Cell Kinetics in Adoptive Cell Therapy

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Cellular Therapies

Cell suspensions used for therapeutic purposes

Examples
• Red Cells
• Platelets
• Granulocytes
• Hematopoietic stem cells
• T cells
• Dendritic cells
• Natural killer (NK) cells
• Bone marrow stromal cells (BMSCs)
• Embryonic stem cells (ESC)
• Induced pluripotent stem cells (iPSC)

What it's not
• Tissue, bone, organs

Blood Products

Maybe autologous or allogeneic
Overview

RBCs – the simplest cell therapy
- Biologic variability

Granulocytes - more complex trafficking
- Cell phenotype effects kinetics

T cells – Adoptive Cell Therapy of Cancer
- Cell expansion effects kinetics
- Genetic engineering to improve kinetics
- Re-programming to change phenotype and improve kinetics
Measuring Cell Kinetics

Radionuclides

- Chromium-51 (27.7 days half-life)
- Technetium-99m (6.0 hrs)
- Indium-111 (2.8 days)

Applications

- Intravascular recovery
- Intravascular survival
- Tissue distribution

Issue = Radionuclides are Difficult Handle
Molecular Biology for Cell Detection

Cell Clones – Same T cell receptor (same peptide and HLA restriction)
- HLA tetramers and peptide antigen
- T cell receptor PCR
- TCR β-chain V region sequencing
- TCR β-chain V region antibodies

Genetically Engineered Cells
- Antibodies
- PCR

Good for Accessing Intravascular Recovery and Survival
Iron Labeling – MRI Detection

- Ferumoxytol
- Heparin
- Protamine

3 FDA Approved Drugs

Self-Assembling Nanocomplexes

Iron Labeled Cells

Infusion of Labeled Cells

MRI Detection

Thu MS et al. Nature Medicine Feb 2012
Iron Labeling – MRI Detection

- Ferumoxytol
- Heparin
- Protamine

3 FDA Approved Drugs

Self-Assembling Nanocomplexes

Bone Marrow Stromal Cells (BMSCs)

Iron Labeled BMSCs

Infusion of Labeled BMSCs

MRI Detection

Thu MS et al. Nature Medicine Feb 2012
Measuring Cell Kinetics:
Summary

• Radionuclide labeling = gold standard but limited availability
• Molecular methods can be useful for cloned T cells or genetically engineered cells

Alternative methods are needed
Red Blood Cells: the simplest cell therapy

Function
• Transport oxygen

Production
• Bone Marrow

Clearance
• Spleen

Distribution
• Extracellular space
• 40 to 45% of blood volume

Life span
• 105-120 days

Well documented with radionuclides
RBC Donation and Transfusion

Manufacturing RBCs for Transfusion
- Remove plasma
- Add anticoagulant / storage solution
Recovery of Transfused RBCs

Expected Recovery = 75%  Expected Half-life = 30 days

Fig 1. Red cell survival after storage for 35 days as whole blood in PVC bags with (●) or without (○) DEHP. Survivals were compared in ten donors after storage in each container type. Note that difference in survival at 24 hours is quantitatively apparent within the first ten minutes after reinfusion. Vertical bars represent SEM; asterisks indicate statistical significance, P < .05.

Variability in RBC Recovery: Intra-donor and Inter-donor variability

Sources of Variability

Assay
• Intra-donor
• Inter-donor

Donor
• Intra-donor
• Inter-donor

Manufacturing

Storage

Recipient factors
• RBC-specific antibodies
• Enlarged spleen

Hess JR. Transfusion 2012

Concept: Cell Therapy Kinetics are Highly Variable

Fig. 1. The variation in the “storability” of RBCs from different donors. This is the distribution of $^{51}$Cr 24-hour in vivo RBC recovery in 27 volunteers whose blood was stored repeatedly in CPD for 3 weeks as originally published by Dern and colleagues. With permission.
Granulocytes
More Complex Trafficking

- Intravascular, organ and tissue distribution
- Marrow reserves
- Transfusion recipient factors affect the survival of transfused cells
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>40 to 50</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>50 to 80</td>
</tr>
<tr>
<td>Monocytes</td>
<td>5 to 10</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1 to 5</td>
</tr>
<tr>
<td>Basophils</td>
<td>0 to 1</td>
</tr>
</tbody>
</table>

Normal White Blood Cell Count = 4 to 5 x10⁹ cell per L
Granulocytes

**Function**
- Antimicrobial

**Production**
- Bone Marrow

**Clearance**
- Spleen
- Tissue
- Sites of infection or inflammation

**Distribution**
- Intravascular
- Spleen, lungs
- Tissue

**Life span**
- 24 hours
Life of Granulocytes

Bone Marrow
- 7 to 10 days
- 100x10⁹ produced per day

Spleen

Lungs
Marginal Pool
25x10⁹ cells in the lungs and spleen

Circulation
- 12 hour
- 25x10⁹ circulating cells

Tissue
- 24 hours
Collection of Granulocyte by Apheresis

Donor

Blood Cell Separator

Whole blood

Granulocytes

Whole blood – Granulocytes
Changes in Donor Granulocyte Trafficking Increases the Concentration of Granulocytes in the Blood and Apheresis Collection yields

Granulocyte in a unit of whole blood = \(2.5 \times 10^9\) cells

**Donors given Dexamethasone**
- Granulocytes in an apheresis concentrate = \(20 \times 10^9\) cells

**Donor given Dexamethasone plus G-CSF**
- Granulocyte in an apheresis concentrate = 40 to \(80 \times 10^9\) cells
Survival of Transfused Granulocytes

**Issue**
- Recovery of transfused granulocytes is less than expected

**Explanation**
- Granulocytes are trapped in the lungs
- Likely due to normal trafficking and activation during collection

**Concept**
Kinetics of even minimally processed cells may be much different than naïve cells
Method of Collection (Mobilization) Effects
Granulocyte Kinetics

Classical Granulocyte Products
- Donors given Dexamethasone
- Collection yield = 20 x 10⁹ cells
- Expected increase in counts = 5 x 10⁹/L
- Actual increase in counts = none
- Can find transfused cells in buccal swabs

G-CSF Granulocyte Products
- Donors given G-CSF + Dexamethasone
- Collection yield = 40 to 80 x 10⁹ cells
- Expected increase in counts = 15 x 10⁹/L
- Actual increase in counts = 1 to 3 x 10⁹/L

Effects of G-CSF
- Improved recovery is due to mild activation and down regulation of adhesion receptor CD62L
- Improved survival is due to the release of slightly less mature granulocytes
Granulocyte Transfusions in a 16 year female with Severe Aplastic Anemia

Sequential CT scans of the thorax and response to granulocyte transfusions

Inflammation increases after initial (4) transfusions due to granulocyte trafficking to the site of infection

Dose of G-CSF+Dex mobilized Granulocytes (x10¹⁰ cells)

<table>
<thead>
<tr>
<th>Grans</th>
<th>7.0</th>
<th>7.7</th>
<th>6.8</th>
<th>5.6</th>
<th>8.6</th>
<th>7.1</th>
<th>6.0</th>
<th>5.9</th>
<th>8.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph</td>
<td>3.4</td>
<td>3.8</td>
<td>3.4</td>
<td>4.0</td>
<td>3.4</td>
<td>4.3</td>
<td>7.2</td>
<td>4.0</td>
<td>3.8</td>
</tr>
</tbody>
</table>

• Weak relationship between and dose and count increment
• Count increments are reduced as more transfusions are given

Effects of Granulocyte Transfusion Cell Dose and the Presence of HLA antibodies on Neutrophil Count Increments

(A) Relationship between ANC increment and granulocyte dose transfused for the first five non-alloimmunized patients who received 69 granulocyte concentrates.

(B) ANC over time for one patient who developed de novo HLA antibodies during the course of granulocyte therapy. Arrows denote granulocyte transfusions.

Quillen K et al. Haematologica 2009:94:1661-1668

©2009 by Ferrata Storti Foundation
Effect of splenomegaly and positive HLA antibody screen on absolute WBC increments 0–4 h post-transfusion

Quillen K et al. Haematologica 2009;94:1661-1668

©2009 by Ferrata Storti Foundation
T cells – Most Common Cell Used For Adoptive Immune Therapy of Cancer and viral infections

**Function**
- Adoptive cytoxicity

**Production**
- Bone marrow
- Lymph nodes

**Distribution**
- Blood
- Lymph nodes
- Thymus
- Tissue

**Life span**
- Short
- Long – months to years
Adoptive Immune Therapy: Antigen Specific T Cell

Antigen specific T cells recognize tumors and viral infected cells using T cell receptor to recognize peptide (antigen)-loaded HLA antigen (MHC)

Clinical Applications: Cancer therapy (melanoma and B cell malignancies) and viral infections (after marrow transplantation)
T Cell Therapy for Melanoma
Tumor Infiltrating Lymphocyte (TIL) Therapy

Patient with Metastatic Melanoma

Transfuse 30 to 60 x $10^9$ cells and administer IL-2

Expanded TIL

Clinical Response Rate: 10 to 70%
Typical TIL Treatment Protocol

- Collect Cells
- Process and Expand Cells
- Infuse Cells

High dose IL-2

Post infusion IL-2 enhances T cell survival

Clinical responses may not occur for several weeks post-therapy
Adoptive Cell Therapy for Metastatic Melanoma: Kinetics and Outcome

Patients: 23 with melanoma
TIL: Clones produced from tumors or peripheral blood leukocytes
Treatment Schedule: Treated with 2 or more times
Average Cell Dose: 10 x 10^9 cells
Measurement of Cell Kinetics: T-Cell receptor PCR
Survival: Concentration of cells in the blood reached a maximum after 1 hour and rapidly declined to undetectable levels by 2 weeks
Clinical outcome: One partial response (PR)

Issues
• Peripheral blood counts should have been much higher
• Cells should have persisted much longer

T-Cell Receptor Specific PCR to Detect TIL Clones in the Blood

Adoptive Transfer of Cloned Melanoma-Reactive T Lymphocytes for the Treatment of Patients with Metastatic Melanoma.
Dudley, Mark; Wunderlich, John; Nishimura, Michael; Yu, David; Yang, James; Topalian, Suzanne; Schwartzentruber, Douglas; Hwu, Patrick; Marincola, Francesco; Sherry, Richard; Leitman, Susan; Rosenberg, Steven
Circulating TIL were Detected 1-day post infusion in approximately 50% of Patients/Cycles

<table>
<thead>
<tr>
<th>PBL sample</th>
<th>By Patient</th>
<th>By Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># positive/</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td># tested</td>
<td>positive</td>
</tr>
<tr>
<td>Preinfusion</td>
<td>0/7</td>
<td>0</td>
</tr>
<tr>
<td>5 min–2 h after infusion</td>
<td>7/7</td>
<td>100</td>
</tr>
<tr>
<td>4–8 h after infusion</td>
<td>6/7</td>
<td>86</td>
</tr>
<tr>
<td>1 day after infusion</td>
<td>4/7</td>
<td>57</td>
</tr>
<tr>
<td>2–5 days after infusion</td>
<td>2/5</td>
<td>40</td>
</tr>
<tr>
<td>2–3 wk after infusion</td>
<td>0/4</td>
<td>0</td>
</tr>
</tbody>
</table>

PBL samples drawn at the indicated times after T-cell transfer were analyzed by clonotype specific PCR. All samples included in this analysis had adequate template cDNA as assessed by beta-actin PCR controls. A sample was considered positive for the presence of transferred clone if a band of the expected size was visible after gel electrophoresis of PCR products. The limit of detection varied for different clones but was within the range of 1 cell per $10^3$ to $10^5$ total lymphocytes.
Why is T cell Survival Limited?

1. Unfavorable recipient environment
2. Inadequate cell dose
3. Expansion changes phenotype to short-lived effector T cells

T Cell Phenotypes

- **Tn (Naïve)**: CD62L+ CD45RO-
- **TCM (Central Memory)**: CD62L+ CD45RO+
- **TEM (Effector Memory)**: CD62L- CD45RO+
- **TEFF (Effector)**
Improving the Survival of Infused Adoptive Cell Therapies

**Modify the Recipient**
- Growth Factors/Cytokines
- Leukocyte Reduction

**Modify the Infused Cells**
- Different Cell Subset/Type
- Shorter Duration of Expansion
**New TIL Treatment Protocol: Recipient leukoduction**

1. Collect Cells
2. Process and Expand Cells
3. Infuse Cells
4. High dose IL-2

**Chemotherapy**

**Total Body Irradiation**

**Autologous CD34+ cells**

**Potential Benefits of Leukocyte Reduction**

- Increased cytokine levels
- Elimination of inhibitory cells
TIL Therapy and Lymphodepleting Chemotherapy

Treatment Protocol

- **Nonmyeloablative lymphodepletion**: cyclophosphamide and fludarabine, no autologous CD34+ cells
- **TIL**: approximately $7 \times 10^9$ cells
- **Analysis of TIL persistence**: by TCR β-chain V region sequencing

Clinical Outcomes

- Objective clinical responses in 13 of 25 patients

TIL Kinetics

- Post-infusion lymphocytosis in 2 patients
- Persistence of cells more for 23 to 62 days
- Greater degree of persistence of TIL in patients with clinical responses

Robbins PF et al. Journal of Immunology 2004;173:7125-7130
# Persistence of T Cell Clones in the Blood Following TIL Therapy

Robbins PF et al. Journal of Immunology 2004;173:7125-7130

<table>
<thead>
<tr>
<th>Responders</th>
<th>PBMC Sample Day</th>
<th>Lymphocyte Number Per mm² (days 5-15)</th>
<th>Persistence Total %a</th>
<th>Lymphocyte Number Per mm² (days 22-50)</th>
<th>Persistence Total %a</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>3.53</td>
<td>2,085</td>
<td>48</td>
<td>1,646</td>
<td>74</td>
</tr>
<tr>
<td>9</td>
<td>9.29</td>
<td>21,436</td>
<td>96</td>
<td>7,086</td>
<td>96</td>
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<tr>
<td>10</td>
<td>7.23</td>
<td>12,150</td>
<td>94</td>
<td>3,610</td>
<td>84</td>
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<tr>
<td>16</td>
<td>7.64</td>
<td>389</td>
<td>80</td>
<td>377</td>
<td>14</td>
</tr>
<tr>
<td>17(2)</td>
<td>8.28</td>
<td>555</td>
<td>43</td>
<td>1,328</td>
<td>10</td>
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<tr>
<td>19</td>
<td>5.33</td>
<td>1,056</td>
<td>57</td>
<td>2,740</td>
<td>12</td>
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<tr>
<td>21</td>
<td>6.27</td>
<td>1,467</td>
<td>45</td>
<td>1,079</td>
<td>15</td>
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<tr>
<td>25</td>
<td>7.31</td>
<td>168</td>
<td>56</td>
<td>455</td>
<td>8</td>
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<tr>
<td>36</td>
<td>8.34</td>
<td>711</td>
<td>44</td>
<td>483</td>
<td>2</td>
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<tr>
<td>38</td>
<td>9.64</td>
<td>576</td>
<td>74</td>
<td>1,382</td>
<td>7</td>
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<tr>
<td>30(2)</td>
<td>5.26</td>
<td>111</td>
<td>61</td>
<td>283</td>
<td>11</td>
</tr>
<tr>
<td>31</td>
<td>7.26</td>
<td>739</td>
<td>36</td>
<td>643</td>
<td>4</td>
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<tr>
<td>34</td>
<td>9.37</td>
<td>82</td>
<td>62</td>
<td>639</td>
<td>31</td>
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</table>

<table>
<thead>
<tr>
<th>Non-Responder</th>
<th>PBMC Sample Day</th>
<th>Lymphocyte Number Per mm² (days 5-15)</th>
<th>Persistence Total %a</th>
<th>Lymphocyte Number Per mm² (days 22-50)</th>
<th>Persistence Total %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>15.37</td>
<td>ND²</td>
<td>9</td>
<td>1,893</td>
<td>&lt;1</td>
</tr>
<tr>
<td>11</td>
<td>8.35</td>
<td>810</td>
<td>4</td>
<td>760</td>
<td>&lt;1</td>
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<tr>
<td>12</td>
<td>8.35</td>
<td>791</td>
<td>5</td>
<td>864</td>
<td>9</td>
</tr>
<tr>
<td>13</td>
<td>8.29</td>
<td>1,075</td>
<td>36</td>
<td>1,027</td>
<td>&lt;1</td>
</tr>
<tr>
<td>14</td>
<td>8.29</td>
<td>532</td>
<td>&lt;1</td>
<td>1,792</td>
<td>&lt;1</td>
</tr>
<tr>
<td>15</td>
<td>7.34</td>
<td>769</td>
<td>66</td>
<td>871</td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>13.29</td>
<td>287</td>
<td>2</td>
<td>385</td>
<td>&lt;1</td>
</tr>
<tr>
<td>20</td>
<td>8.25</td>
<td>548</td>
<td>18</td>
<td>592</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>8.34</td>
<td>365</td>
<td>43</td>
<td>507</td>
<td>1</td>
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<tr>
<td>24</td>
<td>7.29</td>
<td>2,282</td>
<td>87</td>
<td>535</td>
<td>41</td>
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<tr>
<td>27</td>
<td>7.24</td>
<td>2,496</td>
<td>47</td>
<td>200</td>
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<td>29</td>
<td>8.32</td>
<td>164</td>
<td>25</td>
<td>186</td>
<td>&lt;1</td>
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<table>
<thead>
<tr>
<th>Mean</th>
<th>3,258</th>
<th>58</th>
<th>1,754</th>
<th>26</th>
</tr>
</thead>
</table>

* The days given are relative to the date of TIL infusion.
* The values indicate the sum of the percentages of individual clones detected in PBMC samples that were also present in the administered TIL.
* Patients 6, 17, and 30 had received a single prior allogeneic TIL.
* A PBIL count was not carried out for patient no. 7 at this time point.
* The lymphocyte counts and the percentage of persistent clones per patient in responders and nonresponders were compared using the Wilcoxon rank sum test.
T Cell Clones in the Blood Following TIL Therapy: Comparison of Responder and Non-Responders (23-63 days post infusion)

Robbins PF et al. Journal of Immunology 2004;173:7125-7130
Leukocyte Depletion Enhances TIL Therapy Outcomes

Preparative Regimens for Cell Transfer

<table>
<thead>
<tr>
<th>Days</th>
<th>-7</th>
<th>-4</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-myeloablative</td>
<td>Cy</td>
<td>Gy</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>IL-2</td>
<td>IL-2</td>
<td>IL-2</td>
<td></td>
</tr>
<tr>
<td>Ablative (30cGy)</td>
<td>Cy</td>
<td>Gy</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>TBI</td>
<td>IL-2</td>
<td>IL-2</td>
<td>CD34+</td>
</tr>
<tr>
<td>Ablative (100cGy)</td>
<td>Cy</td>
<td>Gy</td>
<td>Flu</td>
<td>Flu</td>
<td>Flu</td>
<td>TBI</td>
<td>TBI</td>
<td>IL-2</td>
<td>IL-2</td>
</tr>
</tbody>
</table>


Survival of Patients with Metastatic Melanoma Treated with Autologous Tumor Infiltrating Lymphocytes and IL-2

Young TIL Telomere Length, CD27 expression and Persistence affect Patient Outcome

Mean telomere length, the number of CD27+CD8+ cells, and the percentage persistence of the infused cells in peripheral blood at 1 month after cell infusion are significantly different in objective responders (CR + PR) compared with nonresponders (all P2 < 0.001)

Genetically Engineered T cells: Improved Kinetics and Improve efficacy

**Why Engineer T cells?**
1. Higher affinity T cell receptors
2. Develop therapies that are not dependent on HLA type and T cell receptor restrictions
3. Improve kinetics
Chimeric Antigen Receptor (CAR): T Cell + Antibody = T Body

Chimeric Antigen Receptor (CAR) T cells

First/Second generation

CD8 or CD28

TCR complex

α-Tumor mouse mAb

Third generation

CD28

4-1BB or OX-40

Production of Autologous Anti-CD19 CAR T Cells

Autologous PBMCs

T cell Stimulation
Anti-CD3/CD28 beads + IL-2

Transduction + Anti-CD19/CD28/CD3 zeta vector

Expansion
Continue culture with anti-CD3/CD28 beads + IL-2

Treat Acute Lymphocytic Leukemia
Clinical Trial with Second Generation CAR T cells

- CAR Directed to the B cell antigen CD19
- CAR contained a co-stimulator molecule domain
- Treated 3 patients with Chronic Lymphocytic Leukemia (CLL)

**Table 1. Patient demographics and response. CR, complete response; PR, partial response; N/A, not available.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>karyotype</th>
<th>Previous therapies</th>
<th>CLL tumor burden at baseline</th>
<th>Total dose of CART19 (cells/kg)</th>
<th>Response day</th>
<th>CR (duration)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>65</td>
<td>male</td>
<td>normal</td>
<td>Fludarabine × 4 cycles (2002)</td>
<td>Hypocellular 70% B + 30% L</td>
<td>N/A</td>
<td>6.1 × 10^7 to 1.0 × 10^8 cells/day</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>male</td>
<td>del (17p)</td>
<td>Rituximab/Fludarabine × 4 cycles (2005)</td>
<td>2.4 × 10^7 cells/day</td>
<td>N/A</td>
<td>6.1 × 10^7 to 1.0 × 10^8 cells/day</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>male</td>
<td>del (17p)</td>
<td>Fludarabine/rituximab (2021)</td>
<td>3.3 × 10^7 cells/day</td>
<td>N/A</td>
<td>6.1 × 10^7 to 1.0 × 10^8 cells/day</td>
<td>37</td>
</tr>
</tbody>
</table>

---

65 year old male, CR (11+ months)

77 year old male, PR (7+ months)

65 year old male, CR (11+ months)
Prolonged Persistence of CD19 CAR Cell in the Blood and Marrow

A. CART 19: Blood

B. WBC and CART 19: Blood

C. CART 19: Blood

D. CART 19: Marrow

Kalos M et al. Sci Transl Med 2011;3:95ra73-95ra73
Summary of the first patients treated on the NCI adult autologous anti-CD19 CAR trial

### Table 2 | Summary of the first patients treated on the NCI adult autologous anti-CD19 CAR trial[^33]

<table>
<thead>
<tr>
<th>Patient*</th>
<th>Age (years)</th>
<th>Malignancy</th>
<th>Number of unique prior therapies</th>
<th>Number of CAR-expressing T cells infused per kg</th>
<th>Response (duration in months after T-cell infusion)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>47</td>
<td>Follicular lymphoma</td>
<td>4</td>
<td>0.3×10^7</td>
<td>PR (7)</td>
</tr>
<tr>
<td>1b</td>
<td>48</td>
<td>Follicular lymphoma</td>
<td>5</td>
<td>1.3×10^7</td>
<td>PR (33)</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>Follicular lymphoma</td>
<td>5</td>
<td>0.3×10^7</td>
<td>NE</td>
</tr>
<tr>
<td>3</td>
<td>61</td>
<td>Chronic lymphocytic leukaemia</td>
<td>3</td>
<td>1.1×10^7</td>
<td>CR (24)</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>Splenic, marginal zone lymphoma</td>
<td>3</td>
<td>1.1×10^7</td>
<td>PR (12)</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>Chronic lymphocytic leukaemia</td>
<td>4</td>
<td>0.3×10^7</td>
<td>SD (6)</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>Chronic lymphocytic leukaemia</td>
<td>7</td>
<td>1.7×10^7</td>
<td>PR (7)</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>Chronic lymphocytic leukaemia</td>
<td>4</td>
<td>2.8×10^7</td>
<td>CR (21+)</td>
</tr>
<tr>
<td>8</td>
<td>63</td>
<td>Follicular lymphoma</td>
<td>7</td>
<td>3.0×10^7</td>
<td>PR (11)[^8]</td>
</tr>
</tbody>
</table>

[^33]: All patients received two cycles of CAR-T cell therapy.

Clinical Responses in 6 of 8 pts


Normal and malignant B-lineage cells were eliminated from the blood and BM of patient 3 with CLL

Peripheral blood B cell counts

CD19 Staining of Marrow

CD20 Staining of Marrow

CD19 Flow cytometry of Marrow

Blood and CSF Levels CD19 CAR T Cells in 2 Children with ALL

Circulating CD19 CAR T Cells

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Day 1</th>
<th>Day 6</th>
<th>Day 10</th>
<th>Day 21</th>
<th>Day 58</th>
<th>Day 153</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL019</td>
<td>0.3%</td>
<td>3.9%</td>
<td>71.5%</td>
<td>65.2%</td>
<td>20.9%</td>
<td>1.4%</td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 2</th>
<th>Infusion Product</th>
<th>Day 3</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 13</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL019</td>
<td>14.4%</td>
<td>0.0%</td>
<td>4.0%</td>
<td>33.6%</td>
<td>12.2%</td>
<td>0.9%</td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD19 CAR T Cells in the CSF

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Day 175</th>
<th>Day 175</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMO</td>
<td>0%</td>
<td>9.7%</td>
</tr>
<tr>
<td>CTL019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 2</th>
<th>Day 23</th>
<th>Day 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMO</td>
<td>43.8%</td>
<td>43.8%</td>
</tr>
<tr>
<td>CTL019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Phase I Study of T Cells Expressing an Anti-CD19 Chimeric Receptor in Children and Young Adults with B Cell Malignancies
Principal Investigators Daniel Lee, MD and Crystal Mackall, MD

Dose Escalation Schedule

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Dose of anti-CD19-CAR T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort Level 1</td>
<td>$1 \times 10^6$ transduced T cells/kg (± 20%)</td>
</tr>
<tr>
<td>Cohort Level 2</td>
<td>$3 \times 10^6$ transduced T cells/kg (± 20%)</td>
</tr>
<tr>
<td>Cohort Level 3</td>
<td>$1 \times 10^7$ transduced T cells/kg (± 20%)</td>
</tr>
</tbody>
</table>

Arm 1 (Low Disease Burden)
• Fludarabine/cyclophosphamide lymphodepleting chemotherapy regimen

Arm 2 (High Disease Burden)
• Intensive standard of care chemotherapy, in lieu of the lymphodepleting chemotherapy regimen, to decrease tumor burden in preparation for the administration of the CAR T cells.

No post-infusion IL-2 therapy

Lee DW et al, Lancet 2014, Oct 10
Results of Treating 21 Pediatric ALL Patients with Anti-CD19 CAR T Cells by the Pediatric Oncology Branch, NCI, NIH


14 CRs
Anti-CD19 T Cells Persist for Approximately 28 Days

Percentage of T Cells Expressing CAR

Absolute Number of CAR T Cells

Number of Copies of CAR Gene

Lee DW et al, Lancet 2014, Oct 10
CD19 CAR T Cells Markers of Better Outcomes

Multiple Studies
• Peak CAR T cell levels

One Study
• CD8:CD4 T cell ratio at time of peak levels

Factors Associated with Higher Peak levels
• Presence of CD19+ cells at time of CAR T cell infusion (Lymphoma)
• Quantities of blast in marrow (ALL)
Greater Numbers of Circulating Anti-CD19 CAR T Cells is Associated with Clinical Responses

Lee DW et al, Lancet 2014, Oct 10
High peak blood levels of anti-CD19 chimeric antigen receptor (CAR19) T cells were associated with remissions of B cell malignancy in Adults.

Jennifer N. Brudno et al. JCO doi:10.1200/JCO.2015.64.5929

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High peak blood levels of anti-CD19 chimeric antigen receptor (CAR19) T cells were associated with the presence of circulating B cells but not the quantity of infused cells: Data from adults with B cell malignancies

Jennifer N. Brudno et al. JCO doi:10.1200/JCO.2015.64.5929
High peak blood levels of CD8 anti-CD19 chimeric antigen receptor (CAR19) T cells were associated with remissions of malignancy.

Jennifer N. Brudno et al. JCO doi:10.1200/JCO.2015.64.5929

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Most blood chimeric antigen receptor (CAR) –positive T cells expressed CD8, and CAR-positive T cells acquired more differentiated phenotype after infusion.

James N. Kochenderfer et al. JCO 2015;33:540-549

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CD19 CAR T Cells are Associated with 3 Types of Toxicities

Tumor lysis syndrome
Cytokine release syndrome
• Fever
• Hypotension
• Multi-organ failure
• Cardiac arrest
Neurotoxicity
• Confusion
• Dysphasia
Standardizing the Assessment and Treatment of CRS

How I Treat

Current concepts in the diagnosis and management of cytokine release syndrome

Daniel W. Lee,1 Rebecca Gardner,2 David L. Porter,3 Chrystal U. Louis,4 Nabil Ahmed,4 Michael Jensen,2
Stephan A. Grupp,3,5,6 and Crystal L. Mackall1

1Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD; 2Seattle Children’s Hospital, Seattle, WA; 3Division of Hematology-Oncology,
University of Pennsylvania, Philadelphia, PA; 4Texas Children’s Hospital, Center for Cell and Gene Therapy, Baylor College of Medicine, Houston, TX; and
5Children’s Hospital of Philadelphia Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

As immune-based therapies for cancer become potent, more effective, and more widely available, optimal management of their unique toxicities becomes increasingly important. Cytokine release syndrome (CRS) is a potentially life-threatening toxicity that has been observed following administration of natural and bispecific antibodies and, more recently, following adoptive T-cell therapies for cancer. CRS is associated with elevated circulating levels of several cytokines including interleukin (IL)-6 and interferon-γ, and uncontrolled studies demonstrate that immunosuppression using tocilizumab, an anti-IL-6 receptor antibody, with or without corticosteroids, can reverse the syndrome. However, because early and aggressive immunosuppression could limit the efficacy of the immunotherapy, current approaches seek to limit administration of immunosuppressive therapy to patients at risk for life-threatening consequences of the syndrome. This report presents a novel system to grade the severity of CRS in individual patients and a treatment algorithm for management of CRS based on severity. The goal of our approach is to maximize the chance for therapeutic benefit from the immunotherapy while minimizing the risk for life-threatening complications of CRS. (Blood. 2014;124(2):188-196)

Blood July 2014
Temperature, LDH and Cytokine Levels in 2 Children with ALL Treated with CD19 CAR T Cells

Cytokine Release Syndrome (CRS) is more likely to Occur in Patients with Great Numbers of Circulating CAR T cells and Greater Tumor Burden

Lee DW et al, Lancet 2014, Oct 10
IL-6 and IFNγ Levels are Associated with Grade 3 or 4 Toxicity

Lee DW et al, Lancet 2014, Oct 10
C-Reactive Protein Levels are Associated with IL-6 Levels and Toxicity

Relationship between IL-6 and CRP levels in 2 Patients

Maximum CPR vs Maximum IL-6

CRP Levels and CRS

Lee DW et al, Lancet 2014, Oct 10
Other CAR T Cells

**In Clinical Trials**
- CD20: B cell lymphoma
- Anti-GD2: neuroblastoma and osteosarcoma
- Anti-ERBB2 (HER-2/neu): colon cancer

**In Development**
- CD22
- CD23
- CD70
- Anti-Immunoglobulin Kappa light chain
- Anti-B cell maturation antigen (BCMA) (Multiple Myeloma)
- Anti-glypican 3 (hepatocellular carcinoma)
- Anti-Erythropoietin-producing hepatocellular carcinoma A (EphA2) (glioblastoma)
Improving T Cell Adoptive Cellular Therapy by Starting with T Cells with Better Survival Kinetics
What Type of T Cells are Best for Adoptive Cell Therapy

Naïve
CD62L+ CD45RO-

Central Memory
CD62L+ CD45RO+

Effector Memory
CD62L- CD45RO+

Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates

Carolina Berger, Michael C. Jensen, Peter M. Lansdorp, Mike Gough, Carole Elliott, and Stanley R. Riddell

Isolation of and labeling of T_{CM} and T_{EM} cells

Better Persistence and Migration of TcM-derived CD8+ Tέ clones


Macaque 1

Macaque 2

**TεM cells**

**TcM cells**

**TcM cells**

**TεM cells**
Memory Stem T Cells (TSCM) Which Have Characteristics of Naïve and Memory Cells are Present in the Peripheral Blood

**Naïve**
- CD62L+ CD45RO-

**Central Memory**
- CD62L+ CD45RO+

**Effector Memory**
- CD62L- CD45RO+

Stem Memory T Cells

*Stem cell characteristics:*
- CD62L+, CD45RO-, CD45RA+, CD27+, CD28+ and IL-7Ra+

*Memory Cell Characteristics*
- CD95+, IL-2Rb+, CXCR3+ and LFA-1+

TSCM Function
- Increased proliferative capacity
- Superior anti-tumor cell responses
More Adoptively Transferred Mesothelin-Specific Human T\textsubscript{SCM} cells are Recovered from NSG Mice

10\textsuperscript{6} cells of each type were transferred to each mouse

Adoptively Transferred Mesothelin-Specific T_{SCM} Cells Have Greater Antitumor Activity

Production of Tscm cells

- Tscm are a good vehicle for T cell therapy using genetically engineered T cells.
- Tscm cells are found in the blood but their quantities are limited.
- Methods to isolate and expand Tscm are being developed.
Wnt/β-catenin Signaling is Involved with T-cell Differentiation, Polarization, and Survival

Wnt/β-catenin signaling

© 2010 American Association for Cancer Research

Production of Memory Stem T Cells (TscM) From Peripheral Blood Leukocytes

**Anti-CD45RA Selection**
- CD45RA+ Lymphocytes
- CD45RA+ CD62L+ CCR7+ CD28+
- Both Naïve and Memory characteristics
- Increased proliferative capacity
- Superior anti-tumor cell responses

**Anti-CD62L Selection**
- CD62L+ CD45RA+ Lymphocytes

**Anti-CD8 Selection**
- CD8+ Lymphocytes

**Anti-CD3/CD28 Beads + TWS119**
- TscM Cells

Peripheral Blood Lymphocytes
Future Directions: Overcoming Cancer-Induced Immune Suppression

Myeloid Derived Suppressor Cells (MDSC)

• Found in the blood and tumor microenvironment of cancer patients
• Suppress T cell proliferation
• Monocyte (CD33+ CD14+ HLA-DR-) and granulocyte (CD33+ CD15+ HLA-DR-) phenotypes
• Presence in patients with hematologic malignancies is not certain

Use of immune check point inhibitors
Conclusions

• Cellular Kinetics are important for the licensure of RBCs and platelets.
• Improvements in cell persistence have improved adoptive T cell therapy for cancer.
• Gene engineering is being used to increase the survival and clinical effectiveness of adoptively transferred T cells.
• T Cell reprogramming may be a useful tool for improving the persistence and effectiveness of adoptive T cell therapy.