Programming tumor-reactive effector memory CD8\(^+\) T cells \textit{in vitro} obviates the requirement for \textit{in vivo} vaccination

Christopher A. Klebanoff, M.D.
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Objective response rates (RECIST) in patients with metastatic melanoma treated at the National Cancer Institute, Surgery Branch

Modified from Rosenberg and Dudley, COI 2009.
Antigen stimulation can potently enhance the function of adoptively transferred T cells in mouse and man.

Antigen stimulation can potently enhance the function of adoptively transferred T cells in mouse and man.

Modified from:
Smith FO et al, JIT 2009.
Translation of concomitant Ag-stimulation following ACT is technically and practically challenging

Table 3. Treatment Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of Patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigens recognized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown/autologous only</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Gp100 only</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>MART-1 only</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>Autologous and gp100</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Autologous and MART-1</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Gp100 and MART-1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Autologous, MART-1 and gp100</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Contains autologous reactivity 55%
Hypothesis

Antigen-experienced CD8$^+$ T cells may be “programmed” to execute an effector response with a limited duration in vitro stimulation, bypassing the requirement for systemic vaccination following ACT for optimal tumor treatment.

Experimental approach

In vitro differentiated Pmel T_{EM}

Stimulation x 12hr $\rightarrow$ 72hr

Autologous hgp100\textsubscript{25-33} pulsed irradiated splenocytes

Adoptive transfer into mice bearing established B16 melenoma:
- Lymphodepletion
- Exogenous IL-2 support

Endpoints:
- In vivo proliferation
- Tumor treatment

In vitro differentiated Pmel T_{EM}

Stimulation x 12hr $\rightarrow$ 72hr
Programmed CD8\(^+\) T cells execute a similar *in vivo* proliferative response compared with vaccine stimulated T cells.
Programming antigen-experienced tumor-reactive
T cells prior to ACT replaces the need for vaccination

- No treatment
- Pmel T<sub>EM</sub>, no stimulation, IL-2
- Pmel T<sub>EM</sub>, peptide program, IL-2
- Pmel T<sub>EM</sub>, rFPhgp100, IL-2
- Pmel T<sub>EM</sub>, αCD3/αCD28 program, IL-2
Delayed transfer of programmed CD8$^+$ T cells impairs anti-tumor treatment efficacy

- No treatment
- Pmel $T_{EM}$, no stimulation, IL-2
- Pmel $T_{EM}$, peptide program, IL-2
- Pmel $T_{EM}$, rFPgp100, IL-2
Programming CD8\(^+\) T cells incites an interval of antigen independent IFN\(\gamma\) release

- □ CM
- ○ EL-4 / \(\beta\text{gal}_{96-103}\)
- ● EL-4 / hgp100_{25-33}
- ▼ B16

IFN-\(\gamma\) (pg ml\(^{-1}\))

Days post re-stimulation
IFNγ production by programmed CD8+ T cells enhances tumor D^b expression and subsequent tumor kill *in vitro*
IFN$_\gamma$ production by activated CD8$^+$ T cells enhances tumor D$^b$ expression \textit{in vivo}.
The *in vivo* therapeutic benefit of *in vitro* programmed CD8$^+$ T cells is IFN$\gamma$-dependent

![Graph showing tumor size over days post treatment with different treatments]
Conclusions

- *In vitro* programming tumor-reactive CD8⁺ T cells prior to ACT with either peptide-pulsed irradiated feeders or combined αCD3/αCD28 stimulation can bypass the requirement for *in vivo* vaccination.

- *In vitro* programming incites an interval of antigen-independent IFNγ release that facilitates tumor recognition both *in vitro* and *in vivo*.

- The benefit of *in vitro* programming CD8⁺ T cells prior to adoptive transfer is entirely dependent on the ability to release IFNγ.
A model for “immunologic sonar”
B16 tumor deposit
B16 tumor deposit
*In vitro* programmed Pmel IFNγ⁻/⁻ T_{EM}
B16 tumor deposit
B16 tumor deposit
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Delayed transfer does not impair the relative engraftment efficiency of transferred CD8⁺ T cells

† = P > 0.05; * = P < 0.05
Antigen stimulation can potently enhance the function of adoptively transferred T cells in mouse and man.