6 expression results in the production of several immunomodulatory cytokines that are known to promote the activation and migration of several classes of immune cells such as T cells, monocytes, and natural killer cells. High USP6 expression correlated with increased immune cell infiltration in mouse xenografts and patient samples. The ability of USP6 to provoke an enhanced immune response could potentially serve as a biomarker for susceptibility to immunotherapy.

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P463

TAM RTKs facilitate MDSC-mediated tumor immunosuppression

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Background

The innate immune-regulatory receptor tyrosine kinases Tyro3, Axl and MerTK (TAM RTKs) and their ligands, Gas 6 and Protein S, suppress the immune response through various mechanisms, including down regulating M1 cytokines, suppressing antigen presenting cell function and decreasing CD8⁺ tumor infiltration. Myeloid-derived suppressor cells (MDSCs) are potently immunosuppressive and inhibit T cell activity via various mechanisms, including increased expression of arginase, inducible nitric oxide synthase (iNOS), and the production of reactive oxygen species (ROS).

Methods

We studied the expression of TAM RTKs in MDSCs and their respective roles in MDSC-mediated immune suppression in the setting of cancer. Using syngeneic murine tumor models implanted into WT and TAM RTK^{-/-} mice, we FACS isolated MDSCs and analyzed the TAM RTKs' effect on MDSC suppressive mechanisms. To complement our genetic models, we employed a pan-TAM RTK inhibitor, UNC4241, and evaluated MDSC function and tumor growth.

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Results

TAM RTKs and their ligands are significantly (M-MDSCs >20 fold, G-MDSCs >15 fold) upregulated in MDSCs from tumor-bearing mice compared to non-tumor bearing controls. MDSCs isolated from tumor bearing TAM RTK^{-/-} mice have significantly diminished arginase, iNOS and ROS activity compared to WT counterparts. Consistent with these findings, M-MDSC functional suppression assays showed that loss of either *mertk* or *tyro3* yielded tumor-elicited M-MDSCs exhibiting reduced suppressive capabilities in T cell proliferation and IFN- γ ELISPOT assays. We performed tumor and MDSC co-implantation studies and demonstrated that WT and *axl*^{-/-} MDSCs migrate readily to the TDLN while *mertk*^{-/-} and *tyro3*^{-/-} MDSCs exhibit a migration defect and remain within the tumor, suggesting that the TAM RTKs play several roles in MDSC-mediated inhibition of T cell priming. Adoptive transfer of TAM RTK^{-/-} MDSCs results in diminished B16 melanoma development in syngeneic hosts relative to those receiving WT MDSCs. To evaluate whether inhibition of TAM RTKs has clinical potential, we employed a pan-TAM inhibitor, UNC4241, and found this agent to suppress MDSC arginase, iNOS and ROS activity, all while increasing T cell proliferation and diminishing the growth of a BRAF^{V600E}PTEN^{-/-} melanoma model. *In vivo* MDSC ablation reversed the effect of UNC4241 indicating that the mechanism of this agent is MDSC-dependent.

Conclusions

These results indicate that coordinate TAM RTK action mediates MDSC suppressive capabilities and that their pharmacologic inhibition reverses MDSC-dependent T cell suppression and diminishes tumor growth. These findings suggest that inhibition of TAM RTKs represents a novel approach for modulating MDSC function and augmenting the efficacy of checkpoint inhibitor therapy.

P464

Use of evofosfamide for targeting immune-suppressive hypoxia in head and neck

squamous cell carcinoma

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Background

Hypoxia is prevalent in head and neck squamous cell carcinoma (HNSCC), where it limits radiotherapy outcomes and may create an immune-suppressive microenvironment. The hypoxia-activated prodrug (HAP) evofosfamide (TH-302) targets hypoxia by undergoing oxygen-sensitive reductive activation (Figure 1). Evofosfamide is being clinically evaluated in combination with ipilimumab in solid tumours, including HNSCC (NCT03098160). The present study investigated the efficacy and sensitivity determinants of evofosfamide in HNSCC.

Methods

Case reports were retrieved from an unpublished phase 2 evofosfamide monotherapy expansion cohort in solid tumours (480 or 575 mg.m² on days 1, 8 and 15 of 28-day cycles). A collection of 22 human papillomavirus-negative HNSCC cell lines derived from lesions of varying TNM stages was assessed for sensitivity to 3 HAPs – evofosfamide, PR-104A and SN30000 – in addition to cisplatin, 5-fluorouracil and the active metabolite of evofosfamide, bromo-*iso*-phosphoramidate mustard

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(Br-IPM). Reductive activation of evofosfamide in cultured cells was measured by LC-MS/MS. The lines were molecularly characterised by RNAseq and whole-exome sequencing and their genomic and transcriptomic features compared to public domain HNSCC clinical samples. Molecular predictors of evofosfamide sensitivity were investigated using hierarchical clustering, differential expression and correlation analyses. The antitumour activity of evofosfamide (50 mg.kg⁻¹ qdx5 for 2-3 cycles with/without 10 Gy radiation on day 5 of cycle 1) was evaluated in two HNSCC xenografts and two HNSCC PDX models derived in-house. Evofosfamide was evaluated in combination with CTLA-4 blockade in the murine SCC-VII model. Hypoxic fraction was assessed using pimonidazole.

Results

Of 5 metastatic or locally-advanced HNSCC patients who received evofosfamide after failing standard-of-care, two showed partial responses and three showed stable disease. Evofosfamide was highly selective for hypoxic HNSCC cells and more potent and selective than PR-104A or SN30000. Cell line sensitivity to evofosfamide was correlated with Br-IPM and cisplatin but not with PR-104A, SN30000 or 5-FU, indicating distinct sensitivity determinants. Evofosfamide sensitivity was associated with the expression of genes relating to proliferation. Accordingly, a proliferation metagene identified subtypes within the cell lines that were differentially sensitive to evofosfamide. Xenografts chosen on the basis of putative predictive biomarkers (tumour hypoxia, proliferation subtype) showed the expected patterns of response. Two PDX models were also highly responsive to evofosfamide. SCC-VII was refractory to evofosfamide monotherapy but showed increased growth delay when evofosfamide was combined with CTLA-4 inhibition.

Conclusions

This study provides a rationale for the clinical evaluation of evofosfamide with immunotherapy and/or radiotherapy in genetically defined subsets of HNSCC.

P465

Characterization of immune cells in the pre-metastatic niche in a murine model of rhabdomyosarcoma

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Background

Tumor metastasis is a critical step in the progression of cancer that is associated with patient mortality. Prior to the arrival of tumor cells at the metastatic site, hematopoietic stem and progenitor cells expand in the bone marrow and mobilize to the pre-metastatic niche, where they differentiate into immunosuppressive myeloid cells [1,2]. Furthermore, recent data has demonstrated that perivascular cells undergo phenotypic changes in the lung that are dependent on the transcription factor KLF4 and enhance metastasis in response to tumor-derived factors [3]. Targeting the pre-metastatic niche by either antibody-mediated depletion of myeloid cells or inhibition of perivascular cell phenotypic switching significantly decreases metastasis [2,3]. However, changes in other immune cell populations in the pre-metastatic niche are not well defined. We hypothesize that primary tumor growth will alter immune cell infiltration and/or function in the pre-metastatic niche.

Methods

Lungs and bone marrow from mice bearing M3-9-M, a metastatic embryonal rhabdomyosarcoma cell line, were harvested at various time points and analyzed by flow cytometry for lymphoid and myeloid populations.

Results

Primary tumor progression was correlated with infiltration of immune cells into the lung before detectable metastasis. There was a dramatic increase of CD11b⁺Ly6G⁺ and CD11b⁺Ly6C⁺ myeloid

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cells in the lung, and a shift in macrophage and dendritic cell populations and phenotypes in the lung and bone marrow. Furthermore, the proportion of both CD4⁺ and CD8⁺ T cells that express PD-1 is significantly enhanced in the lung, as is the number of leukocytes expressing PD-L1.

Conclusions

We demonstrate that the immune compartment in the bone marrow and in pre-metastatic lungs changes significantly in response to primary tumor growth. The differences in immune populations that were observed in the lung and bone marrow were distinct, indicating that the immune changes occurring at these sites in response to tumor progression are specific to their unique microenvironment. We postulate that disrupting the formation of the pre-metastatic niche by targeting stromal cell populations combined with immunotherapy could have anti-metastatic potential. These studies will provide insight into the immune signature of metastatic disease and identify new targets for therapeutic intervention with the potential to reduce metastasis.

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P466

Rational design of immuno-oncology biologics with improved safety and efficacy

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Background

The benefit of enhancing anti-tumor immune response has been demonstrated by the unprecedented clinical responses of immune checkpoint antagonists and cell-based therapies¹. However, the challenge with these therapeutic approaches is that they necessarily involve a systemic activation of the immune system². This leads to treatment-limiting and often debilitating immune related adverse events (irAEs). Attempts to control irAEs include reducing dosing frequency and dosing levels, co-dosing immune suppressants, and drug withdrawal; all of which may limit the utility of immune therapies³. Focusing the activity of immuno-oncology agents to the tumor microenvironment is expected to overcome the limitations posed by irAEs.

Methods

Using anti-mouse CTLA4 antibody 9D9 and anti-mouse PD1 antibody J43 as a proof of concept, we have applied a rational approach to design molecules that are activated in the tumor microenvironment by tumor-associated proteases. *In vitro* testing using antigen binding by Surface Plasmon Resonance (SPR), antigen-capture ELISA and kinetic assays provided important information to support the design process. Candidates were further characterized *in vivo* in C57Bl/6 mice implanted with syngeneic MC38 tumors using tumor regression as a measure of efficacy. The potential for improved safety resulting from inhibition of systemic activity was evaluated using a Fluorescent Activated Cell (FAC) analysis of the T cell population in the spleen for the CTLA4 targeting agents. Induction of diabetes in NOD mice was used to evaluate safety potential for the anti-PD1 agents.

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Results

The tumor-activated antibodies exhibit significantly reduced affinity for their targets without activation, and full binding affinity is restored with proteolytic activation. *In vivo* testing of the tumor-activated antibodies led to tumor reduction similar to the parental antibody. Critically, the tumor-activated antibodies exhibit reduced levels of the systemic activity compared to their parental antibody.

Conclusions

We have developed and demonstrated a rational approach to efficiently design immunotherapeutics that are locally activated in the tumor microenvironment and exhibit reduced systemic activities. Our approach, which we have termed Aklusion™, has the potential to improve the safety and efficacy of immuno-oncology therapeutics.

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P467

CCR4 antagonists inhibit regulatory T cell (T_{reg}) recruitment and increase effector T cell (T_{eff}) numbers in the tumor microenvironment potentiating an anti-tumor response

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Background

Regulatory T cells (T_{reg}) are essential for immune tolerance and T_{reg}-mediated suppression of effector T cells (T_{eff}) is important to control inflammation and prevent autoimmune diseases. However, the presence of T_{reg} in the tumor microenvironment (TME) has been shown to dampen anti-tumor immune responses. Human T_{reg} express CCR4, the receptor for the chemokines CCL17 and CCL22. Preclinical and clinical data supports a role for CCR4-mediated recruitment and accumulation of T_{reg} in the TME which can be associated with poor prognosis. Furthermore, patients receiving immunomodulatory agents demonstrate an influx of T_{reg} in responding patients which may dampen optimal anti-tumor responses. Therefore, CCR4 is an ideal target to selectively block T_{reg} recruitment into the TME.

Methods

We have developed a structurally unique series of small molecule antagonists of CCR4 with cellular potencies in multiple assays (e.g. chemotaxis of primary human T_{reg} in 100% serum) in the low double-digit nM range. Moreover, these compounds have excellent *in vitro* and *in vivo* ADME properties, consistent with convenient oral dosing. These CCR4 antagonists were tested in murine syngeneic tumor models alone and in combination with immunomodulatory agents. During and following treatment, CCR4 ligand levels, tumor infiltrating lymphocytes, and tumor volumes were evaluated.

Results

Preclinically, these CCR4 antagonists block T_{reg} migration and support expansion of activated T_{eff} in the tumor. Our antagonists reduce T_{reg} in the tumor, but not in peripheral tissues such as blood, spleen or skin; which presents a potential safety advantage to the non-selective approach of depleting anti-CCR4 antibodies. In preclinical efficacy studies, treatment with various checkpoint inhibitors and

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immune stimulators (e.g., anti-CTLA-4 or anti-CD137) induce the upregulation of CCR4 ligand expression. Combination therapy with CCR4 antagonist and immunomodulatory agents reduced intratumoral T_{reg} number and increased number of activated and total T_{eff}, resulting in an increase in the intratumoral ratios of both CD4⁺ and CD8⁺ T_{eff} to T_{reg}. The change in these T_{eff} to T_{reg} ratios is greater for our CCR4 antagonist in combination than with the immunomodulatory agent alone and correlates with enhanced tumor growth inhibition and increased tumor regression.

Conclusions

Combination therapy with CCR4 antagonist and immunomodulatory agents overcome T_{reg}-mediated suppression in tumors and tips the balance toward tumor rejection.

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A novel 3D *ex vivo* platform of fresh patient tumor tissue with intact tumor microenvironment for immuno-oncology drug development and biomarker discovery

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Background

Cancer immunotherapy delivers treatment of high specificity, low toxicity and prolonged activity in subsets of patients. There is an unmet need for clinically relevant preclinical models and translational strategies that recapitulate the complexity of tumor immune microenvironment to test the therapeutic potential of immuno-oncology (IO) drugs, identify rational combination therapies and to develop novel predictive biomarkers of clinical response. Here we describe a novel 3D *ex vivo* drug assay utilizing fresh patient tumor samples with intact immune microenvironment to facilitate

oncology and IO drug development and biomarker discovery.

Methods

3D *ex vivo* studies were performed with fresh tumor tissue obtained from consented patients with non-small cell lung, renal, urothelial, head & neck and breast carcinoma. 3D tumor microspheroids (150 μ m in size) prepared according to a proprietary protocol were treated in their intact immune microenvironment with PD1 inhibitors pembrolizumab or nivolumab at 10 μ g/ml for 36 hours. Culture media was collected over the course of the experiments to simultaneously analyze the differential release of cyto- and chemokines. Treatment-mediated changes in T-cell activation and immune cell populations were monitored by flow cytometry while NanoString PanCancer Immune Profiling platform containing probes to quantitate 770 immune function genes was used to determine positive and negative associations between expression of immune function genes and TIL activation by *ex vivo* treatment. Immunohistochemical studies were performed to identify PD-L1 expression and immune cell composition in tumor samples.

Results

3D *ex vivo* samples treated with pembrolizumab or nivolumab demonstrated PD1 occupation in all tumors, while approximately 30% of tumors showed increased CD8 T-cell activation that closely correlated with proinflammatory cytokine release in the conditioned media. Immune gene expression profiling studies revealed increased expression of IFN γ inducible genes in tumors showing activated CD8 population by flow cytometry. No significant correlation was found between tumor PD-L1 expression and *ex vivo* response to treatment. Furthermore, we showed pembrolizumab or nivolumab treatment *ex vivo* led to changes in tumor immune cell composition in specifically including changes in monocyte populations in subset of tumors used in the *ex vivo* experiments.

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Conclusions

Our studies showed that this approach can be used to identify rationale drug combinations and to develop potential companion diagnostics to facilitate biomarker-driven drug development efforts and personalized medicine in immunology.

P469

Tumor oxygenator OMX reverses multiple modes of immunosuppression, activates anti-tumor immunity and cures as a single agent, and in combination with anti-PD-1 in resistant orthotopic tumors

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Background

Hypoxia, a common feature of solid tumors that correlates with poor patient outcomes, is a central mediator of escape from innate and adaptive anti-tumor immune responses. Numerous classes of immunotherapy target specific pathways of hypoxia-driven immunosuppression. Reversing tumor hypoxia, however, would work upstream at one of the primary nodes of immunosuppression to stimulate effective responses. The hypoxic tumor microenvironment arises due to dysfunctional tumor vasculature, and oxygen diffusion is restricted to within ~80 μm of active capillaries. To reverse tumor hypoxia and create an immunopermissive microenvironment, we engineered OMX, a stable, non-vasoactive, high affinity oxygen carrier that preferentially accumulates in solid tumors and specifically re-oxygenates hypoxic microenvironments without affecting normoxic tissues. OMX improves anti-tumor immune responses.

Methods

We used immunohistochemical, flow cytometric, and multiplex cytokine analyses to evaluate OMX's ability to weaken immunosuppression in the tumor microenvironment of orthotopic GL261 brain tumors and promote tumor cures.

Results

A single dose of OMX in GL261 tumor-bearing mice reduces tumor hypoxia, enhances T cell accumulation into the tumor, decreases Tregs and increases activation and proliferation of cytotoxic T lymphocytes (CTLs). Specifically, OMX increases the Teff/Treg ratio by ~3-fold, indicating a switch from an immunosuppressive to an immunopermissive microenvironment. In addition, OMX stimulates secretion of a major chemoattractant for Th1 cells, and increases the accumulation of CD8+ tumor-specific T cells in the tumor tissue. When combined with anti-PD-1, OMX increases CTL infiltration, proliferation and cytotoxic activity, and modulates IFN γ and IFN γ -inducible cytokines that may polarize T cells towards a Th1 phenotype. OMX alone resulted in ~50% tumor cures when treatment is initiated at day 7 in GL261 tumor-bearing mice. Furthermore, the combination of OMX with anti-PD-1 in late-stage GL261 tumor-bearing mice increases mouse survival by ~80% and in large PD-1 resistant GL261 tumors, OMX significantly increases the median survival by ~50%. The survival benefit observed with OMX could be predicted with an identified circulating chemokine signature (post-hoc test).

Conclusions

By delivering oxygen specifically to the hypoxic tumor microenvironment, OMX may restore anti-cancer immune responses in cancer patients through activation of inflammatory cytokine cascades and mobilization and activation of tumor-specific T cells. As a unique, pleiotropic immunotherapy, OMX may enhance immune control of tumors to improve patient outcomes.

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P470

Tertiary lymphoid organs as a good prognostic indicator following neoadjuvant chemo (radio) therapy for pancreatic cancer

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Background

Pancreatic cancer (pancreatic ductal adenocarcinoma; PDAC) is a highly malignant tumor that frequently develops local recurrence and distant metastasis even after a curative resection. Prognosis of PDAC is very poor, and the overall 5-year survival rate for patients with PDAC treated by curative resection is 15-25%. Recently, neoadjuvant chemo (radio) therapy had been reported to improve treatment outcome. Therefore, it is an urgent need to explore surrogate markers predicting patients' outcome. We herein focused on the tumor microenvironment as one of the candidates for monitoring local pathological and immunological reactions, and put a spotlight on tertiary lymphoid organs (TLO, alternatively, ectopic lymphoid tissue) as a biomarker reflecting the effect of preoperative neoadjuvant chemotherapy (NAC).

Methods

In this study, we retrospectively analyzed the area of TLO, dividing 140 patients diagnosed with PDAC from January 2009 to December 2015 into two groups, those who performed upfront surgery (SF group; n=93) and those who underwent NAC (NAC group; n=47). Then we measured the area (mm²) of TLO, the total area (mm²) of the tumor, and calculated the area ratio of total tumor to the total TLO (TLO/ Tumor ratio) by microscopic observation, and statistically analyzed the association between

the level of TLO formation and prognosis in the tumor microenvironment. All the microscopic images were imported as digital photo files using a NanoZoomer Digital Pathology system (Hamamatsu Photonics, Hamamatsu, Japan), and imaging analysis were performed using Image J software (NIH, Bethesda, MD, USA).

Results

The TLO formation was recognized in 128 patients (91.4%; 128/140). There were no significant difference in terms of TLO formation nor TLO/ Tumor ratio between the two groups (94.6%; 88/93 v.s. 85.1%; 40/47, p=0.0575 and 0.66±1.22 v.s. 0.64±8.62, p=0.2342, respectively). The 5-year-overall survival rate of NAC group had been significantly better than SF group (41% v.s.16%, p=0.0203). On multivariate analysis, lymphnode metastasis (HR 0.047, 95%CI: 0.005-0.265, p<0.0001) and high TLO/ Tumor ratio (HR 0.108, 95%CI: 0.010-0.593, p=0.008) were revealed as independent favorable prognostic factors.

Conclusions

Our present results indicate that the level of TLO serves as one of the valuable independent prognostic markers following neoadjuvant chemo (radio) therapy for PDAC.

P471

Patterns of immune cell infiltration in murine models of melanoma: roles of antigen and tissue site in creating inflamed tumors

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Background

Immune cell infiltration is associated with improved survival in melanoma and other cancers. Melanoma

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metastases in humans may be grouped into three Immunotypes representing patterns of immune cell infiltration: A (sparse), B (perivascular cuffing), and C (diffuse)¹. Prior work suggests that anatomical site and intratumoral vascularity can have dramatic effects on T cell responses and immune infiltration. Murine models provide opportunities to understand factors that control immune infiltrates, but Immunotypes have not been defined for common murine models. We hypothesize that patterns of immune cell infiltration can be defined in Braf/Pten and B16 murine models that are similar to Immunotypes observed in human melanoma metastases¹, and that AAD and OVA transfectants and intraperitoneal (i.p.) location will facilitate greater immune infiltration in the B16 model.

Methods

We performed immunohistochemistry for S100, CD31, and CD45, with immune cell enumeration, Immunotyping, and scoring of vascular density in spontaneous melanoma models and in transplantable melanoma models (B16-F1, B16-OVA, and B16-AAD). The transplantable tumors were grown either in subcutaneous (s.c.) or i.p. locations.

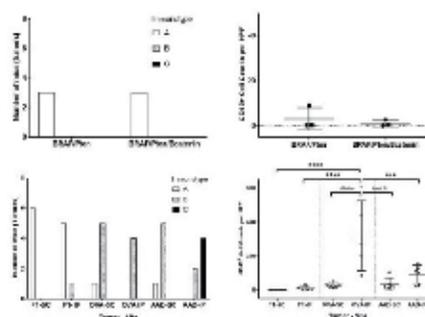
Results

The Braf/Pten and Braf/Pten/ β -catenin tumors had low immune cell counts in 6/6 tumors that were consistent with Immunotype A (Figure 1A, Figure 1B), which was also seen in 11/12 B16-F1 tumors (Figure 1C). In comparison, 9/10 tumors in B16-OVA s.c. and i.p. and 5/6 B16-AAD s.c. tumors were characterized as Immunotype B. Only the B16-AAD i.p. tumors were Immunotype C in 4/6 tumors. The i.p. location was characterized by significantly higher immune cell counts in B16-OVA tumors ($P = 0.0008$, Figure 1D) and higher tumor vascularity. Interestingly, mutated cancer antigens in mutant BRAF models and in B16 melanoma were insufficient to induce significant immune infiltrates, suggesting the existence of barriers to immune infiltration. Addition of model neoantigens (OVA or AAD) overcame existing barriers in a manner that was dependent in part on tumor site.

Conclusions

These murine models may be useful for preclinical studies of combination immune therapy, and suggest that both tumor antigen expression and tumor location contribute to the extent of immune cell infiltration and Immunotype in murine melanoma.

Figure 1: Immunotype and immune cell density of Braf/Pten and B16 murine melanomas.



Spontaneous Braf/Pten and Braf/Pten/ β -catenin dermal melanomas lacked significant intratumoral immune cell infiltrates characteristic of Immunotype A (A), and the mean CD45+ cell counts for 3 mice of each tumor type were all less than 10 per hpf in murine melanoma (B). Associations of B16 tumor cell type and tumor location with immune infiltrate. B16-F1 melanomas (B16-F1), or those transfected with OVA or AAD, were grown in from s.c. or i.p. locations and evaluated for Immunotype (C) and overall CD45+ cell counts (D). Significance is noted by asterisks: < 0.05 , < 0.001 , < 0.0001 .

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DARPin[®]-based fibroblast activation protein-targeted agonists of 4-1BB and OX40 co-stimulate T-cells in a Fc-receptor-independent, tumor-restricted manner

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Background

In addition to check point blockade, immunosuppressive tumor microenvironment can be counteracted by enhanced triggering of T-cell co-stimulatory receptors. In vivo agonistic activity of antibodies against such receptors usually requires Fc-dependent crosslinking and comes at a cost of various systemic, immune related toxicities. We developed a new class of biologics based on the DARPin[®] technology platform which allow tumor-restricted engagement of co-stimulatory molecules independently of Fc-receptors. Our preclinical in vitro and in vivo results indicate that DARPin[®]-based co-stimulatory receptor agonists are comparable to or exceed conventional antibodies in agonistic potency but restrict their activity to the tumor site effectively widening the therapeutic window for compounds exploiting this mode of action.

Methods

We generated multi-specific DARPin[®] therapeutics, which bind to human fibroblast activation protein (FAP) and either to 4-1BB or OX40 co-stimulatory receptor, preferentially expressed on CD8+ and CD4+ lymphocytes, respectively. The agonistic activity of both drug candidates was analyzed using

NFkB-pathway activation reporter cells lines and several types of T-cell activation assays. The 4-1BB-agonist was tested in a xenotransplantation tumor model established in NOG mice humanized by PBMCs.

Results

Both 4-1BB and OX40-specific FAP-targeted DARPin[®] drug candidates efficiently induced activation of the NFkB pathway in 4-1BB or OX40 expressing reporter cells but only after crosslinking on FAP, either surface immobilized or expressed on cells. The capacity of the molecules to enhance lymphokine secretion and upregulate expression of activation markers on T-cells stimulated by polyclonal T-cell receptor crosslinking or specific antigen recognition also strictly required FAP-dependent oligomerization.

Urelumab, a clinically tested 4-1BB agonistic antibody associated with liver toxicity, led to signs of severe graft-versus-host disease (GVHD) and weight loss greater than 20% in the humanized NOG mice transplanted with HT-29 tumor cells at termination of the experiment. In contrast, mice treated with several doses of the 4-1BB-specific DARPin[®] drug candidate showed only minor signs of GVHD and marginal weight loss. Further analysis indicated that urelumab induced CD8+ T-cell expansion both in the peripheral blood and tumor tissue of animals while the 4-1BB-specific DARPin[®] drug candidate triggered a similar change only in the tumor sites.

Conclusions

Thus, tumor targeted DARPin[®] agonists of co-stimulatory molecules can enhance T-cell activity in a manner independent of Fc-crosslinking and limited to the tumor site, thereby avoiding systemic toxicity caused by antibodies against the same immunologic targets. This approach with DARPin[®] therapeutics can be broadly applied to a range of tumor targets and immune receptors.

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Immuno-oncological efficacy of RXDX-106, a novel TAM (TYRO3, AXL, MER) family small molecule kinase inhibitor

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Background

The receptor tyrosine kinases (RTKs), TYRO3, AXL, and MER (TAM), are emerging targets for cancer therapy. Particularly, TAM RTKs play a key homeostatic role as negative regulators of immune responses. RXDX-106 is a potent and selective TAM family inhibitor in preclinical development. Here, we sought (1) to evaluate the efficacy of RXDX-106 as a single agent and in combination with immune checkpoint inhibitors; (2) to identify the immunomodulatory mechanisms of action; and (3) to decipher the reciprocal regulation of TAM expression on cancer and immune cells, and how pharmacological inhibition of TAM signaling would be beneficial, given their complex interaction and regulation in the tumor microenvironment.

Methods

The anti-tumor efficacy of RXDX-106, as single agent or in combination with checkpoint inhibitors, was studied in tumors grown either in syngeneic models or in immunocompromised mice. Immunophenotyping was performed in tissue and tumor samples collected during or at the end of studies by flow cytometry, gene expression and protein analyses, and Luminex bead-based multiplex assays.

Results

Here, we demonstrated that tumor growth inhibition in an MC38 model was associated with increase in intra-tumoral M1 macrophages, CD169^{hi}

antigen presenting macrophages, and PD-L1 expression. Also observed were a higher ratio of CD8+/CD4+ T cells and an increased expression of CD69 and PD-1 on CD8+ T cells, indicative of activation of cytotoxic T cells. Additionally, an increase in Granzyme B and IFN γ with a concomitant decrease in VEGF in tumor cell lysates indicted T cell activation and M1 polarization of macrophages. Furthermore, in a CT26 syngeneic model, we demonstrated that RXDX-106 inhibited tumor growth as a single agent, and the effect was further potentiated by combination therapy with immune checkpoint inhibitors, as evidenced by upregulation of anti-tumor gene expression patterns, upregulation of anti-tumor cytokines in the tumor cell lysates, and an increase in T cell function. Finally, in an AXL-driven tumor model, we demonstrated that AXL expressing tumors induced a pro-tumorigenic immune environment, and treatment with RXDX-106 resulted in complete tumor regression and re-polarization of macrophages towards an M1, anti-tumor phenotype.

Conclusions

RXDX-106 has the potential to restore and enhance immune function in macrophages and T cells, resulting in repolarization of the immune response towards an anti-tumor microenvironment. The unique mechanism of activating both innate and adaptive immunity, plus regulating cross-talk between immune cells and tumor cells by RXDX-106 supports clinical development of RXDX-106 to potentially treat a wide variety of cancers.

P474

Gene expression in MHC II pathway may predict triple negative breast cancer prognosis

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Background

Triple negative breast cancer (TNBC) is a subtype with heterogeneous patient outcomes. It behaves aggressively, and patients are not a candidate for ER or HER2/Neu targeted therapy. Although there have been major research efforts directed at establishing genomic signatures to assess prognosis of breast cancer, prognostic gene expression signatures are not well developed for TNBC. In this study, we investigated twenty-four genes [1] related to the MHC II pathway in 47 TNBC patients and explored gene(s) that can be used to distinguish who will relapse. This work may lead to TNBC management and treatment and could also provide mechanism for the generation of the anti-tumor immune response.

Methods

Forty-seven snap frozen primary TNBC tumor specimens were analyzed using RNA-Seq. Individual gene expressions were transformed to fit a normal distribution. The Logistic regression analysis was used to identify genes that can predict no relapse. The optimal cutpoint was determined from the receiver operating characteristic analysis. This study has the approval of UAB's Institutional Review Board.

Results

CIITA had significantly high expression in TNBC patients who had not relapsed ($P < 0.0001$). With high CIITA expression, TNBC were 10 times more likely to have not relapsed than those with low expression (OR = 10.8, 95% CI 2.8-41.9, $p = 0.006$). The sensitivity and specificity are 75% and 78.3%, respectively. Using multivariable approach with all 24-gene panel, we identified three genes, CIITA, CTSH and KRT14, which together can predict no relapse with 87.5% sensitivity, and 78.3% specificity. Further, at the optimal cut point, we found CIITA alone can predict TNBC no relapse with 84% sensitivity and 77% specificity and accuracy of 81%. It predicted 17/22 relapse, and 21/25 no relapse

correctly. These biomarkers were independent predictors of TNBC prognosis regardless of age, race, tumor grade and stage. The findings were confirmed in public microarray data from 199 patients with TNBC confirmed.

Conclusions

High expression of genes in the MHC II pathway, e.g. CIITA, may predict TNBC prognosis with high sensitivity and specificity regardless of age, race, and tumor grade and tumor stage. This work may lead TNBC management and treatment. Although we explored the optimal point to distinguish patients' outcome, it is important to further establish the threshold in a larger TNBC population.

References

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P475

Profiling Immune Infiltration of Glioblastoma

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Background

The median survival for patients with glioblastoma (GBM) treated with standard of care therapies is 15-20 months. The efficacy of immunotherapy for many types of cancer make this an attractive strategy to improve outcomes for patients with GBM; unfortunately, thus far these have not had demonstrated efficacy in GBM. In other cancers, the presence of immune cell infiltration, the pattern of infiltration, and the expression of immune markers have been associated with survival and response to some types of immunotherapy. However, the immune infiltrate in GBM has not been well-characterized.

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Methods

Multispectral immunohistochemistry images were obtained from 6 GBM specimens stained with CD8, CD20, FoxP3, PNAD, CD83 and Ki67, and analyzed for tumor infiltrating lymphocytes using digital software.

Results

In preliminary studies, we have observed that GBM can have dense B cell infiltrates located near blood vessels; however, the functional role of B cells in GBM, and the prevalence of these cells has been unstudied. Few studies have been performed evaluating B cells in GBM and suggest contradictory roles for B cells in GBM. Candolfi et al. studied the role of B cells in a GBM murine model and concluded that B cells act as antigen presenting cells, lending a critical role in clonal expansion of tumor antigen specific T cells, and brain tumor regression in mice. Conversely, Saraswathula et al. showed that patients with GBM have Bregs in peripheral blood. Bregs downregulate effector T cell responses through the secretion of immunosuppressive cytokines such as IL-10 and through cell-cell contact. Thus, these studies present conflicting roles for B cells in the GBM setting. As a preliminary assessment, we examined the association between survival and overexpression of the MS4A1 gene which encodes for CD20, using RNA-seq gene expression data obtained from TCGA. The expression data showed that patients with overexpression of the gene encoding for CD20 had decreased overall survival ($p = 0.0111$). While these data provide interesting preliminary findings, they need to be further investigated, by evaluating markers of B cell effector functions in GBM and the impact of B cells on T cells infiltrating tumor. However, considering these gene expression data, we hypothesize that CD20⁺ B cells in GBM may negatively impact patient survival, and that these cells may constitute Bregs.

Conclusions

To optimize immunotherapy, there is a necessity to understand the prevalence and the functional role

that B cells play in GBM and their impact on patient survival.

P476

Targeting the IDO/TDO pathway through degradation of the immunosuppressive metabolite kynurenine and inhibition of the downstream AHR pathway

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Background

Checkpoint inhibitors have become the cornerstone of immune-based oncology therapy. Several orthogonal immune pathways are currently being investigated to relieve suppression or boost activity of the innate and adaptive immune system, including the study of the immune-modulating role of metabolites. Both Indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase 2 (TDO2) enzymes metabolize tryptophan forming kynurenine, which binds the aryl hydrocarbon receptor (AHR) in multiple innate and adaptive immune cell types, causing a net immunosuppressive environment. Both enzymes are upregulated across many tumor types, and the IDO1 enzyme has been clinically validated with small molecule inhibitors in combination with checkpoint inhibition, lending credence to the tumor microenvironment containing small molecule metabolites that induce immune cell tolerance, and leaving the opportunity for broader efficacy if both pathways can be targeted. Kynureninase (Kynase), an enzyme degrading kynurenine generated from

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IDO1 and TDO2 pathway, as well as AHR antagonism through small molecule inhibition, can potentially relieve the immunosuppression on multiple cells types and provides novel approaches to inhibiting this pathway in multiple tumor types.

Methods

For Kynase, a pegylated enzyme was used for in vitro and in vivo studies. Pharmacodynamic (kynurenine depletion) and efficacy studies were performed in syngeneic tumor models as single agent or in combination with checkpoint inhibitors (anti-PD1 or anti-CTLA4). Ex-vivo studies were done to analyze the immune cell composition of tumors post-treatment and the effect on AHR signaling. For AHR antagonists, in vitro and in vivo studies were performed to assess kynurenine-dependent signaling through AHR.

Results

Kynase depleted kynurenine produced by IDO1+, TDO2+ and dual positive human cancer cells whereas, the IDO1 inhibitor epacadostat or TDO2 inhibitor 680C91 selectively inhibited Kyn production in IDO1+ or TDO2+ cells, respectively. Kynase ameliorated the suppressive effects of kynurenine on CD4+ and CD8+ T-cells. In B16F10 tumor-bearing mice, a single subcutaneous dose of Kynase depleted kynurenine in the plasma and tumors, leading to an increase in tumor infiltrating CD8+ and CD4+ lymphocytes. Kynurenine activation of AHR in cells, measured by gene expression, is inhibited with AHR antagonists. Kynase demonstrated significant tumor growth inhibition and survival benefit either as a single agent or in combination with checkpoint inhibitors in B16F10, CT26 and 4T1 models. Interestingly, Kynase combined with anti-PD1, showed greater efficacy than epacadostat / anti-PD1 combination.

Conclusions

Our data support clinical development of human Kynase for the treatment of cancers where both IDO1/TDO2 pathways play a significant immunosuppressive role through kynurenine production.

P477

Novel effector phenotype of Tim3+ regulatory T cells leads to enhanced suppressive function in head and neck cancer patients

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Background

Regulatory T (Treg) cells are important suppressive cells among tumor infiltrating lymphocytes. Treg express the well-known immune checkpoint receptor PD-1, which is reported to mark "exhausted" Treg with lower suppressive function. T cell immunoglobulin mucin (Tim)-3, a negative regulator of Th1 immunity, is expressed by a sizable fraction of TIL Tregs, but the functional status of Tim-3⁺ Tregs remains unclear.

Methods

CD4⁺CTLA-4⁻CD25^{high} Treg were sorted from freshly excised head and neck squamous cell carcinoma (HNSCC) TIL based on Tim-3 expression. Functional and phenotypic features of these Tim-3⁺ and Tim-3⁻ TIL Tregs were tested by in vitro suppression assays and multi-color flow cytometry. Gene expression profiling and NanoString analysis of Tim-3⁺ TIL Treg was performed. A murine HNSCC model was used to test the effect of anti-PD-1 immunotherapy on Tim-3⁺ Treg.

Results

Despite high PD-1 expression, Tim-3⁺ TIL Treg displayed a greater capacity to inhibit naïve T cell proliferation than Tim-3⁻ Treg. Tim-3⁺ Treg from human HNSCC TIL also displayed an effector-like phenotype, with more robust expression of CTLA-4, PD-1, CD39, and IFN- γ receptor. Exogenous IFN- γ treatment could partially reverse the suppressive function of Tim-3⁺ TIL Treg. Anti-PD-1

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immunotherapy downregulated Tim-3 expression on Tregs isolated from murine HNSCC tumors, and this treatment reversed the suppressive function of HNSCC TIL Tregs.

Conclusions

Tim-3⁺ Treg are functionally and phenotypically distinct in HNSCC TIL, and are highly effective at inhibiting T cell proliferation despite high PD-1 expression. IFN- γ induced by anti-PD-1 immunotherapy may be beneficial by reversing Tim-3⁺ Treg suppression.

P478  **Abstract Travel Award Recipient**

Formation and functional associations of CD49a-, CD49b- and CD103-expressing CD8 T cell populations in human metastatic melanoma

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Background

Integrins $\alpha 1\beta 1$ (CD49a), $\alpha 2\beta 1$ (CD49b) and $\alpha E\beta 7$ (CD103) mediate retention of lymphocytes in peripheral tissues, and their expression is upregulated on CD8⁺ tumor infiltrating lymphocytes (TIL) compared to circulating lymphocytes. Little is known about what induces expression of these retention integrins (RI). We hypothesized that RI expression marks functionally distinct T cell subsets in the tumor microenvironment (TME) and that these subsets can be induced by selected cytokines and T cell receptor (TCR) stimulation.

Methods

CD8⁺ TIL from 19 human melanoma metastatic lesions were stained for CD49a, CD49b, CD103, and cytokines either directly or after PMA/Ionomycin

stimulation. For in vitro studies, circulating CD8⁺ T cells from normal donors (n=4-7) were cultured with cytokines and/or CD3/CD28 stimulation and evaluated by flow cytometry.

Results

Among CD8⁺ TIL from melanoma metastases, RI^{neg}, CD49a⁺CD49b^{neg}CD103^{neg} and CD49a⁺CD49b⁺CD103^{neg} subpopulations were found in virtually all patients. However, only a subset of patients had CD103⁺ subpopulations (CD49a⁺CD49b^{neg}CD103⁺ and CD49a⁺CD49b⁺CD103⁺). Each subset was assessed for cytokine production. A large fraction of CD49a⁺CD49b^{neg}CD103^{neg} TIL was multifunctional, producing IFN γ , TNF α and IL-2. In contrast, CD49a⁺CD49b^{neg}CD103⁺ TIL only expressed IFN γ , and both CD49a⁺CD49b⁺CD103^{neg} and CD49a⁺CD49b⁺CD103⁺ subsets were incapable of inducing IFN γ , TNF α or IL-2 at comparable levels (Fig.1). Next, we evaluated RI expression of naive T cells in response to different stimuli in vitro. TCR or IL-2 stimulation alone induced two RI⁺ cell populations: one that co-expressed CD49a and CD49b and another that expressed CD49a alone. Adding TNF α to TCR stimulation further induced these populations, whereas TGF β + TCR stimulation generated two additional populations; CD49a⁺CD49b^{neg}CD103⁺ and CD49a⁺CD49b⁺CD103⁺. Each of these subsets was found among melanoma TIL, suggesting that TCR stimulation, IL-2, TNF α and/or TGF β may be responsible for the generation of RI⁺ subsets in the TME.

Conclusions

These observations suggest that CD49b and/or CD103 expression is upregulated as effector TIL lose the capability of producing cytokines and become more exhausted. Generation of CD103⁺ subsets in the TME may be driven by TCR stimulation and TGF β and given their absence in a fraction of tumors, may only be present in tumors producing sufficient TGF β . The other RI⁺ subsets can be induced by IL-2, TCR engagement and/or TNF α , which we hypothesize will be available in all tumors. Together, our findings identify opportunities to modulate RI expression in the TME by cytokine

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therapies and to augment subsets with specific RI expression in the interest of improving immune therapies for cancer or other immune-related diseases.

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MPL-5821, an ESM™-p38 MAPK Inhibitor, modulates macrophage plasticity leading to enhanced IL-12p70 and IFN gamma, reduced IL-10 and the reversal of macrophage induced T-cell suppression.

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Background

Myelomonocytic cells orchestrate both innate and adaptive immune response to tumours but frequently adopt an immunosuppressive, tumor supportive phenotype (M2-like). However, their plasticity offers the opportunity for therapeutic repolarization to one that is non-immunosuppressive and supportive of an anti-tumor immune response (M1-like). A challenge in identifying such pharmaceuticals is their counter-active functionality in other important immune cell types such as lymphocytes. Macrophage Pharma Limited's Esterase Sensitive Motif (ESM™) technology predominantly targets myelomonocytic cells [1]. We describe the preliminary *in vitro* characterisation of MPL-5821, an ESM-p38 MAPK inhibitor, contrasting it with conventional inhibitors, with reference to its cell type specificity, sparing of myelomonocytic – lymphocyte communication and ability to reverse macrophage driven lymphocyte immunosuppression.

Methods

Human Macrophage Assays: M2 polarised macrophages (M-CSF, +/- IL-4, IL-10, TGFβ) were stimulated with LPS/IFNγ +/- compounds overnight. Cytokine production and macrophage markers were measured by ELISA and flow cytometry respectively.

Human PBMC Assay: PBMCs were stimulated with either LPS or anti-CD3 +/- compounds for 72 hours and cytokine production measured by ELISA. CD4 and CD8 T-cell proliferation was measured by flow cytometry.

Macrophage suppression of Human T-cells: M2-polarised macrophages were incubated with autologous PBMCs +/- compound. T cell stimulation was provided by anti-CD3/anti-CD28. Cytokine production was analysed by ELISA and expression of CD4, CD8 and Ki67 by flow cytometry.

Results

Human Macrophage Assays: MPL-5821 and reference p38 MAPK inhibitors exhibited a similar profile in inhibiting IL-10 production and enhancing IL-12p70 production.

Human PBMC Assay: In comparison with reference p38 MAPK inhibitors, MPL-5821 was several orders of magnitude more potent in enhancing IL-12p70 production and was the only inhibitor to enhance IFNγ production under LPS stimulation. Similarly, MPL-5821 was the only inhibitor to enhance IFNγ production in response to anti-CD3 stimulation.

Macrophage suppression of Human T-cells: MPL-5821 reversed the suppressive effects of the M2 macrophages as evidenced by a return of CD4 and CD8 proliferation to control levels accompanied by a concomitant increase in IFNγ and reduction in IL-10.

Conclusions

MPL-5821 modulates the macrophage phenotype to IL-12p70^{hi} / IL-10^{Lo} and reverses the M2 macrophage immune suppression of T-cell functionality. The ESM™ cell selectivity differentiates MPL-5821 from other p38 MAPK inhibitors by its sparing of other

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immune cells such as lymphocytes. Thus MPL-5821 enables the maintenance of the myelomonocytic/lymphocyte IL-12p70/IFN γ axis, key to an effective anti-tumour immune response.

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P480

Tumor infiltrating T regulatory cells in head and neck cancer patients treated with cetuximab demonstrate increased inhibitory receptors and survival.

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Background

Patients with locally advanced head and neck squamous cell carcinoma (HNSCC) incur significant treatment morbidity and have a poor 5 year overall [1]. The idea of treating HNSCC by targeting epidermal growth factor receptor (EGFR) developed from the demonstration of EGFR overexpression correlating with advanced tumor size and decreased survival [2]. Clinical anti-tumor activity of cetuximab, a monoclonal antibody that prevents ligand binding of EGFR, did not correlate with expected response rates despite high HNSCC tumor cell EGFR expression. Further studies to clarify the mechanism of action of cetuximab in HNSCC revealed an increase in CTLA4+ T regulatory cells (Tregs) that suppressed NK cell mediated cytotoxicity, which correlated with poor response to neoadjuvant cetuximab treatment [3].

Methods

We aimed to further measure inhibitory receptor [e.g. PD1, TIM3, TIGIT, CTLA4, neuropilin-1(NRP1)] expression on T cells in tumor and peripheral blood lymphocytes (TIL/PBL) of cetuximab treated patients. Patients with locally advanced HNSCC were

treated with neoadjuvant cetuximab for four weeks prior to definitive surgery with CT scans performed prior to each intervention. Nine samples (TIL and PBL) pre and post cetuximab were analyzed using flow cytometry, and tumor area change was measured using delta CT.

Results

Tumor infiltrating CTLA4+ Tregs had increased expression of NRP1 and TIGIT after cetuximab therapy, specifically in patients that had a poor response to cetuximab. Further, tumor infiltrating CTLA4+TIGIT+ Tregs correlated with patients having larger baseline tumors and minimal change in tumor area post cetuximab. Finally, Tregs from these patients displayed an increase in mean fluorescence intensity of pro-survival markers such as Bcl2.

Conclusions

Increased inhibitory receptors such as TIGIT and NRP1 on CTLA4+ Tregs combined with increased pro-survival markers in patients that had a minimal response to cetuximab suggests that these Tregs maintain suppressive function and survival despite cetuximab treatment. Further characterization of the immune resistance mechanism to cetuximab will provide the basis for designing knowledgeable combinatorial trials with other immunotherapy agents i.e. CTLA-4.

Trial Registration

clinical trials.gov:NCT01218048,NCT0193592

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Non-oncogenic acute viral infection modulates the innate immune response and reduces tumor growth in hosts with established cancer

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Background

The impact of infection on the development and progression of cancer has been a source of debate throughout the past century. Several pathogens have been linked to the development of cancer, while conversely, pathogen components (including Coley's toxins) and oncolytic viruses have demonstrated tumor regression. Since infection and cancer are two often-concomitant challenges to the immune system, we sought to understand the impact of acute, non-oncogenic, non-oncolytic infections – which constitute the majority of infections affecting humans – on tumor growth and host immunity.

Methods

C57BL/6 (B6) or NOD-scid-gamma (NSG) mice were challenged with 1.2×10^5 cells B16-F10 melanoma intradermally (right flank) on day 0 and influenza A/PR8/H1N1 (10,000 PFU) administered intranasally (prior to tumor challenge, after tumor challenge before clinical appearance of the tumor, or once the tumor was established). Tumor growth and influenza infection were monitored via caliper measurement and host weight, respectively, every 2-3 days. Immune cell populations within tumors, lungs, and spleens were analyzed by flow cytometry at day 20. Statistical comparisons between groups were determined using the student's t test with $p < 0.05$ considered statistically significant.

Results

Mice infected with influenza prior to or during the subclinical phase of melanoma development

displayed accelerated tumor growth relative to influenza-naïve mice ($p < 0.01$). Contrarily, mice with established (~3 x 3 mm) tumors displayed slower tumor growth after infection compared to uninfected controls ($p < 0.01$). For the latter, infected mice were found to have a 9-fold reduction of systemic MHC-II⁺ Ly6-G⁺ Ly6-C⁺ myeloid-derived suppressor cells (MDSCs) in the spleen, relative to that observed in influenza-naïve counterparts. Furthermore, the result of curtailed tumor growth in concomitantly challenged mice was observed in immunocompromised NSG mice as well.

Conclusions

Previously we reported that influenza infection in the lung accelerates tumor growth of subclinical melanoma in the flank. However, our recent data demonstrate that the reverse effect is observed if influenza is administered to hosts with an established tumor in the flank. The latter effect is observed also in NSG mice, which lack adaptive immunity and maintain limited innate immunity. These findings, in conjunction with a decrease in MDSCs observed in immunocompetent infected B6 mice exhibiting reduced tumor growth, suggest that the innate immune system may promote anti-tumor immunity in the context of infection. Further research into the mechanisms by which influenza alters tumor growth are being investigated in preparation for clinical trials that will focus on harnessing microbial immunity for the treatment of cancer.

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Somatic TP53 mutations alter the immune micro-environment after chemotherapy in breast cancer

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Background

Neoadjuvant chemotherapy (NAC), followed by surgery, is the standard of care for triple negative breast cancer (TNBC) patients. Unfortunately, only 30% achieve a pathological complete response (pathCR). In patients who do not achieve a pathCR, tumor infiltrating lymphocytes (TILs) after NAC correlate with improved survival. This suggests there is an immune response after NAC which may be augmented by immune checkpoint blockade (ICB). Clinical trials are underway to examine the efficacy of ICB, therefore it is vital to identify biomarkers of response to identify patients who may benefit from these modalities. As more than 80% of TNBC patients harbor somatic *TP53* mutations, we investigated the role of *TP53* mutations on the immune microenvironment of breast cancer after NAC.

Methods

We examined matched pre and post NAC primary breast cancer. TILs were scored by a pathologist. RNA and DNA were extracted and analyzed using Nanostring Pan-Cancer Immune panel (>700 genes) and ImmunoSeq T-cell Receptor (TCR) sequencing respectively. CRISPR technology was applied to mouse tumor cell lines to develop *Trp53* mutations commonly found in TNBC. Cells were treated with chemotherapy *in vitro* to determine tumor specific changes in cytokines and PD-L1 expression. To determine alterations in the immune microenvironment, cell lines were injected into syngeneic mice or genetically engineered mouse models (GEMM) were utilized. Expression of antigen presentation by the tumor as well as immune phenotyping of TIL after NAC was performed.

Results

TILs in residual disease of patients correlated with improved survival. A third of patients had increased

immune gene signatures after NAC which correlated with increased TCR clonality and increased overall survival. CRISPR induced *Trp53* mutant cells were used to elucidate the role of p53. Cells *in vitro* had higher basal levels of immune-recruiting chemokines which were further induced with chemotherapy treatment. *Trp53* mutant cells also exhibited increased antigen presentation (MHC-I/II) and PD-L1 expression after NAC. In a GEMM of breast cancer that had recurred after chemotherapy, TILs had increased expression of CXCR3, LAG3 and PD-1 by flow cytometry.

Conclusions

Our work suggests that NAC induces immune gene signatures in a subset of TNBC which is correlated with a better prognosis. *TP53* mutations in the tumor may contribute to an increased immune infiltrate through upregulating chemokines, antigen presentation, and increased cytokine receptor CXCR3+ T cells that display increased exhaustion marks PD-1 and LAG3. Ongoing studies with ICB will indicate if p53-mutant tumors have a better response to ICB after NAC.

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A novel non-competitive and non-brain penetrant adenosine A_{2A} receptor antagonist designed to reverse adenosine-mediated suppression of anti-tumor immunity

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Background

High levels of extracellular adenosine in the tumor microenvironment promote tumor immune evasion by suppressing Th1 cytokine production and the activation and cytolytic activity of T cells and NK cells.

Methods

We measured extracellular adenosine concentration using intratumoral microdialysis of 11 patient-derived xenografts from various cancer indications. In parallel, we defined the expression of the four adenosine receptors in primary human immune cells using nanostring.

Results

Intratumoral adenosine concentrations reached a range of 1 to 37 μM (mean, 11 μM), significantly higher than the 0.1-0.2 μM reported in normal brain tissue.

A_{2A} was the main adenosine receptor expressed by CD4 and CD8 T lymphocytes and monocytes, and the only one in mature monocyte-derived dendritic cells and NK cells. A_{2B} mRNA were not abundant in T cells and monocytes, while A_1 and A_3 mRNA were not detected in any of the cell types evaluated. We further studied A_{2A} functions in primary human T lymphocytes and monocytes and demonstrated that selective A_{2A} agonists such as CGS-21680 suppressed cytokine production by activated T lymphocytes and monocytes, highlighting the role of A_{2A} as the main receptor mediating adenosine signaling in these cells.

We showed that A_{2A} antagonists initially designed for Parkinson's disease but recently repurposed for immuno-oncology dramatically lost potency in a high adenosine environment. We therefore developed a novel non-brain penetrant and non-competitive inhibitor of A_{2A} with sub-nanomolar Ki and selectivity versus A_1 , A_{2B} , and A_3 receptors. Our compound potently inhibited A_{2A} signaling in human T lymphocytes independently of adenosine concentrations (IC_{50} in T cell cAMP assay = 2.8 and 5.5 nM in 1 and 100 μM adenosine, respectively),

and rescued cytokine production in the presence of high concentrations of A_{2A} agonists. iTeos A_{2A} antagonist potently rescued Th_1 cytokine production in human whole blood treated by A_{2A} agonists, and increased CD8 T cell cytotoxicity in a co-culture assay of effector CD8 T cells and target cancer cells.

Conclusions

iTeos Therapeutics non-competitive A_{2A} receptor antagonist is uniquely designed to address the challenge of counteracting elevated adenosine concentrations in tumors in order to restore antitumor immunity.

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A novel metabolic therapeutic that harnesses the anti-tumor immune response

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Background

In the past decade immunotherapy has shown impressive clinical responses, quickly becoming the first line treatment for cancers such as melanoma. Despite these promising results, all patients do not have the same level of response, and recent efforts have focused on delineating avenues to understand and avoid therapy resistance. Our lab and others have recently proposed that tumor cells may compromise T cell function by generating a metabolically inhospitable microenvironment. Most importantly immune or tumor metabolism can be modified to modulate the T cell response to immunotherapy.

Methods

We hypothesize that leptin, a hormone that affects a wide range of metabolic processes, might be utilized as a means to remodel the metabolic state of the tumor microenvironment, consequently modulating tumor growth and immunotherapy

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response. Previous studies have shown that leptin can enhance T cell proliferation and activation, but the effects of leptin on tumor-infiltrating T cells have not been thoroughly investigated. In order to assess the effects of leptin in the tumor microenvironment, we obtained tumor infiltrating T cells (TIL) from tumor-bearing mice treated with recombinant leptin. Additionally, we generated PTEN/BRAF melanoma cell lines stably overexpressing leptin.

Results

As we have previously shown, glucose uptake in TIL is decreased compared to ndLN. When mice were treated with recombinant leptin, we observed an increase in TIL glucose uptake, suggesting metabolic remodeling. Furthermore, PTEN/BRAF melanoma cells which overexpress leptin have significantly slower growth kinetics compared to control tumors and an increase in overall survival. Leptin overexpressing tumors have increased T cell infiltration compared to control tumors, and these TIL are metabolically and functionally superior.

Conclusions

Taken together, these data suggest leptin can have a direct effect on tumor infiltrating T cell function and tumor growth. Our goal is to further characterize how leptin can metabolically remodel the tumor microenvironment and modulate immunotherapeutic response.

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Use of the NanoString gene expression profiling platform to capture the immunological status of the leukemia microenvironment

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Background

Acute myeloid leukemia (AML) is characterized by clonal expansion of poorly differentiated myeloid precursors and results in impaired hematopoiesis and often bone marrow failure. Immune responses are defective in patients with AML due to powerful immune suppressive circuits that are activated by soluble factors and immune checkpoint molecules, including PD-L1 and IDO1. The development and delivery of new therapeutic strategies for high-risk AML, including immunotherapy, therefore remains a priority.

Methods

This multi-institutional study was undertaken to collect comprehensive immune profiles across the biological heterogeneity of childhood and adult AML, with the aim to implement new molecularly targeted agents for patients with specific immunologic subtypes of AML. We employed the nCounter™ system (NanoString Technologies, Seattle, USA) to characterize bone marrow (BM) specimens from 70 patients with non-promyelocytic AML (42 children and 28 adults). Ninety BM samples (63 *de novo* AMLs, 18 AMLs in complete remission [CR] and 9 relapsed AMLs) were analyzed on the nCounter® FLEX platform, using the RNA Pan-Cancer Immune Profiling Panel. Transcriptomic data were normalized using the nSolver™ software package.

Results

Hierarchical clustering identified patient subgroups with heightened expression of CD8 T-cell, Th1-cell, B-cell and cytotoxicity-related genes, including genes related to NK-cell function, in the leukemia microenvironment (“immune enriched” patients).

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Interestingly, a select group of patients over-expressed genes associated with monocyte/macrophage, neutrophil and mast cell functions, with low expression of T-cell and B-cell genes. AML patients with 'immune enriched' gene profiles also expressed *CD8A*, *IFNG*, *FOXP3*, and inhibitory molecules including *IDO1* and the immune checkpoints *LAG3*, *CTLA4* and *PD-L1*.

Gene set analyses identified differences in immune gene expression levels between childhood and adult AMLs, as well as patients with newly diagnosed AML, relapsed AML or AML in CR. In particular, genes highly expressed in adult AMLs compared with childhood disease included chemokine genes, dendritic cell and macrophage genes and NF- κ B inhibitor- α . In contrast, immune genes down-regulated in adult compared with childhood AMLs included integrin family members, cytokine receptors, TCR complex components and lipocalin-2, a neutrophil gelatinase-associated molecule implicated in suppression of tumor invasiveness.

Conclusions

Our analysis has captured the immunological status of the leukemia tumor microenvironment in children and adults with AML at different disease stages. From a clinical standpoint, 'immune enriched' AMLs might be amenable to immunotherapy approaches tailored to the BM microenvironment.

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Utilization of dual IHC and quantitative image analysis techniques to evaluate LAG-3-positive T cells in the tumor microenvironment of NSCLC tissue

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Background

Non-small cell lung cancer (NSCLC) patients have impaired immune responses, where the expression of inhibitory checkpoint molecules on T cells may lead to reduced immune cell activity. The presence of lymphocyte activation gene-3 (LAG-3)-positive T cells in the tumor microenvironment and their potential impact on prognosis have been investigated over many years. One implication has been that the engagement of LAG-3 inhibits T cell proliferation resulting in T cell suppression and poor prognosis [1]. We aimed to quantify CD3 and LAG-3 immune cell relationships in terms of cell infiltrations and proportions within the tumor microenvironment of NSCLC tissue.

Methods

Ten individual NSCLC clinical tissue samples were immunohistochemically (IHC) stained by Indivumed using the Ventana DISCOVERY XT automated staining platform. The first section was dual-labeled for LAG-3 and CD3 and the second for pan-Cytokeratin (CK). Image analysis was performed by OracleBio using Indica Labs Halo software. Tumor and stroma regions of interest (ROI) were classified using the CK section. ROI classifications were automatically transferred, during analysis, from the sample-associated CK section to the co-registered dual IHC section. Cellular analysis was performed on the ROI using threshold established to identify and count CD3, LAG-3 and dual-labeled cells.

Results

Evaluation of tumor samples demonstrated a mean number of LAG-3/CD3-dual positive cells within the stroma and tumor of 132 ± 59 and 54 ± 20 per mm^2 ROI area, respectively. Interestingly however, when the proportion of LAG-3/CD3-dual positive cells was normalized to the total population of CD3-positive cells within these regions, an opposite relationship was observed where the mean % LAG-3/CD3-positive cells relative to the total CD3 immune cell population was $5 \pm 2\%$ within the stroma and $12 \pm 3\%$ within the tumor.

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Conclusions

These example data highlight the benefits of utilizing dual IHC staining with whole slide image analysis to characterize immune cell relationships within the tumor microenvironment. These techniques can assist in the assessment of changing relationships between proportions of immune cells between tissue compartments and evaluation of LAG-3 as a potential target for modulating T cell responses in NSCLC.

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Multiplex immunofluorescence evaluation of an immune cell marker panel in the tumor microenvironment of NSCLC tissue using tailored analysis of multi-spectral image component data

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Background

Multiplex fluorescence immunohistochemistry (MIF), multispectral imaging and image analysis of immune cells within the tumor microenvironment has been increasingly applied to profile and correlate cell interactions in relation to cancer treatment and predicting patient outcome. Tumor-infiltrating T lymphocytes are known to play an important role in anti-cancer mechanisms where coexisting infiltrations of CD8 and CD4 T cells affords a good prognosis in non-small-cell lung carcinoma (NSCLC) [1]. Here we use CD8/CD4 evaluation to exemplify the use of MIF combined

with multispectral imaging and tailored spectral and spatial quantification to examine relationships between immune cell populations within tumor and stroma.

Methods

An exemplar NSCLC lung tumor section was MIF labelled by Asterand for 5 immune cell markers, including CD4 and CD8, a pan cytokeratin tumor marker and DAPI nuclear marker. The stained slide was digitised using the Vectra Polaris multispectral scanner (Perkin Elmer) and exemplar region of interest (ROI) images exported in component data format. The component data images were analysed by OracleBio using a 3-tiered sequence of tailored applications developed in Visiopharm Oncotopix Software. The first tier enabled the classification of tumor and stroma ROI, the second tier facilitated cell detection, classification and analysis and the third tier refined cell relationships and measured immune cell distances to the nearest tumor cell or between defined immune cells.

Results

Selected ROI displayed a range of tumor and stroma content with varying populations of CD4 and CD8 positive immune cell infiltrations. The mean number of CD4 and CD8 positive cells in tumor was 30 and 250 per mm² respectively and in stroma was 474 and 915 per mm² respectively. Evaluation of tumor-immune cell spatial relationships within the stroma, at a 20µm distance to the tumor border, highlighted that the percentage of CD4 and CD8 positive cells within this stromal border was 33% and 54% respectively and the average distance between the 2 immune cell types was 13µm.

Conclusions

The exemplar data generated provides information relating to the potential influences of immune cell recruitment and activation within the tumor microenvironment, correlation of spatial distributions and the inter-dependencies between immune cells and cancer cells, which may be indicative of response to treatment or predicting patient outcome.

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Single-cell RNA sequencing of CD8+ T cells to prioritize immunotherapy combinations

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Background

Immunotherapy has transformed cancer treatment. To address remaining unmet needs, combinations of checkpoint inhibitors and agonists are under investigation, although prioritization strategies are unclear. We hypothesized that molecular understanding of T-cell phenotypes under chronic stimulation that mimics the tumor microenvironment could aid in optimizing combinations. Additionally, target co-expression is important for designing bispecific monoclonal antibodies (mAbs). Although aggregate gene expression analysis of CD8+ T cells is valuable, it does not show target co-expression at the single-cell level. Hence, we explored single-cell RNAseq to identify targetable CD8+ T-cell subpopulations at various dysfunctional states.

Methods

CD8+ T cells were negatively selected from PBMCs and treated with IL2 (NonStim) or IL2+anti-CD3,antiCD28 antibodies (Stim) for 13 days, including two cycles of treatment separated by a two-day rest in IL2 [1].

Over 1500 Stim and NonStim cells were profiled on 10X Genomics platform and analyzed with corresponding pipeline. Gene expression corresponded to 2 unique molecular indices. Published consensus gene-sets for functional states of CD8+ T cells in cancer [2] were used for Gene-Set

Variant Analysis (GSVA) to identify populations with significant differences in the four states – dysfunctional, senescent, terminal-effector, and stem-like memory – between Stim and NonStim.

Results

GSVA scores indicated significant upregulation of a dysfunction signature and downregulation of terminal-effector function in Stim versus NonStim cells ($P < 0.001$). PD-1 was most differentially expressed between top and bottom tertiles of Stim cells with dysfunctional signature. Similarly, cells with high expression of TIM3, another dysfunction marker, showed downregulation of cytotoxicity and antigen-presentation associated genes, compared to those with low TIM3 expression.

The percentage of cells expressing dysfunction markers increased under chronic stimulation, while those producing effector cytokines decreased. Cells expressing PD-1, CTLA-4 and GATA3 increased from 5%, 3% and 16% to 17%, 80% and 21%, respectively, whereas cells expressing IFN γ decreased from 16% to 7%. Of the Stim cells, 11%, 15% and 71% co-expressed PD-1 and TIM3, PD-1 and CTLA-4, and CTLA-4 and LAG3, respectively. CD137 and TIM3 were co-expressed in >30% of Stim cells, but only 1% of NonStim.

Conclusions

Our results provide new insights into the heterogeneity of activated/exhausted/dysfunctional CD8+ T cells at the single-cell level, which could aid in prioritizing immunotherapy combinations and designing new bispecific mAbs.

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Studying the cellular heterogeneity and spatial arrangement of immune cells in FFPE sections of Hodgkin Lymphoma and normal lymph node using a highly multiplexed Imaging Mass Cytometry

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Background

Clinical successes with immuno-oncology agents have demonstrated the potency of the immune system in controlling cancers, most strikingly in Hodgkin lymphoma (HL), where response rates to PD1/PDL1 inhibitors approach 90%. The majority of tumor bulk in HL is made up of non-malignant immune cells. High parameter methods such as gene expression profiling or flow cytometry have been applied to the study of the tumor immune microenvironment (TME) in Hodgkin disease, however due to the inherent limitations of these techniques, several questions remain unanswered. Data on cellular heterogeneity and rare cells is lost with gene expression studies while the spatial relationship between tumor and immune cells is lost with flow cytometry. The Fluidigm Hyperion imaging mass cytometry (IMC) system combines a CyTOF mass cytometer with a laser ablation system allowing for 40+ parameter simultaneous immunophenotyping on a single slide of FFPE tissue, with sub-cellular resolution. Here we describe our efforts to develop and optimize a 26 parameter antibody panel for the characterization of the lymphoma TME on the IMC platform.

Methods

Literature was reviewed to identify markers relevant to HL TME. Antigen retrieval, blocking, and antibody staining concentrations were optimized to

identify universal staining conditions. Slides were imaged using the Fluidigm Hyperion IMC system and image analysis was performed with MCD viewer (Fluidigm) or CellProfiler/miCAT (<https://doi.org/10.1101/109207>).

Results

We successfully performed IMC imaging of Hodgkin lymphoma, NK lymphoma and normal lymph nodes (LN) using a 26 parameter panel. We were able to clearly identify tissue architecture (Figure 1, normal LN, germinal center(GC), mantle zone(MZ), cortex/paracortex(PC), Figure 2A) and characterize specific cellular populations such as CD8+Tcells, Th₁/Th₂ subsets, FoxP3+T_{reg}, tissue associated macrophages, CD56+NK cells and tumor vasculature. Important immuno-oncology targets such as LAG3, PDL1/PDL2 and PD1 are included. Cellular resolution is similar to IHC, allowing for imaging of tumor, effector and regulatory cell interactions at a single cell level (Figure 2B, Reed Sternberg(RS)cells (red). CD8+(green) adjacent to RS cell flanked by T_{REG} (FoxP3-teal) and Th₂(CCR4-yellow) CD4+(not shown) cells. Abundant CD68+(magenta) cells which co-express PDL1/PDL2 (not shown) in the background.)

Conclusions

IMC allows for successful multiplex imaging of the TME in FFPE tissues. The method does not require special processing and can be applied to archival specimens and tumor microarrays. Methods developed here should be applicable for the study of the TME in other tumor types and could be used to identify additional biomarkers of response to immuno-oncology agents.

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Figure 1. FFPE section of Normal lymph node

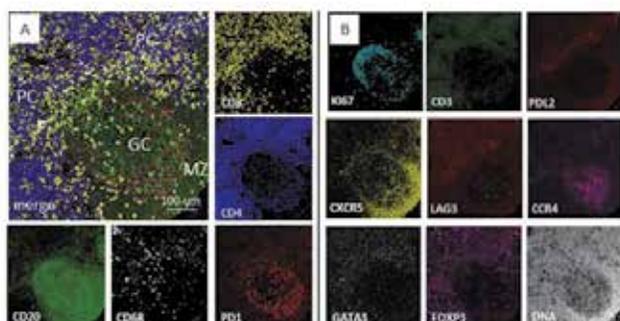


Figure 1

Figure 2. FFPE section of Hodgkin lymphoma

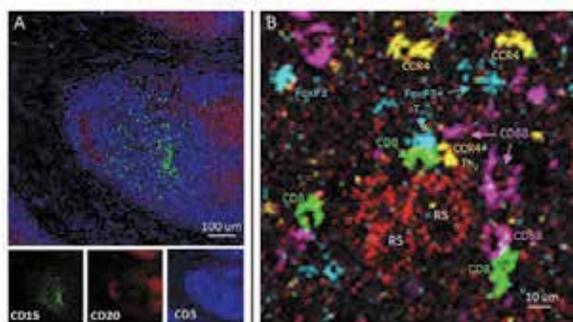


Figure 2

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PSMA-specific CARTyrin T-stem cell memory therapy eliminates solid tumor in subcutaneous prostate cancer model

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Background

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein overexpressed in >80% of metastatic prostate cancer. Although PSMA is a promising target for peptide and antibody drug conjugate therapies, early chimeric antigen receptor

(CAR) T-cell therapies lacked clinical efficacy. Here we developed a novel CAR T-cell product (P-PSMA-101) via piggyBac™ transposition of a tri-cistronic transgene encoding a safety switch, a PSMA-specific Centyrin-based CAR (CARTyrin), and a selection gene, features that may improve safety and efficacy compared with previous anti-PSMA CAR T-cell therapies.

Methods

We developed and identified a lead anti-PSMA CARTyrin from over 250 available Centyrin binders. We also tested the previously clinically-tested anti-PSMA J591 scFv-based CAR for comparability. Initial assessment utilized mRNA delivery of candidate CARTyrins to confirm CAR surface expression and specific T-cell degranulation against PSMA+ prostate tumor cells or PSMA-engineered cells. We then used piggyBac to deliver our tri-cistronic vector system encoding the lead PSMA CARTyrin (P-PSMA-101), J591 scFv CAR, or a BCMA-specific CARTyrin to T-cells, resulting in >95% CAR+ T-cells after selection and expansion. Importantly, our unique production methodology leads to >60% T-stem cell memory (Tscm) cells, an early memory population that correlates with complete responses in CD19 CAR T-cell clinical trials. *In vitro*, P-PSMA-101 and J591 CAR T-cells specifically proliferated, lysed, and secreted IFN-γ against PSMA+ LNCaP or PSMA-engineered K562s. No evidence of tonic signaling or exhaustion was detected.

Results

P-PSMA-101 demonstrated significantly enhanced anti-tumor efficacy and survival in comparison to J591 CAR T-cell-treated mice in a low “stress test” dose of T-cells against established subcutaneous LNCaP(fLuc+) solid tumors in NSG mice. The >60% Tscm P-PSMA-101 expanded *in vivo* and gave rise to differentiated effector CAR+ T-cells that were detected in the peripheral blood concomitant with a decrease in tumor burden below detectable caliper and bioluminescent imaging limits. P-PSMA-101 then contracted, yet persisted in the peripheral blood with >70% of T-cells exhibiting a Tscm phenotype. On the contrary, J591 CAR T-cells did

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not significantly control tumor burden. In a dose titration study, P-PSMA-101 eliminated established LNCaP tumor in 100% of animals for the duration of the studies (42 days post-treatment), while 2/3 low-dose animals remained tumor-free.

Conclusions

P-PSMA-101 is a first-in-class Centyrin-based CAR T-cell therapeutic that exhibits a persistently high frequency of Tscm and mediates durable anti-solid tumor efficacy that surpasses previously established anti-PSMA CAR T-cell therapy in our *in vivo* model. Future efforts will continue towards clinical application of P-PSMA-101 in patients with metastatic castrate resistance prostate cancer.

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Alerting the immune system by removing epigenetic silencing of T_H1 chemokines

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Background

BACKGROUND: Solid tumors employ multiple mechanisms to evade immunologic detection and destruction. However, the potential to enhance the immune response to cancer has been proven in several malignancies and is under investigation in many others, including primary CNS tumors. Brain tumors, in particular, lack robust T-cell infiltration, suggesting poor immune surveillance. Recent studies have found that tumor cells express T-cell attracting chemokines, CXCL9 and CXCL10, in response to interferon gamma (IFN γ). Chemokine expression can be further amplified by drugs that prevent epigenetic silencing, such as histone methyltransferase inhibitors or histone deacetylase (HDAC) inhibitors. This approach increases T-cell trafficking to tumors *in vivo* and enhances the anti-tumor efficacy of checkpoint blockade. We hypothesized that T-cell trafficking to brain tumors

could be increased by epigenetically enhancing CXCL9 and CXCL10 expression.

Methods

Assays were performed on human glioma brain tumor cell lines. CXCL9 and CXCL10 expression were measured by real-time PCR and Western blot. GSK126, a commercially available methyltransferase inhibitor, was utilized to demethylate histone H3 (K9 and K27). LB201 (Lixte Biotechnology, Setatuket, NY) and SAHA were used to inhibit class 1 and class 2 HDACs and Entinostat was used to inhibit class I and class III HDACs. Histone methylation and acetylation status were examined using Western blot analysis. T-cell migration was measured using transwell migration assays. RESULTS: Expression of CXCL9 and CXCL10 was induced by IFN γ in brain tumor lines. Chemokine expression was further increased when cells were treated with GSK126 and HDAC inhibitors compared to IFN γ alone. Migration assays confirmed that tumor cells treated with GSK126 and HDAC inhibitors released chemotactic factors that increased T-cell migration.

Results

Expression of CXCL9 and CXCL10 was induced by IFN γ in brain tumor lines. Chemokine expression was further increased when cells were treated with GSK126 and HDAC inhibitors compared to IFN γ alone. Migration assays confirmed that tumor cells treated with GSK126 and HDAC inhibitors released T-cell chemotactic factors that increased T-cell migration.

Conclusions

These studies demonstrate that brain tumors express T-cell attracting chemokines CXCL9 and 10 in response to IFN γ . Further, single agent and the combination of GSK126 and HDAC inhibitors enhanced expression of CXCL9 and CXCL10, resulting in increased T-cell migration toward tumor cells. Together, these data provide a potential means to enhance the efficacy of immune therapies for brain tumors by promoting T-cell trafficking toward tumor cells.

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Galectin-3 inhibition with GR-MD-02 synergizes with agonist anti-OX40 mAb therapy leading to reduced immune suppression and improved overall survival

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Background

Cancer immunotherapy aims to harness the immune system to destroy tumor cells as well as reverse tumor-induced immune suppression. One such molecule reported to contribute to immune suppression is galectin-3 (Gal3). Indeed, Gal3 is upregulated on a wide variety of tumors and is associated with poor patient prognosis. In addition, increased amounts of Gal3 are found in patients with metastatic disease. Gal3 has been shown to recruit myeloid derived suppressor cells (MDSCs) into the tumor microenvironment, thus aiding in tumor escape. Therefore, we hypothesized that the addition of a galectin-3 inhibitor (GR-MD-02) in conjunction with an agonist anti-OX40 antibody (aOX40) would synergize to promote tumor regression and increased survival via a reduction in tumor-induced immune suppression.

Methods

MCA-205 sarcoma cells or TRAMP-C1 prostate adenocarcinoma cells were implanted into the flank of C57Bl/6 mice; 4T-1 mammary carcinoma cells were implanted orthotopically into the mammary fat pad. GR-MD-02 (2.4 mg/dose) and aOX40 (250 µg/dose) were administered to mice intraperitoneally (ip) either 3x/week for 2 weeks or 2x/week for 1 week, respectively. Tumor growth (area) was assessed 2x/week and mice were sacrificed when tumors exceeded 150 mm². Tumors were either digested and immune cells were

analyzed by flow cytometry or homogenized into tumor lysate was assayed by cytokine bead array.

Results

We observed that combined GR-MD-02/aOX40 therapy significantly improved survival of MCA-205, 4T-1, and TRAMP-C1 tumor-bearing mice, and additionally reduced lung metastases in the 4T-1 model. Further analysis revealed that GR-MD-02/aOX40 therapy significantly reduced the amount of both monocytic (Mo-) and granulocytic (Gr-) MDSCs within the tumor compared to aOX40 alone (p<0.05). In addition, we observed a significant decrease in PD-L1, iNOS, arginase 1 (p<0.05), and CD206 (p<0.001) expression in both Mo- and Gr-MDSCs. Moreover, GR-MD-02/aOX40 therapy led to increased CD8⁺ T cell proliferation in the lymph node and increased intratumoral concentrations of IL-17a and IL-15 (p<0.05), suggesting a potential role in modulating immune infiltrate and/or prolonged T cell survival.

Conclusions

In summary, our data suggests that Gal3 inhibition plus agonist aOX40 therapy may reduce myeloid recruitment to the tumor microenvironment, thus reducing immune suppression and subsequently mediating tumor regression and increased survival.

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LTX-315: A first-in-class oncolytic peptide that reshapes the tumor microenvironment

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Background

The oncolytic peptide LTX-315, which has been de novo designed based on structure-activity relationship studies of host-defense peptides, has

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the ability to kill human cancer cells and induce long-lasting anticancer immune response when injected locally into tumors established in immunocompetent murine models. The oncolytic effect of LTX-315 involves perturbation of the plasma membrane and the mitochondria with subsequent release of danger-associated molecular pattern molecules (DAMPs) such as ATP, Cytochrome C and HMGB1. Furthermore, LTX-315 effectively disintegrates the cellular compartments with subsequent release of tumor antigens as demonstrated by a greater T-cell infiltration (TILs), TILs clonality and the number of clones with greater abundance in the tumor microenvironment. In experimental tumor models, LTX-315 exerts abscopal effects and reshapes the tumor microenvironment by decreasing the local abundance of immunosuppressive cells and by increasing the frequency of effector T-cells. LTX-315's ability to convert immunogenically "cold" tumors to "hot" makes it ideal combination partner with other immunotherapies or immunochemotherapy. Indeed, in preclinical tumor models, a combination of LTX-315 and immune checkpoint inhibitor (anti-CTLA4) demonstrates significant synergy.

Methods

In the present study the antitumor efficacy and potential synergy of LTX-315 in combination with low-dose chemotherapy was investigated in experimental mouse models. Subcutaneously established murine A20 lymphoma was treated with LTX-315 alone, cyclophosphamide alone or LTX-315 in combination with cyclophosphamide. Similarly, orthotopically established 4T1 murine mammary carcinoma was treated with either LTX-315 alone, liposomal doxorubicin (CAELYX) alone or in combination.

Results

LTX-315 showed significantly enhanced anticancer efficacy against A20 lymphomas and 4T1 breast carcinomas when combined with cyclophosphamide and doxorubicin, respectively.

Conclusions

The LTX-315 unique "release and reshape" properties make it a promising candidate for combination with several types of anticancer therapies. Phase 1b study combining LTX-315 with ipilimumab (anti-CTLA-4) in malignant melanoma patients, as well as LTX-315 with Pembrolizumab (anti-PD1) in metastatic breast cancer is ongoing.

P494

Reversal of adenosine-mediated immune suppression by AB421, a potent and selective small-molecule CD73 inhibitor

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Background

Extracellular adenosine-triphosphate (ATP) is efficiently hydrolyzed to adenosine by ecto-nucleotidases CD39 and CD73, which converts adenosine-monophosphate (AMP) into adenosine (ADO). ADO suppresses immune responses including those of T cells, natural killer (NK) cells, and dendritic cells (DCs) through activation of A_{2a}R and A_{2b}R receptors. CD73 inhibition is a promising therapeutic approach for preventing ADO-mediated immunosuppression in the tumor microenvironment.

Methods

Human CD8⁺T cells, CD4⁺T cells, and CD14⁺ monocytes were isolated from buffy coats using various RosetteSep and EasySep Enrichment cocktails. The ability of AB421 to rescue AMP-mediated inhibition of T cell activation was evaluated using CD3/CD28/CD2 stimulation. Mixed lymphocyte reactions (MLRs) were established by mixing GM-CSF and IL-4 differentiated monocytic DCs with allogeneic CD4⁺T cells. AMP and ADO

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levels in C57BL/6J mice were measured by mass spectrometry in plasma samples isolated at various time points after administration of AB421.

Results

We have designed a highly potent and selective small-molecule CD73 inhibitor, AB421, which inhibits endogenous CD73 activity in human CD8⁺ and CD4⁺ T cells with IC₅₀ values of 4.5 pM and 6.2 pM, respectively. AB421 prevents AMP-mediated inhibition of T cell activation in CD3/CD28/CD2 activated human CD4⁺ and CD8⁺ T cells, as well as allogeneic CD4⁺ T cell activation in MLRs. Addition of exogenous AMP abrogated the enhanced allogeneic CD4⁺ T cell activation and IFN- γ production mediated by α -PD-L1 monoclonal antibody (mAb), an effect that was blocked by AB421.

Mechanistically, addition of AMP repressed expression of activation markers (CD25) and immune checkpoint proteins (CTLA-4, PD-1, TIM-3, LAG-3, ICOS, CD28) in the MLR assays. This suggests that activation of the adenosinergic pathway is dominant and may limit the utility of most antibodies targeting immune checkpoint proteins by curtailing their expression and/or upregulation (as was seen with an α -PD-L1 mAb and AMP co-culture systems). Analysis of TCGA databases and tumor microarrays showed differential expression of CD73 across tumor types, with high expression detected in non-small cell lung carcinoma (adenocarcinoma), colorectal, head and neck, esophageal, and stomach cancers. Analysis of dissociated tumor cells by flow cytometry showed CD73 expression in both hematopoietic and non-hematopoietic cells. Finally, we show that AB421 can elevate AMP-to-ADO ratios *in vivo* in a dose-dependent manner, reflecting systemic inhibition of CD73.

Conclusions

AB421 represents a class of potent, reversible and selective CD73 inhibitors that exhibit picomolar potency in primary human immune cells and is currently undergoing preclinical evaluation as a candidate for clinical evaluation.

P495

Separate molecular pathways mediate anti-tumor versus tumor-promoting Aspects of dsRNA signaling in cancer microenvironments

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Background

Infiltration of tumors with cytotoxic T cells (CTLs) predicts improved prognosis and has been shown critical for antitumor effectiveness of checkpoint blockers. In contrast, tumor infiltration with regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) typically predicts rapid progression and poor outcomes. Poly-I:C, a frequently used adjuvant which induces not only the CTL-attracting chemokines but also Treg attractants. Here we evaluated the molecular pathways, which lead to the induction of chemokines by dsRNA in TME and different types of tumor-associated cells, in order to develop improved adjuvants which selectively attract the desirable effector cells rather than suppressive cells.

Methods

Isolated cells or human cancer biopsies were cultured in the absence or presence of one of two synthetic TLR3 ligands Poly-I:C (non-selective activator of TLR3 and helicases) or rintatolimod (selective TLR3 ligand) and in the absence or presence of a COX-1/2 inhibitor, NF- κ B- or TNF α inhibitors. mRNA assays, ELISA, chemotaxis assays and molecular biology assays were used to analyze the chemokine production and tumor-associated suppressive factors. Confocal microscopy of macrophage cultures treated with the TLR3 ligands

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was performed to evaluate the activation of the different signaling pathways by the TLR3 receptor and cytoplasmic helicases.

Results

We observed that poly-I:C induced activation of NF- κ B- and COX-2 pathways leading to induction of COX2-dependent suppressive factors and Treg- and MDSC-attracting chemokines. These undesirable effects were blocked with inhibitors of both NF- κ B- or COX-2 pathways. In contrast rintatolimod selectively induced the desirable chemokines, which were associated with lack of direct activation of NF- κ B- or COX-2 pathways, and strongly suppressed attraction of Tregs and MDSCs, with elevated CTL attraction in *ex vivo* migration assays. Looking at the upstream signaling pathways, both TLR3 ligands induced IRF3 (Type-1 interferon pathway). However, only Poly-I:C induced activation of TRAF3 and RIP-1 (TLR3 dependent NF- κ B pathway) as well as MAVS (cytoplasmic helicases).

Conclusions

Our data implicate an important new role of helicases by the induction of tumor-promoting factors by dsRNA and points out to new targets to enhance the immunogenic and antitumor activities of adjuvants.

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Degradation of hyaluronan (HA) by PEGPH20 promotes anti-tumor immunity and enhances the effect of checkpoint blockade in an HA-accumulating mouse syngeneic tumor model

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Background

The glycosaminoglycan hyaluronan (HA) is abundant in many solid tumors, and its accumulation is often associated with poor patient outcomes. Its

degradation by intravenously administered PEGylated recombinant human hyaluronidase PH20 (PEGPH20) remodels the tumor stroma, reduces intratumoral pressure, decompresses tumor blood vessels, and facilitates drug delivery. Recently, PEGPH20 was shown to enhance tumor growth inhibition induced by the immune checkpoint inhibitor anti-PD-L1 in HA-accumulating pancreatic and orthotopic breast syngeneic tumor models, and to enhance tumor T cell infiltration. Here, the HA content of commonly used syngeneic models was systematically studied, and MC38 (Charles River Laboratories) was identified to further evaluate the combinatorial effect of PEGPH20 and anti-PD-L1 in HA-accumulating tumors.

Methods

Eight syngeneic tumor models were screened for HA content by ELISA, and immune activity by qPCR for a panel of transcripts that included surface markers, cytokines, chemokines, immune checkpoints, and immunomodulatory enzymes. For tumor growth and immunophenotyping studies, MC38 tumors were implanted subcutaneously, and PEGPH20 (37.5 μ g/kg) was administered intravenously 24 h prior to anti-PD-L1 (10F.9G2, 5 mg/kg) using a biweekly dosing regimen. Tumor HA was visualized by immunohistochemistry followed by positive pixel count. Tumor-infiltrating immune cells were evaluated by flow cytometry following tumor dissociation.

Results

Of the eight models tested, MC38 tumors had the highest HA content, and displayed evidence of robust immune activity in the qPCR screen. PEGPH20 reduced MC38 tumor HA by nearly 90% 24 h after either a single dose, or the last of three biweekly doses. In two out of three blinded studies, PEGPH20 significantly enhanced inhibition of MC38 tumor growth induced by anti-PD-L1, and combination treatment also significantly prolonged the survival of tumor-bearing mice compared to either treatment alone. PEGPH20-treated tumors contained significantly higher numbers of CD8+ and CD4+ (both Th and Tregs) T cells, as well as natural

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killer cells, compared to controls. Among CD45+ cells, tumor-associated macrophages (TAMs) were the most abundant cell type; PEGPH20 significantly reduced the frequency of TAMs, while increasing the frequency of dendritic cells.

Conclusions

Collectively, these results suggest that degradation of tumor HA by PEGPH20 can facilitate an anti-tumor immune response by promoting effector cell infiltration and skewing the immune microenvironment toward a more anti-tumor composition, thereby enhancing the effect of anti-PD-L1. These findings support the ongoing clinical evaluation of PEGPH20 in combination with checkpoint inhibitors in HA-accumulating solid tumors, and provide rationale for investigating PEGPH20 in combination with additional immune modulating cancer therapies.

P497

Tumor microenvironment immune gene signature associated with axicabtagene ciloleucel (axi-cel, KTE-C19), an anti-CD19 chimeric antigen receptor (CAR) T cell, in a multicenter trial (ZUMA-1)

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Background

The efficacy of axicabtagene ciloleucel (axi-cel), an autologous anti-CD19 CAR T cell therapy, was evaluated in the multicenter, pivotal ZUMA-1 study in patients with refractory, aggressive non-Hodgkin lymphoma (NHL). In a pre-specified interim analysis, ZUMA-1 met its primary endpoint with an objective response rate of 82% and a complete response rate of 54% [1]. Based on an analysis of patients enrolled in ZUMA-1, we describe for the first time a tumor microenvironment immune gene signature associated with CAR T cell treatment of patients with aggressive NHL.

Methods

In this post-hoc analysis, paired biopsies were taken before and within 3 weeks of axi-cel treatment from 14 patients enrolled in ZUMA-1. These biopsies were analyzed by digital gene expression (Nanostring™) and a pre-specified bioinformatics algorithm was then applied to IGES15 and IGES21 genes, which are hypothesized to be involved in immune-mediated tumor regression (Immunosign®) [2]. The Immunosign® profiles evaluated a pre-defined set of genes for effector T cells, Th1 cells, chemokines, and cytokines. Expression analysis and hierarchical clustering were used to define an axi-cel-related tumor immune gene signature. Wilcoxon signed rank test with multiple test correction by false discovery rate (FDR; Benjamini-Hochberg) was used.

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Results

A comparison of gene expression profiles of biopsies from 14 patients on ZUMA-1 taken before and after axi-cel treatment showed profound changes in gene expression within the tumor environment after infusion. The most upregulated genes post-axi-cel treatment were *CCL5 (RANTES)*, *CTLA4*, and *GZMA* (log₂ fold change >2, *P*<0.05, FDR <0.050). Immune checkpoints *PD-L1* and *LAG3* were also upregulated post-axi-cel treatment (log₂ fold change >1.6, *P*<0.05, FDR <0.055). Other genes associated with T cell proliferation, homing, and effector function that were upregulated included *IL-15*, *GZMK*, *CXC3CL1 (Fractalkine)*, *CD8A*, and *STAT4* (log₂ fold change >1.6; *P*< 0.05, FDR <0.074). Additional baseline tumor characteristics and associative analysis will be presented.

Conclusions

We define a mechanistic tumor immune gene signature in NHL patients associated with axi-cel treatment. This signature comprises upregulation of T cell activation, effector, chemokine, and immune checkpoint genes. These data will potentially lead to rational optimization of T cell interventions in cancer.

Trial Registration

NCT02348216

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AB928, a dual antagonist of the A_{2a}R and A_{2b}R adenosine receptors for the treatment of cancer

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Background

In the tumor micro-environment (TME), extracellular ATP is sequentially hydrolyzed to adenosine by the ecto-nucleotidases CD39 (ATP→AMP) and CD73 (AMP→adenosine), where it acts to suppress immune cell function. A_{2a}R is expressed by a variety of lymphocytes, while myeloid cells express both A_{2a}R & A_{2b}R. A_{2a}R activation results in decreased T cell activation, while binding of adenosine to A_{2b}R on myeloid cells is thought to promote a tolerogenic phenotype. To alleviate immune suppression within the TME, we have designed AB928, a potent and selective dual antagonist of A_{2a}R and A_{2b}R

Methods

Human immune cells were isolated from healthy donor PBMC and cultured as appropriate for the cell type. pCREB assays were performed using freshly collected whole mouse blood.

Results

AB928 inhibits both A_{2a}R and A_{2b}R with similar potencies (K_B: 1.4 nM and 2 nM, respectively). In PBMC sorted from healthy donors, similar levels of A_{2a}R mRNA were observed in naïve and central memory CD4 and CD8 T cells, as well as NK and B cells; A_{2a}R was also expressed in both dendritic cells and monocytes. In contrast to this, A_{2b}R was absent from lymphocytes but was present in both dendritic cell and monocyte populations. Adenosine-mediated suppression of CD8 T cell activation was reversed by AB928, which restored IFN-γ and granzyme B production from these cells. Adenosine-mediated suppression of NK lytic activity was inhibited by AB928, resulting in a significant increase in cell killing. Monocyte-derived dendritic cells (moDC) express high levels of A_{2b}R and those cells, when cultured in the presence of adenosine, had lower levels of costimulatory molecules upon

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activation and a reduced capacity to stimulate CD4 T cells in a mixed-lymphocyte reaction (MLR). Inhibition of adenosine-mediated signaling by AB928 resulted in a significant increase in the frequency of activated T cells in the MLR assay. Adenosine receptor activation leads to phosphorylation of CREB, which can be detected in circulating leukocytes, allowing for the establishment of PK/PD relationships in mice. AB928 exhibited a dose dependent inhibition of pCREB in whole blood.

Conclusions

In conclusion, AB928 is a potent dual inhibitor of A_{2a}R and A_{2b}R receptors with the potential to block all immunosuppressive effects of extracellular adenosine in the TME. This molecule is anticipated to enter clinical development later in 2017.

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Expression pattern and prognostic value of IL-36g in colorectal cancer

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Background

IL-36g/IL-1F9 is a member of the IL-1 cytokine superfamily and a mediator of Type 1 immunity. It was recently reported to be a necessary mediator of tertiary lymphoid organ (TLO) formation in a murine model of colon carcinoma [1]. Specifically, when IL-36g was introduced therapeutically into the tumor microenvironment of MC38-bearing mice, tumors exhibited delayed progression in correlation with TLO formation; this was not observed in control-treated mice. Co-treatment with the antagonist to IL-36g, IL-1F5, blocked the efficacy of the therapy. CD11c+ dendritic cells (DC) were observed to be a

major source of IL-36g in the tumor microenvironment (TME), and signaling through IL-36R+ host cells (including those of the vasculature) was necessary for the efficacy of IL-36g-based treatment. In the present study, we sought to identify a role for IL-36g in the *de novo* formation of TLO.

Methods

In a cohort of 33 primary colorectal cancer patients, the histologic localization and magnitude of expression of IL-36g, IL-1F5/IL-36RA, CD20, CD138, CD8, CD31, PNA^d, DC-LAMP, CD68, and Tbet were determined using immunohistochemical and immunofluorescent imaging. Images were analyzed using CaloPix and Visiopharm software packages.

Results

Both IL-36g and IL-1F5 can be expressed by vasculature-associated smooth muscle cells/myofibroblasts as well as immune cells. Expression of IL-36g by the tumor vasculature positively correlates with CD20+ B cell density in TLO, but not the number of TLO observed in the tumor; neither observation holds for IL-1F5. Some IL-36g+ vessels appear to be high endothelial venules, specialized vessels that aid in recruiting lymphocytes to TLO. Interestingly, while B cells are the primary cellular component of TLO in this model, they do not produce IL-36g. Within the immune compartment, CD8+ T cells (including those expressing Tbet) and CD68+ macrophages are major sources of IL-36g in the TME, while DC-LAMP+ DC are less so.

Conclusions

These results support that the pro-inflammatory conditions of colorectal cancer can promote a Type 1-polarized immune microenvironment; that IL-36g is secreted by diverse cell types within the TME; and that IL-36g expression may play a role in the maintenance of TLO and in B cell recruitment to these structures, within the immune contexture of colorectal cancer.

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P500 Abstract Travel Award Recipient

CD73 blockade restores the abscopal response to radiation therapy and anti-CTLA-4 in cGAS-deficient tumors

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Background

We have recently shown that in poorly immunogenic tumors resistant to immune checkpoint blockers (ICB), radiation therapy (RT) used at 8GyX3 induces cancer cell-intrinsic interferon type I (IFN-I) through cGAS-activation. This process is required for recruitment of BATF3-dependent CD103⁺ dendritic cells (DCs) and for priming of tumor-specific CD8⁺ T cells that mediated abscopal responses in the presence of ICBs [1]. Importantly, knockdown of cGAS in the cancer cells at the irradiated tumor site abrogates the induction of abscopal responses by RT+ICB. Extracellular ATP, which is released from irradiated cancer cells, provides an alternative signal for recruitment and activation of DCs. However, rapid conversion of ATP into immunosuppressive adenosine effectively abrogates this process. Therefore, we hypothesized that antibody blockade of the adenosine-converting ectoenzyme CD73 could restore abscopal responses by RT+ICB in cGAS-deficient tumors by augmenting ATP-mediated DC recruitment.

Methods

Mice bearing subcutaneously inoculated flank tumors (cGAS-silenced TSA^{shcGAS} or mock-silenced TSA^{shNS} as primary tumors and TSA^{shNS} as abscopal tumors) were treated with (1) control antibody; (2) RT; (3) RT+anti-CD73; (4) RT+anti-CTLA-4 or (5) RT+anti-CD73+anti-CTLA-4. Anti-CD73 was administered on day 11, 14, 17 and 20 and anti-CTLA-4 on day 14, 17 and 20. RT was administered to the primary tumor in doses of 8 Gy on day 12, 13 and 14. Tumors were analyzed for DC infiltration on day 18.

Results

In mice bearing primary TSA^{shNS} tumors, RT+anti-CTLA-4 elicited abscopal responses. In mice bearing primary TSA^{shcGAS} tumors, RT+anti-CTLA-4 did not induce abscopal responses, while RT+anti-CTLA-4+anti-CD73 did (Tumor size day 37: 873±533 mm³ in control; 827±732 mm³ in RT+anti-CTLA-4; 131±169 mm³ in RT+anti-CTLA-4+anti-CD73, p<0.01). Treatment of mice bearing TSA^{shcGAS} tumors with RT+anti-CD73 resulted in significantly increased tumor infiltration of CD103⁺ DCs among intratumoral leukocytes compared to mice receiving RT alone (1.2±0.8% in RT v. 2.5±0.7% in RT+anti-CD73, p<0.05).

Conclusions

Our results demonstrate that blocking CD73 recovers the ability of RT to induce recruitment of CD103⁺ DCs to cGAS-deficient tumors and restores the abscopal anti-tumor responses elicited by RT+ICB.

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P501

Involvement of local renin-angiotensin system in immunosuppression of tumor microenvironment and its modulation for augmentation of cancer immunotherapy

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Background

To improve the current cancer immunotherapies, strategies to modulate various immunosuppressive stromal cells need to be developed. Local renin-angiotensin system (RAS) in cancer tissues has been reported to be involved in cellular migration, proliferation, inflammation, and angiogenesis in the tumor and supporting stromal cells, few studies have evaluated the effect of local RAS on anti-tumor immune responses. In this study we have evaluated the role of local RAS in the tumor immune-microenvironment.

Methods

We have evaluated the effect of administration of angiotensin receptor blockers (ARBs) on phenotypes of CD11+ myeloid cells and cancer associated fibroblasts (CAF) in cancer microenvironments, induction of tumor antigen specific T cells, and therapeutic effects of PD-1/PD-L1 immune checkpoint blockade therapies, using murine models bearing tumor cell lines in which RAS was not involved in their proliferation and angiogenic ability.

Results

Administration of ARBs to tumor-bearing mice resulted in significant enhancement of tumor antigen specific T cells. The ARB administration did not change the numbers of CD11b+ myeloid cells in

tumors, but significantly reduced their T-cell inhibitory ability along with decreased production of various immunosuppressive factors including IL-6, IL-10, VEGF, and arginase by CD11b+ cells in tumors. ARB also decreased expression of immunosuppressive factors such as chemokine ligand 12 and nitric oxide synthase 2 in CAFs. Lastly, combination of ARB and anti-programmed death-ligand 1 (PD-L1) antibodies resulted in significant augmentation of anti-tumor effects in a CD8-positive T cell-dependent manner.

Conclusions

These results demonstrated that RAS is involved in generation of immunosuppressive tumor microenvironments caused by myeloid cells and fibroblasts, other than the previously shown proliferative and angiogenic properties of cancer cells and macrophages, and that ARB can transform the immunosuppressive properties of MDSCs and CAFs and could be used in combination with PD-1/PD-L1 immune checkpoint blockade therapies.

P502

IDO1, an immune checkpoint with distinct mechanisms and prognostic significance in glioblastoma

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Background

It is commonly accepted that indoleamine 2, 3 dioxygenase 1 (IDO1), a pivotal immune checkpoint, mediates potent immunosuppression in cancers through its tryptophan (Trp)-to-kynurenine (Kyn) catabolizing activity. However, this enzymatic-dependent mechanism of IDO1 has not been established in glioblastoma (GBM), the most common malignant brain tumor in adults. Additionally, the basis for elevated IDO1 expression in GBM is also poorly understood. The major objective of this study is to address this gap in our understanding of how IDO1 contributes to the biology of GBM and immunotherapy of this deadly malignance, as well as whether its level of expression is a determinant of GBM patients' outcome.

Methods

Freshly resected human GBM, human T cell:GBM co-cultures, HPLC analysis, CTLA-4 and PD-L1 immune checkpoint blockade, IDO1^{-/-} and IDO1^{+/+} C57BL/6 mice, spontaneous GBM [GFAP(ERT2)→Cre^{+/-};pTEN^{fl/fl};Rb^{fl/fl};p53^{fl/fl};(+/- IDO1^{fl/fl});(+/-IDO1^{-/-})] mice, nu/nu mice, NOD-scid and humanized (NSG-SGM3-BLT) mice engrafted human GBM as well as the cancer genome atlas form the basis of our investigation.

Results

(1) Depletion of GBM-derived IDO1 did not affect brain tumor Trp catabolism, while systemic knockout of IDO1 resulted in decreased Trp→Kyn conversion in both naïve and tumor-bearing mice; (2)CTLA-4 and PD-L1-based dual immunotherapy did not alter Trp/Kyn level in IDO1^{-/-} and IDO1^{+/+} mice, but only reached to maximal treatment effect in IDO1^{+/+} mice; (3) In situ hybridization for IDO1 revealed consistent transcript expression in all human GBM samples (n=172), whereas immunohistochemical IDO1 expression was highly variable; (4) Multivariate analysis revealed that higher levels of IDO1 transcript predicted/correlated with shorter survival in the TCGA dataset (P=0.0076) and IDO1 mRNA levels positively correlate with increased gene expression

for markers of cytolytic and regulatory T cells, but no correlation with IFNs gene expression; (5) Humanized mice intracranially-engrafted with human GBM revealed an IFN γ -dependent and T cell-mediated increase of intratumoral IDO1 expression.

Conclusions

Our data indicate that tumor cell-derived IDO1 mediates its immunosuppression in GBM *via* a non-enzymatic mechanism, while the non-tumor IDO1 dominantly contributes to its Trp catabolic function and is essential for the immune checkpoint blockade therapy. TCGA analysis also revealed high intratumoral IDO1 mRNA levels correlate with inferior GBM patient outcome and infiltrating T cells. Future immunotherapeutic efforts aiming to increase T cell-mediated immune response should consider combinatorial approaches that inhibit potential T cell-mediated IDO1 upregulation during therapy.

P503

Comprehensive characterization of immune microenvironment of cholangiocarcinoma

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Background

Background: Biliary tract cancers (BTC) are groups of tumors that include both intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC) and gallbladder carcinoma (GC). BTC comprise an uncommon cancer type that is currently on the rise with a high mortality rate. New modalities of treatment for BTC are highly demanded. Immunotherapy has emerged as a promising treatment for many malignant diseases. Although many risk factors of BTC are associated with chronic inflammatory conditions,

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the immune microenvironment of BTC has been examined to a very limited extent. Therefore, we performed a comprehensive analysis of tumor microenvironment of BTCs to identify immune mechanistic targets for immunotherapy and identify optimal strategies of combinational immunotherapy.

Methods

Materials and methods: Surgically resected tumors from 31 cases of ECC and 24 cases of ICC were stained with multiplex immunohistochemistry of 14 immune cell markers including CD3, CD4, CD8, CD20, CD45, CD68, Cd163, CSF1R, DC-LAMP, DC-Sign, Foxp3, Ki-67, PD-1, PD-L1 on single slide section and analyzed by the Halo software. Nanostring of immune panels and cytokine arrays PCR were also performed by RNA purified from FFPE slides.

Results

Results: Higher density of CD8 T cells is significantly associated with longer overall survival ($p=0.024$). Higher densities of PD-1+ T cells and PD-L1+ myeloid cells are significantly associated with longer overall survival in ECC (PD-L1, $p=0.019$; PD-1, $p=0.018$) (Figure 1, Figure 2), but higher density of PD-L1+ cells are significantly associated with shorter overall survival in ICC ($p=0.003$) (Figure 3, Figure 4). CD68 is not prognostic. However, higher density of CD163+CD68+ M2 macrophages is associated with poorer survival in ECC (Figure 5). Nanostring and cytokine array PCR results are being analyzed. More cases are being stained by multiplex immunohistochemistry.

Conclusions

Conclusion: Our study represents so far the most comprehensive characterization of immune cell subtypes in the tumor microenvironment of cholangiocarcinoma. The results suggest that PD-1/PD-L1 is not a good target in ECC, but a potentially good target in ICC. An ideal macrophage-targeting agent would be the one that depletes the M2 macrophages more specifically.

Figure 1.

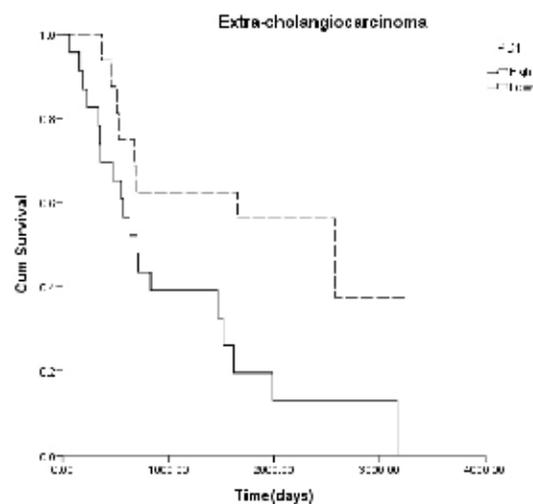
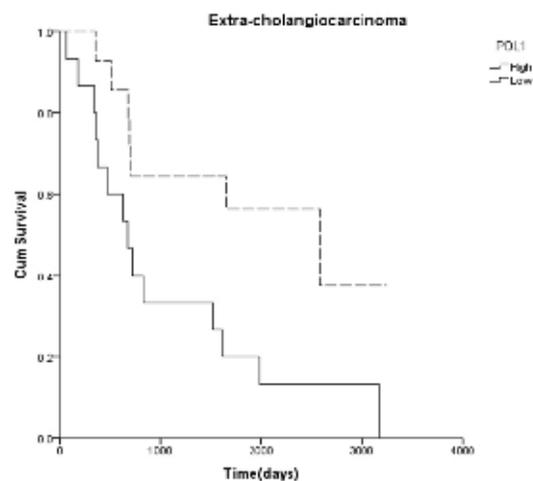


Figure 2.



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Figure 3.

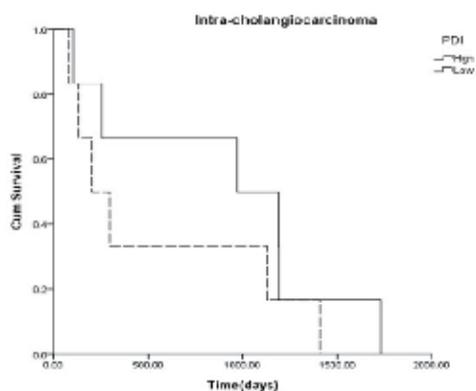


Figure 4.

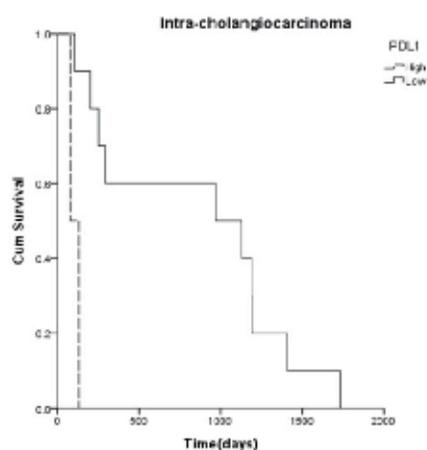
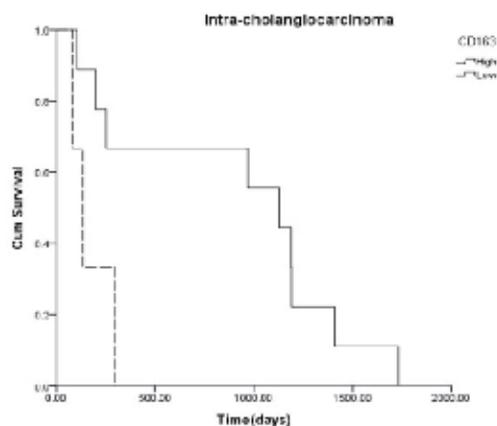


Figure 5.



Various

P504

Enhancement of CAR-T cell tumor tropism through expression of CXCR2 chemokine receptors

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Background

Chimeric Antigen Receptor (CAR)-T cell-based immunotherapies are novel approaches to cancer therapy. With the expected FDA approval of an anti-CD19 CAR for B-cell malignancies, adoptive immunotherapies will become the standard of care for a large number of patients. However, the clinical success of CAR-T cells beyond hematological malignancies has been scarce. We hypothesize that this is in part due to suboptimal bioavailability of CAR-T cells in solid tumors, and we propose to manipulate the expression of chemokine receptors to enhance the CAR-T cells homing and increase their therapeutic efficacy against the tumor.

Methods

We first analyzed the expression of chemokines by human lung tumors, using publicly available databases of RNA expression (TCGA). In addition, we measured their expression in the supernatants of short-term cultures of human lung tumors. Retroviral vectors were generated for the expression of chemokine receptors, in combination with an anti-mesothelin (MSLN) second generation CAR. T cell migration and function were assessed using migration transwell and cytotoxic assays. Anti-tumor efficacy was assessed using a xenograft model of lung cancer.

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Results

IL-8 (CXCL8) was consistently expressed by lung tumors, and secreted by short-term cultures of surgically-removed lesions. Co-expression of an anti-MSLN CAR and CXCR2 or CXCR1 increased the migration of human T cells towards medium containing recombinant IL-8, or conditioned media from lung cancer cell cultures, in transwell assays. Importantly, co-expression of chemokine receptors did not compromise CAR expression or function. Furthermore, expression of CXCR2 (but not CXCR1) in CAR T cells increased the number of intratumoral T cells, and significantly enhanced antitumor efficacy in a xenograft tumor model based on H2110 cells.

Conclusions

Tumor homing and therapeutic efficacy of CAR-T cells can be improved through co-expression of chemokine receptors. This approach may apply to other types of T cell-based immunotherapies, for the treatment of lung cancer, and other solid tumors.

P505

First in human, single ascending dose study in healthy volunteers of SNDX-6352, a humanized IgG4 monoclonal antibody targeting colony stimulating factor-1 receptor (CSF-1R)

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Background

CSF-1R is expressed on immunosuppressive tumor associated macrophages (TAMs). High numbers of TAMs in tumors are associated with poor prognosis. SNDX-6352 is a humanized IgG4 monoclonal antibody with high affinity against CSF-1R and has demonstrated antitumor activity in preclinical tumor models by enhancing the immune response.

Methods

Study SNDX-6352-0001 is a first-in-human, double-blind, randomized, placebo-controlled, single ascending dose, phase I trial in healthy volunteers. The planned dose levels were 0.15, 1.0, 3.0, 6.0, and 10.0 mg/kg. All treated subjects were observed for 72 hours post-infusion and underwent follow-up evaluations for 12 weeks. Safety assessments included adverse event (AE) monitoring, clinical laboratories, vital signs, 12-lead electrocardiogram (ECG), eye examination, and physical examination. Blood samples were collected to characterize the pharmacokinetics (PK) and pharmacodynamics (PD; change in plasma colony stimulating factor-1 [CSF-1] and interleukin-34 [IL-34]), as well as to evaluate changes in circulating classical and non-classical CD16⁺ monocytes in blood and change in CSF-1 receptor occupancy (RO).

Results

The first three placebo-controlled cohorts were completed with 14 subjects receiving SNDX-6352 (2 at 0.15mg/kg, 6 at 1mg/kg and 6 at 3mg/kg) and 6 subjects receiving placebo (2 in each cohort). Dosing up to 1.0 mg/kg was tolerated well with the most common complaint being mild to moderate itching (lasting 2 or 3 days). At the 3 mg/kg dose, similar itching complaints were observed. At this dose, mild to moderate eyelid swelling was observed in all subjects (median duration of 40 days), with no impact on vision. Dose escalation was then terminated. In blood, a transient reduction in non-

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classical CD16+ monocytes was observed, and an upward trend in LDH and ASAT. Cmax increases corresponded with dose, while AUC increases were greater than dose proportional, indicating non-linear PK. Increased CSF-1 concentrations were observed at all dose levels, peaking at 914.5 ng/ml approximately 20 days after the 3mg dose. Increased IL-34 was detected after the 1 mg and 3 mg dose in a pattern consistent with what was seen for CSF-1.

Conclusions

The observed increases for both CSF-1 and IL-34 are consistent with the mechanism of action of SNDX-6352, along with transient suppression of circulating monocyte levels. The PK/PD concentration-time profiles are characteristic of drugs that exhibit target-mediated drug disposition. Consistent with other agents in this class, eyelid swelling/periorbital edema was observed, limiting dose escalation in healthy volunteers. These data support initiation of clinical studies in cancer patients.

P506

A Phase 1, First-in-Human, Dose Escalation, Dose Expansion Study of MSC-1, A Humanized anti-LIF Monoclonal Antibody, in Patients with Relapsed/Refractory Metastatic Solid Tumors

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Background

Leukemia Inhibitory Factor (LIF) is a pleiotropic cytokine involved in many physiological and pathological processes. LIF is highly expressed in a subset of tumors across multiple tumor types and has been shown to correlate with poor prognosis. LIF is hypothesized to contribute to tumor growth and progression by acting on multiple aspects of cancer biology, including immunosuppression of the tumor microenvironment and is a key regulator of cancer initiating cells (CICs), which are thought to underpin tumor growth, metastasis, and resistance to therapy. MSC-1, a first-in-class, humanized monoclonal antibody (IgG1), is a potent and selective inhibitor of LIF. MSC-1 leads to inhibition of STAT3 phosphorylation by disrupting LIF signaling through the LIF receptor (LIFR). Blocking LIF with MSC-1 decreased tumor growth in multiple mouse tumor models and drove reprogramming of the tumor microenvironment through modulation of immunosuppressive macrophages and of several immune cell types. These findings form the basis of a robust therapeutic hypothesis, whereby MSC-1 treatment may lead to clinical activity in multiple cancer indications.

Methods

The Phase I dose-escalation, dose-expansion study of MSC-1 will enroll patients with metastatic relapsed/refractory solid tumors. The study will employ an accelerated 3+3 design to explore the safety, PK, immuno-regulatory activity and preliminary anti-tumor activity of MSC-1. Patients will receive once every three-week treatment until confirmed disease progression or intolerable toxicity. Multiple dose expansion cohorts will be initiated once dose and schedule are established from dose escalation. Response will be assessed every 6 weeks per RECIST v1.1.

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P507

Cancer patient blood plasmablasts reveal functional antibodies with anti-tumor activity

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Background

The role of B cells and antibodies in anti-cancer immune responses is complex. In humans, both the degree of B-cell infiltration and the expression of certain B-cell genes in tumors correlate with improved prognosis in several types of cancer. Indeed, tumor-reactive antibodies are detected in the blood of cancer patients, and tumor-infiltrating B-cells have been shown to produce tumor-reactive antibodies. Moreover, administration of tumor-reactive antibodies resulted in tumor regression in several mouse models. These findings provide a rationale for characterizing the anti-tumor antibodies generated in effective anti-cancer responses, and further defining their targets and mechanisms by which they contribute to tumor control. Here we use our cell-barcoding technology to analyze the anti-tumor antibody response in patients with non-progressing metastatic cancer.

Methods

To better understand the B-cell response in cancer patients, we analyzed plasmablasts circulating in the patients' blood. Plasmablasts were collected from patients with non-progressing metastatic cancer

and antibody sequences obtained from individual cells using Atreca's Immune Repertoire Capture (IRC™) technology. Antibodies representing clonal families were expressed recombinantly and analyzed for binding to tumor cells and tissue and the ability to mediate anti-tumor activity in syngeneic mouse tumor models.

Results

We observed elevated plasmablast levels in individuals with non-progressing metastatic cancer, an indication that these patients have ongoing, active B cell responses. Analysis of these plasmablast antibodies revealed clonal families of B cells that persist over time and show hallmarks of affinity maturation and class switching. We identified antibody sequences with features common to more than one patient consistent with convergent antibody selection in the plasmablast populations across cancer patients. Antibody 013-1, derived from NSCLC patient 031014, bound strongly to human tumors and mouse EMT6 tumor. Antibody 013-1 expressed as a chimera on mouse IgG2a constant region showed efficacy at reducing tumor volume and increasing survival in the mouse EMT6 model. Anti-tumor activity was observed in a dose-dependent manner as both monotherapy and in combination with checkpoint inhibitors. We feel, based on these data, Antibody 013-1 could be a very important therapeutic.

Conclusions

Using Atreca's IRC™ technology we used plasmablasts circulating in cancer patients to identify antibodies that bind to non-homologous tumor types. We demonstrate that recombinant anti-tumor antibodies derived from plasmablast clonal families induce tumor regression, and for certain antibodies durable anti-tumor immunity, in a heterologous, syngeneic, mouse cancer model. Antibodies identified using IRC™ technology represent a new paradigm for the discovery of potentially therapeutic anti-tumor antibodies.

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P508

Analytical validation of reproducibility and precision for automated multiplex immunofluorescence staining

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Background

Recent advances in the field of immunohistochemistry have led to the development of automated fluorescence multiplex technologies focused on interrogating multiple protein markers in a single tissue section. Analytical validation of assay reproducibility is an important parameter for the technology success.

Methods

Automated multiplex staining of five immunology markers CD3, CD4, CD8, CD68 and FoxP3 was performed on tonsil tissue with the VENTANA DISCOVERY ULTRA platform and analyzed with the PerkinElmer Vectra 3 multispectral imaging system. The multiplex staining involves five rounds of sequential incubation of primary antibodies with a heat deactivation step between each round and tyramide signal amplification of Coumarin, FAM, R6G, Red610 and Cy5 fluorophore. Vectra 3 hardware and software configured to support new sets of fluorophores and create a new spectral library. Slides were analyzed for assay reproducibility with PerkinElmer's Vectra 3.0 imaging platform and inForm image analysis software. Twenty tonsil slides (4 batches of 5 slides) were processed. Staining performance was assessed

for precision (defined as the coefficient of variation (CV) of signal intensity within batches), and reproducibility, (defined as CV across batches). In addition, three batches of six tonsil tissue slides, each consisting of five single-plex and one multiplex, were stained to compare cell count. Data was analyzed with inForm's phenotyping cell classification function from fields captured from matched morphological contexts across tissue sections.

Results

Optimizing the 5-plex staining panel involved: (1) individual antibody titer (2) selecting fluorophores for each antibody and staining sequence, (3) adjusting staining parameters to balance signals and (4) further adjusting staining parameters to minimize signal cross-talk. InForm's cell phenotyping and scoring algorithms determined percentage CV of CD3 as 23.3%, CD4 15.7%, CD68 18.2%, CD8 25.4% and FoxP3 as 28.7% indicating robust reproducibility. Agreement between single-plex and multiplex slides for all markers was determined to be within +/- 20% in intensity and 15% in counted cell types.

Conclusions

We demonstrate an effective and reproducible automated multiplexed immunofluorescence staining method using the DISCOVERY RUO 5-plex staining technology and analyzed with the PerkinElmer Vectra3 quantitative slide analysis system. This data validates the VENTANA DISCOVERY IF staining workflow and support a) applications requiring any set of 5 or less validated antibodies, and b) translational study workflows in immuno-oncology research.

Late-Breaking Abstract Poster Presentations

Biomarkers and Immune Monitoring

P509

Modelling oscillatory human immune system dynamics of point-of-care biomarkers for targeting/ sequencing vaccine immuno-chemotherapy in advanced melanoma

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Background

Vaccine immunotherapy for advanced melanoma has demonstrated long-term survival (>15 years) and Complete Responses (CR=17%)¹. Recent clinical responses from 3-5 years using anti-PD-1, anti-CTLA4 and other immunotherapies represent major advances over standard cytotoxic chemotherapies with 1-2% ≥1 year survivals. However, even with the best approved immunotherapies most patients still do not obtain CR's or achieve long-term survival². Toxicity remains a serious problem². Pre-treatment identification of responders and non-responders remains truly enigmatic. Optimal coordination with the individual patient's dynamic immune response has been proposed to better target treatment³.

Methods

Patients with advanced melanoma received vaccine immunotherapy alone, or immuno-chemotherapy, and daily immune point-of-care monitoring with serum C-reactive protein as an inflammatory biomarker. The null hypothesis was stability. Immune oscillatory behavior was tested mathematically. Advanced mathematical analysis was performed to determine the validity of the null hypothesis.

Results

Oscillatory biochemical inflammatory marker behavior was identified in most patients during

therapy, and investigated for correlation with clinical outcome. Monitoring periods containing ≥5 measurements, and of at least 3 in number, are required for statistically defining oscillatory cyclical behavior in humans with cancer.

Conclusions

The implications of these findings are that immunomodulatory therapies (eg. pathway inhibitors, cytotoxics, radiation & perhaps surgery) may require individualized tailoring to coordinate with immune system phase dynamics at delivery to direct immune control and influence clinical efficacy. These findings of immune fluctuation might explain why predictive biomarker identification has been so elusively problematic, and why toxicity is often variable and unpredictable.

Trial Registration

Australian Clinical Trials Registry [ACTRN] 12605000425695

Consent

N/A

References

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Late-Breaking Abstract Poster Presentations

Multitumor profiling of lymphocyte activation gene 3 (LAG-3) and association with immune cell phenotypes

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Background

LAG-3 negatively regulates T-cell activation, is expressed on exhausted T cells, and may promote regulatory T-cell activity. By limiting antitumor T-cell activation, LAG-3 may contribute to immunotherapy nonresponsiveness, as observed in patients with melanoma who progressed during prior anti-PD-(L)1 therapy [1]. Here we describe first results from comprehensive multitumor profiling using quantitative immunohistochemistry (IHC) to characterize expression of LAG-3 and its ligand, MHCII, in the context of inflammation markers, as well as a bioinformatic investigation of LAG-3 using The Cancer Genome Atlas (TCGA).

Methods

Urothelial, gastric, non-small cell lung cancer, renal cell carcinoma (RCC), squamous cell carcinoma of the head and neck, and melanoma tumor specimens (N=245) were stained by IHC for LAG-3, CD8, FOXP3, CD68, CD163, PD-L1, and MHCII. The proportion of total nucleated cells in the tumor microenvironment expressing a given marker was determined using image analysis, and unsupervised clustering was

used to identify subgroups within tumor types. A 160-gene T-cell-inflamed signature was applied to TCGA RNA-sequencing data to assess correlations between LAG-3 and IFN γ -induced gene expression.

Results

Unsupervised clustering of IHC results revealed inflammation-high, -moderate, and -low subgroups, and LAG-3 expression generally correlated with the level of inflammation: CD8 ($r=0.65$); CD68, CD163, and FOXP3 ($r=0.49-0.53$). MHCII tumor-cell expression was observed in inflammation-high and -low tumors and did not correlate with PD-L1 positivity, whereas LAG-3 was significantly higher in tumors with MHCII expression $\geq 1\%$ vs $< 1\%$ ($P=0.001$). In 6 individual tumors with heterogeneous MHCII tumor-cell expression, LAG-3 was higher in MHCII^{hi} ($>70\%$) vs MHCII^{lo} ($<10\%$) regions (P range=0.001-0.070). TCGA analysis was consistent with IHC analyses, demonstrating a strong correlation of LAG-3 mRNA expression with CD8, PD-1, and CTLA-4 ($r=0.81$; $r=0.87$; $r=0.69$), moderate correlation with PD-L1 and MHCII ($r=0.47$; $r=0.58$), and correlation of LAG-3, CD8, and PD-1 mRNA expression with T-cell-inflamed gene signatures across tumor types. Exploratory analyses of clinical trials in RCC and melanoma showed increased mean LAG-3 mRNA expression after nivolumab (anti-PD-1) treatment.

Conclusions

LAG-3 expression correlates with tumor inflammation and is enriched in tumors with MHCII^{hi} tumor cells. Preliminary data suggest that preferential localization of LAG-3-expressing leukocytes to MHCII^{hi} tumor regions potentially serves as a mechanism for LAG-3 checkpoint pathway activation. These findings, and the observation that nivolumab may induce LAG-3 expression, underscore the importance of studies to define predictive biomarker profiles for relatlimab (anti-LAG-3) therapy in PD-1-naive and -progressed patients.

Consent

N/A

Late-Breaking Abstract Poster Presentations

References

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P511

Evaluating immune responses of patients receiving the DPV-001 cancer vaccine

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Background

DPV-001 DRibble[®] is a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and >130 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. We hypothesize that the efficacy of DRibbles' vaccination can be attributed to tumor-derived short-lived proteins (SLiPs) and defective ribosomal products (DRiPs). SLiPs and DRiPs are typically not processed and presented by professional antigen presenting cells therefore the host may be less tolerant. The large number of potential antigens in the vaccine necessitate new techniques to monitor responses.

Methods

Patients received induction cyclophosphamide, then 7 vaccines at 3-week intervals. First vaccine was given intranodally; subsequent vaccines intradermally. Patients were randomized to receive DRibble alone (A), or with imiquimod (B) or GM-CSF (C). PBMCs and serum were collected at baseline and at each vaccination to assess changes in antibodies (Protoarray, microsphere affinity proteomics (MAP)) and cytokines (Quanterix), peripheral lymphocytes populations (flow cytometry) and TCR repertoires (Adaptive immunoSEQ).

Results

13 pts were enrolled (Arm A: 5; B: 4; C: 4). Serum cytokines (IL1 β , IL8, IFN α , IFN γ , IL6, IL17 and TNF α) were measured and normalized and the sum plotted against time. The slope of the resultant trend line was used as an indicator of either increased (positive slope) or decreased (negative slope) systemic inflammation. DPV-001 alone did not change net cytokine load while the addition of the adjuvant imiquimod increased, and the addition of GM-CSF significantly lessened the slope. Vaccination induced or increased IgG Ab responses against targets over-expressed by NSCLC, correlating with activated Th1 cells in whole blood samples. New or augmented Ab responses were observed with continued vaccination. Pts receiving DPV-001 had a significant ($p < 0.04$) increase in total (CD4 + CD8) TCRs that increased 10 fold over baseline compared to normal controls (independent from trial, $n=3$) and the increase in CD4 clones was similar to that seen following Ipilimumab (melanoma pts, independent from trial, $n=9$). Patients receiving DPV-001 alone had the largest increase in CD8 T cell clones.

Conclusions

Vaccination with DPV-001 increased the number of strong antibody responses to antigens commonly over-expressed in NSCLC and expanded populations of T cells. DPV-001 alone provided the greatest increase in CD8 TCRs. Interval monitoring of PBMCs/serum identified the complexity of the

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immune response to this vaccine and suggests possibilities to boost or sustain immunity.

Trial Registration

NCT01909752

Consent

N/A

P512

Deep immunoprofiling of rare T-cell populations from clinical samples

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Background

The complexity and heterogeneity of the immune system combined with its central role in tumor biology necessitates sophisticated analytical approaches to reveal molecular mechanisms, novel therapeutic targets and clinically relevant biomarkers. T-cells have significant functional variation in activation states. However, the rare frequency of antigen-specific CD8+ cells, for example, limits transcriptomic and proteomic analysis to identify biomarkers of exhaustion and activation.

Methods

Utilizing a novel integrated workflow, we performed both proteomic and transcriptomic analysis of very rare populations of T-cells. Negatively selected CD3+ cells were derived from whole PBMCs and stimulated *in vitro* with allogeneic, CD40L-activated, viral-antigen presenting B cells for 8 days. The stimulated cell population was stained with HLA-A02:01 MHC Pentamers specific for Influenza A M1₅₈₋₆₆ (GILGFVFTL). The cells were then simultaneously labeled with fluorescent markers and 30 different DNA barcoded antibodies. Using the fluorescent markers, antigen-specific and naïve

CD8+ T-cells were sorted, lysed, and then the antibody-bound DNA barcodes and the released cellular RNA's were simultaneously measured using the NanoString nCounter® system and analyzed using the nSolver™ software.

Results

By integrating flow cytometry with downstream analysis on the NanoString nCounter system, 30 proteins and 770 RNAs were quantitatively measured on the nCounter from as few as 400 pentamer-positive T-cells. Using the nSolver Advanced Analysis software, differences in gene and protein expression between Influenza A M1₅₈₋₆₆ specific CD8+ T cells and a pentamer-negative CD8+ T cell population were quantitatively measured.

M1₅₈₋₆₆ specific cells showed upregulation of extracellular markers of exhaustion and activation consistent with similar proteomic studies including 4-1BB, CD27, CD45, and ICOS. Additionally, normalized mRNA counts from each population revealed increased presence of transcripts coding for Granzyme B, CD225, Interleukin-32, STAT1, and TCF7 in the Influenza-specific CD8+ cells.

Conclusions

The rarity and functional importance of immune cell subsets in clinical samples has necessitated the development of new analytical methodologies that permit quantitative multiplexed immunoprofiling of RNA and protein expression. Using the nCounter platform downstream of cell sorting uniquely allows simultaneous high-plex analysis of protein and RNA from small numbers of targeted cells. In addition to the data we present on pentamer-positive antigen-specific T-cells, this method can be applied to any number of immune cell populations.

Consent

Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Late-Breaking Abstract Poster Presentations

Cancer Vaccines

P513

Sustained complete response to Nivolumab in a HPV16+ head and neck cancer patient after treatment with MEDI0457 (INO-3112), a DNA immunotherapy targeting HPV16/18

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Background

We report outcomes of a patient (pt) with HPV16+ head and neck squamous cell cancer (HNSCCa) who underwent treatment with 4 doses of MEDI0457 as part of a pilot prospective clinical trial. Disease progression was noted approx. 7 months after completion of adjuvant chemoradiation (due to extracapsular extension) following definitive surgery. The pt was subsequently treated with nivolumab (nivo) and was noted to have a Complete Response (CR) at 6 weeks by RECIST. We performed correlative immune analysis for this pt to understand the mechanism underlying this response.

Methods

This Phase I/IIa trial included pts with p16+ locally advanced HNSCCa. MEDI0457 was delivered IM followed by electroporation with the CELLECTRA[®] device, Q3 weeks x 4 doses. Trial methods and results have been previously reported (Aggarwal C et al *J Immunother Cancer*. 2015;3(Suppl2):P426). Humoral and whole PBMC immune responses were assessed by ELISA and IFN γ ELISpot, respectively. CD8+T cell activity and PD1 were

assessed by flow cytometry (FC). Tissue immune responses were assessed by IHC.

Results

Pt is a 66 yr old Caucasian male with HPV16+ Stage IVA (T2N2b) tonsillar SCCa. He received one dose of MEDI0457 before definitive surgery, and three doses post-operatively. Tissue immune assessment showed decrease in both CD8+ and FoxP3+ infiltrates. Assessment of peak antibody and IFN γ ELISpot responses showed titers of 1:150 and 0 for HPV16 E6 and E7 antigens, respectively, and an elevation of 7 SFU/10⁶ PBMC for each antigen. However, analysis of HPV16 specific CD8+T cells prior to and post dosing with MEDI0457 showed *de novo* induction of CD8+ T cells cells expressing PD-1 (1.8% of all CD8+ T cells), as well as cells co-expressing PD-1, granzyme A, granzyme B and perforin (0.70% of all CD8+ T cells). The pt was noted to have CR after 4 nivo doses, and remains in complete clinical remission 14 months after initiation of nivo.

Conclusions

The data above suggest that the pt responded immunologically to treatment with MEDI0457 as evidenced by the expansion of antigen specific CD8+T cells noted by FC. The expression of PD1 on the CD8+ T cells may have allowed them to be subsequently inhibited by binding to tumor cells expressing PD-L1. Nivo may have relieved this inhibition, allowing for an outgrowth of functional HPV16-specific CTLs, contributing to the sustained CR. An ongoing trial with MEDI0457 and durvalumab in HPV+ HNSCCa is evaluating the clinical and immunologic efficacy of the combination treatment. Clinical trial information: NCT02163057

Consent

N/A

Cellular Therapy Approaches

P514

Late-Breaking Abstract Poster Presentations

Utilizing T-cell activation signals 1, 2 and 3 for tumor-infiltrating lymphocytes (TIL) expansion: the advantage over the sole use of interleukin-2 in cutaneous and uveal melanoma

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Background

MDACC has been conducting clinical trials using adoptive transfer of autologous tumor-infiltrating lymphocytes (TIL) in the context of metastatic melanoma for more than a decade [1]. The art of TIL expansion lies in a two-phase process. The initial phase utilizes high-doses of IL-2 (signal 3) and results in a 62% successful TIL growth rate averaged over the past 11 years (n=1135) with 68% for the last 5 years. The second phase is the rapid expansion process (REP) and relies on TCR activation (signal 1) and co-stimulation (signal 2) followed by high-doses of IL-2 (signal 3). This leads to successful expansion in the vast majority of cases.

Methods

It was recently demonstrated that uveal melanoma is infiltrated with CD8⁺ TIL. However, the initial TIL expansion (pre-REP) from uveal melanoma tumors does not lead to comparable TIL growth with IL-2 alone as seen from cutaneous melanoma [2]. Once cultures from both types of melanoma reach the REP phase, our anecdotal observations concluded that there was no difference in expansion. Therefore, we hypothesized that TCR activation in the 1st phase of expansion combined with an agonistic stimulation of CD137/4-1BB (Urelumab) to protect the TIL from over differentiation in this initial TCR stimulation would favor reliable expansion of CD8⁺ TIL.

Results

This novel 3-signal approach resulted in a faster and more consistent expansion of TIL, up to 100% for both types of melanoma. For cutaneous melanoma, numbers were comparable to or higher than the traditional high-dose IL-2 method and favored expansion of CD8⁺ TIL. Importantly, this new method allowed for better/enhanced pre-REP expansion of TIL from uveal melanoma which, in turn, would allow for this patient population to have access to TIL therapy. Finally, providing the 3-signal attributed to T-cell activation led to expansion of TIL capable of recognizing their tumor counterpart in cutaneous as well as uveal melanoma as determined using IFN γ ELISPOT.

Conclusions

This new methodology for the initial phase of TIL expansion addresses one of the major critiques for TIL therapy - the time needed for proper expansion of a suitable product. It brings consistency in successful growth as well as a new opportunity for challenging malignancies such as uveal melanoma.

Trial Registration

N/A

Consent

N/A

References

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P515

Novel cryopreserved tumor infiltrating lymphocytes (LN-144) administered to patients with metastatic melanoma demonstrates efficacy and tolerability in a multicenter Phase 2 clinical trial

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Background

The safety and efficacy of adoptive cell therapy (ACT) with non-cryopreserved tumor infiltrating lymphocytes (TIL) has been studied in hundreds of patients with metastatic melanoma. This multicenter clinical trial was initiated with centrally manufactured TILs (LN-144) as non-cryopreserved and cryopreserved infusion products. Our novel manufacturing process for the non-cryopreserved LN-144 is used in Cohort 1, and a shortened 3 weeks, cryopreserved LN-144 is used in Cohort 2. The Cohort 2 manufacturing offers a significantly shorter process, which allows for flexibility of patient scheduling and dosing. The shorter manufacturing process reduces the wait time for the patient to receive their LN-144 product and adds convenience to logistics and delivery to the clinical sites.

Methods

C-144-01 is a prospective, multicenter study evaluating metastatic melanoma patients who receive LN-144. Following a non-myeloablative lymphodepletion with Cy/Flu preconditioning regimen, patients receive a single infusion of LN-144 followed by the administration of IL-2 (600,000 IU/kg) up to 6 doses. Patients are evaluated for objective response as a primary endpoint for up to 24 months.

Results

We characterize the cryopreserved LN-144 administered to a second cohort of patients, Cohort 2 following the same pre- and post-TIL infusion treatment regimen as used for Cohort 1.

Cohort 2 patients were heavily pretreated with increased number of prior lines with all patients having anti-CTLA-4 and anti-PD-1 therapies, and larger tumor burden (mean SOD: 15.3, 10.9 cm for Cohorts 2, 1). Median number of prior systemic therapies were 4 and 3 for Cohorts 2 and 1, respectively. An initial analysis of safety data demonstrates comparable tolerability of cryopreserved LN-144. The safety profile for Cohort 1 patients receiving the non-cryopreserved LN-144 continues to be acceptable for this late stage patient population. The most common TEAEs observed in both cohorts by frequency are nausea, anaemia, febrile neutropenia, neutrophil count decreased, platelet count decreased. Early review of efficacy data indicates anti-tumor activity, including PRs, to the TIL therapy observed in patients treated in Cohort 2.

Conclusions

This represents the first clinical trial in a multicenter setting with centrally manufactured TIL assessing a novel process for cryopreserved product with a significantly shorter process (~3 weeks). Preliminary results indicate cryopreserved LN-144 as a novel, well tolerated therapeutic option for patients with metastatic melanoma who have failed multiple prior therapies, including checkpoint inhibitors. The cryopreserved LN-144 provides greater flexibility for patients and caregivers and allows for more immediate treatment for patients with such high unmet medical need.

Trial Registration

NCT02360579

Consent

N/A

P516

Late-Breaking Abstract Poster Presentations

An evaluation of autologous tumor-reactive TIL generation from head and neck squamous cell cancers.

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Background

Head and neck squamous cell carcinoma (HNSCC) remains a significant unmet medical need. While checkpoint blockade has provided improved outcomes for some patients, the majority of patients do not benefit. We hypothesize that the lack of clinical benefit is secondary to the absence of tumor-specific T cells that can expand and mediate tumor destruction. To begin to address this hypothesis we have investigated the percentage of HNSCC from which we can generate TIL reactive with the autologous tumor.

Methods

Over the past 5 years we have collected and processed more than 300 HNSCC specimens. When sufficient tumor material was available, tumor-infiltrating lymphocytes (TIL) and primary tumor cultures were initiated from enzyme digests (collagenase, thermolysin and DNAase) of freshly resected surgical samples. TIL cultures were assessed for growth and autologous tumor reactivity measured by IFN-g release. IFN-g was measured by ELISA. Once established, tumor cell lines were characterized for phenotypic markers by flow cytometry.

Results

For 242 tumor samples with sufficient tumor, 51 (21%) were identified as being contaminated. TIL were generated from 82 (42%) of the remaining 191

tumors. Of the 59 where testing is complete, 46 TIL (77.9%) were autologous tumor-reactive. Overall, we were able to generate TIL from 33% of tumors tested. We identified TIL cultures with a range of autologous tumor reactivity that ranged from very strong to no cytokine-release following stimulation with autologous tumor. At least 20 (10.2%) cell lines were established from the HNSCC samples. A majority of these tumor cell lines contain cells that express CD44, a marker of cancer stem cells.

Conclusions

Tumors from one-third (33%) of the 191 patients evaluated contained TIL that could be expanded and recognize autologous tumor cells. This may correspond to the patients that benefit from immunotherapy with checkpoint blockade or T cell agonists. The remaining patients may require vaccines or other therapies that will prime T cells that can recognize autologous cancer cells. Our group is preparing to undertake a clinical trial of vaccine plus anti-OX40 in patients with HNSCC. Current efforts are examining whether multiplex IHC will be useful in identifying tumors that contain tumor-reactive TIL

Consent

N/A

P517

Regional intraventricular delivery of HER2-specific CAR T cells targets breast cancer metastasis to the brain

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Background

Metastasis to the brain from breast cancer remains a significant clinical challenge, and may be targeted with CAR-based immunotherapy. CAR design optimization for solid tumors is crucial due to the absence of truly restricted antigen expression and potential safety concerns with “on-target off-tumor” activity. Here, we have optimized human epidermal growth factor receptor-2 (HER2)-CAR T cells for the treatment of breast to brain metastases, and determined optimal second generation CAR design and route of administration for xenograft mouse models of breast metastatic brain tumors, including multifocal and leptomeningeal disease.

Methods

HER2-CAR constructs containing either CD28 or 4-1BB intracellular co-stimulatory signaling domains were compared for functional activity *in vitro* by measuring cytokine production, T cell proliferation, and tumor killing capacity. We also evaluated HER2-CAR T cells delivered by intravenous, local intratumoral, or regional intraventricular routes of administration using *in vivo* human xenograft brain metastatic breast cancer models.

Results

Here, we have shown HER2-CARs containing the 4-1BB intracellular co-stimulatory domain confer improved antigen-selective tumor targeting with reduced T cell exhaustion phenotype and enhanced antigen-dependent proliferative capacity compared to HER2-CARs containing the CD28 co-stimulatory domain. Local intracranial delivery of HER2-CARs showed *in vivo* anti-tumor efficacy in an orthotopic xenograft model using a tumor line generated from a breast cancer patient with brain metastasis. Importantly, we demonstrated robust anti-tumor activity following regional intraventricular delivery of HER2-CAR T cells for treatment of multifocal brain metastases and leptomeningeal disease.

Conclusions

Our study shows the importance of CAR design in defining an optimized CAR T cell, and highlights

intraventricular delivery of HER2-CAR T cells for treating multifocal brain metastases.

Consent

N/A

Combination Therapy (IO/IO, IO/Standard of Care, IO/Other)

P518

Generation of non-reprogrammable, dysfunctional CD8⁺ T-cells following anti-PD-1 therapy in the presence of low antigen priming is a cause of failure of the treatment.

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Background

Currently, several clinical trials utilizing anti-PD-1 for cancer therapy are ongoing either alone or in combination with other immune modulators [1]. However, despite a durable response in some patients, monotherapy with anti-PD-1 fails in significant number of patients. Hence, understanding of the mechanisms that lead to failure of anti-PD-1 as an anti-cancer agent would help to harness its full potential.

Methods

Effects of PD-1 blockade, prior or together with tumor-specific vaccine, on tumor growth and survival were evaluated in TC-1 and B16 tumor mouse models. Immune responses were determined in TC-1 tumors in a time-dependent manner. *In vitro* mechanistic studies were carried out in pMel-1 CD8⁺ T-cells.

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Results

We show that in the immunosuppressive microenvironment of the tumor, anti-PD-1 treatment leads to induction of PD1^{high}CD38^{high} non-reprogrammable CD8⁺ T-cells [2]. These cells are generated early after first anti-PD-1 treatment and remain unresponsive to subsequent antigenic stimulation indicating their dysfunctional state. Using *in vitro* assays, we found that treatment with anti-PD-1 prior to T-cell priming abrogates the ability of T-cells to upregulate CD40L and increase IFN γ , and leads to significant cell death by apoptosis and failure to generate memory cells. On the other hand, anti-PD-1 therapy given concomitantly with strong T-cell antigen priming by vaccine produces a robust anti-tumor immune response accompanied with the generation of reprogrammable (plastic) PD1^{high}CD38^{high} cells that get activated, generating effector functions and immune memory.

Conclusions

Our data provide a plausible explanation for the inability of anti-PD-1 therapy to generate effective anti-tumor effects in some patients especially with non-immunogenic tumors. This suggests that the development of combination therapies that would increase the immunogenicity of tumors might enhance the efficacy of anti-PD-1 therapy. These results also suggest that recurrent patients that have undergone anti-PD-1 therapy earlier may not respond to combination therapies that further activate/stimulate the TCR signaling. Therefore, these findings have important implications in the design of future trials employing combination therapy with anti-PD-1 to achieve clinical success.

Consent

N/A

References

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Emerging Models and Imaging

P519

Artificial intelligence augmented phenotypic screens rapidly reveal novel macrophage biology

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Background

Tumor Associated Macrophages have long been recognized as a key component of the tumor microenvironment and play a critical role in tumor progression. However, our ability to fully leverage macrophages as treatment targets is hampered by the complexity of immune phenotypes and low-dimensional screening practices. At Recursion Pharmaceuticals, we combine target-agnostic phenotypic imaging experiments with artificial-intelligence and advanced data analytics to interrogate complex biology in high-dimensional screens. We describe the application of our platform to the study of macrophage polarization states to deliver novel and actionable insights for immuno-oncology drug discovery.

Methods

PMA activated THP1 cells were treated with the relevant polarizing cytokines for 48h prior to staining with the Cell Painting method, which fluorescently labels 9 cellular components to provide a morpho-functional snapshot of a cellular state in the form of ~1000 unique features. Cells were then imaged for fluorescence across 5 channels to generate ~200 images per perturbation and processed through a custom cloud-based software pipeline enabled to run CellProfiler and proprietary artificial intelligence algorithms at scale. Secretome and transcriptome profiling were performed using a standard 65-plex Procartaplex panel and a custom 50-plex Quantigene Plex assay kit respectively (ThermoFisher).

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Results

Our data demonstrate unique, sensitive, and functionally meaningful high-dimensional phenotypes for each of the M0, M1, M2a, M2c, and M2-like (M-CSF) macrophage polarizations (Figure 1, Figure 2). Principal component analysis on the phenotypic features further suggests that the M1-M2-like-M2a-M2c macrophage states are distinct polarizations and not part of an axis, as current dogma holds. All polarizations were validated in orthogonal tumor relevant functional assays. Importantly, when compared to transcriptome and secretome analyses, our phenotypic approach is able to better differentiate between the different polarization states. We demonstrate near perfect distinguishability of the different macrophage classes by a Machine Learning classifier trained on phenotypic features (Figure 3). A phenotypic screen of ~2000 diverse small molecules rapidly uncovered at least 1 novel drug class that modulates M-CSF induced polarization, and at least 3 drug classes that outperform CSF-1R inhibition alone for prevention of a M2-like phenotype. We also identified, in the same screen, putative compounds that could cause a M1-like repolarization in M-CSF treated macrophages. Our entire screening campaign described here was executed in 3 weeks, highlighting the efficiency with which imaging and artificial intelligence can be used in drug discovery.

Conclusions

In conclusion, artificial intelligence augmented phenotypic approaches allow facile interrogation of complex phenotypic states at scale to drive rapid discovery.

Figure 1: Cell painting reveals rich morphological profiles of macrophage polarizations

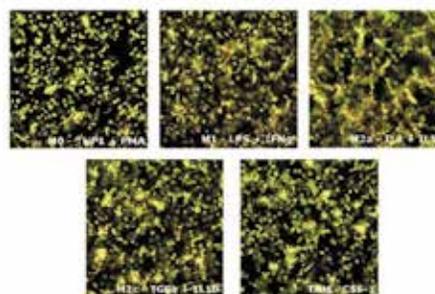


Figure 1: Cell Painting™ images of macrophage polarizations induced by treatment of PMA activated THP1 cells with the various cytokine combinations (as indicated) for 48h allowed computer-vision mediated extraction of ~1000 morpho-functional features that allow sensitive differentiation of the polarizations even when not apparent to the naked eye.

Figure 2: Principal component analysis of phenotypic features reveals meaningful clustering and novel relationships between polarizations

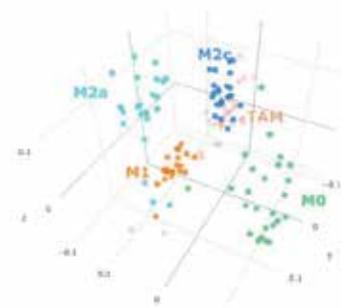


Figure 2: Principal Component Analysis of the cell-level morpho-functional features reveals meaningful clustering of the polarizations in phenotypic space and suggests novel relationships between the macrophage polarization states.

Figure 3: Machine learning can be leveraged to classify macrophage phenotypes with high

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confidence

	PREDICTED				
ACTUAL	M0	M1	M2I	M2a	M2c
M0	25	0	7	0	0
M1	0	35	0	0	0
M2I	6	0	19	0	0
M2a	0	0	0	18	0
M2c	0	0	0	0	25

Figure 3: A confusion matrix depicting classification of different macrophage polarizations by a Machine Learning classifier trained on ~1000 cellular phenotypic features.

Consent

N/A

Mechanisms of Efficacy or Toxicity

P520

Tuberculosis following PD-1 blockade in a patient with Merkel cell carcinoma (MCC): coincidence or causality?

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Background

Preclinical data suggest that PD1 blockade may assist in eradicating a variety of infections. In contrast, PD1 knockout mice are hyper-susceptible to mycobacterium tuberculosis (Mtb) infection, developing necrotic pulmonary lesions with high bacterial loads. We report tuberculosis in a patient treated with pembrolizumab for Merkel cell carcinoma (MCC). We also characterize Mtb-specific immunity and bacteria isolated from a pulmonary lesion that arose after initiating PD1 therapy.

Methods

An 83-year old man began a clinical trial of pembrolizumab in June 2015 for advanced MCC. The patient had no risk factors and no testing for latent tuberculosis was performed. CT scan after 12 cycles revealed known sites of MCC decreasing in size or remaining stable, and a new right lower lobe pulmonary nodule was noted (1.1 x 1.6 cm). The patient underwent excision of the nodule in January 2016. Pathology revealed necrotizing granulomas staining for acid-fast bacilli. Cryopreserved PBMC obtained immediately prior to pembrolizumab and at cycles 5, 8, 11, and 14 were analyzed for antigen-specific CD4 and CD8 T cell responses by intracellular cytokine staining after stimulation with PPD. Serum samples were also analyzed for IgG responses to a panel of different Mtb antigens. Mtb genotyping was performed.

Results

PD1 blockade in this individual was associated with significantly increased circulating Mtb-specific Th1 responses prior to development of the necrotic pulmonary tuberculoma. However, neither Th17 cells nor CD8 T cells specific to Mtb were detectable in PBMC at any time. Circulating Foxp3+ Tregs did not change in number during pembrolizumab treatment in this individual. Mtb-specific IgG levels, although detectable, did not display significant changes before the development of the necrotic granuloma. TB genotyping also did not correlate with any known new clusters of TB in North America in the recent past. Collectively, these data show that the development of tuberculosis following PD1

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blockade in this individual was selectively associated with increases in Mtb-specific Th1 responses.

Conclusions

Four previous cases of TB have been reported following PD-1 blockade. In this case, Mtb-specific Th1 responses increased after PD1 blockade was initiated, and clinical tuberculosis arose subsequently. Importantly, this nodule was assumed to be an MCC metastasis, and would not have been recognized as due to Mtb if an excisional biopsy had not been performed. In conjunction with animal model data suggesting a plausible mechanism, and the prior reported cases, these findings suggest that Mtb is possibly a concern following PD1 blockade.

Trial Registration

NCT02267603

Consent

N/A

Mechanisms of Resistance to Immunotherapy

P521

Imprime PGG, a novel phase 2 immunotherapeutic, enhances the anti-tumor activity of checkpoint inhibitors (CPI) and suppresses CPI-induced Indoleamine 2, 3-dioxygenase (IDO) expression

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Background

Although checkpoint inhibitors (CPI) have shown unprecedented efficacy in cancer treatment, a significant fraction of patients eventually develop resistance to CPI. Therefore, there is a growing need

to identify resistance mechanisms as well as rational combination strategies to combat this resistance. Imprime PGG (Imprime), a novel yeast derived β -glucan pathogen-associated molecular pattern (PAMP), is being developed as a combination agent with CPI in patient populations who have failed single-agent CPI therapy. In pre-clinical mechanistic studies, Imprime has been shown to reprogram the immuno-suppressive myeloid cells in the microenvironment and enhance the effector functions of tumor infiltrating T cells. The objective of this study was to focus on IDO1, one of the critical resistance mechanisms in the microenvironment that hinders T cell anti-tumor immunity.

Methods

The anti-tumor efficacy of Imprime in combination with anti-PD-1 was evaluated in the murine colon cancer model MC38. Transcriptional changes in the tumor were assessed by QuantiGene Multiplex platform. IDO1 gene expression in IFN- γ -stimulated human whole blood post Imprime treatment was assessed by qRT-PCR. Tryptophan and kynurenine levels were measured in the serum by LC/MS.

Results

In the MC38 model, Imprime in combination with anti-PD-1 resulted in significantly reduced tumor growth as compared to anti-PD-1 monotherapy. Consistent with our previous results, transcriptional analyses of tumor tissues showed that Imprime alone induced a M1 skewing gene expression profile by modulating several genes including iNOS, TNF, CXCL10, Arg1, and CCL17. Anti-PD-1 treatment alone up-regulated several genes affecting T cell functionality, such as IFN- γ , PD-L1 and GzmB. Interestingly, anti-PD-1 treatment also resulted in increased expression of several immuno-suppressive genes, such as IL10, Arg1, and most notably, IDO1. Furthermore, IDO1 expression was inversely correlated with tumor volume, suggesting IDO1 up-regulation is a counter-regulatory mechanism induced in the tumor and/or myeloid cells in response to enhanced IFN- γ production by anti-PD-1-treated tumor-infiltrating T-cells.

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Interestingly, this anti-PD-1 mediated IDO1 induction was dampened significantly by the addition of Imprime to anti-PD-1. Flow cytometry showed that Imprime treatment affected IDO expression in the Ly6C^{hi} monocytes and macrophages but not in tumor cells. In human whole blood, IFN- γ treatment increased the transcriptional level of IDO1 and the ratio of tryptophan to kynurenine, but Imprime treatment significantly inhibited this IFN- γ -induced IDO1 increase.

Conclusions

These results collectively demonstrate that Imprime treatment can enhance efficacy of anti-PD-1 treatment and may do so by restricting compensatory immuno-suppressive mechanisms mediated by myeloid cells.

Consent

N/A

Microbiome

P522

Clostridium species control primary liver cancer and liver metastasis via bile acids/CXCL16/CXCR6 mediated NKT cell immunity

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Background

Gut commensal bacteria have been described as important regulators of anti-tumor immunity. Primary liver tumors and liver metastasis represent the leading cause of cancer-related death [1]. The liver is exposed to gut bacteria, and gut sterilization has profound effects on hepatocellular carcinoma (HCC) development [2, 3]. However, the role of gut bacteria in anti-tumor surveillance in the liver is poorly understood.

Methods

Gut commensal bacteria were depleted by feeding mice with antibiotic cocktail in drinking water, or using Germ-free mice. Primary HCC was induced in liver-specific MYC transgenic mice. Liver metastasis was induced by intrasplenic injection of B16 melanoma tumors cells or *i.v.* injection of A20 lymphoma or EL4 thymoma tumor cells into C57BL/6 or BALB/c mice. Immune cell monitoring was performed by flow cytometry analysis. Stool bacteria was analyzed by 16S rRNA sequencing. Patient liver bile acids were measured by Metabolon's Discover HD4 Platform.

Results

Depleting gut commensal bacteria induced a liver-selective anti-tumor effect using both primary MYC-HCC model or A20 or EL4 liver metastasis models. An increase of hepatic CXCR6⁺ NKT cell number and function was observed, independent of mouse strain, gender or presence of liver tumors. *In vivo* functional studies confirmed that NKT cells mediated a tumor inhibition in the liver. Further investigation showed that NKT cell accumulation was regulated by CXCL16 expression of liver sinusoidal endothelial cells, which was controlled by *Clostridium* species-mediated primary-to-secondary bile acid conversion. Feeding mice with secondary bile acid w-MCA reversed both the NKT accumulation and the inhibition of liver tumor growth caused by depleting gut microbiome. In human livers, primary bile acid CDCA levels correlated with CXCL16 expression, and the opposite was found with the secondary bile acid glycolithocholate (GLCA).

Conclusions

Gut bacteria such as *Clostridium* species control liver anti-tumor immunosurveillance by altering bile acid composition, which regulates CXCL16 expression in LSECs, thus affects NKT cell level in the liver. This study shows that the gut microbiome utilizes bile acid metabolism to control anti-tumor immunity in the liver and opens novel opportunities to treat primary liver cancer as well as liver metastasis.

Late-Breaking Abstract Poster Presentations

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Oncogenetics and Immunogenomics

P523

Gene expression profiling of dermatologic toxicities from immune checkpoint therapy

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Background

Cancer patients receiving antibodies abrogating immune checkpoints antibodies may develop a diverse array of histologic skin reactions to these therapies that can include immunobullous, spongiotic, and lichenoid dermatitis (LD), and infrequently Steven Johnson Syndrome/Toxic Epidermal Necrolysis. The development of any type of these adverse immune-related cutaneous reactions may be sufficiently severe to warrant cessation of potentially efficacious treatment regimen. There is therefore a critical need to understand the pathogenesis of dermatologic toxicities in order to devise rational therapies to manage them more effectively.

Methods

Total RNA from formalin-fixed paraffin, embedded tissue from patients who developed LD skin toxicity

[n=3; mean age (range) = 60.7 years] while receiving immune checkpoint therapy (nivolumab =1, pembrolizumab = 1, nivolumab + ipilimumab = 1) for metastatic melanoma and from benign lichenoid keratosis (BLK) in control patients [n=3; mean age (range) = 48.7 (37-56) years] were profiled with the NanoString nCounter PanCancer Immune Profiling Panel interrogating the mRNA levels of 770 genes. Fold differences in mRNA transcript levels were compared between the two groups using two-sample tests and p-value < 0.05 were considered significant.

Results

Of the 770 genes, significant log fold difference in gene expression was observed between the two groups in 167 genes. Compared to the BLK control group (Figure 1), the LD skin toxicity group (Figure 2) showed down-regulation of 93 mRNAs (p < 0.05) and up-regulation of 74 mRNAs (p < 0.05). The ten most significantly down- and up-regulated transcripts in the LD skin toxicity group are listed (Table 1). Among the down-regulated genes are CCL27, CCL18, CD83, ILIRN, and IL2RA (Log₂ fold change range: -1.81 to -1.17; all p values < 0.05). Among the up-regulated genes were CD14, CXCL12, and CCL14 (Log₂ fold change range: 1.28-2.52, all p values < 0.04).

Conclusions

LD skin toxicity from immune checkpoint therapy exhibits an mRNA gene expression profile distinct from BLK. Despite showing histopathologically identical reaction patterns between the LD skin toxicity from immune checkpoint therapy and BLK, we observed differences in the mRNA transcript levels of 167 genes. Down-regulation of CCL27, CCL18, XCL2, CD83, ILIRN, and IL2RA genes implies dysregulation of normal homeostasis and immune regulation in the skin. Up-regulation of genes that encode chemotactic molecules (e.g. CXCL12 and CCL14) functions to recruit distinct subsets of skin immunocytes specific to immune checkpoint mediated LD and further suggest that abrogation of these signaling pathways may spare patients from developing this type of skin toxicity.

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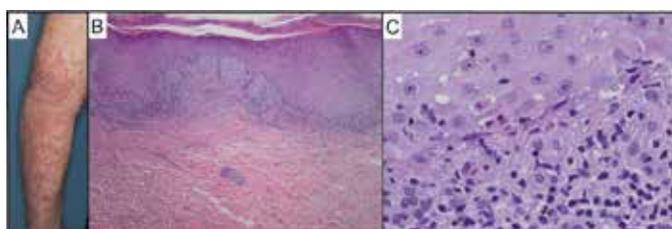
Table 1. Most highly down-regulated and up-regulated genes in LD skin toxicity from immune checkpoint therapy.

Gene	Log2 fold change	P-value	FDR
CCL27 (2-C motif ligand 27)	-2.21	<0.001	1
CDLY (translocin)	-2.06	<0.001	0.347
CCL18 (2-C motif ligand 18)	-1.55	<0.001	0.347
XCL2 (2-C motif ligand 2)	-1.49	<0.001	0.347
CD81	3.3	<0.001	0.456
CD101	3.27	<0.001	0.956
CD1C	-3.26	<0.001	0.621
KLDC2 (keratin-like receptor subfamily C member 2)	-3.24	<0.001	0.242
IL1RN (interleukin 1 receptor subunit 1)	-3.22	<0.001	0.061
IL2RA (interleukin 2 receptor alpha)	-3.17	<0.001	0.689
CXCL2 (C-X-C motif ligand 2)	2.52	<0.001	0.582
DMB1 (dimeric protein 1)	2.17	<0.001	1
CDP (chemokine factor D)	2.07	<0.001	0.621
EXL14 (2-C motif ligand 14)	1.98	<0.001	0.242
CTSG (cathepsin G)	1.32	>0.01	0.85
CD14	1.26	>0.01	1
PDGFB (platelet-derived growth factor beta)	1.26	<0.001	0.6
ESRA1 (elginate-1, mouse ortholog)	1.21	>0.02	1
IL13P1 (interleukin 13 receptor 1, accessory subunit 1)	1.26	<0.001	0.6
EXL15 (2-C motif ligand 15)	1.24	<0.001	0.242

Figure 1. Representative case of lichenoid dermatitis (LD) skin toxicity to immune checkpoint therapy.



Figure 2.



Consent
N/A

Oncolytic Viruses and Intratumoral Therapies

P524

Clinical and biomarker analyses of a phase II study of intratumoral tavokinogene telseplasmid (pIL-12) plus pembrolizumab in stage III/IV melanoma patients predicted to not respond to anti-PD-1

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Background

Melanoma patients with a low frequency of PD-1^{hi}CTLA-4^{hi}TIL are predicted to not respond to pembrolizumab, yet our previous interim analysis demonstrated that the combination of intratumoral (IT) plasmid IL-12 (tavokinogene telseplasmid; TAVO) and pembrolizumab yields robust clinical responses with an excellent safety profile. Updated clinical analyses (locked August 2017) from this multi-center, phase II, open-label trial including 2-year progression free survival (PFS) and DOR are presented. New biomarker data reveals coordinated anti-tumor immunological mechanisms in both the tumor microenvironment (TME) and in the peripheral blood.

Methods

Melanoma stage III/IV patients with a low CD8⁺TIL status (<25% PD-1^{hi}CTLA-4^{hi}) were treated with pembrolizumab (200mg every 3 weeks)

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concurrently with electroporation of IT-TAVO on days 1, 5 and 8 (6 week cycles). Tumor samples were profiled with multispectral immunohistochemistry (mIHC) and NanoString's Human Immunology and the PanCancer IO360 Beta Version panels. PBMC were analyzed for immune phenotype (flow cytometry) as well as NanoString's PanCancer Immune Profiling RNA and Protein panels.

Results

Progression free survival (PFS) rates for this treatment were 62% at 6 months and 57% at 18 months (median PFS not reached at 24 months) with a 48% BORR. DOR was not assessable as no responders have progressed and no safety signals were observed with only 2/22 grade 3 treatment-emergent adverse events. In responding patients, significant post-treatment increases were observed in both the Th1-associated gene expression of *STAT4* and *IL-12RB* in biopsies, and frequencies of CD8⁺PD-1⁺TIGIT⁺ and proliferating CD8⁺PD-1⁺ peripherally. Additionally, responding patients had a significant increase of TCR clonality in the tumors compared to PBMCs with a reversed relationship in non-responding patients. Spatial analysis by mIHC revealed a significant increase of both PD-L1⁺ and FoxP3⁺ cells <15um from CD8⁺ T cells in non-responders. Exploratory analysis with Nanostring's IO360 Beta Version panels highlighted underexpression of *WNT2B* and overexpression of *MICB* in the pretreatment responder biopsies.

Conclusions

Durable responses and favorable PFS rates in likely PD-1 non responders continues to suggest that combination IT-TAVO-EP with pembrolizumab is an effective therapeutic modality with an excellent safety profile. Associated biomarker data highlights connected immunological mechanisms, whereby intratumoral Th1-polarization, associated TCR clonality and limited suppressive cell types can drive robust anti-tumor responses (intratumoral and systemic) that positively impact this difficult to treat patient population.

Trial Registration

NCT02493361

Consent

N/A

Tumor Microenvironment (Mechanisms and Therapies)

P525

Exploring tumor microenvironment and human bone marrow stromal cells by single cell sequencing

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Background

Proinflammatory stimulation can lead to phenotype changes in marrow mesenchymal stromal cells (MSCs). We have been using MSCs to study the role of stromal cells in the tumor microenvironment. Our previous study showed that IFN- γ (Interferon Gamma) and TNF- α (Tumor Necrosis Factor Alpha) together result in the synergist uniform polarization of MSCs toward primarily Th1 phenotype, suggesting that tumor associated stromal cells may contribute to immune-mediated tumor killing. MSCs are heterogeneous and contain both stromal cells and skeletal stem cells that are responsible for osteogenesis. Prolonged passages of MSC can result in a change in phenotype associated with the loss of the skeletal stem cells. In this study, we used IFN- γ and TNF- α to stimulate different passages MSCs. We hypothesize that if stromal cells remain in late passage and if late passage Th1 polarization is similar to that of early passage, Th1 polarization is likely an intrinsic property of MSCs.

Methods

MSCs from bone marrow of one healthy donor were cultured and treated by IFN- γ (6.5ng/ml) and TNF- α

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(1.5ng/ml) at passages 2 (Bulk only), 3 (Single cell only), 4, 6, 8, 9 and 10(Bulk only) for 24 hours. RNA or cells were harvested from treated and control MSCs for mRNA Next Generation Sequencing (NGS) respectively.

Results

Control and stimulated MSCs NGS analysis found that the transcriptome of all passages of stimulated MSCs changed. Differences in the transcriptome of stimulated MSCs among different passages were observed, however, the expression by stimulated MSCs of important immune modulatory genes such as CXCL9, HLA-DRA, IL15 and IDO1 were up-regulated in all passages but their expression levels varied among passages.

Conclusions

Results show that after stimulation, different passages have similar but not identical gene expression changes. This suggests that immune modulation is an intrinsic property of MSCs, immune modulation may vary among MSCs types and some variability may exist among MSCs in different cancer types.

Consent

Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.



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