Jensen

COI Disclosure:

- scientific co-founder of Juno Therapeutics, Inc. (JTI)
- equity holder in JTI
- inventor of IP licensed to JTI
- SAB/consultant to JTI
CD19-Specific CAR T Cells as a Post-Allo HSCT Relapse Salvage Therapy

Michael Jensen, MD
Sinegal Endowed Professor of Pediatrics, UWSOM
Director, Ben Towne Center for Childhood Cancer Research
# Pediatric Leukemia CD19CAR Adoptive Therapy Trials

<table>
<thead>
<tr>
<th>Site</th>
<th>Defined Cells</th>
<th>Vector</th>
<th>scFv</th>
<th>ECD Spacer</th>
<th>Co-stim</th>
<th>Selection/Suicide</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSKCC</td>
<td>No</td>
<td>Retro</td>
<td>SJ251</td>
<td>CD28partial</td>
<td>CD28</td>
<td>No/No</td>
</tr>
<tr>
<td>CHOP</td>
<td>No</td>
<td>Lenti</td>
<td>FMC63</td>
<td>CD8hinge</td>
<td>4-1BB</td>
<td>No/No</td>
</tr>
<tr>
<td>NCI</td>
<td>No</td>
<td>Retro</td>
<td>FMC63</td>
<td>Short</td>
<td>CD28</td>
<td>No/No</td>
</tr>
<tr>
<td>Baylor</td>
<td>EBV</td>
<td>Retro</td>
<td>FMC63</td>
<td>Full IgG1</td>
<td>CD28</td>
<td>No/No</td>
</tr>
<tr>
<td>SCRI</td>
<td>CD4:CD8</td>
<td>Lenti</td>
<td>FMC63</td>
<td>IgG4hinge</td>
<td>4-1BB</td>
<td>Yes/Yes</td>
</tr>
</tbody>
</table>
PLAT-02: A Phase 1/2 Trial of Defined Composition CD19CAR T Cell Adoptive Therapy For Refractory Relapsed and Post-HSCT Recurrent Pediatric ALL

<table>
<thead>
<tr>
<th>Protocol PI:</th>
<th>Rebecca Gardner, MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Site:</td>
<td>Seattle Children’s Hospital Phase I Program, Julie Park, MD</td>
</tr>
<tr>
<td>cGMP Facility:</td>
<td>Therapeutic Cell Production Core, Seattle Children’s Research Institute</td>
</tr>
<tr>
<td>IND Sponsor:</td>
<td>Program in Gene and Cell Therapy, SCRI</td>
</tr>
</tbody>
</table>
PLAT-02: Second Generation (4-1BB) CD19 Specific CAR-EGFRt Lentiviral Vector

**PLAT-02**

- EF1p
- Leader sequence
- VH
- linker
- VL
- CD19 scFv
- IgG1 murine monoclonal FMC63
- Spacer domain
- Signaling domain
- CD28tm
- 41BB
- CD3ζ
- T2AhuEGFRt
- Transduction marker
- 3’ LTR
- 2nd gen
Construction of truncated human EGFR (huEGFRt) with retention of Cetuximab binding epitope
Pediatric Leukemia Adoptive Therapy (PLAT-02) Trial

- Age 12mo-26yrs
- Refractory Dz (No Prior AlloHSCT)
- Post AlloHSCT Relapse (no active GVHD)
- ALC >100 (no in vitro lymphocyte prolif screening assay)

Phase I/2 Dose-escalation Safety Study

<table>
<thead>
<tr>
<th>Dose level</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>cells/kg</td>
<td>0.5x10^6</td>
<td>1.0x10^6</td>
<td>5.0x10^6</td>
<td>10.0x10^6</td>
</tr>
</tbody>
</table>
Creating a homogeneous product from a heterogeneous population of patients/T cells

<table>
<thead>
<tr>
<th>Age</th>
<th>12 y (1-25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>M:F</td>
</tr>
<tr>
<td>Disease Status</td>
<td>n=41</td>
</tr>
</tbody>
</table>

- Primary refractory
- 1st relapse
- 2nd relapse
- 3rd relapse
- 4th relapse

Prior Transplant | n=41
- No HSCT
- 1 HSCT
- 2 HSCT

Time from HSCT: 596 days (167-1853)

Status at time of infusion | n=37
- M1 MRD-
- M1 < 5%
- M2 5-25%
- M3 > 25%
T Cell Subset Set Repertoires of PLAT-02 Apheresis Products

**CD8 Memory Subsets**

**CD4 Memory Subsets**

HD = healthy donor (age 18-26)
PLAT-02 Defined Product Composition:

- PBMC
- Immunomagnetic Selection
- Lentivirus Transduction
- Expansion
- Formulation

FORMULATED CAR T CELL PRODUCT

100% CAR<sup>+</sup>CD8<sup>+</sup> 1 100% CAR<sup>+</sup>CD4<sup>+</sup> 1
MANUFACTURING CAR T CELL PRODUCTS OF DEFINED COMPOSITION:

**DAY 1**
- ACTIVATION OF T CELLS (ANTI-CD3/CD28 BEADS)

**LEUKAPHERESIS/PBMC ISOLATION**

**DAY 1**
- PURIFICATION OF CD4 and CD8 SUBSETS

**DAY 2**
- LENTIVIRUS TRANSDUCTION
- EXPANSION IN CYTOKINE COCKTAILS

**Mid-Process**
- Bead Removal/EGFRt Positive Selection

**DAY 14-21**
- CRYOPRESERVATION
Superior In Vivo Anti-tumor Activity of Defined Composition CD19CAR T Cell Products (1:1 CD4/CD8 Cell Dose, 100% CAR+)

(Compared to Undefined or Single Parameter Selected Products)
Defined Composition CAR T Cell Product
Uniformity Compared to Unformulated Products

- Percent of CD4+CD8- (of CD3)
- Percent of EGFRt+ cells

PLAT-01 and PLAT-02 comparisons.
Phenotype of Expanded Defined Composition CD19CAR T Cell Products At Time of Cryopreservation (Day 11-18):

CD4+

- CD19CAR/EGFRt+
  - CD4-EGFRt- 4.7% (97.5%)
  - CD4-EGFRt+ 0.7% (0.2%)
  - CD4+EGFRt+ 95.6% (54.4%)

CD62L/CD28+

- CD28-CD62L- 0.2% (44.7%)
- CD28-CD62L+ 0.7% (0.7%)
- CD28+CD62L- 24.3% (23.8%)
- CD28+CD62L+ 35.6% (23.8%)

CD27+

- CD27- 18.6% (97.0%)
- CD27+ 96.1%
Limited Product-Product Variability In Phenotypic Markers

CD4

- Percent of CD127
- Percent of CD45RA
- Percent of CD62L
- Percent of CD27

CD8

- Percent of CD127
- Percent of CD45RA
- Percent of CD62L
- Percent of CD27
PLAT-02 Product Manufacturing Stats:

• Intent to treat assessment
  - Overall Product Released: 40/41
  - Autologous Patients: 14/15
  - Post AlloHSCT Patients: 26/26
PLAT-02: Post-alloHSCT ALL Relapse/Pt Derived Donor Origin T Cells
CD4/CD8 1:1 AntiCD19CAR(4-1BBzeta)-EGFRt
Dose 250,000 cells/kg of CD4 product and CD8 product

Peripheral Blood Day +14:

CD3+CD8+

CD3+CD4+

DAY + 14 CSF:

CD3+CD8+ 78.8%

CD3+CD4+ 23.1%
PLAT-02 Product Safety Profile

• With Cy alone lymphodepletion
  MTD = Dose Level 2 ($10^6$ cells/kg)

• Flu/Cy MTD confirmation in process at DL2

• No toxic deaths
Intent to treat MRD-neg Remission

- Remission Criteria: MPF

Overall: 33/36 (91%)

Post AlloHSCT Patients: 24/26 (92%)
Overall survival: 6 months 85% (CI: 74%, 98%), 12 months 73% (59%, 91%).
Leukemia-free survival: 6 months 66% (CI: 52%, 84%), 12 months 50% (35%, 72%).
CAR T Engraftment Magnitude And Duration of Persistence Correlates With Patient’s CD19 Antigen Burden (Malignant + Nonmalignant B Cells in Marrow)

CAR+CD4+

CAR+CD8+
Marrow CD19 Antigen Load and Duration of B Cell Aplasia

The graph shows the persistence of CD19 aplasia (%) over months post T-Cell Infusion. The black line represents patients with >15% CD19 Load (n=21), and the red line represents patients with <15% CD19 Load (n=12). The p-value for the difference is 0.013.
Day 63 B Cell Aplasia and Risk of Leukemic Relapse

![Graph showing Leukemia-Free Survival over time](image)

- **B-cell aplasia persists at Day 63 (n=14)**
- **B-cell aplasia lost by Day 63 (n=13)**

As of 2016-02-01

Significance level: **p = 0.016**
Post alloHSCT Patients Similar Duration of Functional CAR T Cell Persistence Compared to Autologous Patients

No longer significant

[Graph showing persistence of CD19 Aplasia (%) over months post T-Cell Infusion]
Transplant Type vs D63 BCA and CAR T Cell Persistence
Post alloHSCT Patients Exhibit Superior Leukemia-Free Survival Compared to Autologous Patients

\[ p = 0.03 \]
De Novo Acute Skin GVHD post-CRS

Peripheral Blood:
70% of CD3$^+$ T cells are EGFRt$^+$

Skin Bx:
7% of CD3$^+$ T cells are EGFRt$^+$

CD3$^+$EGFRt$^-$ = RED
CD3$^+$EGFRt$^+$ = Yellow
CD3$^-$EGFRt$^+$ = Green
Strategies to Enhance CAR T Engraftment Magnitude and Duration (AUC):

1. Intensify lymphodepletion regimen (fludarabine + cytoxan)

2. Humanize CAR to diminish incidence of immune (cellular) CAR T rejection

3. Vaccine to reactivate memory CAR T cells for proliferation
PLAT-02: Post-alloHSCT ALL Relapse/Pt Derived Donor Origin T Cells
CD4/CD8 1:1 AntiCD19CAR(4-1BBzeta)-EGFRt
Dose 250,000 cells/kg of CD4 product and CD8 product

Peripheral Blood Day +14:

- CD3+CD8+
- CD3+CD4+

DAY + 14 CSF:

- CD3+CD8+ 78.8%
- CD3+CD4+ 23.1%
PLAT-03: PLAT-02 CAR T Cell Product + CD19t T-APC Vaccination

CAR T CELL PRODUCT

100% CAR⁺CD8⁺  100% CAR⁺CD4⁺

Autologous CD4⁺/CD8⁺ CD19⁺ T-APC’s

Polyclonal CD3⁺CD19⁺

CAR T Cells

B Cells

Months: 1 2 3 4 5 6
PLAT-02 Phase I Toxicity Assessment:

S8/Dz 75%/Toci
S14/Dz MRDneg/Supportive
S20/ Dz 21%/Supportive
S22/Dz 99%/Toci

S2/Dz .34%/Toci+Dex
S19/Dz 76%/Toci+Dex
S12/Dz neg/Dex
S13/Dz 8%/Supportive
M3 MARROW/CNS1
CY/FLU
5x10^6 CAR T CELLS/KG
TOCI 500mgx2 (63KG)
DEX 10mg IV THEN 5mg D2,3

DAY 21 MRD-NEG REMISSION
PERSISTENT B CELL APLASIA
ATTENUATED CRS <24HR SINGLE PRESSOR/NO NEUROTOX

M3 MARROW/CNS2
CY
1x10^6 CAR T CELLS/KG
TOCI 150mg (18.2KG)
DEX 5mg q12x2

DAY 21 MRD-NEG REMISSION
PERSISTENT BCA
MILD CRS/1 SZ WHEN KEPPRA STOPPED ON D14
Pre-Emptive Tocilizumab & Dexamethasone

- Tocilizumab for first fever (sustained)
- Dexamethasone 5-10 mg/day x 2 days for pts. at time of pressor therapy initiation

Flu/Cy Conditioning DL1 and DL2:

6/6 pts MRD-neg remission

0/6 pts Requiring Pressors for CRS

0/6 pts Exhibiting CNS symptoms
Rebecca Gardner, MD (PLAT-01/-02 PI)
Julie Park (ENCIT-01 PI)
Annette Kuenkele, MD (L1-CAM CAR)
Kaileen Rohr & Tracy Ooi (TamR-Tg)
Adam Johnson & Anne Silva, MD (spacer/bispecific)
Adam Johnson (Lead- TgX Project)
Jensen Lab
SCRI TCPC Staff
SCH Clinical Research Team

Paulina Paszkiewicz (Busch Lab)
(EGFRt/Erbitux ablation)

Xiuli Wang, MD,PhD
(human Tcm/EGFRt)
Stephen Forman, MD

Supported by:
RO1 CA136551
COH Lymphoma SPORE
Alex’s Lemonade Stand Fnd.
SU2C/St. Baldrick’s Dream Team
LSDF Opportunity Grant
Extra graphs
CD3 engraftment – all graphs
CD8 engraftment — all graphs

![Graphs showing CD8 engraftment](image)

- **Dose Level**
  - 1
  - 2
  - 3
  - 4

- **Disease Burden**
  - MRD−
  - MRD+
  - M2
  - M3

- **CD19 Load**
  - <5%
  - 5−25%
  - >25%
CD4 engraftment – all graphs

- **Mean CAR CD4 Proportion (%)**
  - Dose Level 1, 2, 3, 4
  - Days post T-cell Infusion 0, 20, 40, 60
  - p = 0.96

- **Disease Burden**
  - M3, M2, MRD+, MRD−
  - Disease Burden 0, 3, 6, 9, 0, 20, 40, 60
  - p = 0.458

- **Patient's Peak CAR CD4 Proportion (%)**
  - Dose Level 1, 2, 3, 4
  - Days post T-cell Infusion 0, 20, 40, 60
  - p = 0.828

- **Patient's Peak CAR CD4 Proportion (%)**
  - Disease Burden MRD−, MRD+, M2, M3
  - Days post T-cell Infusion 0, 20, 40, 60
  - p = 0.685

- **CD19 Load**
  - CD19 Load <5%, 5−25%, >25%
  - Days post T-cell Infusion 0, 20, 40, 60
  - p = 0.685

- **CD19 Load**
  - CD19 Load 0, 3, 6, 9, 0, 20, 40, 60
  - p = 0.065