Adoptive Immunotherapy

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Cell Therapy

- Red cells
- Stem cells
- Platelets
Adoptive T Cell Transfer

• Potent therapy
• Unique research tool
  – Host lymphodepletion
  – Lymphocyte attributes
  – Tumor antigen targets
## Examples of Successful Adoptive Immunotherapy

<table>
<thead>
<tr>
<th>Target</th>
<th>T cells</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTLD</td>
<td>Viral antigen specific</td>
<td>Heslop, 2009</td>
</tr>
<tr>
<td>Viral infection</td>
<td>Viral specific (uncultured)</td>
<td>Feuchtinger et al, 2010</td>
</tr>
<tr>
<td>B cell tumor</td>
<td>Genetically retargeted</td>
<td>Kalos et al 2011</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Tumor infiltrating lymphocytes</td>
<td>Rosenberg et al 2011</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Genetically retargeted</td>
<td>Robbins et al 2011</td>
</tr>
</tbody>
</table>
Adoptive Cell Therapy with TIL for Melanoma

Dudley et al, Nat Rev Ca, 2003
ACT with Lymphodepletion

- Expand T cells to large numbers in the absence of potentially suppressive tumor environment
- Selection of optimal T cell attributes
- Manipulation of host immune system without damaging the effector T cells
Ensuring a Safe Product for ACT

All cells undergo extensive safety and quality testing to produce a certificate of analysis (COA) for each final product prior to infusion.

**COA Criteria:**

- Viable cell count
- Tumor recognition
- Gram stain
- Fungal testing
- Mycoplasma testing
- Bacterial testing
- Endotoxin testing
- Cytological analysis
- TCR expression
- RCR

www.piercenet.com/media/cytokine_kit.gif
www.daigger.com http://isips.org/products/graphics/12a.gif
• ACT with lymphodepletion
  – Responses can be rapid
  – No relation between bulk of disease and response
  – Impact metastatic disease at any site
Adoptive T Cell Transfer

- Potent therapy
- Unique research tool
  - Host lymphodepletion
  - Lymphocyte attributes
  - Tumor antigen target
Adoptive Cell Therapy
Selected T cells
Lymphodepletion

![Graph showing lymphocyte levels over time with treatment phases including Bulk TIL, start, Rapid Expansion, harvest, Fludarabine, and IL-2.](image)
Mechanisms underlying the impact of lymphodepletion on adoptively transferred T cells

Chemotherapy or Radiation therapy
Lymphodepletion

• Elimination of T\textsubscript{REG}
• Increased homeostatic cytokines
• APC activation through TLR
• Stem cell “facilitation”
T cells are active in vivo

Specifically lytic

Phenotypically active

Effector: target ratio

Specific lysis

526 mel (HLA-A2+)

938 mel (HLA-A2-)

- pre
- pt 7
- TIL

Patient 10

938 mel (HLA-A2-)

526 mel (HLA-A2+)
Persistence of Transferred TIL

- A2/MART tetramer
- TCR VB12
- TCR VB14
- average of all other VB

percent of CD8+ lymphocytes

PRE 35 85 135
Transferred T cells up-regulate expression of HLA by tumor cells *in vivo*

- CD8
- MHC Class I
- MHC Class II

Pre treatment

56 days post
Impact of Lymphodepletion

- Functional and phenotypic activation of transferred T cells
- Proliferation and persistence
- Traffic to tumor and up-regulation of HLA on tumor cells
- Regression of bulky tumor masses
Adoptive immunotherapy – a unique research tool

- Host Conditioning
- T cell attributes
- Target selection
Attributes of T cells Associated with Response

- Minimum time in culture
- Persistence in vivo after transfer
- Replicative potential
  - Long telomeres
- Less differentiated phenotype
  - High expression of CD27, CD28
Potent immune responses require CD8+ and CD4+ cells

- Antigen Presenting Cells (APC) activate both CD8+ CTL and CD4+ T_{helper} cells.
- At a site of inflammation, T_{helper} cells secrete IL-2 to further activate and cause proliferation of CTL.
- T_{REG} dampen immune responses and inactivate CTL.
- In ACT, chemotherapy eliminates endogenous T cells. Tumor reactive CTL are administered with exogenous IL-2.
What is the role of CD4 cells

- CD4+ T-regs limit CD8+ ACT effectiveness in mouse models
- Anecdotal examples of CD4+ T cells correlated potent in vivo tumor rejection
- CD8+ TIL number is associated with response
CD8 enrichment of TIL for Therapy

Young TIL
Optimized Clinical scale CD8+ enrichment of TIL using CliniMACS
CD8+ Cells can Mediate Tumor Regression

- 56 patients treated
  - 33 with chemotherapy lymphodepletion
  - 23 with TBI and chemo lymphodepletion
- 29 (52%) objective responders
Adoptive immunotherapy – a unique research tool

- Host Conditioning
- T cell attributes
- Target selection
TCR-Transduced Peripheral Blood Lymphocytes (PBL)

- HLA-A2 patients only
- Uses PBMC pheresis prior to start
- Defective virus “infects” patient lymphocytes
- Engineered genes insert into lymphocyte genome
- New protein receptors are expressed (TCR)
- T cells start hunting tumor cells

PBMC Pheresis → Transduce cells → Expand and test

http://flickr.com/photos/jepoirrier/385575933/
Development of TCR gene therapy

From Rosenberg et al, Nat. Rev. Cancer April 2008
Anti-tumor antigen receptor containing retroviral vectors

Target Tumor Antigen

- gp100:154
- MART-1 (DMF5)
- NY-ESO-1

MART-1 and GP100

- Melanocyte differentiation antigens
- Expressed by tumors and normal melanocytes
  - Skin, eyes, ears
- TIL – low/no toxicity
- Moderate affinity “F4” TCR – no toxicity

- “F5” and g154 TCRs selected for high affinity antigen recognition
Day 8: CD8 positive cells
Melan-A positive control
Day 8: Melan-A (rare specific staining)
## SB Clinical Trials with MDA TCR

### Table: Response and Toxicity

<table>
<thead>
<tr>
<th>TCR</th>
<th>Total</th>
<th>OR</th>
<th>Skin</th>
<th>Uveitis</th>
<th>Auditory</th>
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</thead>
<tbody>
<tr>
<td>MART-1TCR (DMF5)</td>
<td>20</td>
<td>6 (30%)</td>
<td>11/3/0</td>
<td>2/9/0</td>
<td>2/0/7</td>
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<tr>
<td>gp100TCR (gp154)</td>
<td>16</td>
<td>3 (19%)</td>
<td>11/4/0</td>
<td>0/4/0</td>
<td>2/2/3</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>9 (25%)</td>
<td>22/7/0 (81%)</td>
<td>2/13/0 (42%)</td>
<td>4/2/3 (25%)</td>
</tr>
</tbody>
</table>

*Trials performed at the Surgery Branch, NCI. Response based on RECIST. Toxicity graded as shown below:

**Grade 1**
- Skin: Erythema
- Eye: No symptoms
- Ear: 15–25 dB, 2 freq.

**Grade 2**
- Skin: Desquamation ≤50%
- Eye: Anterior
- Ear: >25 dB, 2 freq.

**Grade 3**
- Skin: Desquamation >50%
- Eye: Pan uveitis
- Ear: >25 dB, 3 freq.

* Morgan et al. *The Cancer Journal* • Volume 16, Number 4, July/August 2010

### Notes
- 20-30% objective Response
- ~70% Grade 2-3 Toxicity
Anti-tumor antigen receptor containing retroviral vectors

Target Tumor Antigen

NY-ESO-1 TCR

- NY-ESO-1 – Cancer/Testes antigen
- Not expressed in normal adult tissues except testes (no class I expression)
- Expressed by 10-50% of tumors of multiple histologies including melanoma, breast, prostate, thyroid and ovarian.
- Expressed by ~90% of synovial cell sarcomas
- Eso TCR has an alpha chain CDR3 modification
Patients and outcomes for Eso TCR therapy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Sites of Disease</th>
<th>Response†</th>
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<tbody>
<tr>
<td><strong>Melanoma</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>52</td>
<td>M</td>
<td>ln</td>
<td>PR (8)</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>sc, lu</td>
<td>PD</td>
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<tr>
<td>3</td>
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<td>F</td>
<td>bo, ln, panc, sb</td>
<td>PD</td>
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<tr>
<td>4</td>
<td>56</td>
<td>M</td>
<td>lu, ki</td>
<td>CR (22+)</td>
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<tr>
<td>5</td>
<td>32</td>
<td>M</td>
<td>ln</td>
<td>CR (20+)</td>
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<tr>
<td>6</td>
<td>38</td>
<td>M</td>
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<td>PR (3)</td>
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<tr>
<td>7</td>
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<td>PD</td>
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<td>11</td>
<td>46</td>
<td>M</td>
<td>lu, li</td>
<td>PR (9+)</td>
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<td><strong>Synovial cell sarcoma</strong></td>
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<tr>
<td>12†</td>
<td>20</td>
<td>M</td>
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<tr>
<td>13†</td>
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<td>F</td>
<td>lu</td>
<td>PR (18)</td>
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<td>15†</td>
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<td>pl, hi</td>
<td>PR (8)</td>
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<tr>
<td>17</td>
<td>40</td>
<td>M</td>
<td>pl, hi</td>
<td>PD</td>
</tr>
</tbody>
</table>
ESO TCR

• 5/11 responses in melanoma
• 4/6 responses in synovial sarcoma
• No toxicity related to cells
Adoptive T Cell Transfer

• Potent therapy
  – Clinical responses in refractory disease
  – Break tolerance to self antigens

• Unique research tool
  – Patient, T cells, tumor