Mutation of the CD28 costimulatory domain confers enhanced CAR T cell function

Justin C Boucher, PhD

Department of Blood & Marrow Transplantation and Cellular Immunotherapy, Division of Clinical Sciences

H. Lee Moffitt Cancer Center and Research Institute

Marco L Davila Lab
Next-generation CAR designs

CAR, chimeric antigen receptors; TCR, T cell receptor.
Persistence of CAR T cells is a major clinical problem

<table>
<thead>
<tr>
<th>Trial</th>
<th>CD19 CAR Construct</th>
<th>Relapse Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children’s Hospital of Philadelphia phase I</td>
<td>FMC63-41BBz</td>
<td>36% (20/55)</td>
</tr>
<tr>
<td>Novartis phase II</td>
<td>FMC63-41BBz</td>
<td>33% (20/61)</td>
</tr>
<tr>
<td>Seattle Children’s Research Institute phase I</td>
<td>FMC63-41BBz</td>
<td>45% (18/40)</td>
</tr>
<tr>
<td>Fred Hutchinson Cancer Center phase I</td>
<td>FMC63-41BBz</td>
<td>31% (9/29)</td>
</tr>
<tr>
<td>NCI phase I</td>
<td>FMC63-CD28z</td>
<td>29% (8/28)</td>
</tr>
<tr>
<td>Memorial Sloan Kettering phase I</td>
<td>SJ25C1-CD28z</td>
<td>57% (25/44)</td>
</tr>
</tbody>
</table>

Maude et al, *NEJM* 2014

Majzner and Mackall, *Cancer Discovery* 2018
CAR T cells after EARLY or LATE challenge with Eμ-ALL

EARLY CHALLENGE

- EARLY CHALLENGE
- CAR T iv
- CAR T cells
- WK 1
- WK 2
- WK 5
- WK 6
- Eμ-ALL challenge
- Sac & Analyze

LATE CHALLENGE

- LATE CHALLENGE
- Rag1−/−
CAR T cell immune phenotype in BM 6 weeks after adoptive transfer

<table>
<thead>
<tr>
<th></th>
<th>m19Δz</th>
<th>m19z</th>
<th>m1928z</th>
<th>m19-humBBz</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD44</td>
<td>2.56</td>
<td>8.49</td>
<td>6.39</td>
<td>15.3</td>
</tr>
<tr>
<td>CD62L</td>
<td>0.15</td>
<td>0.070</td>
<td>1.22</td>
<td>2.10</td>
</tr>
<tr>
<td>PD1</td>
<td>44.7</td>
<td>30.9</td>
<td>22.5</td>
<td>47.6</td>
</tr>
<tr>
<td>CD62L</td>
<td>18.8</td>
<td>7.29</td>
<td>19.5</td>
<td>23.0</td>
</tr>
</tbody>
</table>

0-1 Exhaustion Markers:
- m19Δz: 21.6/78.4%
- m19z: 23.3/76.7%
- m1928z: 34.7/65.3%
- m19-humBBz: 54.0/46.0%
How does CD28 contribute selectively to exhaustion?

Moran et al., JEM 2011
How do these subdomains contribute to CAR T cell exhaustion?

- APC:
  - CD80/CD86

- T cell:
  - CD28
  - PI3K
  - Itk
  - NFAT
  - PLCγ
  - Lck
  - ZAP70
  - Erk

- Cellular Processes:
  - Cell Differentiation
  - IL-2 Production
  - Cell Proliferation
  - Anti-apoptosis
Mutated m1928z CAR Constructs

- m1928z
- mut04 (YMNM)
- mut05 (PRRP)
- mut06 (PYAP)
Mutations that disrupt signaling in CD28 preserve in vitro CAR T cell function

Transduction efficiency

Cytokines

Cytotoxicity
Mut06 enhances *in vivo* CAR T cell function

- **Eμ-ALL iv**
- **Cytoxan**
- **Day -7**
- **Day -1**
- **Day 0**
- **CAR T iv**

**Graph:**
- **Percent survival**
- **Day**
- **N = 8 mice per group**

- **m19dz**
- **m1928z**
- **mut06**
Mut06 decreases CAR T cell exhaustion

**Eμ-ALL challenge**

- DAY 0
- WK 1
- WK 2

**Sac & Analyze**

- CAR T iv
- Rag1⁻/⁻

**Restimulate** splenocytes with target cells expressing CD19 and PDL1 for 4hr

- Analyze cytokines by FACS

- **CAR**
  - m19dz
  - m1928z
  - m19hBBz
  - mut06
  - Percent +

- **PD1**
  - Percent +
  - **m19dz**
  - **m1928z**
  - **m19hBBz**
  - **mut06**

- **IL2**
  - Percent +
  - **m19dz**
  - **m1928z**
  - **m19hBBz**
  - **mut06**

- **IFNY**
  - Percent +
  - **m19dz**
  - **m1928z**
  - **m19hBBz**
  - **mut06**
Mut06 preserves pZap70 signaling while decreasing Nur77 and pAkt
Decreased signaling causes less NFAT expression resulting in decreased CAR T cell exhaustion.
How does co-stimulation regulate CAR T cell function in relevant pre-clinical models

• Mutation of CD28 co-stimulatory subdomains (YMNM and PRRP) preserves \textit{in vitro} and \textit{in vivo} function, while reducing signs of exhaustion

• These \textit{in vitro} results have been validated in human CARs

• Our hypothesis is reducing CAR signaling with mut06 favors transcription factor induction that reduces upregulation of exhaustion related genes (Martinez et al, \textit{Immunity} 2015)

• Future Directions:
  • Genomic studies
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