Umbilical Cord Blood-Derived T Regulatory Cells

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Outline

• Overview of T regulatory (T<sub>R</sub>) cells
• Potential for clinical application
• Summary of efforts to isolate and expand T<sub>R</sub> cells at the U of MN
  – Peripheral blood
  – Umbilical cord blood
• Current trials
T Regulatory Cells

- Naturally arising $T_R$ cells are minor population (~5-10%) of CD4$^+$ T cells
- Crucial for control of autoreactive T cells in vivo; contribute to maintenance of immunologic self-tolerance
- CD4$^+$ and CD8$^+$ T cell responses (auto-, allo-, and anti-tumor- immunity) can be inhibited by $T_R$ cells
- Exact mechanism of suppressive effects unknown (cell-cell contact in vitro/in vivo)
T Regulatory Cells

- Main mechanism of suppression seems to be inhibition of transcription of IL-2 in responder cells
- Express high levels of immune suppressive molecules (e.g., CTLA-4 and TGF-β)
- Originally characterized in mice; more recently in humans
- Generated through central thymic development and poorly defined peripheral mechanisms
T Regulatory Cells

- IL-2 = key growth/survival factor for $T_R$ cells
- $T_R$ cells co-express high levels of IL-2 receptor $\alpha$-chain (CD25)
- Foxp3 = master control gene for development and function of natural $CD4^+/CD25^+$ $T_R$ cells
- Foxp3 transcription factor: controls expression of CD25 in natural $CD4^+/CD25^+$ $T_R$ cells, but not in activated T cells in general
### Potential Clinical Applications of CD25⁺CD4⁺ Tregs

<table>
<thead>
<tr>
<th>Enhancement of CD25⁺CD4⁺ Treg function</th>
<th>Target condition</th>
<th>Potential therapeutic approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ transplantation, autoimmune disease, allergy</td>
<td>Transfer of Tregs or enhancement of their function allows specific suppression of immune responses; e.g., ex vivo gene transduction of Foxp3; ex vivo generation of regulatory cells using cytokines, pharmacological agents, or modified DCs</td>
<td></td>
</tr>
<tr>
<td>Reduction of CD25⁺CD4⁺ Treg function</td>
<td>Cancer, infectious disease</td>
<td>Removal of Tregs or blocking of their function boosts immune responses; anti–CTLA-4, -CD25, -GITR mAbs</td>
</tr>
</tbody>
</table>

Clinical Application in BMT

- $T_R$ cells essential for ex vivo induction of tolerance
- Addition of B6 $T_R$ cells resulted in dose-dependent suppression of allo-responses in MLR (responders = B6 CD4$^+$/CD25$^-$ and stimulators = irradiated bm12)
- CD25 depletion of CD4$^+$ T cells resulted in heightened MLR responses
- Role for $T_R$ cells in allo-responses in vivo (e.g., GVHD)?

Depletion of CD4⁺CD25⁺ Cells Accelerates GVHD Lethality

A) Dose = 10⁵ cells  
Donor = B6 T cells  
Recipient = bm12 (subleth. irr.)  
P = 0.024

B) Dose = 0.5 x 10⁵ cells  
Donor = B6 T cells  
Recipient = bm12 (subleth. irr.)  
P = 0.0068

Depletion of CD4$^{+}$CD25$^{+}$ Cells Accelerates GVHD Lethality in a Different Strain Combination

Dose = 2 x $10^6$ T cells  
Donor = B6 BM and T cells  
Recipient = BALB/c (leth. irr.)  
P = 0.016

Depletion of CD25⁺ Cells from a Whole T Cell Inoculum Accelerates GVHD in a Non-irradiated SCID GVHD Model

Dose = 10⁶ T cells
Donor = B6 T cells
Recipient = BALB/c SCID
P = 0.021

Pre-transplantation In Vivo Depletion of CD25\(^+\) Cells Accelerates GVHD

Dose = 15 x 10\(^6\) splenocytes
Donor = BALB/c BM and splenocytes
Recipient = thymectomized, leth. irr. B6
P = 0.0063

Taylor PA, Lees CJ, and Blazar BR. The infusion of ex vivo activated and expanded CD4\(^+\)CD25\(^+\) immune regulatory cells inhibits GVHD lethality. Blood (2002); 99: 3493-3499.
Ex Vivo Expanded and Activated CD25^+ Cells Inhibit GVHD

Dose = 2 \times 10^6 fresh CD4^+ T cells +/- 2 \times 10^6 activated cells

Donor = B6

Recipient = non-irr., NK-depleted BALB/c SCID

P = 0.022

Non-Sorter Bead-Based Human $T_R$ Isolation from PB

1. PBMC (Apheresis)
2. CD25 positive selection
   *Miltenyi beads (low titer) x 2
3. CD8,14,19,20,56 negative selection
4. CD4 positive selection
   Irradiate, use as feeders
5. Anti-CD3/28 beads
   IL-2
6. CD4 $^{+25+}$
Non-Sorter Bead-Based Human $T_R$ Isolation from PB

Pre-purification

After purification

CD3/28 Beads Augment CD4$^{+}$25$^{+}$ Cell Expansion

Purified Cultured CD4+CD25+ Cells Markedly Suppress MLRs

- MLR
  - 1 week
  - 1:2 suppressor:responder

-Suppression
  - >50% (15/22=68%)
  - >95% (7/22=32%)

- More potent than fresh
- CD62L+/CD27+ subset

most potent

UCB as a $T_R$ Cell Source

• Would UCB be a better source of $T_R$ cells?
  • Low exposure to prior antigenic stimulus
  • UCB T cells have defects in T cell activation
  • Is the reduced GVHD risk of UCB transplants potentially due to higher $T_R$ cell number or function?

• More distinct CD4$^+$CD25$^{++}$ population
  • Reduce the likelihood of isolating or expanding CD4$^+$25$^-$ cells?
Adult PB Has More CD25\textsuperscript{lo} Cells Than Cord Blood

Uniform Suppression by UCB $T_R$ v. Adult PB $T_R$

UCB CD25$^+$ cells have higher CD25 and L-selectin levels as compared to adult cells

Adult CD25$^{++}$Lin- Tregs were more rigorously purified than adult CD25$^+$

Clinical Production of UCB-Derived $T_R$ Cells

University of Minnesota Cancer Center

Minnesota Molecular & Cellular Therapeutics Facility

Cancer Center photo courtesy of Chris Gregersen; [http://www.phototour.minneapolis.mn.us](http://www.phototour.minneapolis.mn.us); Phototour of Minneapolis; MMCT Facility photo courtesy of Diane Kadidlo
• Enrich for CD4+/CD25+ T regulatory cells using the CliniMACS® device, CD25 MicroBeads.

• Culture in X-VIVO 15 supplemented with 10% human AB serum, L-glutamine, n-acetylcysteine and 2.5 mL gentamicin in a tissue culture flask.

• Anti-CD3/antiCD28-coated beads (provided by Carl June and Bruce Levine, University of Pennsylvania).

• The cell culture is supplemented with 300 IU/mL IL-2 starting on the third day of culture.

• Cells are cultured as described above in an appropriately sized culture flask(s)/cell factory for 18 (±1) days.

• Lot release testing
### UCB T-Reg Processing Summary

#### Cell Therapy Translational Laboratory

<table>
<thead>
<tr>
<th>Lot Release Information</th>
<th>Spec</th>
<th>C070581</th>
<th>C070615</th>
<th>C070634</th>
<th>C070659</th>
<th>Average</th>
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<td>Test</td>
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<td></td>
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<tr>
<td>% CD4+/CD25+</td>
<td>&gt; 70.0%</td>
<td>70.7%</td>
<td>92.7%</td>
<td>73.8%</td>
<td>95.4%</td>
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<tr>
<td>% CD4-/CD8+</td>
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<td>3.0%</td>
<td>1.2%</td>
<td>2.7%</td>
<td>0.5%</td>
<td>1.9%</td>
</tr>
<tr>
<td>7AAD</td>
<td>&gt; 70.0%</td>
<td>99.3%</td>
<td>99.5%</td>
<td>96.8%</td>
<td>75.9%</td>
<td>92.9%</td>
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<tr>
<td>CD3/28 Bead Ct</td>
<td>&lt;100 / 3.0E+06</td>
<td>6 / 3.0E+06</td>
<td>0 / 3.0E+06</td>
<td>3 / 3.0E+06</td>
<td>0 / 3.0E+06</td>
<td>2 / 3.0E+06</td>
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<td>EU/Kg</td>
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<td>&lt; 2.2 EU/Kg</td>
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<tr>
<td>Gram Stain</td>
<td>No Organisms Seen</td>
<td>No Organisms Seen</td>
<td>No Organisms Seen</td>
<td>No Organisms Seen</td>
<td>No Organisms Seen</td>
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</tr>
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</table>

#### Initial Cord Blood Unit

| TNC                     | 2.74E+09   | 2.00E+09  | 1.32E+09  | 8.00E+08  | 1.72E+09  |

#### Initial Product Information (Post-Wash)

| TNC                     | 6.51E+06   | 4.25E+06  | 7.26E+06  | 2.50E+06  | 5.13E+06  |
| % CD4+/CD25+            | 43.8%      | 61.3%     | 49.1%     | 70.9%     | 56.3%     |
| % CD4-/CD8+             | 0.8%       | 1.9%      | 1.4%      | 0.1%      | 1.0%      |
| 7AAD                    | 92.6%      | 99.3%     | 98.1%     | 98.7%     | 97.2%     |
| Recovery                | 0.2%       | 0.2%      | 0.6%      | 0.3%      | 0.3%      |

#### Final Product Information (Pre-Processing)

| TNC                     | 7.61E+09   | 1.19E+09  | 5.45E+09  | 1.47E+08  | 3.60E+09  |
| Expansion               | 1169       | 280       | 751       | 59        | 565       |

#### Final Product Information (Post-Processing)

| TNC                     | 4.35E+09   | 5.29E+08  | 3.82E+09  | 3.36E+07  | 2.18E+09  |
| Recovery                | 57%        | 44%       | 70%       | 23%       | 49%       |
| CFSE (1:X)              | 16         | 64        | 64        | 16        | 40        |
| MLR (1:X)               | 8          | 32        | 32        | 32        | 26        |

#### Infused Product

| Cell Volume (ml)        | 0.14       | 1.6       | 0.8       | 31        |
| TNC                    | 5.91E+06   | 1.07E+07  | 2.30E+07  | 1.71E+07  |
| Dose (NC/Kg)           | 1.00E+05   | 1.00E+05  | 3.00E+05  | 3.00E+05  |

1/24/2008
UMBILICAL CORD BLOOD TRANSPLANT WITH CO-INFUSION OF UCB-DERIVED T REGULATORY CELLS

Hypothesis

We hypothesize that the infusion of CD4+CD25+ Treg cells is safe and will not be associated with excessive toxicity.
Regulatory T cell unit and UCB graft selection

- HLA A & B: Ag level
- HLA DRB1: Allele level
Study Design

Phase I semi-log dose escalation:

– $1 \times 10^5$/kg
– $3 \times 10^5$/kg
– $10 \times 10^5$/kg and
– $30 \times 10^5$/kg

Planned Accrual: 12 to 24 patients
Endpoints

Primary: Determine the MTD of UCB-derived Treg cells.

Secondary:
1. Determine speed of neutrophil and platelet recovery at day 42.
2. Determine incidence of ‘double chimerism’ (e.g., engraftment of both UCB units) at day 21.
3. Estimate the risk of severe grade III-IV acute GVHD at day 100.
4. Estimate the risk of chronic GVHD at 1 year.
5. Estimate probability of survival at 100 days and 1 year.
Stopping Rules

- Graft Failure
- Grade III-IV Acute GVHD
- Transplant Related Mortality
Monitoring

UCB-derived Treg cells Production

UCB-derived Treg cells

FLU FLU FLU

1320 cGyTBI

Double UCBT

Monitoring

MMF CSA

G-CSF

Treatment Plan

UCB-derived Regulatory T Cells
Nonmyeloablative UCB-Derived Treg Study

UCB-derived Treg cells

Production

Conditioning

UCBT

Monitor

MMF

CSA

G-CSF

UCB-derived Treg cells

-18 .... -6 -5 -4 -3 -2 -1 0 1 2 3..... 15 16
## Patient Activities

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Test</th>
<th>Days</th>
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</thead>
<tbody>
<tr>
<td>Molecular Diagnostics Laboratory</td>
<td>Engraftment</td>
<td>1(±2)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7(±2)*</td>
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<tr>
<td>CancerCenter Cell Therapy Core Laboratory</td>
<td>Cytokine levels</td>
<td>14(±2)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28(±2)</td>
</tr>
<tr>
<td></td>
<td>Flow cytometry</td>
<td>60(±7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100(±7)</td>
</tr>
</tbody>
</table>

* Research only samples
<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Flow Cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ</td>
<td>CD4</td>
</tr>
<tr>
<td>IL-7</td>
<td>CD25</td>
</tr>
<tr>
<td>IL-15</td>
<td>Foxp3</td>
</tr>
<tr>
<td>TGFβ</td>
<td>CD19 or 20</td>
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<tr>
<td>IL-10</td>
<td>CD27</td>
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<tr>
<td>IL-17</td>
<td>CD62L</td>
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<tr>
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<td>CD127</td>
</tr>
<tr>
<td></td>
<td>CD45RA</td>
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<td></td>
<td>CD45RO</td>
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</table>
Conclusion

• Double UCB is efficacious but we can improve on engraftment and GVHD

• Animal data shown potent effect of ex vivo expanded activated Tregs on GVHD and engraftment

• Clinical scale production shows consistent expansion and function

• Clinical protocols are accruing patients
Future Directions

• $T_R$ cells will undergo extensive phase I testing in the next few years in a variety of clinical settings (e.g., BMT)

Will $T_R$ cells be well-tolerated and efficacious in suppressing GVHD and BM graft rejection?

To what extent will efficacy be dependent upon the clinical venue to be used for testing of $T_R$?

How will immune suppressive agents alter $T_R$ efficacy?

Will infection risk or tumor recurrence be increased?
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