Memory CD8\(^+\) T cells induce precocious effector differentiation of naïve CD8\(^+\) T cells in a FasL-Fas dependent manner

Youth corrupted.

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Exposing T cells to Fas Ligand (FasL)-Fas Receptor (FasR) Antagonists Withholds Differentiation and Increases Expansion Making T cells More Suitable for Use in Cancer Immunotherapy
**CD8⁺ T cells move through progressive stages of differentiation**

![Diagram showing the stages of CD8⁺ T cell differentiation](image)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>TN</th>
<th>TSCM</th>
<th>TC</th>
<th>TEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR7</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>CD62L</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>CD27</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>CD28</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>CD45RA</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+/-</td>
</tr>
<tr>
<td>CD45RO</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD122</td>
<td>–</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>CD95</td>
<td>–</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>KLRG-1</td>
<td>–</td>
<td>–</td>
<td>+/-</td>
<td>++</td>
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</tbody>
</table>

CD8+ T cell differentiation status is highly correlated with anti-tumor efficacy in mice

A. Tumor area (mm^2)

B. Tumor slope (mm^2/day)

“At present, there is no evidence that the presence of “older” cells with limited potential for clonal expansion within cell grafts containing “younger” cells is detrimental.”

Central experimental question:

- Is it strictly necessary to physically isolate naïve CD8+ T cells from other T cell subsets, or is their presence sufficient to convey optimal *in vivo* expansion, persistence, and anti-tumor function?
Generation, isolation, and expansion of \textit{in vivo} generated tumor-reactive CD8$^+$ T cell subsets for ACT

- Pmel Thy1.1 or Ly5.1
- rVVhgp100 (2e7 pfu)
- 28d

**Isolation by FACS sort**

Gated on live$^+$, CD8$^{\alpha^+}$:

- $\alpha$CD3$\alpha$CD28 + IL-2 (4 ng/mL)

- $T_{\text{effAg}}$
- $T_{\text{effMix}}$
- $T_{\text{effNaive}}$

**TeffMix**

<table>
<thead>
<tr>
<th>$T_{\text{EM}}$</th>
<th>$T_{\text{CM}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve/</td>
<td></td>
</tr>
<tr>
<td>$T_{\text{SCM}}$</td>
<td></td>
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</tbody>
</table>
Indelible fate tracking of CD8^+ T cells subsets using congenic markers

\[ \alpha CD3/\alpha CD28 + IL-2 (4\text{ng/mL}) \]

FACS,
Magnetic bead or
FACS sort isolations

T Naïve
Pmel Thy1.1

\[ T_{\text{eff} \text{Naive}} \]

\[ T_{\text{eff} \text{Mix}} \]

\[ T_{\text{eff} \text{Ag}} \]

T Memory
Pmel Ly5.1

\[ T_{\text{eff} \text{Naive}, \text{mix}} \]

\[ T_{\text{eff} \text{Ag}, \text{mix}} \]

\[ T_{\text{eff} \text{Ag}} \]
Naïve CD8$^+$ T cells differentially lose CD62L and CCR7 when primed with Ag experienced CD8$^+$ T cells

Gated on live, naïve (Ly5.1) or Ag experienced (Thy1.1) Pmel-1 CD8$^+$ T cells

Day 0

Day 7

$T_{eff}Ag$

$T_{eff}Naive$ mix

$T_{eff}Naive$
Naïve CD8+ T cells acquire greater effector functions when primed with Ag experienced T cells

Gated on live, naïve (Ly5.1) or Ag experienced (Thy1.1) Pmel-1 CD8+ T cells

**A**

- Naïve, alone
- Naïve, mix
- Ag experienced, mix

**B**

- Naïve, alone
- Naïve, mix
- Ag experienced, mix

*** ns

**CD62L**

**CD44**

**IFNγ+CD8α+ T cells (%)**

**T_EM**

**T_CM**

**Naïve/ T_SCM**
Naïve-derived CD8+ T cells cluster genetically with Ag experienced T cells when expanded together.
Naïve CD8+ T cells primed in vitro with Ag experienced T cells have impaired expansion and anti-tumor efficacy

** = $P < 0.05$

** = $P < 0.01$
What factor(s) are responsible for inducing precocious differentiation?
Precocious effector differentiation of naïve-derived effector cells can be retarded by blockade of FasL

Gated on live\(^+\), CD8\(\alpha^+\), naïve-derived cells:

**Naïve alone**

**Naïve mix**

**Naïve mix, anti-FasL**
FasL trimer can induce precocious differentiation of naïve CD8+ T cells independently of Ag experienced T cells

Modulation of Fas-signaling causes dynamic changes in the phenotypic and functional qualities of CD8\(^+\) T cells

A

- No treatment
- \(T_{\text{EFF}}^{\text{Naive}}\) alone
- \(T_{\text{EFF}}^{\text{Naive}} + \alpha\text{-FasL Ab}\)
- \(T_{\text{EFF}}^{\text{Mix}} + \text{Ctrl Ab}\)
- \(T_{\text{EFF}}^{\text{Mix}} + \alpha\text{FasL Ab}\)

B

- CD62L\(^+\) (%) on transfer

Tumor area (mm\(^2\))

- Tumor slope (mm\(^2\)/day)

\(R^2 = 0.797\)

\(P < 0.0001\)
FasL induces dose-dependent activation of the pro-differentiation AKT pathway in naïve CD8\(^+\) T cells

<table>
<thead>
<tr>
<th>Fas Ligand (ng/mL)</th>
<th>T(_N)</th>
<th>0</th>
<th>5</th>
<th>15.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-AKT (Thr 308)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AKT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td></td>
<td></td>
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Naïve enriched Pmel-1 CD8\(^+\) T cells
Expanded with \(\alpha\)CD3/\(\alpha\)CD28 x 24h +/- lz-FasL
Inhibition of AKT blocks precocious differentiation induced by signals delivered through FasL

**A**

Naïve alone

Naïve alone + lz-FasL (50ng/mL)

Naïve alone + lz-FasL (50ng/mL) + AKTi

**B**

<table>
<thead>
<tr>
<th>CD44</th>
<th>CD62L</th>
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<tbody>
<tr>
<td>9.5</td>
<td>64</td>
</tr>
<tr>
<td>62</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>90</td>
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<table>
<thead>
<tr>
<th>CD8α</th>
<th>IFNγ</th>
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<tbody>
<tr>
<td>53</td>
<td>47</td>
</tr>
<tr>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>51</td>
<td>49</td>
</tr>
</tbody>
</table>
Summary and conclusions

Preservation of youth

More effective cells

Less effective cells

\[ T_N \rightarrow T_{SCM} \rightarrow T_{CM} \]

\[ T_{EM} \rightarrow T_{EFF} \]

\[ \ldots \text{‘younger’ cells remain in contact with ‘older,’ more differentiated cells} \]

\[ \ldots \text{younger cells are physically separated away from ‘older’ cells} \]

Fas/FasL
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