Transitioning Engineered T Cells From Discovery to Manufacturing to Regulatory Approval

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Conflicts of Interest Statement

- Declaration of financial interest due to intellectual property and patents in the field of cell and gene therapy.
- Univ. Penn. Alliance with Novartis
- Consultant for GE Healthcare
- Consultant for Brammer Bio
- Founder Tmunity Therapeutics
- Conflict of interest is managed in accordance with University of Pennsylvania policy and oversight
The Fall of a Wall, Navy Research Priorities, and HIV Led to the First Cellular Gene Therapy BLA Accepted by the FDA
Goal: Improved T Cell Culture System for Adoptive Immunotherapy
Result: 10-100X Improved Growth and Function

J Immunol 1997; 159: 5921
Science 1997; 276: 273
Mol. Ther. 2004; 9; 902
Exp. Opin. Biol. Ther. 2008; 8: 475
T Cells Embrace Enhanced Stimulation
If we could design T cells for the treatment of disease, they would be:

• Be Numerous (Effector to Target Ratio)
• Potent
• Be Persistent
• Have a Good Memory

But, in HIV and Cancer →
The Chimera + The Trojan Horse = The CAR T

- T cell
- CTL019 cell
- Native TCR
- Anti-Tumor Ag CAR construct
- Tumor Antigen
- Dead tumor cell
- Tumor cell

- Ligand binding domain
- Signalling domains
  - CD16
  - CD27
  - CD28
  - ICOS
- 4-1BB
- OX40

CAR
First CAR Clinical Trial (Was in HIV!)

Persistence of CD4z-modified CD8 CAR T Cells in Blood

Anti-CD3/IL-2 stimulation and selection

PBMCs

• Purification
  – α-CD8 MAb

CD8⁺ T cells

• Stimulation (OKT3 + IL2)
  • retroviral transduction: CD4ζ

CD4ζ CD8⁺ T cells

• Purification
  – α-CD4 MAb: CD4ζ

Expansion in vitro 30 - 60 days

• High dose IL2

Infusion

Walker et al. Blood 2000

Long-term in vivo survival of receptor-modified syngeneic T cells in patients with human immunodeficiency virus infection


BLOOD, 15 JULY 2000 VOLUME 96, NUMBER 2
Improved Persistence of CD4z-modified CD8 CAR T Cells in Blood
Second Generation Trial Manufacturing

Prolonged survival and tissue trafficking following adoptive transfer of CD4\(\zeta\) gene-modified autologous CD4\(^+\) and CD8\(^+\) T cells in human immunodeficiency virus–infected subjects

Ronald T. Mitsuyasu, Peter A. Anton, Steven G. Deeks, David T. Scadden, Elizabeth Connick, Matthew T. Downs, Andreas Bakker, Margo R. Roberts, Carl H. June, Sayeh Jalali, Andy A. Lin, Rukmini Pennathur-Oas, and Kristen M. Hegge

BLOOD, 1 AUGUST 2000 • VOLUME 96, NUMBER 3
Translation of Targeted Gene Editing to Humans (circa 2004)
What are Zinc Finger Proteins?
-specific DNA binding proteins, e.g. transcription factors and other regulatory proteins

Dale Ando, MD

Why Target CCR5 in HIV?

• HIV (R5 virus) targets CD4 T-cells by binding to CCR5, one of the major co-receptors for HIV entry
• CCR5 delta-32 mutation produces a nonfunctional protein
  • Homozygotes are resistant to HIV infection
  • Heterozygotes have slower disease progression

Scientific American, March 2012
What unique challenges have you faced as you prepared your product for clinical application?

- Research Lab Process 1: electroporation of plasmid DNA
  - feasible but toxic, low cell yield, viability
- Research Lab Process 2: adenovirus transduction
  - very efficient in research media, bombs in clinical media
- Clinical Process 1: serum free during adenovirus transduction
  - very efficient

Follow Safety, CD4/HIV, CCR5 selection

Clinicaltrials.gov NCT00842634

Maier et al., Hum Gene Ther 2013: 24(3):245
• Engraft, expand, and persist (>1 yr) in circulation
• CCR5-modified CD4 T cells detected in gut mucosa, demonstrating homing and persistence
• Survival advantage of CCR5-modified cells during ARV treatment interruption
2004- present: Clinical Translation of HIV CAR’s in 1990’s Informs Cancer CAR’s

2010 CLL trial
Advanced, chemotherapy-resistant CLL, 2 of 3 patients had p53-deficient CLL
1 Partial & 2 Complete Responses in Relapsed/Refractory CLL


(Baseline total body tumor burden in kg)
Dismal Outcomes for 2\textsuperscript{nd} Relapse \textit{ALL}

Resimuller et al. JPHO 2013
CART19 in Ped ALL: Response similar at high and low disease burdens

Patient population

- ≥ 2\textsuperscript{nd} relapse or refractory
- Majority refractory to multiple prior therapies

* <0.01% MRD by flow cytometry
** \( \frac{1}{3} \) CD19+, \( \frac{2}{3} \) CD19-

MRD- by 3 months without further therapy

Abbreviations: BM, bone marrow; CR, complete response, MRD, minimal residual disease; NR, no response
Successful Technology Transfer of CAR T Cell Processing from Academia to Industry Enabled Scale-Up to Support Global Clinical Trials

- Further enhancement in control and consistency of the process
  - Closing of process steps
  - Some manual processes replaced with automation solutions

- Analytics - validation and implementation of more robust and/or faster methods
  - New quantitation method for expression of CTL019 transgene
  - Rapid mycoplasma testing
Final product control through Analytical Specifications

Product Release Tests

- **Identity**
  - Appearance
  - Vector integration

- **Purity**
  - %T Cells (Flow)
  - Cell viability
  - Transduction efficiency

- **Impurities**
  - Residual beads
  - B cells
  - Vector residuals

- **Potency**
  - CAR expression
  - Cytokine production

- **Safety**
  - Sterility
  - Endotoxin
  - Mycoplasma
  - RCL / vector residuals

Release specifications (and in-process controls) ensure:

- ✔ Consistency of manufacturing
- ✔ Adherence to Health Authority requirements
- ✔ Appropriate safety profile
- ✔ Desired product CQAs
What challenges do you face when defining source cell populations?

High variability of (patient derived) raw material

Apheresis products that resulted in failed MFG runs due to poor growth had significantly lower %CD3/45+ (via FACS) and %LY (via Multisizer) and significantly higher %MO (via Multisizer).

Solution: Conditional manufacturing pathways
Consistent CTL019 T-cell product from individual patient material

- T cells
- NK cells
- Monocytes
- B cells

Incoming leukapheresis material

Patient 1
Patient 2
Patient 3
Patient 4

Transduced viable T cell product (CTL019) ~97% T cells

Patient 1
Patient 2
Patient 3
Patient 4
Determine Efficacy and Safety of CTL019 in Pediatric Patients with Relapsed and Refractory B-cell ALL (ELIANA)

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<td>Australia</td>
<td>Royal Childrens Hospital VIC</td>
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• Scheduling
• Collection
• Shipping – cold chain management
• Manufacturing – supply chain management
• Testing
• Shipping – cold chain management
• Administration
ELIANA: CTL019 Phase II global trial in Ped ALL

Overall remission rate (CR+CRi) within 3 mos 82 (41/50)

Best overall response (BOR)
CR 68
CRi 14

- Novartis announced PedALL BLA accepted by FDA 3/29/17
- Granted Priority Review (FDA goal – 6 months)
- FDA ODAC Ad Comm 7/12/17
- PDUFA Date – not yet announced
- Filings for DLBCL in US and EU later in 2017

6-month OS\(^{^}\)
89% (95% CI: 76%, 95%)

Overall Survival (%)

Kaplan-Meier medians
All patients: NE months, 95% CI [8.6, NE]

Patients still at risk
62 57 50 44 34 29 23 17 16 11 8 6 4 2 1 0

Censoring Times\(^{+}\)
All Patients (N=62)

Number of Events (n=9)

\(^{^}\)Full analysis set
All patients infused with CTL019 were included. Time is relative to CTL019 infusion
Humanized CART19 (CTL119) Response Rate

CAR-naïve cohort: 22/22 CR (100%)

Relapse-free Survival

- 6-mo RFS: 86% (63,95)
- 12-mo RFS: 86% (63,95)
- Median f/u: 10 mo

Presented at ISCT 2017
http://dx.doi.org/10.1016/j.jcyt.2017.02.011

Shannon Maude
Stephan Grupp
US sites
• Emory Winship Cancer Institute
• University of Chicago
• University of Kansas
• University of Michigan
• University of Minnesota
• Duke University
• Ohio State James Cancer Hospital
• Oregon Health Sciences University
• MD Anderson Cancer Center

Ex- US
(Canada, Japan, EU)
• Montreal
• Sapporo
• Oslo

Clinicaltrials.gov NCT02445248
Single Arm, Open-Label, Multi-Center, Phase II Study of Efficacy and Safety of CTL019 in Adult DLBCL Patients (JULIET)

- 27 sites in 10 countries across North America, Europe, Australia, and Asia

### Primary Endpoint Was Met

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<th>Response Rate</th>
<th>Patients (N = 51)</th>
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<td>Best overall response (CR + PR)</td>
<td>59%</td>
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<td>CR</td>
<td>43%</td>
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<td>PR</td>
<td>16%</td>
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<td>SD</td>
<td>12%</td>
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<td>PD</td>
<td>24%</td>
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\(^{a}\) Probability = \(P < 0.001\)^
\(^{b}\) (95% CI, 44-72)

Duration of Response:
79% Relapse-free at 6 Months

- All responses at 3 months were ongoing at the time of cut-off
  - No responding patients went on to SCT
- Median DOR and OS not reached
(Some) Lessons Learned in Developing First in Human Clinical Trials

- Cells from healthy humans are not the same as cells from patients with advanced disease
- Demand your research laboratories begin working with clinical grade materials and methods early
- Currently, variable cell material requires human judgement and intervention
- More human intervention = opportunity for deviations = more training required
- A few very well studied patients can radically accelerate clinical development
- An academic scientist can learn to speak Novartian
(Some) Critical Path Issues for Commercialization and Wider Patient Access

- Securing Supply Chain – hundreds of complex components
  - Serum free media with comparable growth/potency
- Reducing COGS and Labor
  - Viral vector → electroporation/nanoparticle delivery
- Increasing Consistency, Comparability, managing challenging cases
- Rapid and Modified Release Test Development
  - Bacterial, Fungal, Mycoplasma
  - RCR, RCL testing of vector lots
- Recruiting, Training, Retaining Skilled Technologists/Engineers
- Near Term Clinical Trial/Post-Approval Allocation Ethics
  - AZT, Imatinib, Zmapp (Ebola)
- Near Term Outscaling, Mid to Long Term Automation