Enhancing the IQ of CAR T Cells

Michael Jensen, MD
Sinegal Endowed Professor of Pediatrics, UWSOM
Director, Ben Towne Center for Childhood Cancer Research
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
CAR T Cell Therapy Version 1.1 - 
Empiric Designs, Trial and Error, Luck

Limited Engineering Of Optimized Binding Domain/Spacer Tuning

Vector Transduction Variable Efficiency, Expression Levels, Unregulated Constitutive Promoter

Variable Cell Product Composition/ Differentiation Status

Sadelain et al
Synthetic Biology:

- The re-design of existing, natural biological systems for useful purposes.

or

- The design and construction of new orthogonal biological parts, devices, and systems.  
  (from SyntheticBiology.org)
Synthetic Biology’s (Genetic) Engineering Approach -

Designing new molecular parts, device modules, circuits, and networks:

- modeling the designed systems & predicting their properties
- making & testing the designs
- updating our understanding from the model/test agreement
Vocabulary of Synthetic Biologists-

**PARTS**- DNA sequences encoding some component of the genetic machinery. (e.g. promoter, cDNA, riboswitch, transcription regulator, IRES, etc)

**DEVICES**- A group of parts that work together to perform a specific function. (exp. small molecule regulated promoter for controlled transgene expression)

**CHASSIS**- Organism (host) containing the device(s)

![Diagram of a TATA box and transcription factors regulating gene expression]

![Diagram of a transgene expression system controlled by Tet-On/Tet-Off system]
**Current State:** Polyclonal T Cells/
Constitutively Expressed CAR/No Suicide Mechanism
T Cells Fail to Engraft to Therapeutic Levels / Engrafted T Cells Fail To Retain Anti-tumor Functional Outputs
I. FORMULATING CAR T CELL PRODUCTS OF DEFINED COMPOSITION FOR IMPROVED REPRODUCIBLE ENGRAFTMENT, EFFICACY, AND SAFETY
PLAT-02 Defined Product Composition:

PBMC

Immunomagnetic Selection
Lentivirus Transduction
Expansion
Formulation

FORMULATED
CAR T CELL
PRODUCT

100% CAR⁺CD8⁺  100% CAR⁺CD4⁺
1 : 1
MANUFACTURING CAR T CELL PRODUCTS OF DEFINED COMPOSITION:

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

**DAY 2**
- PURIFICATION OF CD4 and CD8 SUBSETS
- LENTIVIRUS TRANSDUCTION
- EXPANSION IN CYTOKINES
- Mid-Process Bead Removal/EGFRt Positive Selection

**DAY 14-21**
- CRYOPRESERVATION
Defined Composition CAR T Cell Product Uniformity Compared to Unformulated Products

**Product CD4+**

![Graph showing the percentage of CD4+ cells for different products](image)

**Product EGFRt+ %**

![Graph showing the percentage of EGFRt+ cells for different products](image)

- PLAT-01: 13835
- PLAT-02: 14602
Phenotype of Expanded Defined Composition CD19CAR T Cell Products At Time of Cryopreservation (Day 11-18):
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)
CURRENT STATE: Version 2.0 (SCRI PLAT-02)

Parts List-
- EF1α-promoter
- T2A Linker
- FMC63 scFv
- 4-1BB
- CD3zeta

Devices-
- G3 SIN Lenti

Chassis-
- Defined combinations of T cell subsets

References:
- Wang X et al. J Immunother 2013

Jensen Lab
Advanced Lymphocyte Engineering
## 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></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
PLAT-02: Post alloHSCT Patient Profile

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Patient ID</th>
<th>Age (y)</th>
<th>Relapse#</th>
<th>Amount of disease in BM at enrollment by MPF</th>
<th>Current progress in study/off study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>14602-S01</td>
<td>21</td>
<td>2</td>
<td>90</td>
<td>In long term follow up</td>
</tr>
<tr>
<td>1A</td>
<td>14602-S02</td>
<td>22</td>
<td>2</td>
<td>0.4</td>
<td>In long term follow up</td>
</tr>
<tr>
<td>1A</td>
<td>14602-S03</td>
<td>21</td>
<td>2</td>
<td>90</td>
<td>In long term follow up</td>
</tr>
<tr>
<td>1A</td>
<td>14602-S04</td>
<td>11</td>
<td>2</td>
<td>0.04</td>
<td>In long term follow up</td>
</tr>
<tr>
<td>1A</td>
<td>14602-S05</td>
<td>19</td>
<td>2</td>
<td>2</td>
<td>In long term follow up</td>
</tr>
<tr>
<td>1A</td>
<td>14602-S06</td>
<td>4</td>
<td>2</td>
<td>54</td>
<td>D+42</td>
</tr>
<tr>
<td>1B</td>
<td>14602-S07</td>
<td>1</td>
<td>2</td>
<td>69</td>
<td>D+21</td>
</tr>
<tr>
<td>1B</td>
<td>14602-S08</td>
<td>23</td>
<td>1</td>
<td>98.6</td>
<td>D+7</td>
</tr>
<tr>
<td>1B</td>
<td>14602-S09</td>
<td>6</td>
<td>2</td>
<td>30</td>
<td>Pre T cell infusion</td>
</tr>
<tr>
<td>TBD</td>
<td>14602-S10</td>
<td>17</td>
<td>2</td>
<td>1.94</td>
<td>Pre T cell infusion</td>
</tr>
<tr>
<td>TBD</td>
<td>14602-S11</td>
<td>15</td>
<td>2</td>
<td>23</td>
<td>Pre T cell infusion</td>
</tr>
<tr>
<td>TBD</td>
<td>14602-S12</td>
<td>12</td>
<td>3</td>
<td>0.04</td>
<td>Pre T cell infusion</td>
</tr>
</tbody>
</table>

Update 10-18-2014: 15 pts enrolled, 11 pts infused
S09 and S10 Infused
S09 PR after first dose, received second dose, no response.
S10/S11 MRD- CR
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+

CD3-CD19- 2.92

CD3 96.9

CD19 FITC

B cells 0

CD3 PC5.5

EGFRt+

EGFRt- 77.2

EGFRt+ 22.8

CD45RO

CD62L+

CD45RO

CD62L+

EGFRt+

EGFRt+

78.8%

23.1%

Jensen Lab
Advanced Lymphocyte Engineering
PLAT-02: Day +7 Tumor Burden vs Response

Marrow Dz Burden

Response

Dose Level 3
(5.0x10^6/kg)

Dose Level 2
(1.0x10^6/kg)

Dose Level 1
(0.5x10^6/kg)

100%  75%  50%  25%

PD  PR  CR

Dex
PLAT-02: Remission Duration
(Intent to Treat/Post-HSCT Relapse)

Complete Response (MRD-neg by MPF)

- Dose Level 1 (0.5x10^6/kg): Ongoing
- Dose Level 2 (1.0x10^6/kg): Ongoing
- Dose Level 3 (5.0x10^6/kg): Ongoing

Relapse indicated at 4 months for Dose Level 1.
PLAT-02: Day +7 Tumor Burden vs Engraftment

Marrow Dz Burden

Peak EGFRt+ T Cells in PB (cells/μl)

- Dose Level 2 (1.0x10^6/kg)
  - 100%: 500
  - 75%: 512
  - 50%: 304
  - 25%: 353

- Dose Level 1 (0.5x10^6/kg)
  - <1 (Dex)
  - 1,288
  - 118
  - Pend.
  - 77
  - 63

100% 75% 50% 25%
PLAT-02: Magnitude and Duration of CAR/EGFRt⁺ T Cell Persistence
PLAT-02: Duration of B Cell Aplasia

Complete Response // B Cell Aplasia

Dose Level 3 (5.0x10^6/kg)
Dose Level 2 (1.0x10^6/kg)
Dose Level 1 (0.5x10^6/kg)

(Months) 1 2 3 4 5 6 7 8

Toci + Dex
Toci
Pred.
Summary I:

1. Feasible (100% based on intent to treat) to manufacture defined composition products.

2. Bioactive against ALL, high peak engraftment, duration of engraftment is heterogeneous.

3. Products have high frequencies of CD62L/CD28/CD27⁺ T Cells.
LIFE THREATENING TOXICITIES ARISING FROM UNREGULATED CAR T CELL FUNCTIONAL OUTPUTS
CURRENT STATE:  Version 2.0 (JCAR14, 17)

Parts List-
- EF1α-promoter
- T2A Linker
- 4-1BB
- CD3zeta

Devices-
- G3 SIN Lenti

Chassis-
- Defined combinations of T cell subsets
CAR T Cells Are Constitutively “ON”-

S02 CAR T Cell Engraftment -

Severe Sx’s/Pressors, Toci, Dex
II. CLINICAN CONTROLLED CAR T CELLS THROUGH REGULATED TRANSGENE EXPRESSION
The ideal system for regulating transgene expression in CAR T cells

- A clinically relevant transgene regulatory system should:
  - Demonstrate selective and specific regulation by ligand
  - Stringent OFF state *
  - High inducibility *
  - Non-immunogenic
  - Regulation by a safe, well tolerated ligand

- While other transcriptional regulatory system exits for transgene regulation, few possess sufficient number of these key attributes to permit clinical application
bioDEVICE ASSEMBLY: Transgene Expression Rheostats For Regulated TgX Expression:

TamR Transcriptional Regulatory System
TamR-Transcription Platform - *Parts I*

- Human Estrogen Receptor LBD Tuned For Tamoxifen Binding
- Human Hepatocyte Nuclear Factor-1α DNA Binding Domain
- Human RelA Transactivation Domain
- TamR-TF Responsive Synthetic Promoter
- 7X huAlb promoter HNF-1α Binding Motif
- Adenovirus E1b mp/TATA

Prototype described by Roscili et al., 2002
TamR Transcriptional Control System
TamR-Transcription Platform - LV Device

LV Transfer Plasmid #1

EF1ap → Tam-R-TF → EGFRt

LV Transfer Plasmid #2

7xHBD/mE1b → CD19CAR → Her2t

“Dual Packaged LV”

>15Kb Payload Capacity
TamR-Transcription Platform- *Parts II*

Cell Surface Barcoding Tags

![Diagram of cell surface barcoding tags](image-url)
TamR-LV Transcription Platform-
Performance in Primary Human T Cells
TamR-LV CAR Functional Outputs

“ON (+Tam)”

“OFF”

Raji

% Specific Lysis

Effector:Target Ratio

50 kDa

15 kDa

Parental Jurkat
Jurkat-h427
No 4OHT +4OHT
TamR-LV tf Tuning For High Versus Low Regulated Outputs

TamR-tf$^{\text{high}}$

TamR-tf$^{\text{low}}$

% of maximal ZsGreen induction

4-OHT nM

Jensen Lab
Advanced Lymphocyte Engineering
T Cell Activation Amplifies Tam-Dependent TamR LV Transgene Expression Outputs
T Cell Activation Amplifies Tam-Dependent CAR Expression

- CD4 Mock transduced
- CD4 + TamR CD19CAR + Vehicle
- CD4 + TamR CD19CAR + 4OHT

**HER2T**
TamR-LV Formats For Regulated Expression of TransgeneX by CAR T Cells
Summary II:

1. TamR-LV transgene expression regulation system displays favorable attributes for clinical application.

2. System has tunable features for output states (4-OHT sensitivity).

3. System exhibits context specific (T cell activation) positive feedback.
Cell Mass in Patient

Time

Selection of Antigen Loss Tumor Escape Variants
Aggregate data from CHOP, NCI, Seattle suggest CD19 epitope escape loss as etiology of treatment failure in approx. 10% of relapsing patients.
STRATEGIES TO GENERATE CAR T CELL PRODUCTS WITH 2X SPECIFICITIES

MIXING

- cDNA CAR A
- cDNA CAR B

CAR A Vector
CAR B Vector
CAR A Cell Product
CAR B Cell Product

ADDING

- cDNA CAR A
- cDNA CAR B

CAR A+B Vector
CAR A+B Cell Product

COMBINING

- cDNA CAR A/B

CAR A/B Vector
CAR A/B Cell Product
Dual CAR LV’s (“Adding”)

**CD19CAR/HER2t**

- 5’
- EF1p
- CD19CAR
- T2A
- HER2t
- Stop
- 3’

**CD20CAR/EGFRt**

- 5’
- EF1p
- CD20CAR
- T2A
- EGFRt
- Stop
- 3’

**EGFRt/HER2t Expression by Transduced Human CD8+ CTLs**

**Redirected CD19 and/or CD20 Cytolysis by Human CD8+ CTLs**

- Mock
- CD19CAR-Her2t
- CD20CAR-EGFRt
- CD19CAR-Her2t/CD20CAR-EGFRt
- CD19CAR-EGFRt

![Graphs showing redirected cytolyis](image)
BiSpecific CAR ("Combining")

Schematic of Bispecific antiCD19xCD20 Chimeric Antigen Receptor

Bispecific Anti-CD19xCD20 CAR Components:

CD19 RV_l → CD19R V, → Gly4Ser → CD20R V, → CD20R V, → IgG₄-CD28-Zeta

Complete cDNA packaged into epHIV-7 lentivirus vector transfer plasmid:
4-hr Chromium Release Assay: Anti-CD19xCD20 CAR+ T Cells Kill Both CD19+ and CD20+ Target Cells

**Effector T Cells:**
Anti-CD19xCD20 CAR+ EGFRt+

**Effector T Cells:**
Anti-CD19 CAR+EGFRt+
Anti-CD20 CAR+EGFRt+
CAR- (Mock Transduced)
Summary III:

1. Multiplexed antigen specificity is feasible and can be accomplished in a single LV vector.

2. Targeting 2 antigens on tumor cells expected to diminish antigen escape as etiology of treatment failure.
Cell Mass in Patient

Complications of Prolonged B Cell Aplasia & Chronic Activation of CAR+ T Cells
IV. Control of CAR T Cell Persistence
Construction of truncated human EGFR (huEGFRt) with retention of Cetuximab binding epitope
huEGFRt can be incorporated into lenti-viral vector for co-expression with CD19 chimeric antigen receptor (CD19CAR)
huEGFRt sensitizes huEGFRt+ human T cells to Cetuximab mediated ADCC

Targets: $^{51}$Cr-labeled huEGFRt+ T cells;

Effectors: GM-CSF stimulated huPBMC

Mixed with 1µg/mL Cetuximab or Rituximab (anti-CD20) for 4hr
**In vivo: Depletion of EGFRt+ cells**

Frequency of transferred cells in blood (FACS analysis)

24h post Erbitux

24h post Rituximab

<table>
<thead>
<tr>
<th>EGFR mAb + SA-PE</th>
<th>Number of tEGFR+ cells from spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.35</td>
<td>5.44e-3</td>
</tr>
<tr>
<td>9</td>
<td>4.93</td>
</tr>
</tbody>
</table>

- **Erbitux**
  - 0.0
  - 0.5
  - 1.0
  - 1.5

- **Rituximab**
  - 1.0
  - 1.5

- **control**
  - 0.0
  - 0.5
  - 1.0
  - 1.5
**Summary IV:**

1. EGFRt can serve as a suicide construct based on in vitro and murine models.

2. The efficacy of cetuximab mediated ablation of EGFRt$^+$ T cells in humans is unknown.
SynBio T Cells FUTURE STATE: Version 3.0

Parts List-

Devices- Expression Rheostats, Sensors, Logic Gated Bio-Circuits

Chassis- Defined combinations of T cell subsets
**Future State:** Defined Cellular Composition/Multiplexed Antigen Recognition/Clinician Controlled Regulation/Effective suicide ablation

![Graph showing cell mass over time with different categories and regulatory mechanisms.](image-url)
Rebecca Gardner, MD (PLAT-01/-02 PI)
Julie Park, MD (ENCIT-01 PI)
Annette Kuenkele, MD (L1-CAM CAR)
Kaileen Rohr (TamR-Tg)
Anne Silva, MD (spacer/bispecific)
Cindy Chang (mouse models)

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

Xiuli Wang, MD, PhD
(human Tcm/EGFRt)
Hao Hong PhD L1CAM
Stephen Forman, MD

Supported by:
RO1 CA136551
COH Lymphoma SPORE
SU2C/St. Baldrick’s Dream Team
LSDF Opportunity Grant