Treatment of Non-Hodgkin Lymphoma with Central Memory Derived CD19-Specific CAR-Transduced T Cells

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Disclosures

• M. Jensen is an inventor of licensed patents and equity holder in ZetaRx, Inc., a licensee of these patents.

• All other authors have no conflicts of interest to disclose.
Adoptive T Cell Therapy for Cancer

- **T Cell Donor** (Autologous or Allogeneic)
- **Isolate Cytotoxic T Lymphocyte**
- **Engineer Cytotoxic T Cells to Express Tumor Specific Chimeric Antigen Receptors**
- **Patient Recipient**
- **Adoptive T cell Transfer**
- **Expand Tumor Specific T Cells Ex Vivo**
The Chimeric Antigen Receptor (CAR)

CD19-specific scFv

huIgG4 hinge-Fc

huCD4<sub>TM</sub>

huCD3-ζ<sub>cyto</sub>
Persistence of Transferred T Cells Correlates with Cancer Regression

**Strategies to Improve T Cell Persistence:**

- Incorporate lymphodepletion regimens prior to ACT
- Optimize CAR design for improved co-stimulatory signaling
- Reduce transgene immunogenicity
- **Engineer T cell subsets with the propensity for long-term persistence (i.e., memory T cells)**
Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates

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J. Clinical Investigation 2008

Engraftment of human central memory-derived effector CD8+ T cells in immunodeficient mice

Xiuli Wang,1 Carolina Berger,2 ChingLam W. Wong,1 Stephen J. Forman,1 *Stanley R. Riddell,2 and *Michael C. Jensen1,3

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Blood 2010
Distinct Fate of Effector T Cells Derived from $T_{EM}$ vs $T_{CM}$ Following ACT in Non-Human Primate

Berger et al J Clin Invest 2008
$T_{CM}$ Derived Human Effectors Exhibit Superior Engraftment to $T_{EM}$ Derived Counterparts Following Adoptive Transfer

Wang et al. Blood 2011
Engraftment Fitness and Anti-Lymphoma Activity of CD19CAR+ T Cells Derived from T_{CM} vs T_{EM}

**A**

% human CD45+ cells

- Blood
- BM
- Spleen

<table>
<thead>
<tr>
<th>TcmCD19R</th>
<th>TemCD19R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Blood</td>
</tr>
<tr>
<td>BM</td>
<td>BM</td>
</tr>
<tr>
<td>Spleen</td>
<td>Spleen</td>
</tr>
</tbody>
</table>

**B**

Phontons/sec

- TcmCD19R T cells
- Control

Days

- CD19+ Tumor i.v.
- T cell i.v.

City of Hope
Platform for Manufacturing
\( T_{CM} \) Derived CD19CAR\(^+\) T Cells

Day 1: Leukapheresis

Day 2: CliniMACS Selection of Tcm; Dynabead stimulation

Day 5: Lentiviral Transduction; Initiate Expansion

Day 14-30: Dynabead Removal

Day 26-40: Cryopreservation
Enrichment of CD8+ $T_{CM}$

Day 2: CliniMACs
1) CD45RA/CD14/CD4 Depletion
2) CD62L Positive Selection
Development of $T_{CM}$ Derived CD19CAR+ Cells
(Qualification Runs)

*HD 106 and 108 were from Lymphoma donors

Wang et al. J Immunother 2012
Freshly thawed qualification run T cell products were stained with the IOTest® Beta Mark TCR Vβ Repertoire Kit.
• Relapsed B Cell Lymphoma: Induction failure, recurrence of large cell, mantle cell lymphoma

• Poor prognosis with transplant

• Infuse cells on day +2 after transplant

• Lymphodepletion, homeostatic expansion

• Engraft cells as a component of the reconstituted immune system
Phase I/II study of cellular immunotherapy using central memory-enriched CD8+ T cells lentivirally transduced to express a CD19CAR following HSCT for patients with high risk intermediate grade B lineage NHL.

**Enrollment: Relapsed B Cell Lymphoma** (recurrent large cell & mantle cell lymphoma); Poor prognosis with auto-transplant.

**Dose Schedule**

<table>
<thead>
<tr>
<th>Starting Dose</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>50M</td>
<td>100M</td>
<td>500M</td>
<td>1000M</td>
</tr>
</tbody>
</table>

Infuse cells on day +2/+3 after HSCT
- Lymphopenic environment for homeostatic expansion
- Engraft cells as a component of the reconstituted immune system
FDA limit to one patient at a time in groups of 3 for each cell dose escalation; Starting dose = $5 \times 10^7$

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Prior Therapy</th>
<th>Salvage Prior to Leuk</th>
<th>Leukapheresis Date</th>
<th>Salvage Post Leuk</th>
<th>Disease Status at Time of HSCT</th>
<th>HSCT Date</th>
<th>T Cell Infusion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN 043</td>
<td>68</td>
<td>F</td>
<td>diffuse large B-cell lymphoma, Relapsed 10/2011 - mass on right upper back, as well as possibly the uterus</td>
<td>6 cycles R-CHOP including CNS prophylaxis x 2 with high-dose MTX as an inpatient</td>
<td>No</td>
<td>10/20/11</td>
<td>2 cycles R-ICE</td>
<td>2nd Remission</td>
<td>1/16/12</td>
<td>1/18/12</td>
</tr>
<tr>
<td>UPN 047</td>
<td>74</td>
<td>M</td>
<td>diffuse large B-cell lymphoma, Induction failure 03/2012 – mass in abdomen</td>
<td>6 cycles of R-CHOP</td>
<td>2 cycles of R-ICE</td>
<td>3/29/12</td>
<td>No</td>
<td>Partial Remission</td>
<td>6/5/12</td>
<td>6/7/12</td>
</tr>
<tr>
<td>UPN 048</td>
<td>49</td>
<td>F</td>
<td>diffuse large B-cell lymphoma, Relapsed 03/2012 – mass in thyroid</td>
<td>6 cycles of R-ICE</td>
<td>No</td>
<td>5/17/12</td>
<td>2 cycles R-ICE</td>
<td>2nd Remission</td>
<td>9/21/12</td>
<td>9/24/12</td>
</tr>
</tbody>
</table>
IRB 09174/BB-IND 14645: First Three Clinical Products

T Cell Product Expansion:

CD19CAR Expression:

CD19-Specific Cytolytic Activity of T Cell Products:
Impact of CD19CAR+ T Cell Infusion on HSCT Engraftment

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>HSCT Date (Day 0)</th>
<th>T Cell Infusion Date (Day +2/+3)</th>
<th>ANC &gt; 500</th>
<th>Platelets &gt;20K</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPN 043</td>
<td>1/16/12</td>
<td>1/18/12</td>
<td>Day +10</td>
<td>Day +15</td>
</tr>
<tr>
<td>UPN 047</td>
<td>6/5/12</td>
<td>6/7/12</td>
<td>Day +12</td>
<td>Day +18</td>
</tr>
<tr>
<td>UPN 048</td>
<td>9/21/12</td>
<td>9/24/12</td>
<td>Day +11</td>
<td>Day +16</td>
</tr>
</tbody>
</table>

No Infusional Toxicities
Serum Cytokine Levels Following CD19CAR+ T cell Infusion in UPN 043

** TNF below level of detection for all points**
Serum Cytokine Levels Following CD19CAR+ T cell Infusion in UPN 043

Day after UCPIN 043 Infusion

Rash

IFN-γ (pg/ml)

Day after UCPIN 043 Infusion

0 1 2 14 15 21 28 64 (PB)

-12 (pre-HCT cond) 0 (pre-T cell)

City of Hope
** Preliminary qPCR data confirms CD19CAR+ T cell persistence:**

<table>
<thead>
<tr>
<th>UPN043 PBMC</th>
<th>gDNA (ng)</th>
<th>WPRE copy#</th>
<th>WPRE Copy# /100ug gDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 23</td>
<td>200</td>
<td>2.398</td>
<td>1210</td>
</tr>
<tr>
<td>Day 28</td>
<td>200</td>
<td>2.534</td>
<td>1267</td>
</tr>
<tr>
<td>Day 64</td>
<td>200</td>
<td>1.172</td>
<td>586</td>
</tr>
</tbody>
</table>

** anti-CD19R-CAR antibody kindly provided by Dr. Laurence Cooper, MD Anderson Cancer Center, Houston, TX
CD19+ B Cell Aplasia in Blood (PBMC) of UPN043

<table>
<thead>
<tr>
<th>Time</th>
<th>CD19-PE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>7.35%</td>
</tr>
<tr>
<td>Day 28</td>
<td>0.00%</td>
</tr>
<tr>
<td>Day 64</td>
<td>0.01%</td>
</tr>
<tr>
<td>Day 96</td>
<td>0.00%</td>
</tr>
<tr>
<td>Day 118</td>
<td>0.01%</td>
</tr>
</tbody>
</table>
**Serum Rituxan Levels in UPN 043**

[Graph showing the decrease in serum Rituxan levels over time after CAR+ T cells i.v.]
Clinical Research Plan

• Complete 1\textsuperscript{st} cohort at $5 \times 10^7$ CD19CAR\(^+\) CD8\(^+\) $T_{CM}$-derived cells
  – Amendment to normalize for CAR\(^+\) recently approved by FDA

• Continue dose escalation with $1 \times 10^8$ CD19CAR\(^+\) CD8\(^+\) $T_{CM}$-derived cells

• Work with FDA to liberalize accrual design

• Repeat $1 \times 10^8$ dose with CAR\(^+\) Bulk $T_{CM}$-derived cells
  – i.e., remove CD4-depletion step in $T_{CM}$ selection strategy

• Initiate IND with second generation, costimulatory CAR (CD19R:CD28:ζ)
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- Winnie Wong

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- Joel Conrad, MS
- Larry Couture, PhD
- Catherine Matsumoto
- Yasmine Shad
- Suzette Blanchard, PhD
- Simon Lacey, PhD

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**Michael Jensen, MD**
*Seattle Children’s Research Institute*

**Stanley Riddell, MD & Carolina Berger, PhD**
*Fred Hutchinson Cancer Research Center*
CD19+ B Cells in the Bone Marrow of UPN043

Majority of BM CD19+ cells are CD10+ Pro-B and Pre-B cells

CD19+CD10- cells are mature B cells

 Majority of BM CD19+ cells are CD10+ Pro-B and Pre-B cells
# CD19-Targeted Clinical Trials Using CAR T cells

<table>
<thead>
<tr>
<th>Center</th>
<th>Disease</th>
<th>CAR endodomain</th>
<th>Vector to express CAR</th>
<th>Conditioning regimen</th>
<th>Target</th>
<th>Status</th>
<th>ClinicalTrials.gov NCT no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSKCC</td>
<td>CLL-refractory</td>
<td>ζ/28</td>
<td>RV</td>
<td>None vs. cyclophosphamide</td>
<td>CD3-selected</td>
<td>Open 6 treated</td>
<td>NCT00466531</td>
</tr>
<tr>
<td>MSKCC</td>
<td>B ALL-relapsed</td>
<td>ζ/28</td>
<td>RV</td>
<td>Cyclophosphamide</td>
<td>CD3-selected</td>
<td>Open 1 treated</td>
<td>NCT01044069</td>
</tr>
<tr>
<td>BCM</td>
<td>B NHL and CLL</td>
<td>ζ/28 vs. ζ</td>
<td>RV</td>
<td>None</td>
<td>PBMCs (OKT3 and IL-2)</td>
<td>Open 5 treated: 4 DLBCL 1 B-CLL</td>
<td>NCT00586391</td>
</tr>
<tr>
<td>BCM</td>
<td>B NHL and CLL</td>
<td>ζ/28 vs. ζ-EBV</td>
<td>RV</td>
<td>None</td>
<td>PBMCs and EBV CTLs</td>
<td>2 treated</td>
<td>NCT00608270</td>
</tr>
<tr>
<td>BCM</td>
<td>B ALL, S/P HSCT</td>
<td>ζ/28</td>
<td>RV</td>
<td>+30 Days after allo-HSCT</td>
<td>Multivirus CTL</td>
<td>Open</td>
<td>NCT00709033</td>
</tr>
<tr>
<td>NCI</td>
<td>Lymphoma, CLL</td>
<td>ζ/28</td>
<td>RV</td>
<td>Fludarabine and cyclophosphamide</td>
<td>PBMCs (anti-CD3 + REP)</td>
<td>Open 4 treated</td>
<td>NCT00924326</td>
</tr>
<tr>
<td>U Penn</td>
<td>Refractory B</td>
<td>ζ/41BB vs. ζ</td>
<td>LV</td>
<td>Variable</td>
<td>Auto PBMCs (CD3/CD28 beads)</td>
<td>To open</td>
<td>NCT00891215</td>
</tr>
<tr>
<td>U Penn</td>
<td>leukemia/lymphoma</td>
<td>ζ/41BB</td>
<td>LV</td>
<td>Variable</td>
<td>Allo DLI</td>
<td>To open</td>
<td></td>
</tr>
<tr>
<td>MDACC</td>
<td>B-NHL, S/P autologous HSCT</td>
<td>ζ/CD28</td>
<td>Electroporation/SB plasmids</td>
<td>BEAM-R</td>
<td>Auto PBMCs (± IL-2)</td>
<td>To open</td>
<td>NCT00968760</td>
</tr>
<tr>
<td>MDACC</td>
<td>B-lineage malignancy, S/P allogeneic HSCT</td>
<td>ζ/CD28</td>
<td>Electroporation/SB plasmids</td>
<td>Conditioning regimen for HSCT</td>
<td>Allo PBMCs or umbilical cord blood</td>
<td>To open</td>
<td></td>
</tr>
<tr>
<td>COH</td>
<td>Recurrent LCL-MCL, S/P autologous HSCT</td>
<td>ζ</td>
<td>Plasmid</td>
<td>Fludarabine or +28 days S/P HSCT</td>
<td>PBMCs</td>
<td>Closed 3 treated</td>
<td>NCT00182650</td>
</tr>
<tr>
<td>COH/FHCRC</td>
<td></td>
<td>ζ</td>
<td>LV</td>
<td>Tcm: CD8+/CD4 / CD45RA-/CD62+</td>
<td></td>
<td>To open</td>
<td>NCT01318317</td>
</tr>
</tbody>
</table>

*+2 Days after auto-HSCT*