REMEMBERING

Dr. E. Donnall Thomas

1920 - 2012

1990 Nobel Laureate
Father of Bone Marrow Transplantation
Adoptive T Cell Therapy: *Faster, Higher, Stronger*

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Program in Immunology  
Clinical Research Division  

Professor  
University of Washington  
Division of Oncology  
Department of Medicine  

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Tumor Immune Surveillance

Normal

Tumor

CD4

CD8

DC

DC

DC

DC
Possible Reasons For Failure of Tumor Immune Surveillance

Immune evasion

Low numbers
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Immune evasion

Low numbers
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells

Low numbers
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells

More of them
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells - stronger

More of them - higher
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells - *stronger*

More of them - *higher*

Expedience - *faster*
Peripheral Blood Source > 80 %

Enrichment

APC + peptide

> 80 %

Expansion > 80 %

12-14 weeks

10^6

10^{10}
Adoptive T Cell Therapy: Basic Protocol

**Isolate/Enrich**

**Clone/Select**

**(Genetically Modify)**

**Expand**

**Pre-treatment**

**Staging CT**

**LD IL-2**

**Follow-up**

**Staging CT**

Interval blood draws for persistence

*PNAS Yee et al. 2002*
Adoptive CD8 T Cell Therapy for Melanoma

Safety

Persistence

Efficacy

PNAS 2002
Adoptive T Cell Therapy: Basic Protocol

**Isolate/Enrich**

**Clone/Select**

**Genetically Modify**

**Intrinsic**

**Extrinsic**

Pre-infusion Immunomodulation

Post-infusion Immunomodulation

PNAS Yee et al. 2002
Adoptive T Cell Therapy: Extended Protocol

**Isolate/Enrich**
- Cytokine modulation

**Clone/Select**
- Phenotype
  - CD8/CD4
  - Memory phenotype

**Genetically Modify**
- TCR
- Chimeric receptor
- Costimulatory/Inhibitory modification
- Suicide gene

**Pre-infusion Immunomodulation**
- Lymphodepletion
  - Chemotherapy/TBI

**Post-infusion Immunomodulation**
- Cytokine help
  - Low-dose IL-2
  - High-dose IL-2
  - Other γ-chain receptor cytokines
- Anti-CTLA4, Anti PD-1
- Vaccine + adoptive therapy

Lymphoid Homeostasis
Lymphodepletion
building a better environment

Increase 'space' for transferred T cells
Eliminate 'suppressor cells'
Supply Growth Factors

Increase 'space' for transferred T cells
Eliminate 'suppressor cells'
Supply Growth Factors
Adoptive Therapy following Lymphodepletion

- CY: 60 mg/kg x 2
- FLU: 25 mg/m² x 5
- TBI
- High-Dose IL-2 (600,000 u./kg q8)
Adoptive Therapy following Lymphodepletion

- **CY**
  - 60 mg/kg x 2

- **FLU**
  - 25 mg/m² x 5

- **TBI**

- **Low-Dose IL-2**
  - 250,000 U s.c q12 h
Adoptive Therapy following Lymphodepletion

**Objectives:**
- Evaluate Safety
- Evaluate T Cell Persistence
- Evaluate anti-tumor efficacy

**Eligibility Criteria:**
- Stage IV (Metastatic)
- HLA-A2

**T Cell Infusion:**
- Antigen-specific CD8+ T cell clones
- Targeting MART-1, gp100
- Dose: $10^{10}$ cells / m²

**CY**
60 mg/kg x 2

Low-Dose IL-2 (250,000 U s.c q12 h)
Transferred melanoma-specific CD8⁺ T cells persist, mediate tumor regression, and acquire central memory phenotype

Aude G. Chapuis, John A. Thompson, Kim A. Margolin, Rebecca Rodmyre, Ivy P. Lai, Kaye Dowdy, Erik A. Farrar, Shailender Bhatia, Daniel E. Sabath, Jianhong Cao, Yongqing Li, and Cassian Yee

Program in Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; General Oncology and Hematology, Seattle Cancer Care Alliance and University of Washington, Seattle, WA 98109; and Department of Laboratory Medicine, University of Washington, Seattle, WA 98195

Proc Natl Acad Sci USA
March 5, 2012
## Target and Disease Sites

<table>
<thead>
<tr>
<th>Patient</th>
<th>Target</th>
<th>Toxicity</th>
<th>Persistence</th>
<th>Disease Sites</th>
<th>Response</th>
</tr>
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<tbody>
<tr>
<td>2140-1</td>
<td>Tyrosinase</td>
<td></td>
<td></td>
<td>Cervical, supraclavicular LN, Chest Wall, Breast Pulmonary nodules</td>
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<td>Mediastinal, Pulmonary nodules</td>
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<tr>
<td>2140-3</td>
<td>gp100</td>
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<td>Mesenteric LN, scapular subcutaneous dz</td>
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<tr>
<td>2140-4</td>
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<td>Pulmonary, inguinal, subcutaneous</td>
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<tr>
<td>2140-5</td>
<td>MART-1</td>
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<td>Right and left kidneys, adrenal, liver</td>
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<tr>
<td>2140-6</td>
<td>MART-1</td>
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<td></td>
<td>Mediastinal, supra clavicular, mammary chain, periportal, portacaval nodes.</td>
<td></td>
</tr>
</tbody>
</table>
Toxicity
Toxicity

- “interstitial inflammatory infiltrate consisting predominantly of lymphocytes and plasma cells with admixed eosinophils, focal neutrophils and melanophages.”
- CD20 CD20 [L26] Rare positive cells
- CD3 CD3 [LN10] Positive, 3+ (> 75% of cells)
- CD4 CD4 [1F6] Minority of lymphocytes in epidermis
- CD8 CD8 [4B11] Positive, 2+ (25 - 75% of cells) Majority of lymphocytes in epidermis
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<td>2140-1</td>
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Persistence

2140-1

2140-2
Persistence

CD45 RO+ CD28++ CD127-hi

CD45 RO+ CD28- CD127-lo
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<td></td>
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### Clinical Response

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<th>Patient</th>
<th>Target</th>
<th>Toxicity</th>
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<th>Disease Sites</th>
<th>Response</th>
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<td>&gt;290 days</td>
<td>Cervical, supraclavicular LN, Chest Wall, Breast Pulmonary nodules</td>
<td>MR</td>
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<td>Tyrosinase</td>
<td>F</td>
<td>16 days</td>
<td>Mediastinal, Pulmonary nodules</td>
<td>PD</td>
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<td>2140-3</td>
<td>gp100</td>
<td>F,N,R</td>
<td>&gt;85 days</td>
<td>Mesenteric LN, scapular subcutaneous dz</td>
<td>CR (&gt; 12 mths)</td>
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<td>MART-1</td>
<td>F, N, R</td>
<td>&gt; 30 days</td>
<td>Pulmonary, inguinal, subcutaneous</td>
<td>SD</td>
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<td>2140-5</td>
<td>MART-1</td>
<td>F, N,R</td>
<td>&gt; 30 days</td>
<td>Right and left kidneys, adrenal, liver</td>
<td>PR</td>
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<td>2140-6</td>
<td>MART-1</td>
<td>F, N, R</td>
<td>&gt; 30 days</td>
<td>Mediastinal, supra clavicular, mammary chain, periportal, portacaval nodes.</td>
<td>PR</td>
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</tbody>
</table>
CD8 T Cell Therapy Following Cytoxan Conditioning

• Extended duration of in vivo persistence
  – High-dose Cytoxan
  – CD8+ T cell clones
  – IL-2

• Durable Clinical Responses?
Persistence

2140-1

0  50  100  150  200  250  300

Days post Infusion

0  50000  100000  150000  200000  250000

Persistence of CD45 RO+ CD28++ CD127-hi
Can we enrich for or modulate the phenotype of antigen-specific CTL during in vitro priming?

- CTL (CD62L+ 45RA+)
- Dendritic Cells
- MART-1 peptide
- +IL-2
- +IL-7
- +IL-15
- +IL-21

- STIM 1
- IL-2 +IL-7

- STIM 2
- IL-2 +IL-7

Frequency
Surface Phenotype
Functional Assays
Affinity
Greater Frequency
(ABS# 20-30-fold)

Improved Function
(by peptide titration and Tetramer dissociation)

Li et al, J Immunol 2005
Li et al, Blood 2008
CTRL | IL-21

0.34 | 19.0

CTRL IL-21

CD28

CD28 (D27)
<table>
<thead>
<tr>
<th></th>
<th>CD45R0</th>
<th>CD62L</th>
<th>CD127</th>
<th>CD28</th>
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<tr>
<td>Naïve</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Central M.</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Effector M.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

**Helper-Independence**

![Graph showing interleukin-2 levels](image)

- **T2 unpulsed**
- **T2 + M27pept**
- **T2 + M27pept + CTLA4-Ig**
Synergism with CD25 depletion

A

Control

IL-21

B

<table>
<thead>
<tr>
<th></th>
<th>Experiment. 1</th>
<th>Experiment. 2</th>
<th>Experiment. 3</th>
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<tr>
<td></td>
<td>Absolute number (x 10⁶)</td>
<td>Absolute number (x 10⁶)</td>
<td>Absolute number (x 10⁶)</td>
</tr>
<tr>
<td></td>
<td>/ Fold increase ( vs control)</td>
<td>/ Fold increase ( vs control)</td>
<td>/ Fold increase ( vs control)</td>
</tr>
<tr>
<td>Control</td>
<td>1.45 / 1</td>
<td>1.68 / 1</td>
<td>0.45 / 1</td>
</tr>
<tr>
<td>IL-21</td>
<td>17.14 / 11.82</td>
<td>23.17 / 13.79</td>
<td>8.37 / 18.60</td>
</tr>
<tr>
<td>CD25 depletion</td>
<td>8.20 / 5.66</td>
<td>12.64 / 7.52</td>
<td>4.35 / 9.67</td>
</tr>
<tr>
<td>CD25 depletion + IL-21</td>
<td>244.94 / 168.92</td>
<td>462.65 / 275.38</td>
<td>141.00 / 313.33</td>
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</tbody>
</table>
Figure 2

A

WT-1 (WT126)

NY-ESO1 (NY157)

B

<table>
<thead>
<tr>
<th></th>
<th>WT-1 (WT126)</th>
<th>NY-ESO1 (NY157)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute number (x 10^6)</td>
<td>/ Fold increase (vs control)</td>
</tr>
<tr>
<td>Control</td>
<td>0.023 / 1</td>
<td>0.558 / 1</td>
</tr>
<tr>
<td>IL-21</td>
<td>0.413 / 17.96</td>
<td>5.144 / 9.21</td>
</tr>
<tr>
<td>CD25 depletion</td>
<td>0.203 / 8.83</td>
<td>6.659 / 11.93</td>
</tr>
<tr>
<td>CD25 depletion + IL-21</td>
<td>5.928 / 257.74</td>
<td>56.78 / 101.76</td>
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</tbody>
</table>
IL-21 modulation of CTL

- higher in vitro frequency of rare TAA-specific CTL
  - Synergistic increase with in vitro CD25 depletion
- enhanced affinity/cytolytic function
- central memory/ helper-independent phenotype
- active on naïve CTL → program stable phenotype/early exposure, dose-dependent
Addressing Possible Reasons For Failure of Tumor Immune Surveillance

Highly functional T cells - *stronger*

More of them - *higher*

Expedience - *faster*
Clinical Grade pMHC-multimer-based Sorting
Expedience

Clones

Patient

Isolate/Enrich (2-4 weeks)

Cloning (2 weeks)

Expand (4 weeks)

10-12 weeks

Patient

Polyclonal lines

Patient

Isolate/Enrich (~2 weeks)

Multimer Sort Influx BD (Hours)

Expand (2-4 weeks)

4-6 weeks

Patient

Clones

Polyclonal lines
IL-21 modulation generates population of TAA-specific Tcm
Adoptive T Cell Therapy: Extended Protocol

**Isolate/Enrich**
- Cytokine modulation

**Clone/Select**
- Phenotype
  - CD8/CD4
  - Memory phenotype

**Genetically Modify**
- TCR
- Chimeric receptor
- Costimulatory/Inhibitory modification
- Suicide gene

**Pre-infusion Immunomodulation**
- Lymphodepletion
  - Chemotherapy/TBI

**Post-infusion Immunomodulation**
- Cytokine help
  - Low-dose IL-2
  - High-dose IL-2
  - Other γ-chain receptor cytokines
- Anti-CTLA4, Anti PD-1
- Vaccine + adoptive therapy

2. Anti-CTLA4 (Ipilimumab)

Mechanism of action of anti-CTLA-4 on CD8 T cells:
Phase I/II Clinical Trial of Adoptive Therapy using IL-21-treated Antigen-specific CTL In combination with anti-CTLA4 Antibody Treatment

Primary Aims
• Safety of anti-CTLA4 and CTL
• Influence of anti-CTLA4 and CTL on persistence and anti-tumor efficacy

Secondary Aim
• Influence of anti-CTLA4 and adoptively transferred CTL targeting melanoma on epitope-spreading

Eligibility
• Metastatic melanoma – measurable disease
• Expression of HLA A*0201

CTL Infusion (D0) Anti-CTLA4 (D1) Anti-CTLA4 (D22/W3) Anti-CTLA4 (D43/W6) Anti-CTLA4 (D64/W9) D85/W12

Low-dose CY Low-dose IL-2 (14 days)
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Sex</th>
<th>Previous Treatments</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>59 M</td>
<td>Surgery, IFN, HD IL-2, T-cell clones (#2179), <em>ipilimumab</em>.</td>
</tr>
<tr>
<td>2</td>
<td>66 F</td>
<td>Surgery, HD IL-2, Cisplatin + ALT 801 (anti-p53 antibody linked to IL2), <em>ipilimumab</em>.</td>
</tr>
<tr>
<td>3</td>
<td>33 M</td>
<td>Surgery, HD IL-2, <em>ipilimumab</em> x 2.</td>
</tr>
<tr>
<td>4</td>
<td>39 M</td>
<td>Surgery, HD IL-2.</td>
</tr>
<tr>
<td>5</td>
<td>46 M</td>
<td>Surgery x 2.</td>
</tr>
<tr>
<td>6</td>
<td>63 F</td>
<td>Surgery x 2.</td>
</tr>
<tr>
<td>7</td>
<td>68 F</td>
<td>Surgery x 2</td>
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</table>
# IL-21- CTL + anti-CTLA4

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, Sex</th>
<th>Previous Treatments</th>
<th>Disease Site</th>
<th>Prior Ipiillumumab Failure</th>
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<tr>
<td>1</td>
<td>59 M</td>
<td>Surgery, IFN, HD IL-2, T-cell clones (#2179), <strong>ipilimumab</strong>.</td>
<td>Subcarinal, hilar, paratracheal LAD.</td>
<td>YES</td>
</tr>
<tr>
<td>2</td>
<td>66 F</td>
<td>Surgery, HD IL-2, Cisplatin + ALT 801 (anti-p53 antibody linked to IL2), <strong>ipilimumab</strong>.</td>
<td>Pulmonary nodes</td>
<td>YES</td>
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<tr>
<td>3</td>
<td>33 M</td>
<td>Surgery, HD IL-2, <strong>ipilimumab x 2</strong>.</td>
<td>Pre-tracheal, hilar, pulmonary, liver, pancreatic, pelvic LAD.</td>
<td>YES</td>
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<tr>
<td>4</td>
<td>39 M</td>
<td>Surgery, HD IL-2.</td>
<td>Subcutaneous neck, gluteus, left flank and lung nodes.</td>
<td>NO</td>
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<tr>
<td>5</td>
<td>46 M</td>
<td>Surgery x 2.</td>
<td>Cutaneous lower left extremity</td>
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<tr>
<td>6</td>
<td>63 F</td>
<td>Surgery x 2.</td>
<td>Lung nodes, pelvic LAD.</td>
<td>NO</td>
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<tr>
<td>7</td>
<td>68 F</td>
<td>Surgery x 2</td>
<td>Left inguinal LAD, cutaneous nodes.</td>
<td>NO</td>
</tr>
</tbody>
</table>
IL-21- CTL + anti-CTLA4

• Treatment has been overall well tolerated
• 5/6 patients developed mild rashes within 1 week of CTL infusion and 1 patient developed vitiligo at ~12 weeks.
• Main side effects observed have been related to ipilimumab (dry skin, GI symptoms, elevated liver enzymes).
• 1 patient received Vemurafenib for progressive disease 2 weeks after last dose of ipilimumab and developed fevers/rash necessitating hospitalization.
IL-21-derived polyclonal CTL persist in vivo \( \geq 42 \) days (multimer)
Assessment of epitope spreading: method

- Designed peptide libraries spanning MART-1, NY-ESO1, Gp100, Tyrosinase, Mage A3
- 15mers with 4 amino-acid offset: Long peptide sequence with short offset number = most chances of multiple epitope hits.
- Arranged in matrix (Eg. NY-ESO-1):

```
+---+---+---+---+---+---+---+
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
+---+---+---+---+---+---+---+
| 8 | 9 |10 |11 |12 |13 |14 |
+---+---+---+---+---+---+---+
|15 |16 |17 |18 |19 |20 |21 |
+---+---+---+---+---+---+---+
|22 |23 |24 |25 |26 |27 |28 |
+---+---+---+---+---+---+---+
|29 |30 |31 |32 |33 |34 |35 |
+---+---+---+---+---+---+---+
|36 |37 |38 |39 |40 |41 |42 |
+---+---+---+---+---+---+---+
```

- PBMC reactivity to individual peptide pools tested by IFNγ Elispot.
- Results expressed spots/100,000 PBMC
Assessment of epitope spreading: results pt 1

PATIENT #1 – 50% clinical response at 24 weeks

Mart1

Spots/100,000 PBMC

PATIENT #1 – 50% clinical response at 24 weeks
Conclusions/Future Directions

- No immediate toxicities were observed with LD CY, CTL, low-dose IL-2 and Ipilimumab (not enough patients to reliably establish safety).
- Polyclonal CTL generated in the presence of IL-21 and infused in patients receiving anti-CTLA4 persist and upregulate/acquire characteristics associated with the establishment of long-lived memory T-cells.
- Persistent tumor-specific CTL (with characteristics of long-lived memory) may not be sufficient to induce tumor regression in all patients.
- Evidence of epitope spreading was observed in patients with tumor regression/stable disease. Results need to be compared to patients receiving ipilimumab alone.
Adoptive T Cell Therapy: Extended Protocol

**Isolate/Enrich**
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**Clone/Select**
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**Genetically Modify**
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- Vaccine + adoptive therapy

• Therapy Lab
  – Erik, Ivy, Rebecca
• Shared Resources (FHCRC)
  – Jianhong Cao
  – Andrew Berger

• YongQing Li (IL-21)
• Aude Chapuis (CTL + aCTLA4)
• Sylvia Lee (CD25 depletion)
• Erik Farrar (Cell Sorting)

• Burroughs Wellcome Fund
• Damon Runyon
• Cancer Research Institute
• Edson Foundation
• Bezos Immunotherapy Fund
• Walker Fellowship
• GCRC/ITHS

cyee@fhcrc.org