Computational Approaches to Target Identification for Cellular Therapy
Benjamin Vincent, MD
UNC Hematology | Microbiology & Immunology | Bioinformatics & Computational Biology | Computational Medicine
Cancer specific therapy is an obvious goal...

“We must learn to aim and to aim in a chemical sense.” - Paul Ehrlich (1854 - 1915)
Survival in the Age of Immunotherapy

Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma

Wolchok J et al. (2017) NEJM 377:1345

Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia

Park JH et al. (2018) NEJM 378:449
Of course, we don’t want to overpromise and give people, especially patients, false hope. But too many from my generation are afraid to be optimistic, too sheepish to ever use the word “cure.” But that’s what we want to do, cure our patients. We are, in fact, curing patients right now, more than ever, including those with metastatic cancer.
Thank you SITC

"Cure" T-Shirt

https://sitc.sitcancer.org/donate/
Two broad classes of cellular therapy

Endogenous T cells
- Select and expand to $10^{10}$ cells
- Assay for specific tumor recognition
- Culture with 6000 IU/mL IL-2

Engineered T cells
- Autoologous CAR T-Cell Therapy Process

https://www.lls.org/treatment/types-treatment
<table>
<thead>
<tr>
<th>Car-T</th>
<th>TCR-like CAR-T</th>
<th>Conventional CAR-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>T-cell receptor heterodimer</td>
<td>Single-chain variable fragment (scFv) from antibody</td>
</tr>
<tr>
<td>Target antigen</td>
<td>Peptide/MHC complex</td>
<td>Peptide/MHC complex</td>
</tr>
<tr>
<td>MHC restriction</td>
<td>Dependent</td>
<td>Dependent</td>
</tr>
<tr>
<td>Minimal number of antigen per cell</td>
<td>1</td>
<td>Not fully studied, but 100&lt;</td>
</tr>
<tr>
<td>Range of receptor affinity (Kd)</td>
<td>$10^4$ ~ $10^6$ M</td>
<td>$10^6$ ~ $10^8$ M</td>
</tr>
<tr>
<td>Costimulatory molecules</td>
<td>CD28, CD137</td>
<td>Linked directly to scFv (CD28 and/or CD137 in combination with CD3ζ)</td>
</tr>
</tbody>
</table>

**Coreceptors**
- CD4 for MHC-II, CD8 for MHC-I
- Unknown, some involvement of CD8 for MHC-I
- Not fully studied

**Serial killing function**
- Yes
- Yes
- Yes

**Administration**
- One infusion
- One infusion
- Once infusion

**Challenges**
- Cell manufacturing, competition to endogenous TCR
- Cell manufacturing
- Cell manufacturing

**Advantages**
- **TCR-T**
  - Target antigen
  - MHC restriction
  - Minimal number of antigen per cell
  - Range of receptor affinity
- **Car-T**
  - Costimulatory molecules

**References**
Need to solve three problems for curative immunotherapy

**TARGET ANTIGEN(S)**

- In vivo response monitoring
- Broadening antigen prediction
- Optimizing immunogenicity prediction

**CURE**

- Neoantigen Vaccines
- Vaccine &/or cell therapy?
- “Off-the-shelf” priming agent as induction?
- NeoAg-specific mAbs?

**IMMUNOSUPPRESSION REVERSAL**

- Checkpoint Blockade
- Discover resistance mechanisms
- Overcome resistance mechanisms

**THERAPEUTIC AGENT(S)**

Image credits: www.wikipedia.org
Impressive results with CD19-directed CAR-T therapy

Long-Term Follow-up of CD19 CAR Therapy in Acute Lymphoblastic Leukemia

D Overall Survival, According to MRD Status and Response

<table>
<thead>
<tr>
<th>No. at Risk</th>
<th>Months since T-Cell Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRD-negative complete response</td>
<td>32</td>
</tr>
<tr>
<td>MRD-positive complete response or no response</td>
<td>21</td>
</tr>
</tbody>
</table>

Park JH et al. (2018) NEJM 378:449
Finding good cell surface targets for CAR-T therapy

Potential cell surface target expression in AML

Potential cell surface target expression in AML
CAR-T target search in triple negative breast cancer

Unpublished data
CAR-T target search in triple negative breast cancer

Protein and RNA Expression in Normal Tissue for Two Source Genes

Target genes / proteins

Best Surface Expressed

Best Non Surface Expressed

Benchmarks

Normal Protein Tissues

Normal RNA Tissues

Unpublished data
Basic process for personalized neoantigen vaccine design

1. Tissue procurement
2. Antigenic target selection
   - DNA and RNA sequencing to identify tumor-specific mutations
   - HLA-typing
   - Prediction of personalized HLA-binding peptides
3. Preparation of personalized vaccine
   - Synthesis of mutated peptides
   - (1) NeoORFs
   - (2) Missense mutations
4. Therapeutic immunization
   - Combine mutated peptides with:
     - Strong adjuvant
     - Checkpoint-blockade inhibitor

TCR-dependent target identification empowers personalized neoantigen vaccines

**LETTER**

cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoeediting


**LETTER**

a dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells


**LETTER**

personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

Ugur Sahin, Evtynna Derhovanessian, Matthias Miller, Björn-Philipp Kloke, Petra Simon, Martin Löwer, Valesca Bukun, Arne Tiede, Ulrich Lünzenburger, Barbara Schreier, Tino Oesnoko, Matthias Vormehr, Christian Albrecht, Anna Parayandeh, Andor N. Kuhn, Anna Bück, Sandra Hesse, Katharina H. Schrickel, Felicitas Miller, Ingo Osterle, Isabel Vogler, Eva Godehnert, Sebastian Artigas, Richard Haie, Andrea Breitkreutz, Claudia Tollinger, Martin Sachau, Gerhard Mariot, Alexander Hohberger, Patrick Sorn, Jan Diekman, Janos Cziga, Olga Wackermann, Alexander Kramer-Bricke, Meike Witt, Martina Zellbro, Andre Hoffmann, Barbara Kasemann, David Lange, Stefanie Bolte, Matthias Hilsen, Sebastian Kröter, Bernhard Neumann, Christoph Gebhardt, Stephan Grubmüller, Christoph Höhler, Joachim Utkal, Christoph Huber, Carmen Logu, Ildikó Tóth

Sahin et al. (2017) Nature 547:222

**LETTER**

systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia

Mohini Rajasagi, Sachet A. Shukla, Edward F. Fritsch, Derin B. Keskin, Elisee Carmone, Wanli Zhang, Carine Sougnez, Kristian Cibulskis, John Sidney, Kristen Stevenson, Jerome Rizzi, Donna Neuberg, Vladimir Bratic, Stacey Gabriel, Eric S. Lander, Gad Getz, Nir Hacohen, and Catherine J. Wu

Rajasagi et al. (2014) Blood 124(3):453

**LETTER**

an immunogenic personal neoantigen vaccine for patients with melanoma


First open source neoantigen prediction workflows

**pVACtools**

1. Input samples
2. Somatic variant detection
3. Neocartogen identification and characterization
4. Epitope prediction
5. Peptide processing
6. Vaccine creation and delivery

**OpenVax**

1. Normal DNA Reads
2. Tumor DNA Reads
3. Alignment to Reference
4. Spliced Alignment
5. Post-Processing
6. Alignment to Reference
7. Post-Processing
8. Alignement
9. Class I MHC Alleles
10. Vaccine Peptide Selection

Richters M et al. (2019) Genome Medicine 11:56
Rubinstein A et al. (2018) Front Immunol 8:1807
Targeting mutant KRAS with tumor-infiltrating T cell therapy


Tran E et al (2016) NEJM
Targeting mutant KRAS with tumor-infiltrating T cell therapy

Tran E et al (2016) NEJM 375(23):2255
Targeting mutant KRAS with TCR-engineered T cell therapy

Leidner R et al (2022) NEJM 386(22):2112
Personalized TCR-T therapy (PACT Pharma)

Personalized TCR-T therapy (PACT Pharma)

Personalized TCR-T therapy (PACT Pharma)

## Personalized TCR-T therapy (PACT Pharma)


### Extended Data Table 3 | Patient and disease characteristics, adverse events and response assessment

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Patient ID</th>
<th>Age</th>
<th>Cancer</th>
<th># Prior regimens</th>
<th># TCRs</th>
<th>Conditioning regimen</th>
<th>Total NeoTCR+ cell dose</th>
<th>Any AEs ≥ grade 3 and SAEs</th>
<th>TCR-related AEs</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>DL 1</td>
<td>0010</td>
<td>38</td>
<td>HR+ Breast</td>
<td>7</td>
<td>3</td>
<td>Cy 300 mg/m² x3d Flu 30 mg/m² x3d</td>
<td>4 × 10⁶</td>
<td>G3 neutropenia</td>
<td></td>
<td>PD</td>
</tr>
<tr>
<td>DL 2</td>
<td>0050</td>
<td>70</td>
<td>Ovarian</td>
<td>6</td>
<td>3</td>
<td>Cy 300 mg/m² x3d Flu 30 mg/m² x3d</td>
<td>4 × 10⁶</td>
<td>G3 neutropenia</td>
<td></td>
<td>PD</td>
</tr>
<tr>
<td>DL 3</td>
<td>0030</td>
<td>45</td>
<td>HR+ Breast</td>
<td>5</td>
<td>3</td>
<td>Cy 600 mg/m² x3d Flu 30 mg/m² x3d</td>
<td>1.3 × 10⁴</td>
<td>G4 neutropenia</td>
<td></td>
<td>PD</td>
</tr>
<tr>
<td>NeoTCR-P1 + IL-2</td>
<td>0417</td>
<td>38</td>
<td>MSS-CRC</td>
<td>4</td>
<td>1</td>
<td>Cy 600 mg/m² x3d Flu 30 mg/m² x3d</td>
<td>5.4 × 10⁴</td>
<td>SAE: G4 Hypertension</td>
<td></td>
<td>PD</td>
</tr>
</tbody>
</table>

MSS-CRC: Microsatellite Stable Colorectal Cancer; HR: Hormone Receptor; G: Grade; SAE: Serious Adverse Event; CRS: Cytokine Release Syndrome; SD: Stable Disease; PD: Progressive Disease; Y/N: Yes/No.

No clinical responses

SD (target lesions ≤ 17%) for 4m
Challenge #1: Not all antigen-specific TCRs are good therapeutics
Challenge #2: Some tumors have relatively few neoantigens
Challenge #3: Shared somatic mutation-derived neoantigens are rare

Challenge #4: Most predicted neoantigens are not presented in vivo

Immunity

Atypical acute myeloid leukemia-specific transcripts generate shared and immunogenic MHC class-I-associated epitopes

Graphical Abstract

Authors
Grégory Ehx, Jean-David Larouche, Chantal Durette, ..., Sébastien Lemieux, Pierre Thibault, Claude Perreault

Correspondence
pierre.thibault@umontreal.ca (P.T.), claude.perreault@umontreal.ca (C.P.)

In brief
The lack of suitable targets is the main obstacle to immunotherapy of acute myeloid leukemia (AML). Ehx et al. reveal the structure and genomic origin of 58 AML-specific antigens. Epigenetic changes and intron retention are instrumental in the biogenesis of these antigens that represent attractive targets for AML immunotherapy.
Challenge #5: Most predicted neoantigens are not immunogenic \textit{in vivo}

**Bladder cancer**

- **mBLCA Neoantigen ELISpot Screen**
  - Neoantigen peptide
  - IFN-γ spots

**Breast cancer**

- **T11 C3TAg**
  - Neoantigen peptide
  - IFN-γ spots

**Melanoma (B16F10)**

- Neoantigen peptide
  - IFN-γ spots

**Myeloid leukemia (P815)**

- Neoantigen peptide
  - IFN-γ spots

---

Smith C et al. (2019) \textit{Cancer Immunology Research} 10:1591
Challenge #5: Most predicted neoantigens are not immunogenic \textit{in vivo}

- 25 teams
- Submissions ranged from 7 to 81,904 TSAs per tumor sample.

6\% (37) were immunogenic among the 608 highly ranked peptides selected and tested for immunogenicity.
Challenge #5: Most predicted neoantigens are not immunogenic in vivo

Improving neoantigen immunogenicity prediction

<table>
<thead>
<tr>
<th>Model</th>
<th>Haplotype</th>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16F10</td>
<td>b</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>BBN603</td>
<td>b</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>M849</td>
<td>b</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>UPPL1541</td>
<td>b</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>P815</td>
<td>d</td>
<td>96</td>
<td>N/A</td>
</tr>
<tr>
<td>T11</td>
<td>d</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

A: Table demonstrating various models and their associated haplotypes and class values.

B: Graphs and data visualizations showing various features and their impact on immunogenicity prediction.

C: Diagrams illustrating the process and steps involved in improving neoantigen immunogenicity prediction.

Smith C et al. (2019) Cancer Immunology Research 10:1591
Improving neoantigen immunogenicity prediction

C

BBN963

subcutaneous

1st vaccine
- 30 μg peptide
- 50 μg poly:C

D0

D12

2nd vaccine
- 30 μg peptide

Ulceration > 5 mm²

200 mm²

or

E

2nd vaccine
- 100 μg peptide
- 50 μg poly:C

Antigen-specific

cells

BALB/c donor

1st vaccine
- 100 μg peptide
- 50 μg poly:C

3 x 10⁶ T cells

3 x 10⁶ Bone marrow cells

3 x 10⁶ P815 tumor

Transplant

DBA/2 recipient

Death or paralysis

Transplant

D7

D0

D14

D21

3rd vaccine
- 100 μg peptide
- 50 μg poly:C

D

Percent survival

High immunogenicity (n = 30)

Low immunogenicity (n = 20)

No-peptide (n = 9)

0 20 40 60 80

Days after tumor injection

F

Percent survival

Bone marrow only (n = 5)

No-peptide (n = 6)

Low immunogenicity (n = 6)

High immunogenicity (n = 6)

0 20 40 60 80

Day post transplant
1. Identify neoantigen coding sequence variants
2. Predict MHC-binding neoantigen peptides
   - Confirm with mass spectrometry / immunopeptidomics
   - Understand mechanisms of peptide selection
3. Predict elicitation of neoantigen-specific T cells
   - Confirm with any T cell quantitation &/or functional assay
   - Understand mechanisms of antigen-specific T cell generation
4. Predict neoantigen-specific T cell capacity to kill cancer cells
   - Confirm with T cell cytotoxicity assay
   - Understand mechanisms of resistance to T cell cytotoxicity

Landscape of Effective Neoantigen Software (LENS)

LENS is modular, extensible, and reproducible through nextflow DSL2.

Sample Manifest (.txt)
DNA Normal FASTQs
DNA Tumor FASTQs
RNA Normal FASTQs
RNA Tumor FASTQs

Reference Genome (.fasta)

Gene Annotations (.gtf)

Read Trimming

Reference Indexing

Read Alignment
- BWA for DNA
- STAR for RNA

Transcript Quantification

RNA Alignments
DNA Alignments

MHC Typing

Alignment Sanitization
- InDel Realignment
- Mark Duplicates
- Base Quality Recalibration

Tumor Antigen Workflows

Gene Annotations (.gtf)

Sample Manifest (.txt)

Reference Genome (.fasta)

Read Trimming

Reference Indexing

Read Alignment
- BWA for DNA
- STAR for RNA

Transcript Quantification

RNA Alignments
DNA Alignments

MHC Typing

Alignment Sanitization
- InDel Realignment
- Mark Duplicates
- Base Quality Recalibration

Tumor Antigen Workflows
Phasing of multiple variants including germline variants

Vensko et al. (2023) Bioinformatics 39(6):btad322
Harmonized antigen coding transcript expression quantification

**SNV & InDel**
- Somatic variant detected in DNA
- Fetch all reads covering region of interest from RNA tumor BAM
- Filter to reads that are able to contain the entire peptide coding sequence
- Count reads that contain variant peptide coding sequence

**Splice Variant**
- Splice variant detected in RNA
- Fetch all reads covering region of interest from RNA tumor BAM
- Filter to reads that are able to contain the entire peptide coding sequence
- Count reads that contain splice peptide coding sequence

**Gene Fusion**
- Gene fusion detected in RNA
- Fetch all reads covering region of interest from RNA tumor BAM
- Filter to reads that cover fusion junction (shown) or map discordantly
- Count reads that contain fusion peptide coding sequence

**Virus, ERV & CTA**
- ERV, CTA, or self-antigen predicted targetable peptide discovered in RNA
- Fetch all reads covering region of interest from RNA tumor BAM
- Filter to reads that are able to contain the entire peptide coding sequence
- Count reads that contain ERV, CTA, or self-antigen peptide coding sequence

Vensko et al. (2023) *Bioinformatics* 39(6):btad322
Predicted tumor antigen features in Acute Myeloid Leukemia

Vensko et al. (2023) Bioinformatics 39(6):btad322
Long read sequencing for improved antigen detection
Long read sequencing for improved antigen detection

Unpublished data
Long read sequencing for improved antigen detection

Tracks (from the outermost band)
1. Cytobands
2. mRNA expression in TPM (log10)  
3. Inter-mutation (SNV) distance (log10)  
4. Sequencing depth  
5. Translocations

SBS Types
- C>A
- C>G
- C>T
- T>A
- T>C
- T>G

Translocations
- Long-read only
- Short and long read

Unpublished data
1. Identify neoantigen coding sequence variants

2. Predict MHC-binding neoantigen peptides
   - Confirm with mass spectrometry / immunopeptidomics
   - Understand mechanisms of peptide selection

3. Predict elicitation of neoantigen-specific T cells
   - Confirm with any T cell quantitation &/or functional assay
   - Understand mechanisms of antigen-specific T cell generation

4. Predict neoantigen-specific T cell capacity to kill cancer cells
   - Confirm with T cell cytotoxicity assay
   - Understand mechanisms of resistance to T cell cytotoxicity

Thank you!

Personalized Immunotherapy Research Lab (PIRL)

https://pirl.unc.edu/