T Cell Receptor Affinity and Avidity Defines Antitumor Response and Autoimmunity in T Cell Immunotherapy

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Challenges in immunizing against cancer

- Most tumor antigens are, although over-expressed on the tumor, non-mutated self (host) proteins.
- Our immune system is educated to tolerate self proteins.
- T-cells that express high affinity TCRs specific for self/tumor antigens are deleted in the thymus by negative deletion.

Morgan, RA. Cancer J. (2010)
Yee, C. Cancer J. (2010)
Adoptive cell transfer (ACT) of antigen specific T-cells

- ACT with TILs achieves 49-72% objective response rate.
- Generation of tumor-specific T cells in this mode of immunotherapy is often limiting.
- ACT with TCR-engineered cells is promising but less efficient (25%).
- Not all cases result in complete and durable responses.

Dudley et al., J Clin Oncol, 2008
Yee, C. Cancer J. (2010)
Morgan, RA. Cancer J. (2010)
Can higher affinity TCRs render ACT more effective?

- Substantial evidence indicate a correlation between T cell functional activity and TCR affinity.

- Correlation remains controversial as higher affinity TCRs can lead to:
  - Stronger (Varela-Rohena, 2008).
  - Plateaued (Schmid et al. 2010, Tian et al., 2007)
  - Attenuated (Corse et al., 2010; Irving et al. 2012; McMahan, 2006)

- ACT using CD8+ T-cells is often associated with autoimmunity in mouse and humans (Palmer et al., 2008; Johnson et al., 2008; Yeh et al., 2009).
**A panel of A2/gp100-specific TCRs isolated from melanoma patients**

- Melanoma patients vaccinated with gp100 (2M) peptide + IL-2
- Isolated A2-gp100-specific TCRs by A2-gp100 tetramer sorting
- Cloned and sequenced TCR by RACE

<table>
<thead>
<tr>
<th>TCR Name</th>
<th>TCR gene (IMGT®)</th>
<th>Source</th>
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<tbody>
<tr>
<td>19LF6</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>16LD6</td>
<td>3</td>
<td>19</td>
</tr>
<tr>
<td>R6C12</td>
<td>41</td>
<td>12-3</td>
</tr>
<tr>
<td>K4H5</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>5CE2</td>
<td>12-1</td>
<td>27</td>
</tr>
<tr>
<td>L2G2</td>
<td>12-2</td>
<td>7-9</td>
</tr>
<tr>
<td>W2C8</td>
<td>2</td>
<td>6-2</td>
</tr>
</tbody>
</table>
Generation of human/mouse chimeric TCRs

**Retroviral Constructs**

<table>
<thead>
<tr>
<th>5’ LTR</th>
<th>Ψ⁺</th>
<th>Vα</th>
<th>Cα</th>
<th>P2A</th>
<th>Vβ</th>
<th>Cβ</th>
<th>IRES</th>
<th>Blastcidin</th>
<th>3’ LTR</th>
</tr>
</thead>
</table>

**Transfection**

Phoenix Retrovirus Producer Cells

**Retroviral Particles**

A2-K\(^b\) naïve mouse CD8+ T-cells

**Infection**

A2-K\(^b\) naïve mouse CD8+ T-cells
Distal T-cell signaling events are correlated with tetrramer binding affinities
Tetramer binding threshold for cytotoxicity

- **Graph 1:** Percentage Specific Killing vs. Effector:T-cell Ratio
- **Graph 2:** Percentage Specific Killing vs. Tetramer MFI
- **Graph 3:** KD (μM) vs. MFI
Lack of tumor regression not due to lack of accumulation of T-cells in spleen and tumor
Can higher affinity TCRs render ACT more effective?

Transduce A2-gp100 TCRs of different affinity into primary CD8+ T-cells

ACT

B16-A2/k^b

IL-2 gp100

Tumor rejection

Tumor Area (mm^2)

500

50

Avidity

R6C12

W2C8

No Treatment

Choroid

Photoreceptors

Iris

Cornea

Ocular Autoimmunity

50

500

Avidity
A TCR affinity threshold defines T cell functional activities induced by self-ligands.
Affinity threshold has clinical implications

<table>
<thead>
<tr>
<th>TCR</th>
<th>KD (µM)</th>
<th>Objective cancer regressions</th>
<th>Cellular infiltrate into the eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF4</td>
<td>29</td>
<td>13% (n=17)</td>
<td>0%</td>
</tr>
<tr>
<td>DMF5</td>
<td>5.6</td>
<td>30% (n=30)</td>
<td>55%</td>
</tr>
<tr>
<td>gp154</td>
<td>0.38</td>
<td>19% (n=16)</td>
<td>25%</td>
</tr>
</tbody>
</table>

Autoimmune response is directly correlated with \textit{in vivo} tumor rejection and plateau at the same affinity threshold (KD =10 µM).

Krogsgaard et al., \textit{submitted}
Specific increase in potency via structure-based design of a T cell receptor

ICM Published
Other safety concerns for engineered TCRs

MAGE-A3 engineered TCR (a3a) reacts with Titin to mediate cardiac arrest in patients

wt TCR: 500 µM

a3a TCR: 2.3 µM

Cameron et al., Sci Transl Med, 2013
Conclusions

• T-cells with relative “high” affinity to self exist in the periphery.

• Above a certain T-cell affinity threshold increased activation is observed \textit{in vitro} but plateau \textit{in vivo}.

• Autoimmune response is directly correlated with \textit{in vivo} tumor rejection and plateau at the same affinity threshold (KD =10 μM).

• Strategies focusing on TCRs in the intermediate range (KD ~10 μM) or targeting shared antigens could dampen the potential for autoimmunity during ACT.
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