Adoptive T-cell Therapy

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Stimulating the body’s immune system against cancer: T-cells can kill tumor cells
Cytotoxic T-lymphocytes Can Recognize and Kill Tumor Cells

(From UVA)
Interleukin-2, a natural protein produced by T-helper cells, can stimulate cytotoxic T-cells to kill tumor cells.
Metastatic melanoma treated with IL-2
Response to high dose IL-2

Pre IL-2

Post IL-2
Response to high dose IL-2
IL-2 therapy is effective in some patients with metastatic melanoma

<table>
<thead>
<tr>
<th>Total Number of Patients</th>
<th>Clinical Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>134</td>
<td>23 (17%)</td>
</tr>
</tbody>
</table>
Vaccines stimulate the proliferation of T-cells in vivo
# Active Immunization of Patients with Metastatic Melanoma

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Total (number of patients)</th>
<th>Objective response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recombinant viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus (MART-1 or gp100)</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Vaccinia (MART-1 or gp100)</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Fowlpox (MART-1 or gp100)</td>
<td>48</td>
<td>1</td>
</tr>
<tr>
<td>Fowlpox (ESgp100:209-2M)</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Vaccinia + Fowlpox (tyrosinase)</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Naked DNA</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Dendritic cells (IV; peptide pulsed)</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><strong>Peptides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MART-1</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>gp100 (154, 209, 280)</td>
<td>28</td>
<td>1</td>
</tr>
<tr>
<td>gp100:209-2M*</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td>Her-2/neu</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>gp100:ES-209-2M</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Non A2 peptides (A1, A3, A24, A31, Cw7)</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Class I &amp; II gp100</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Telomerase</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>TRP-2</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>MART-1 + gp100 (multiple)</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>gp100 + MART + Flt3L</td>
<td>31</td>
<td>0</td>
</tr>
</tbody>
</table>

*alone or with GMCSF or IL-12

Total: 570

12 (2.1%)
## Potentially Targetable Immunoregulatory Molecules

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Cellular Expression</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTLA-4</td>
<td>Helper T, Cytotoxic T</td>
<td>Provides co-inhibitory signaling during naïve T-cell priming</td>
</tr>
<tr>
<td></td>
<td>T-reg</td>
<td>Induces local tryptophan metabolism by DCs, inhibiting T-cell proliferation</td>
</tr>
<tr>
<td>PD-1</td>
<td>Helper T, Cytotoxic T</td>
<td>Inhibits T-cell proliferation, cytokine production and cytotoxicity</td>
</tr>
<tr>
<td>IL-10</td>
<td>Tumor, TR1</td>
<td>Regulates growth and differentiation of a wide variety of immune cells</td>
</tr>
<tr>
<td>IL-13</td>
<td>iNKT</td>
<td>Induces immature myeloid cells to produce TGF-β</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Tumor, TR1, Treg, Immature myeloid</td>
<td>Directly suppresses proliferation of antigen-activated T cells</td>
</tr>
<tr>
<td>VEGF</td>
<td>Tumor</td>
<td>Blocks DC differentiation and maturation, leading to accumulation of iDC and iMC</td>
</tr>
<tr>
<td>IDO</td>
<td>Tumor, Dendritic</td>
<td>Depletes local tryptophan, inhibiting T-cell proliferation</td>
</tr>
<tr>
<td>ARG1</td>
<td>Tumor, Immature myeloid</td>
<td>Depletes local arginine, inhibiting CD3ζ expression and T-cell activation</td>
</tr>
<tr>
<td>iNOS</td>
<td>Tumor, Immature myeloid</td>
<td>Generates nitric oxide, inhibiting T-cell priming, proliferation, and cytotoxicity</td>
</tr>
</tbody>
</table>
T-cell Therapy

Infusion of T-cells that are first manipulated in the laboratory.

– Activation
– Expansion
– Subset selection
– Gene transduction
Advantages of T-cell Therapy

• Avoids immunoregulatory environment present in cancer patients
  – Use of T-cells from donor
  – T-cells manipulated ex-vivo

• Increases number of antigen-specific T-cells
  – Post transplant T-cell recovery can be slow
  – A high level of expansion is possible ex-vivo

• Allows control over phenotype of cells that are infused
  – Antigen specificity
  – Activation state
Evidence that T-cell therapy is effective

- Prevention of viral infection post transplant
- Donor Lymphocyte Infusion (DLI) to enhance graft vs tumor effect
- Treatment of melanoma with TIL therapy
Generation of virus-specific CTL

After 3rd or 4th stimulation analyze CTL lines ---> Freeze & QA/QC testing

CM Bollard, MD
Baylor College of Medicine
Houston, Texas
Clinical Outcome - CMV

CMV copies/ml

-3wk -2wk CTL +1wk +4wk +6wk +7wk +8wk +9wk

%pp65 tetramer positive CD8+ve T cells

CMV PCR
CMV pentamer

CM Bollard, MD
Baylor College of Medicine
Houston, Texas
Reduction in EBV load post-CTL and rise in EBV CTLp

CM Bollard, MD
Baylor College of Medicine
Houston, Texas
Diagnosis of PTLD 2 months later

Resolution of liver lesion – no further therapy required

CM Bollard, MD
Baylor College of Medicine
Houston, Texas
Evidence that T-cell therapy is effective

- Prevention of viral infection post transplant

- Donor Lymphocyte Infusion (DLI) to enhance graft vs tumor effect

- Treatment of melanoma with TIL therapy
Traditional Myeloablative Stem Cell Transplant

Conditioning + Donor Graft

Recipient Donor

Recipient Donor

Post-SCT

K. Komanduri
T cell depletion decreases GVHD incidence, but increases risk of relapse

### Response of Chronic Myeloid Leukemia to DLI

<table>
<thead>
<tr>
<th>Disease Stage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetic relapse</td>
<td>43/53 (81%)</td>
</tr>
<tr>
<td>Hematologic relapse</td>
<td>113/148 (76%)</td>
</tr>
<tr>
<td>Transformed phase</td>
<td>18/54 (33%)</td>
</tr>
<tr>
<td>All</td>
<td>174/255 (68%)</td>
</tr>
</tbody>
</table>

Luznik and Fuchs. Cancer Control 9(2):123-137
### Response of Acute Myeloid Leukemia, Acute Lymphocytic Leukemia, and Myelodysplasia to DLI Alone

<table>
<thead>
<tr>
<th>Disease</th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Myeloid Leukemia</td>
<td>18/81</td>
<td>(22%)</td>
</tr>
<tr>
<td>Acute Lymphocytic Leukemia</td>
<td>3/37</td>
<td>(8%)</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>5/14</td>
<td>(36%)</td>
</tr>
</tbody>
</table>

Luznik and Fuchs. Cancer Control 9(2):123-137
GVHD and response of chronic phase Chronic myeloid leukemia to DLI

<table>
<thead>
<tr>
<th>Grade of GVHD</th>
<th>Studied</th>
<th>Responding</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>93</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td>I</td>
<td>38</td>
<td>29</td>
<td>76</td>
</tr>
<tr>
<td>II</td>
<td>51</td>
<td>46</td>
<td>90</td>
</tr>
<tr>
<td>III</td>
<td>19</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>6</td>
<td>75</td>
</tr>
</tbody>
</table>

P ≤ .0001

Luznik and Fuchs.
Cancer Control 9(2):123-137
Strategies to Separate GVHD from GVL

- Infuse antigen-specific T-cells
- Deplete alloreactive T-cells from infused cells
- Insert suicide gene
Evidence that T-cell therapy is effective

- Prevention of viral infection post transplant
- Donor Lymphocyte Infusion (DLI) to enhance graft vs tumor effect
- Treatment of melanoma with TIL therapy
Adoptive Cell Therapy (ACT) with antigen specific T-cells

- Surgical Removal of Cancer Nodule
- Single Cell Suspension Incubated with IL-2
- T Cells Proliferate
- Cancer Cells Die
- IL-2
- T Cells
Growing melanoma metastasis in gall bladder fossa, resected for TIL
MART-1 and gp100 reactive TIL in fresh melanoma biopsy DE

**MART-1 tetramer**

**gp100 tetramer**

**CMV tetramer (control)**

**CD8+ CD27+**

**CD8+ MART-1+**

**CD8+ CD27+ MART-1+**
Producing an effective cancer vaccine will require a deep understanding of interactions between the “Players” that make up the immune system “Team”
Melanoma tumor-infiltrating Foxp3+ cells

Foxp3 - Histology
(MT #712)
Multiple regulatory immune cell subsets have been shown to suppress antitumor immunity

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Effector functions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-reg (CD4+CD25+)</td>
<td>Inhibition of CD4(^+) and CD8(^+) T-cell proliferation via direct cell-to-cell interactions (involving CTLA-4, GITR?)</td>
<td>Sakaguchi, <em>Nature Immunology</em> 2005</td>
</tr>
<tr>
<td>Tr1 (CD4+CD25-)</td>
<td>Suppression of naïve and memory T-cell responses through production of high levels of IL-10 and TGF-β</td>
<td>Levings et al., <em>J Experimental Medicine</em> 2002</td>
</tr>
<tr>
<td>Immature myeloid</td>
<td>Inhibition of IFN-γ production by CD8(^+) T cells mediated by reactive oxygen species (eg. H(_2)O(_2))</td>
<td>Grabilovich, <em>Nature Rev Immunology</em> 2004</td>
</tr>
<tr>
<td>Invariant NKT</td>
<td>Cytokine release (diverse Th1 and Th2) <em>May prevent or activate antitumor immunity</em></td>
<td>Wilson and Delovitch, <em>Nature Rev Immunology</em> 2003</td>
</tr>
</tbody>
</table>
Elimination of regulatory cells with chemotherapy prior to T-cell transfer

Tumor Reactive Cytotoxic T-Lymphocytes

Chemotherapy

Normal Lymphocytes and Treg
Infused T-lymphocytes persist when administered following lymphodepletion with chemotherapy.
Clinical response following lymphodepletion + T-lymphocyte infusion
Clinical response following lymphodepletion + T-lymphocyte infusion
Clinical response following lymphodepletion + T-lymphocyte infusion

<table>
<thead>
<tr>
<th># Patients Enrolled</th>
<th>CR</th>
<th>PR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>4</td>
<td>14</td>
<td>18 (51%)</td>
</tr>
</tbody>
</table>

Science. 2002 Oct 25;298(5594); J Clin Oncol 2005 April; 23(10):2346-57
Challenges of adoptive cell therapy

- Rigorous therapy requiring excellent performance status
- Accessible tumor required to generate TIL
- Adequate numbers of tumor specific T-cells are only generated in approximately 40% of patients
- 4 – 6 weeks are required for the generation of T-cells
- Migration of T-cells to the tumor is suboptimal
Improving adoptive immunotherapy

• Enhance T-cell persistence
• Improve migration to tumor
• Improve recognition of the tumor
DCs Increase the Numbers of Infused T-Cells in Blood and Spleen
Survival is Increased in a Mouse Model by Combining Dendritic Cells & T-cells

% survival

days after treatment

caption: no treatment

pmel-1 + DC/hgp100 + IL-2

pmel-1 + DC/np + IL-2

DC/hgp100 + IL-2

pmel-1 + IL-2

pmel-1 + DC/hgp100
Improving adoptive immunotherapy

- Enhance T-cell persistence
- Improve migration to tumor
- Improve recognition of the tumor
Transduction of T-cells with receptors to enable them to “See” tumor vasculature
Improving adoptive immunotherapy

- Enhance T-cell persistence
- Improve migration to tumor
- **Improve recognition of the tumor**
Transduction of T-cells with receptor genes to direct T-cell specificity
Cancer regression in patients after transfer of genetically engineered lymphocytes

Morgan RA, et al.  
Science 314:126-129
Summary

• The infusion of antigen specific T-cells can:
  - be effective in patients to induce tumor regression.
  - decrease viral infections post-transplant.

• T-cell therapy can be more potent than cytokine or vaccine therapy, possibly because expansion and activation of T-cells can be controlled in the laboratory in a non-immunosuppressive environment.

• Future studies will rely on rational combinations of adoptive therapy with active immunization, as well as with other immune adjuvants and targeted therapies.