Precision CAR T Cell Therapeutics

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I have the following financial relationships to disclose:
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Stockholder in: Tmunity Therapeutics, Inc.
Honoraria from:
Employee of:

- and -

I will discuss investigational use in my presentation: CTL019
CAR T Cell Overview

- Autologous T cells
- Allo 3rd party T cells
  - Cord blood
  - Healthy donor
  - iPSC
Using Synthetic Biology to Overcome Tolerance
Creation of Bi-specific CAR T cells

First Generation CD4 / CD8z CARs

First Generation scFv CARs

Second Generation scFv CD28z CARs

Second Generation scFv BBz CARs

Second Generation scFv CD27z CARs

Extracellular

Intracellular

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axicabtagene ciloleucel
tisagen lecleucel

Irving & Weiss, 1991
Letourneur, 1991
Romeo, 1991

Kuwana, 1987
Eshhar, 1993

Roberts, 1995
Finney, 1998
Maher, 2002

Finney, 2003
Imai, 2004
Milone, 2009
Carpenito, 2009

Song, 2012
Guedan, 2014
Duong, 2013

Design of CAR T Cells
Design Features of tisagenlecleucel and axicabtagene ciloleucel: Differential Persistence

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Tisagen lecleucel</th>
<th>Axicabtagene ciloleucel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector</td>
<td>Lentiviral</td>
<td>Retroviral</td>
</tr>
<tr>
<td>Promoter</td>
<td>EF1α</td>
<td>MSCV</td>
</tr>
<tr>
<td>ScFv (CD19)</td>
<td>FMC63</td>
<td>FMC63</td>
</tr>
<tr>
<td>Signaling Domain</td>
<td>4-1BB zeta</td>
<td>CD28 zeta</td>
</tr>
<tr>
<td>Hinge and TM</td>
<td>CD8α</td>
<td>CD28</td>
</tr>
<tr>
<td>Cell Culture</td>
<td>Frozen CD3/28 beads</td>
<td>Fresh PBMC/CD3</td>
</tr>
<tr>
<td>CAR T Persistence</td>
<td>Long term &gt; 1-7 years</td>
<td>Short term (&lt; 6 weeks)</td>
</tr>
</tbody>
</table>

The diagrams illustrate the CD28 and 4-1BB signaling domains for each CAR construct.
CT019 (tisagenlecleucel): 

Patient Donates Cells

Genetic engineering

Expand T cells

T cell transfusion

Synthetic Biology

Cytokine Release Syndrome

Pediatric Oncology: ALL

Adult Oncology: ALL, CLL, DLBCL, MCL, Myeloma

July 31, 2010
1st CART19 Infusion

Porter, 2011
Grupp, 2013
Maude, 2014
Garfall, 2015
Schuster, 2017
Maude, 2018

FDA approval August 30, 2017
Long Term Persistence of CTL019 in CLL patients

Note: For Flow $0 = 0.01$; qPCR $0 = 1$

1. 7 year survival and function of CTL019 in patients 1 and 2
2. First “living drug”

Porter, Fraietta, Melenhorst, Nat Med 2018
Characterization of Long Term CAR T Cells in CLL Patients

• Transcriptomic profiling revealed that CAR T-cells from complete responding CLL patients were enriched in memory-related genes, including IL-6/STAT3 signature

• CAR T-cells from non-responders upregulated programs involved in effector differentiation, glycolysis, exhaustion, and apoptosis.

• Sustained remission was associated with an elevated frequency of CD27+CD45RO- CD8+ T-cells in baseline blood samples

• CD27+PD-1- CD8+ CAR T-cells expressing high-levels of IL-6R predicts therapeutic response and is responsible for tumor control.

Nature Medicine, 2018
Lessons
1. You can learn a lot from one patient!
2. Cute kids attract media attention…
Subject #1: 7yoF pre-B ALL. Karyotype: high risk
Dx May 2010: standard COG ALL induction
Relapse #1: 10/2011
Relapse #2: 2/2012
3/2012: high dose cytoxan/clofaribine: persistent ALL
Marrow 4/16/2012: 60% blasts w/kidney, liver, spleen lesions
Autologous CART19 4/17/2012
Total dose CART19: 1.2 x10^7 CAR cells/Kg
CAR T cells infused with no additional chemotherapy
First Pediatric ALL patient

- Deep remission induced in 23 days
- 0% blasts seen
- Flow MRD negative
- CR maintained >5yrs

Grupp et al, NEJM 2013
Pediatric CART19 ALL Study
High Response Rate (92%, n=12)

Duration of Response (as of 18 May 2013)

- ORR: 11/12 (92%)
- CR: 10/12 (84%)
- PR: 1/12 (8%)
- NR: 1/12 (8%)

Stephan Grupp, NEJM 2013
Tocilizumab

- Interleukin 6 (IL-6) Receptor antagonist
- Blocks IL-6 mediated effects
- Indicated in:
  - Juvenile idiopathic arthritis (JIA)
  - Rheumatoid arthritis (RA)
  - In Japan, indication for Castleman’s Disease
- Typically given monthly
- Rare side effects of transaminitis and neutropenia
- Co-labeled with CD19 CAR T
Efficient Trafficking of CTL019 T Cells to CNS in ALL

Blood

- CHOP-100
- CHOP-101
- 1% marking

Days after Infusion
Copies/µg gDNA

Blood Day 10

CSF

Days after Infusion
Copies/µg gDNA

CSF Day 23

Stephan Grupp, NEJM 2013
Cellular Kinetics of tisagenlecleucel

- CTL019 transgene levels were observed to undergo significant expansion and demonstrated measurable persistence in vivo >1 year

- Persistence and expansion similar to ALL and CLL patients

\[a\] Includes all patients with \(\geq 1\) quantifiable transgene level post infusion (\(N = 83\)).
## Modelling the PK of a “Living Drug”

<table>
<thead>
<tr>
<th>Property</th>
<th>Small Molecule</th>
<th>CAR-T Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ability to proliferate</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Reason for α and β phase</td>
<td>Distribution and elimination</td>
<td>Contraction and persistence</td>
</tr>
<tr>
<td>Terminal half-life time scale</td>
<td>Hours, days, or weeks</td>
<td>Years</td>
</tr>
<tr>
<td>Applicability of clearance and volume of distribution</td>
<td>Applicable</td>
<td>Not applicable due to the ability of CAR-T cells to proliferate</td>
</tr>
<tr>
<td>Variability in product from patient to patient</td>
<td>None</td>
<td>Variability exists due to variability in patient immune systems</td>
</tr>
<tr>
<td>Clear relationship between dose and exposure</td>
<td>Yes, although it may be nonlinear</td>
<td>No relationship between dose and exposure detected</td>
</tr>
<tr>
<td>Kinetic equation following small molecule oral dose or intravenous CAR T cell dose</td>
<td>$A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t) - (A + B) \cdot \exp(-k_{at})$</td>
<td>$f(t) = \begin{cases} R_0 e^{\rho t} &amp; , t &lt; T_{\text{max}} \ A e^{-\alpha(t-T_{\text{max}})} + B e^{-\beta(t-T_{\text{max}})} &amp; , t \geq T_{\text{max}} \end{cases}$</td>
</tr>
<tr>
<td>Sign of exponent for initial increase after dosing</td>
<td>Negative ($-k_a$)</td>
<td>Positive ($+\rho$)</td>
</tr>
</tbody>
</table>
Modeling the Cellular Kinetics of tisagenlecleucel

- Model adapted from DeBoer and Perelson work on LCMB and Listeria infections
- \( \alpha \) slope corresponds to a rapid contraction due to AICD of CAR T effector and the \( \beta \) slope corresponds to a gradual decrease of memory CAR T

Andrew Stein, unpublished
• Blood samples from 90 patients with r/r ALL analyzed

• For tisagenlecleucel, the initial doubling time ($\ln 2/\rho$) was 0.78 days, the half-life for the initial rate of decline ($\ln 2/\alpha$) was 4.3 days, and the terminal half-life ($\ln 2/\beta$) was 220 days

• Unlike a traditional drug, no relationship was detected between the dose of tisagenlecleucel and $C_{\text{max}}$ or any other model parameter

• $C_{\text{max}}$ was associated with more severe CRS

Andrew Stein, Karen Thudium et al, unpublished
Long term persistence and expression of CTL019 is associated with durable remission in leukemia: Predictive Biomarker CAR T Persistence for first year after infusion

Delayed Tumor Lysis Syndrome in Pt #10

- Presented with fevers 45 days after infusion
- Day 50 definitive evidence of TLS and MAS
  - Fevers, hypoxia (intubated), hypotensive (pressors)
  - Treated with tocilizumab
  - Expanding CART19 cells became detectable
Rapid massive expansion of clonal CART cell population in patient #10

Fraietta, Nature in press 2018
TET Proteins

- Ten-eleven translocation (TET) proteins were first discovered to convert 5mC to 5hmC.
- All TET enzymes contain a C-terminal catalytic domain (CD) that belongs to the dioxygenase superfamily and oxidizes 5mC in a 2-oxoglutarate- (2-OG) and Fe(II)-dependent manner.
- TET2 mutations frequently occur in hematological malignancies, including myeloid malignancies, T cell lymphomas and adult T cell leukemia.
- TET2 mutation not sufficient for transformation.
- TET2 LOF mutations frequent in clonal hematopoiesis.
Lessons Learned from Tet2 Disruption in CLL Patient #10

- Progeny derived from a single CTL019 TCRVβ5.1+ CD8+ T cell were responsible for the eradication of massive tumor burden in patient #10.
- Can the lowest effective dose of CAR T be a single cell?
- Since Tet2 can increase HSC stem cell renewal, would inhibition or intentional disruption of Tet2 increase CAR T cell proliferation and/or function?

WILL CAR T CELLS HAVE A ROLE IN SOLID TUMORS?

**CAR**

1st
- CD3ζ

2nd
- CD28 or 4-1BB
- CD3ζ

3rd
- CD28 or OX-40
- CD3ζ

4th
- 4-1BB
- NFAT

**Armored CAR**

- Additional Features
- IL-12
- ScFv
- IL-12
- IL-18

Cyotoxicity
Proliferation
Persistence

Marco Ruella
Prostate cancer is the most common cancer in men
PSMA protein is expressed on cell membrane and functions in glutamate and folate metabolism, releasing N-acetylaspartate and glutamate
PSMA expression increases with advancing tumor grade and stage
TGFβ is an immune checkpoint
  highly-expressed in prostate tumor microenvironment
  Negative regulator of T cell proliferation, effector T cell function
TGFβ in prostate tumors likely reduces anti-tumor activity of PSMA CAR T cells

PSMA dnTGFβRII Armored CAR T: Design

pTRPE-dnTGFβRII-T2A-anti-PSMA-BBz

Plasmid map

Specific lysis prostate tumor cells Expressing PSMA

Chris Kloss and “Sloan”

Plasmid map

Effector:Target Ratio

Mock

Pbbz

dnTGFβRII-T2A-Pbbz

Effector:Target Ratio
PSMA-TGFB-RDN CAR T Cells Are Resistant to Exhaustion

- PSMA CAR T cells expressing TGFB-RDN have sustained proliferation in presence of chronic antigen exposure
- PSMA TGFB-RDN CAR T are resistant to exhaustion
- Proliferation of CAR T cells is the best surrogate biomarker of clinical activity

Christopher Kloss and Jihyun Lee, Mol Therapy 2018

Transfer 1e6 T cells to 0.2e6 IrrPC3-PSMA
Clinical Trial #NCT03089203
Confirm metastatic castrate resistant prostate cancer and tumor PSMA expression

Screening
Apheresis (-3-4 weeks)

T cell manufacture
Disease staging

Cohort 1*

Cohort 2

Cohort 3

Day -3 CTX 1.0g/m²

*Enrollment will follow in succession from Cohort 1 to Cohort 3

CAR T cell Infusion
1-3x10⁷/m² Day 0

CAR T cell Infusion
1-3x10⁸/m² Day 0

CAR T cell Infusion
MTD Day 0

Naomi Haas, PI
Vivek Narayan, Co-I
Superior antitumor efficacy in NSG mice with disseminated adenocarcinoma

Substantial proliferative advantage in vitro and in vivo in presence of chronic surrogate antigen PSMA

Network analysis: Th1 and Th2 genes; transcription factors and cell cycle genes

Resistance to exhaustion

Phase I clinical trial open and accruing cohort 2

#NCT03089203

Detection of CART-PSMA-TGFβRII dn by qPCR in peripheral blood of prostate cancer subjects.

Cohort 1
Health Care Challenges
Engineered T Cell Therapies

Issues
- Patient specific “n of 1”
- Blood bank model?
- Central manufacturing?

Chris Mason et al, Regen Med. 2011
Levine and June, Nature. 2013
Combinatorial Cancer Immunotherapies: Many possibilities

- Vaccines
- Oncolytic viruses
- CAR T Cell Based Therapies
- Antibodies
- Checkpoints, ADC, etc
- Targeted Small Molecule Drugs
- Cytokines
- Chemotherapy
- Ionizing Radiation