Adoptive transfer of T cells genetically modified using the Sleeping Beauty system

Laurence J.N. Cooper
Adoptive Transfer session
Saturday, October 31st
9:45 AM to 10:15 AM
24th iSBTc
Immunotherapy options for B-lineage (CD19\(^+\)) ALL and lymphoma

- T-cell therapy
- NK-cell therapy
- Antibody therapy
- Immunocytokines
- Vaccination
Tumor-specific T cells

- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor (CAR) independent of MHC
- T cells that recognize TAA through endogenous $\alpha\beta$ T-cell receptor (TCR) in context of MHC
Clinical studies using CAR$^+$ T cells

<table>
<thead>
<tr>
<th>Target</th>
<th>CAR specificity</th>
<th>Major toxicity</th>
<th>References</th>
</tr>
</thead>
</table>
Rationale

Targeting CD19 determinant on B cells

- CD19 antigen is a 95 kDa B lineage-specific membrane glycoprotein, found on >95% of B-cell lymphomas and B-ALL cells;
- CD19 is rarely lost during the process of neoplastic transformation, but disappears upon differentiation to mature plasma cells;
- CD19 is not expressed on hematopoietic stem cells, nor on normal tissues outside the B lineage;
- CD19 is not shed into the circulation.
Improve T-cell therapeutic potential

Improve persistence

- Proliferative potential
- CAR
  - 1\textsuperscript{st} generation
  - 2\textsuperscript{nd} generation
  - 3\textsuperscript{rd} generation
- Improve culturing environment
  - aAPC
  - Cytokines
- Type of T-cell
  - Memory
  - Naïve
Most clinically-effective T-cell therapies includes \textit{ex vivo} antigen-dependent proliferation

- Therefore we developed culture systems \textit{ex vivo} that select for T cells that can sustain CAR-dependent proliferation \textit{in vivo}
Experimental design
Programming the μenvironment
Numerically expand T cells under polarizing conditions
Sleeping Beauty Transposition

Transposon (Donor) sequences flanked by inverted repeats are integrated into genome.

Transposase (Helper) expression is transient.
Numeric expansion of CAR$^+$ T cells on aAPC
Relative efficiency of stable gene expression by SB system

<table>
<thead>
<tr>
<th>DNA plasmid</th>
<th>Day 0</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4⁺CAR⁺</td>
<td>CD8⁺CAR⁺</td>
</tr>
<tr>
<td>No DNA</td>
<td>0.63</td>
<td>0.18</td>
</tr>
<tr>
<td>SBases</td>
<td>0.96</td>
<td>0.75</td>
</tr>
<tr>
<td>SBon</td>
<td>15.9</td>
<td>9.5</td>
</tr>
<tr>
<td>SBon &amp; SBases</td>
<td>13.29</td>
<td>7.87</td>
</tr>
</tbody>
</table>

71 fold
SB T-cell data
CD19-dependent cytotoxicity

% Specific Lysis

Effector:Target Ratio
Improve T-cell therapeutic potential
Improve persistence

• Proliferative potential
• CAR
  – 1st generation
  – 2nd generation
  – 3rd generation
• Improve culturing environment
  – aAPC
  – Cytokines
• Type of T-cell
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CD19-specific CARs
Relative *in vivo* T-cell persistence

![Graph showing relative in vivo T-cell persistence over time](image)

- **CD19R**
- **CD19RCD28**

*Background luminescence*
Relative anti-tumor effect
Improve T-cell therapeutic potential
Improve persistence

• Proliferative potential
• CAR
  – 1st generation
  – 2nd generation
  – 3rd generation
• Improve culturing environment
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• Type of T-cell
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Which T-cell sub-population to genetically modify?
Outgrowth of CAR\(^+\) T cells with memory cell phenotype
Safety of transposition
Translation to clinical trials
Applied Cellular Therapy (ACT)

How the Cooper explained it
How the technicians heard it
How the graduate students heard it
How the post-docs heard it
How the faculty heard it
How the project was documented
How the IRB heard it
How the patient heard it
How the project was funded
What the patient really needed
Translational pipeline

- Trials implementation
- Manufacture
- Correlative studies
- Regulatory affairs
- Development
- Research
Schematic
Trial design

Principal Investigator = Dr. Partow Kebriaei
## T-cell trials at MDACC

<table>
<thead>
<tr>
<th>Trial</th>
<th>Agent</th>
<th>Preclinical</th>
<th>Phase I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>Autologous CD19-specific T cells</td>
<td>→</td>
<td>X</td>
</tr>
<tr>
<td>ALL and lymphoma</td>
<td>Allogeneic CD19-specific T cells</td>
<td>→</td>
<td>X</td>
</tr>
<tr>
<td>ALL and lymphoma</td>
<td>Allogeneic CD19-specific UCB-derived T cells</td>
<td>→</td>
<td>X</td>
</tr>
<tr>
<td>Trial</td>
<td>Agent</td>
<td>Preclinical</td>
<td>Phase I</td>
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</tr>
<tr>
<td>Neuroblastoma</td>
<td>Haploidentical NK cells</td>
<td>→</td>
<td>X</td>
</tr>
<tr>
<td>ALL</td>
<td>Haploidentical NK cells and Epratuzumab</td>
<td>→</td>
<td>X</td>
</tr>
<tr>
<td>AML</td>
<td>Haploidentical NK cells</td>
<td>→</td>
<td>→</td>
</tr>
</tbody>
</table>
Future developments

• Altering the host environment to facilitate persistence and function of transferred T cells
• Altering intrinsic properties of T cells that are selected or engineered for therapy
Lessons and Take Home Messages

• Key points
  – SB system can be used to genetically modify human T cells

• Potential impact on the field
  – SB system can be adapted for human application

• Lessons learned
  – Genetically modified T cells can be generated with improved persistence and therapeutic potential
THANKS!