Session I
Update on Current Approaches and Trials

MDACC EXPERIENCE
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September 10, 2013
Session begins 8:40 AM
MDACC’s approach

• Treat patients with B-cell malignancies at high risk of relapse recognizing that:
  – Infusing CAR\(^+\) T cells in patients with minimal residual disease (MRD) will avoid cytokine storm and concomitant toxicities
  – Such patients should receive CAR\(^+\) T cells after HSCT since the latter is standard-of-care and thus likely needed at this stage in the development of CAR-based therapies
MDACC’s approach (cont.)

• To reduce the burdens and costs of T-cell therapies
  – Avoid cost and complexity of recombinant viral vectors
  – Propagate T cells *ex vivo* in a physiologic CAR-dependent manner
  – Simplify manufacturing and delivery including infusion of cryopreserved products
Implementation of T-cell trials

Begun by testing three new technologies in humans

1. Our CD19-specific CAR (designated CD19RCD28) that co-activates T cells via chimeric CD3-ζ and CD28 to deliver signal 1 and signal 2, respectively.

2. The genetic modification of T cells to express CAR from DNA plasmids derived from Sleeping Beauty (SB) system.

3. Selective activation and propagation of CAR⁺ T cells on γ-irradiated engineered artificial antigen presenting cells (aAPC).
A new approach to manufacturing CAR\(^+\) T cells

Combining two platform technologies we adapted for human application

• *Sleeping Beauty* (SB) transposon/transposase system
  – Non-viral approach to gene therapy using DNA plasmids
    • Inexpensive
    • Facile

• Engineered artificial antigen presenting cells (aAPC)
  – Selective propagation of CAR\(^+\) T cells
SB system to genetically modify T cells to target CD19

- T cells targeting CD19 feasible gene therapy approach
  - Successful infusions of genetically modified T cells
  - Tolerable “on target” side-effects

- Compelling patient population
  - Patients with advanced B-cell malignancies high rate of relapse despite hematopoietic stem-cell transplantation (HSCT)

2nd generation CD19-specific CAR (CD19RCD28) signaling through CD28 and CD3-ζ

![Diagram of CD19RCD28 CAR structure]

Shown as a homo-dimer
<table>
<thead>
<tr>
<th>MDACC / NCI #</th>
<th>Agent</th>
<th>Dose of CD19RCD28⁺ T cells</th>
<th>Enrolled</th>
<th>Products made</th>
<th>Infused</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007-0635/00968760</td>
<td>CD19-specific T cells derived from patient combined with autologous HSCT</td>
<td>5x10⁷/m² to 5x10⁹/m² (IL-2 last 2 cohorts)</td>
<td>9 (all NHL)</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>2009-0525/01497184</td>
<td>CD19-specific T cells derived from donor combined with allogeneic HSCT</td>
<td>10⁶/m² to 10⁸/m²</td>
<td>18 (ALL, n=11; NHL, n=6; CLL, n=1)</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>2010-0835/0136452</td>
<td>CD19-specific T cells derived from umbilical cord blood (UCB) donor combined with UCB transplantation</td>
<td>10⁶/m² to 10⁸/m²</td>
<td>4 (ALL, n=3; NHL, n=1)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2011-1169/01653717</td>
<td>CD19-specific T cells from CLL patients after chemotherapy (non-HSCT)</td>
<td>10⁷/m² to 5x10¹⁰/m²</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
**2007-0635: Autologous CAR\(^+\) T cells**

*Initial clinical data. Trial is accruing and scheduled dose escalations are planned*

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age</th>
<th>Histology at Diagnosis</th>
<th>Stage at SCT</th>
<th>Dose Level</th>
<th>T cells Infused (x10^8)</th>
<th>CAR expression (%)</th>
<th>Response</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P446</td>
<td>61</td>
<td>Follicular, mixed</td>
<td>Transformed DLBL, CR2</td>
<td>5x10^8/m^2</td>
<td>10.0</td>
<td>87.5</td>
<td>CCR, 6 mo.</td>
<td>None</td>
</tr>
<tr>
<td>P458</td>
<td>58</td>
<td>Nodular pred. HL</td>
<td>Transformed DLBL, CR2</td>
<td>5x10^8/m^2</td>
<td>10.6</td>
<td>77.2</td>
<td>CCR, 4 mo.</td>
<td>None</td>
</tr>
<tr>
<td>P468</td>
<td>48</td>
<td>Follicular, mixed</td>
<td>Follicular, mixed, Rel1</td>
<td>5x10^8/m^2</td>
<td>11.4</td>
<td>85.5</td>
<td>CR, 4 mo.</td>
<td>None</td>
</tr>
<tr>
<td>P471</td>
<td>55</td>
<td>DLBL</td>
<td>DLBL, Rel1</td>
<td>5x10^8/m^2</td>
<td>11.1</td>
<td>90.4</td>
<td>CR, 3 mo.</td>
<td>None</td>
</tr>
<tr>
<td>P509</td>
<td>58</td>
<td>CNS NHL</td>
<td>CR2</td>
<td>5x10^8/m^2</td>
<td>2.2</td>
<td>95.9</td>
<td>Too Early</td>
<td>None</td>
</tr>
</tbody>
</table>

*Rituximab omitted in conditioning therapy due to recent atrial fibrillation in patient*
Persistence of autologous CAR⁺ T cells

DAYS AFTER CAR⁺ T-CELL INFUSION

P446: No circulating CD19⁺ B cells at 6 months
CAR$^+$ T cells emerged at 6 months after infusion

**Q-PCR**

**P446**

Transgene copies (no./μg of gDNA)

Days after CAR$^+$ T-cell infusion

**Flow cytometry**

**P446**

Number of cells per μL of blood

Days after CAR$^+$ T-cell infusion

- CD19CAR$^+$ T-cells
- CD19$^+$ B cells
CD3 FITC
CD19CAR APC
10^0 10^1 10^2 10^3 10^4
0.00% 69.65%

CD4 PE
CD19CAR APC
10^0 10^1 10^2 10^3 10^4
0.08% 99.87%

CD8 PerCP
CD19CAR APC
10^0 10^1 10^2 10^3 10^4
0.04% 54.78%

Infused CAR+ T cells

1 week post infusion

6 months post infusion

Gated on CD3+ population
CD3
CD4
CD8

P446

Infused CAR+ T cells

Gated on CD3+ population

Infused CAR+ T cells
## 2009-0525: Allogeneic CAR⁺ T cells

*Initial clinical data. Trial is accruing and scheduled dose escalations are planned*

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<th>CAR-T source</th>
<th>T cells Infused (x10⁸)</th>
<th>CAR expression (%)</th>
<th>Response</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P396</td>
<td>23</td>
<td>B-ALL</td>
<td>CR2, *MRD⁺</td>
<td>10⁶/m²</td>
<td>MSD donor</td>
<td>0.02</td>
<td>96.5</td>
<td>Progressed</td>
<td>None</td>
</tr>
<tr>
<td>P396-</td>
<td></td>
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</tr>
<tr>
<td>P411</td>
<td>50</td>
<td>DLBL</td>
<td>Refractory, 8 lines Rx</td>
<td>10⁶/m²</td>
<td>MSD donor</td>
<td>0.03</td>
<td>70.5</td>
<td>Progressed</td>
<td>None</td>
</tr>
<tr>
<td>P410</td>
<td>21</td>
<td>B-ALL</td>
<td>CR3, MRD⁺</td>
<td>10⁶/m²</td>
<td>MSD donor</td>
<td>0.02</td>
<td>96.8</td>
<td>Progressed</td>
<td>None</td>
</tr>
<tr>
<td>P459</td>
<td>25</td>
<td>B-ALL</td>
<td>CR2, MRD⁻neg</td>
<td>10⁷/m²</td>
<td>MSD donor</td>
<td>0.28</td>
<td>90.5</td>
<td>CCR at 6 mo.</td>
<td>GVHD?</td>
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<tr>
<td>P513</td>
<td>25</td>
<td>B-ALL</td>
<td>Refractory, auto-SCT</td>
<td>10⁶/m²</td>
<td>Haplo donor</td>
<td>0.02</td>
<td>93.3</td>
<td>Too Early</td>
<td>None</td>
</tr>
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<td>MSD donor</td>
<td>0.02</td>
<td>96.5</td>
<td>Progressed</td>
<td>None</td>
</tr>
<tr>
<td>P396-CIND</td>
<td>23</td>
<td>B-ALL</td>
<td>CR3, MRD⁺</td>
<td>5x10^7/m²</td>
<td>MSD donor</td>
<td>0.95</td>
<td>96.5</td>
<td>Progressed</td>
<td>None</td>
</tr>
<tr>
<td>P411</td>
<td>50</td>
<td>DLBL</td>
<td>Refractory</td>
<td>10^6/m²</td>
<td>MSD donor</td>
<td>0.03</td>
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<tr>
<td>P410</td>
<td>21</td>
<td>B-ALL</td>
<td>CR3, MRD⁺</td>
<td>10^6/m²</td>
<td>MSD donor</td>
<td>0.02</td>
<td>96.8</td>
<td>Progressed</td>
<td>None</td>
</tr>
<tr>
<td>P410-CIND</td>
<td>21</td>
<td>B-ALL</td>
<td>CR4, MRD⁻⁻</td>
<td>10⁷/m²</td>
<td>MSD donor</td>
<td>0.17</td>
<td>96.8</td>
<td>CCR, relapse</td>
<td>None</td>
</tr>
</tbody>
</table>

P410 relapsed only in calf
Persistence of allogeneic CAR$^+$ T cells

Days after CAR$^+$ T-cell fusion

Transgene copies (no./ug of gDNA)

- P 39 6
- P 39 6 CI ND
- P 41 0
- P 41 0 CI ND
- P 45 9

Persistences of transgene copies over days.
Lessons Learned infusing patients in MRD

• Summary of Adverse Events
  – No infusion-related toxicity
  – One patient developed acute GVHD of liver, steroid refractory, in setting of concurrent drug-induced liver injury
• Patient-derived T cells can be generated from autologous blood of heavily pre-treated recipients
• Donor-derived T cells can be generated from allogeneic blood, including umbilical cord blood
• Successfully infuse patient-and donor-derived CD19-specific T cells after autologous and allogeneic HSCT, respectively
• Initial clinical data infusing SB-modified and aAPC-propagated CAR⁺ T cells demonstrate safety, feasibility, and persistence
Lessons Learned (cont.)

• Successfully manufacture T-cell products from:
  – Small fraction of umbilical cord blood at time of transplantation
  – 200 mL of peripheral blood and avoid costs and inconvenience of apheresis
  – From heavily pre-treated patients
• No immediate or late toxicities
  – T cells now administered as outpatient infusions
  – No GVHD attributed to infused allogeneic T cells
• Can safely re-infuse CAR⁺ T cells from patient-specific cryopreserved banks
Summary to date

• First-in-human application of SB system
• Human application of K562-derived aAPC
• Human application of CD19RCD28 CAR
• Infusions after autologous and allogeneic HSCT, including umbilical cord blood transplantation
• Persistence of CAR$^+$ T cells in peripheral blood
• Increase in presence of CAR$^+$ T cells in peripheral blood months after infusion
Plans

• Low costs of SB system enable:
  – Change of CAR design and specificity
  – Co-expression of co-stimulatory molecules with CAR

• Use aAPC to propagate CAR\(^+\) T cells with specificity other than CD19
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Thank You

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