Maintenance of CD27+ Effector Memory T Cells During ex vivo Expansion of Melanoma TIL for Adoptive T-cell Therapy

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CD8+ T-cell differentiation:
T-effector memory → T-effector transition

Tumor microenvironment

- $T_N$: CD27+, CD28+, CD62L++, GB-
- $T_{EM}$: CD27+, CD28+/-, CD62L-, GB+/++
- $T_E$: CD27-, CD28-, CD62L-, GB+++
Adoptive Cell Therapy (ACT): Current State-of-the-Art

- Cyclophosphamide
- Fludarabine
- anti-CD3 Ab + IL-2 + allogeneic feeders
- 500X to 1000X expansion
- 5 to 100 billion cells infused
- The more cells infused the better the CR

Typical TIL REP

- Rapid Expansion Protocol (REP)
- High-dose IL-2
- Lymphodepletion

Graph:
- TIL number vs. Day of Culture
- Typical TIL REP
- anti-CD3 Ab + IL-2 + allogeneic feeders
- 500X to 1000X expansion
- 5 to 100 billion cells infused
- The more cells infused the better the CR
Clinical efficacy of metastatic melanoma treatment with adoptive cell therapy

<table>
<thead>
<tr>
<th># Patients enrolled</th>
<th>CR</th>
<th>PR</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>35</td>
<td>4</td>
<td>14</td>
<td>18 (51%)</td>
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Dudley et al, J Clin Oncol 2005 April; 23(10):2346-57
Current Issues with ACT in Melanoma

1. Rapid Expansion Protocol (REP)
2. Choice of cytokine co-therapy

- Need for large cell numbers: (>10 billion for CR) → WHY?
- Cell expansion during REP may negatively affect ability of cells to respond to Ag re-stimulation in vivo.
- Little is known on TIL phenotypes and how they further differentiate and persist following melanoma Ag contact.
- Is IL-2 the best cytokine to work with?
Adoptive Cell Therapy (ACT): DC co-vaccination

- Lymphodepletion
- Rapid Expansion Protocol (REP)
- Ag-pulsed autologous mDC
- High-dose IL-2
Stimulation of Pre-REP and Post-REP TIL with Peptide-pulsed Mature DC

- Tumor
- Pre-REP
  - Medium + IL-2
  - FACS
- Post-REP
  - 200 U/ml IL-2
  - 7 days
  - FACS
  - Function
- DC + MART-1
- 7 days
Post-REP TIL proliferate less after Ag re-stimulation

CFSE labeling $\rightarrow$ DC + MART-1 stimulation $\rightarrow$ FACS analysis
$T_{EM} (CD8^{+}CD27^{+})$ lost upon REP
Post-REP MART-1-specific TIL: Loss of expansion after DC re-stimulation

Representative results of 3 independent TIL lines
Post-REP MART-1-specific TIL: Loss of expansion after DC re-stimulation
MART-1-specific CD27$^+$ cells expand following Ag-specific re-stimulation

Before DC stimulation

After DC stimulation

<table>
<thead>
<tr>
<th>CD27+ cells (x10$^5$)</th>
<th>CD27- cells (x10$^5$)</th>
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<tbody>
<tr>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>2.7</td>
<td>1.9</td>
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$\Rightarrow$ 4.5X

$\Rightarrow$ 1.3X
TIL after REP:

• Loss of $T_{EM} (CD27^+, CD28^+, CD57^-)$ phenotype.

• Loss of proliferative capacity after Ag stimulation.
IL-15 instead of IL-2?

- Common γc cytokine: Signaling through unique IL-15Rα.
- Supports survival of TEM and homeostatic expansion of memory CD8+ T cells.
- Can induce Granzyme B and perforin expression.
- Inefficient at T-reg cell expansion versus IL-2.
- Not used clinically so far.

DC stimulation of melanoma TIL with IL-15 versus IL-2 as cytokine support

DC + MART-1

200 U/ml IL-2 or 20 ng/ml IL-15

Medium + IL-2

200 U/ml IL-2 or 20 ng/ml IL-15

DC + MART-1
IL-15 is superior to IL-2 in expanding MART-1-specific TIL

- Pre-REP TIL.
- Stimulation with MART-1 peptide-pulsed mDC.
- 7-day incubation
IL-15 is superior to IL-2 in expanding MART-1-specific TIL.
IL-15 induces superior effector cells following melanoma Ag re-stimulation

IFN-γ secretion

![Bar chart showing IFN-γ secretion levels](chart)

- Pre-REP: IL-2 (433 pg/ml log), IL-15 (3221 pg/ml log)
- Post-REP: IL-2 (2 pg/ml log), IL-15 (182 pg/ml log)
IL-15 induces superior effector cells following melanoma Ag re-stimulation
IL-15 is superior:

- Expands and maintains $T_{EM}$ better than IL-2.
- Enhances CTL activity (more GB+ T cells).
- Capable of long-term expansion following DC re-stimulation (>3 week continued expansion).
- Effects are Ag-specific.
Proposed adoptive cell therapy approach with pre-REP TIL and DC co-vaccination

- TIL grown from tumor fragments
  - High % TEM (CD27+)
  - Better expansion \textit{in vivo}
  - Better CTL activity

\textit{in vitro REP}

- High-dose IL-2
- IL-15
- Dendritic cells + Ag

\textit{“in vivo REP” in the patient}
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