Cellular Therapy: Pharmacokinetics

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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

What it's not
• Tissue, bone, organs

Maybe autologous or allogeneic

Overview

RBCs – the simplest cell therapy
• Biologic variability
Granulocytes - more complex trafficking
• Cell phenotype effects kinetics
T cells – persistence is important
• Cell expansion effects kinetics
Engineered T cells
• Genetic engineering to improve kinetics
Reprogramming T cells
• Getting the best phenotype to improve kinetics
Measuring Cell Kinetics

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

Applications
- Recovery processed cells
- Intravascular survival
- Tissue distribution

Issue = Radionuclides are Difficult Handle

Molecular Biology for Cell Detection

Cell Clones – Same T cell receptor
- 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

Not Quantitative

Iron Labeling – MRI Detection

Ferumoxytol + Heparin + Protamine 3 FDA Approved Drugs

Ferumoxytol-Heparin-Protamine Nanocomplexes

Self-Assembling Nanocomplexs

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)

Infusion of Labeled BMSCs

MRI Detection

Thu MS et al. Nature Medicine Feb 2012

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

RBC Donation and Transfusion

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

RBCs
Recovery of Transfused RBCs

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

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

Circulating Leukocytes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>(%)</th>
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<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 x10^9 cell per L
Granulocytes

Function
• Antimicrobial Production
• Bone Marrow Clearance
• Spleen
• Tissue
• Sites of infection or inflammation

Distribution
• Intravascular
• Spleen, lungs
• Tissue

Sites of infection or inflammation

Life span
• 24 hours

Life of Granulocytes

Bone Marrow
• 7 to 10 days
• 100x10^9 produced per day

Spleen

Lungs

Marginal Pool
25x10^9 cells in the lungs and spleen

Circulation
• 12 hour
• 25x10^9 circulating cells

Tissue
• <24 hours

Granulocyte Transfusion Outcomes

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
• Granulocytes are affected by G-CSF; mild activation and down-regulation of adhesion receptor CD62L

Concept
Kinetics of even minimally processed cells may be much different naive cells
Granulocyte Transfusion Outcomes

**Classical Granulocyte Products**
- Donors given Dexamethasone
- Collection yield = 20 x 10^9 cells
- Expected increase in counts = 5 x 10^9/L
- Actual increase in counts = none
- Can find transfused cells in bucal swabs

**G-CSF Granulocyte Products**
- Donors given G-CSF + Dexamethasone
- Collection yield = 40 to 80 x 10^9 cells
- Expected increase in counts = 15 x 10^9/L
- Actual increase in counts = 1 to 3 x 10^9/L

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

Weak relationship between 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.
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

T cells – Most Common Cell Used For Adoptive Immune Therapy

Function
- Adoptive cytotoxicity

Production
- Bone marrow
- Lymph nodes

Distribution
- Bone marrow
- 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 viral infections (after marrow transplantation)
**T Cell Therapy for Melanoma**

**Tumor Infiltrating Lymphocyte (TIL) Therapy**

- **Patient with Metastatic Melanoma**
- **Collect Cells**
- **Process and Expand Cells**
- **Infuse Cells**
- **Time**
- **Clinical Response Rate: 10 to 70%**

**Typical TIL Treatment Protocol**

- **Collect Cells**
- **Process and Expand Cells**
- **Infuse Cells**
- **High dose IL-2**

**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:** $10 \times 10^9$ 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

3A

BV762
\text{N} \text{region}
R255
ASCQ/CAMKIVATATATGATGTTGAMCAGCCTCCTA
GCTGCTGGTCTGG
S2/C2/K2 Clone Specific Primer

3B

Control

CSP

CIF

BV7 Clone

Circulating TIL were Detected 1-day post infusion in approximately 50% of Patients/Cycles

<table>
<thead>
<tr>
<th></th>
<th>By Patient</th>
<th>By Cycle</th>
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<tbody>
<tr>
<td></td>
<td># positive</td>
<td># tested</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>1 min 2-h after infusion</td>
<td>7/7</td>
<td>100</td>
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<tr>
<td>4-8 h after infusion</td>
<td>10/17</td>
<td>98</td>
</tr>
<tr>
<td>2-3 days after infusion</td>
<td>2/5</td>
<td>40</td>
</tr>
<tr>
<td>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 10^5 to 10^7 total lymphocytes.

Why is T cell Survival Limited?

1. Inadequate cell dose
2. Prolonged expansion leads to apoptosis
3. Phenotype changes to a short-lived effect 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 Infused Cells
- Different cell subset/type
- Shorter Duration of Expansion

Modify the Recipient
- Growth Factors/cytokines
- Leukocyte Reduction

New TIL Treatment Protocol: Recipient leukoduction

- Collection of cells
- Process and expand cells
- Infusion of cells
- High dose IL-2
- Time
  - 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 x 10^6 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

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**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

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**Leukocyte Depletion Enhances TIL Therapy Outcomes**

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 P < 0.001).


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

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

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

Chimeric Antigen Receptor (CAR) T cells

Clinical Trial with first generation CAR T cells
- Receptor Directed to an ovarian cancer-associated antigen: α-folate receptor (FR)
- Treated 14 patients with metastatic ovarian cancer
**Anti-Folate Receptor CAR T Cells For Ovarian Cancer**

**Kinetics**
- PCR analysis of gene modified cells
- 111In-labeling

**Clinical Outcomes**
- No responses

**Treatment Protocol**
- Phase I/II
- 1 or 2 cycles of Anti-FR CAR

<table>
<thead>
<tr>
<th>Table 2: Summary of treatment regimen and toxicity for patients receiving gene-modified T cells.</th>
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<tbody>
<tr>
<td>Patient</td>
<td>Cycle</td>
<td>No. of cycles</td>
<td>No. of T cells</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1x10^9</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1x10^9</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1x10^9</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1x10^9</td>
</tr>
</tbody>
</table>

Cell dose = 4 to 7x10^9


**Biodistribution**

Anti-Folate Receptor CAR T Cells for Ovarian Cancer

Biodistribution of radiolabeled T cells.


©2006 by American Association for Cancer Research

**Anti-Folate Receptor CAR T Cells For Ovarian Cancer: Little Persistence Beyond 5 days**

Addition of co-stimulatory molecules to CARs enhances the persistence of infused cells.

Anti-Folate Receptor CAR T Cells: Adding Co-Stimulatory Domain Improves Anti-Tumor Response

Anti-FRα CAR Vectors

Control Vectors

MOV19-Δζ
MOV19-ζ
MOV19-BBC

Anti-FRα CAR with 4-1BB

CD137 = 4-1BB co-stimulatory domain

Only Anti-FRα CAR with 4-1BB Reduce Tumor Volume

Effectiveness of Anti-FRα CAR plus 4-1BB is not affected by route of injection

Cell persistence with Anti-FRα CAR plus 4-1BB
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)

Anti-CD19 CAR Treatment Protocol:
Chemotherapy followed by 3 daily doses

Kalos M et al. Sci Transl Med 2011;3:95ra73-95ra73

T Cells with Chimeric Antigen Receptors Have Potent Anti-tumor Effects and Can Establish Memory in Patients with Advanced Leukemia

Kalos M et al. Sci Transl Med 2011;3:95ra73-95ra73

Published by AAAS
Prolonged Persistence of CD19 CAR Cell in the Blood and Marrow

Clinical Responses After CD19 CAR Therapy

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

<table>
<thead>
<tr>
<th>T Cells</th>
<th>CD62L</th>
<th>CD45RO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Central Memory</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Effector Memory</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Hinrichs C S et al. Blood 2011;117:300-314
**Better Persistence and Migration of TCM-derived CD8+ Tc clones**

Macaque 1

Macaque 2

**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+

TSCM

- 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 TSCM cells are Recovered from NSG Mice**

10^6 cells of each type were transferred to each mouse

Adoptively Transferred Mesothelin-Specific Tscm Cells Have Greater Antitumor Activity

Production of Tscm cells

- Tscm cells are found in the blood but their quantities are limited
- Cell re-programming methods are being tested for the expansion of Tscm cells

Wnt/β-catenin Signaling is Involved with T-cell Differentiation, Polarization, and Survival
Wnt Signaling

TWS119 Activates Wnt Signaling by Inhibiting GSK-3β

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

- Anti-CD8 Selection
- Anti-CD45RO Depletion
- Anti-CD62L Selection

Peripheral Blood Lymphocytes → CD8+ Lymphocytes → CD45RO+ Lymphocytes → CD62L+ CD45RO+ Lymphocytes → Tscm Cells

- CD45RO- CD62L+ CCR7+ CD28+
- Both Naïve and Memory characteristics
- Increased proliferative capacity
- Superior anti-tumor cell responses

Tscm Cells + Anti-CD3/CD28 Beads + TWS119
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.