**Clinical Trials: Identifying the role of TREGs**

Sang-Mo Kang, MD  
Division of Transplantation  
Department of Surgery  
University of California, San Francisco

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**The Reality of Immunosuppression**

Triple  
Immunosuppression to Prevent Graft Rejection

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**Immunosuppression**

- Non-specific inhibition of immune responses  
  - against transplanted organ  
  - against pathogens  
  - against cancer  
- Numerous immunosuppression related metabolic complications  
- Long term outcomes have largely plateaued  
- The most pressing need in transplantation is the induction of tolerance

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**Overview**

- Brief background of regulatory T cells  
- Role of Regulatory T cells (Treg) in transplantation tolerance  
- Considerations for therapeutic use of Treg in transplantation  
- Treg Manufacturing  
- Ongoing/planned clinical trials
The Emergence of Tregs in Transplant Tolerance

Immune tolerance

Identity of suppressor T cells in Transplantation

CD4+CD25+

Treg therapy in GvHD

CD4+CD25+ Tregs in autoimmunity

Foxp3

Foxp3+ Tregs are essential for immune homeostasis

The mutation in the FOXP3 gene leads to massive immune dysregulation (autoimmune polyendocrinopathy; autoimmune diabetes; hypothyroidism; autoimmune hemolytic anemia; autoimmune thrombocytopenia lymphadenopathy)

Elimination of Tregs Leads to Rapid Death

Kim JM et al. Nature Immunology 2007

Immune system control of autoimmunity depends on a professional regulatory T cell

General Immune Homeostasis

Local Regulation

Treg Therapy in Transplantation: Bench to Bedside
Role of Treg in Immunity

- Tregs are critical to maintaining homeostasis and preventing autoimmunity
- Treg infiltration into tumors appears to provide a “privileged” microenvironment
- Tregs have been shown to be critical to the development and maintenance of allospecific graft tolerance in numerous animal models
  - Δ Spontaneous liver transplant tolerance

Growing evidence that Tregs are potential therapeutics in transplantation

- Tregs have been shown to prevent and even reverse autoimmunity in animal models
- Tregs have been shown to be effective in preventing graft versus host disease in humans
- Donor-specific Treg therapy does not appear to inhibit responses to viral pathogens or vaccines in mouse models
- Treg therapy for graft versus host disease does not appear to inhibit anti-tumor responses in bone marrow transplant models
  - Can Treg therapy be applied to reduce or eliminate non-specific immunosuppression in humans?

Donor-reactive Tregs have limited capacity to prolong allogeneic graft survival in normal hosts

- BALB/c → B6 heart transplantation
- Donor-reactive Tregs have limited capacity to prolong allogeneic graft survival in normal hosts

Polyclonal vs Antigen Reactive Tregs

- Polyclonal Tregs are “unselected”, easy to expand
- “donor antigen reactive” Treg (DAR Treg) have been selected for reactivity to the donor
- Approximately 1 in 10 polyclonal Tregs will have donor reactivity
  - Δ Therefore at least 10 times less potent on a cell per cell basis
Why Don’t Treg work well in normal hosts?

- Almost all examples of transferring transplantation tolerance with Treg has been in the setting of co-adoptive transfer into lymphopenic hosts (very few lymphocytes), with a limited number of T effector cells (Teff)
- This suggests that the balance of Treg to Teff is important
- 5-10% of ALL T cells are reactive to a fully mismatched donor
  - Compared to approximately 1 in $10^6$ for a conventional antigen
  - is there a role for depletion of Teff?

Depletion of donor-reactive Teff cells is critical to efficacy of Treg

Depletion + donor-specific Tregs
Depletion + polyclonal Tregs

Days after iTx

Graft Survival (%)

0 7 14 21 28 35 42 49 56 63 70

None

Depletion

Treg Therapy Increases Treg Frequency in the Allografts

Deletion alone
Deletion + Treg

CD4 Foxp3 Ly5.1

% Tregs

General Principles of Treg Rx from Mouse Models

- Donor-specific Tregs are more effective than polyclonal, unselected Treg
  - 5-10% of polyclonal Tregs are donor reactive
- Depletion of the donor-specific T effector cells is required for optimal efficacy of Treg therapy
Early adoptive co-transfer studies in mice showed that a ratio of at least 1:3 Treg/Teff ratio is needed.

Tolerogenic treatments, such as sirolimus and anti-CD40L, leads to early accumulation of 30% Tregs in grafts.

Alloantigen-specific Treg protected grafts have 30% Tregs in the first two weeks after transplant.

30% Tregs in immunosuppressive tumor micro-environment

How many Tregs are needed to block transplant rejection in humans?

- If a ~1:3 Treg/Teff ratio is necessary for efficacy, how many Tregs do you need to give?

Numbers of CD4+ T cells and Tregs in humans

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Lymphocytes</th>
<th>%CD4</th>
<th>Total CD4</th>
<th>%Treg</th>
<th>Total Treg</th>
<th>% Total Treg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>10 x 10⁹</td>
<td>50%</td>
<td>5 x 10⁹</td>
<td>5%</td>
<td>0.25 x 10⁹</td>
<td>1.9%</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>190 x 10⁹</td>
<td>50%</td>
<td>95 x 10⁹</td>
<td>8%</td>
<td>7.6 x 10⁹</td>
<td>57.8%</td>
</tr>
<tr>
<td>Spleen</td>
<td>70 x 10⁹</td>
<td>20%</td>
<td>14 x 10⁹</td>
<td>5%</td>
<td>0.7 x 10⁹</td>
<td>5.3%</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>50 x 10⁹</td>
<td>20%</td>
<td>10 x 10⁹</td>
<td>25%</td>
<td>2.5 x 10⁹</td>
<td>19%</td>
</tr>
<tr>
<td>Thymus</td>
<td>50 x 10⁹</td>
<td>10%</td>
<td>5 x 10⁹</td>
<td>9%</td>
<td>0.45 x 10⁹</td>
<td>3.4%</td>
</tr>
<tr>
<td>Lung</td>
<td>30 x 10⁹</td>
<td>40%</td>
<td>12 x 10⁹</td>
<td>7%</td>
<td>0.84 x 10⁹</td>
<td>6.4%</td>
</tr>
<tr>
<td>Liver</td>
<td>10 x 10⁹</td>
<td>25%</td>
<td>2.5 x 10⁶</td>
<td>2%</td>
<td>0.05 x 10⁹</td>
<td>0.38%</td>
</tr>
<tr>
<td>Intestines</td>
<td>50 x 10⁹</td>
<td>30-50%</td>
<td>17 x 10⁹</td>
<td>3%</td>
<td>0.5 x 10⁹</td>
<td>3.8%</td>
</tr>
<tr>
<td>Others</td>
<td>10 x 10⁹</td>
<td>50%</td>
<td>5 x 10⁹</td>
<td>5%</td>
<td>0.25 x 10⁹</td>
<td>1.9%</td>
</tr>
<tr>
<td>Total</td>
<td>460 x 10⁹</td>
<td>8%</td>
<td>165.5 x 10⁹</td>
<td>8%</td>
<td>13.1 x 10⁹</td>
<td>100%</td>
</tr>
</tbody>
</table>

How to get Treg to 30%?

- Infuse Tregs after ex vivo expansion
- Lymphodepletion + non-expanded Tregs
- Lymphodepletion + expanded Tregs
- Lymphodepletion + expanded Tregs (Donor Specific)
- Lymphodepletion + expanded Tregs (Donor Specific)

<table>
<thead>
<tr>
<th>Approaches</th>
<th>Endogenous CD4</th>
<th>Endogenous Tregs</th>
<th>Type of therapeutic Tregs</th>
<th>Number to infuse</th>
<th>% Tregs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infuse Tregs after ex vivo expansion</td>
<td>165.5 x 10⁹</td>
<td>13.1 x 10⁹</td>
<td>Polyclonally expanded</td>
<td>52 x 10⁹</td>
<td>30%</td>
</tr>
<tr>
<td>Lymphodepletion + non-expanded Tregs</td>
<td>16.5 x 10⁹</td>
<td>1.3 x 10⁹</td>
<td>Isolated, banked without expansion</td>
<td>0.2 x 10⁹</td>
<td>9%</td>
</tr>
<tr>
<td>Lymphodepletion + expanded Tregs</td>
<td>16.5 x 10⁹</td>
<td>1.3 x 10⁹</td>
<td>Polyclonally expanded</td>
<td>1.4 x 10⁹</td>
<td>16%</td>
</tr>
<tr>
<td>Lymphodepletion + expanded Tregs (Donor Specific)</td>
<td>1.65 x 10⁹</td>
<td>.13 x 10⁹</td>
<td>Donor antigen expanded</td>
<td>0.4 x 10³</td>
<td>32%</td>
</tr>
</tbody>
</table>

** delete 90% of all T cells, leaving 1% donor reactive T effector cells
Considerations for Treg Therapy in Human Transplantation

- Treg therapy with unexpanded Treg will not achieve high enough levels of Treg
- Treg therapy with polyclonal Treg will also be difficult
  - Also potential for non-specific suppression
- Expansion of donor-specific Treg along with lymphodepletion will be necessary for clinical translation

Large Scale Expansion of Donor-Reactive Tregs

Clinical donor-reactive Treg manufacturing approved by FDA

Treg Therapy in Transplantation: Bench to Bedside

Donor-reactive Treg expansion

Antigen-specific primary expansion
- Donor B cell activation: 10 days
- Polyclonal secondary expansion using anti-CD3/28 beads: 5 days
- Harvest & Release assays: 10-12 hrs

Phenotype of Expanded Tregs - Examples

Un-gated Treg culture
- Gated on CD4+ cells

Treg by TSDR: Treg-specific demethylated region

Treg Therapy in Transplantation: Bench to Bedside
Conclusions I

- Lymphodepletion combined with infusion of approximately half billion donor-reactive Tregs may be effective at preventing transplant rejection
- Billions of donor-reactive Tregs can be selectively expanded in short-term cultures under GMP conditions
  - High purity, potency
  - Stable
  - Can be shipped
- Several clinical trials underway

Why propose to test Tregs in liver Tx

- The liver is known to be a “tolerogenic” organ in animal models
- Many liver transplant recipients become spontaneously tolerant after >6 yrs from transplant
- Importantly, rejection is readily treated in those who “fail” withdrawal with minimal long-term sequelae
- If Phase I safety studies are successful, a phase II withdrawal trial is possible
  - Potential availability of tolerance “signatures”
- Need to give immunosuppression: what kind?

deLTa: Donor Reactive T cells in Liver Transplantation

PIs: Feng, Kang, Tang, Bluestone

Treg Therapy in Transplantation: Bench to Bedside

Treg “friendly” immunosuppression?

- mTOR inhibitors (sirolimus, everolimus) preferentially inhibits conventional T cells (Teff) and promotes outgrowth of T cells (Treg) during in vitro expansion
- Tolerance induction depends on de-bulking allogeneic responses (Strom, Turka and others) - Thymoglobulin is effective “de-bulker”
- Thymoglobulin preserves Tregs and increases the proportion of Treg:Teff in patients
  - Thymo has been shown to favor Treg growth in vitro
Treg Therapy in Transplantation: Bench to Bedside

**deLTa Study Description**

A two-center, open-label, dose escalation, pilot study in which subjects undergoing primary cadaver liver transplantation will receive a single infusion of increasing doses of autologous, donor-reactive T regulatory cells in the context of Treg supportive immunosuppression [rabbit Thymoglobulin (rATG) and everolimus (EVR)]

**Thymo + EVR Immunosuppression**

<table>
<thead>
<tr>
<th>Transplant</th>
<th>Out-patient Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTx / Treg supportive IS</td>
<td>Everolimus conversion</td>
</tr>
<tr>
<td>D0</td>
<td>D3</td>
</tr>
<tr>
<td><strong>Pred (mg/d)</strong></td>
<td>500</td>
</tr>
<tr>
<td><strong>MMF (mg/d)</strong></td>
<td>1000</td>
</tr>
<tr>
<td><strong>Tac (µg/L)</strong></td>
<td>Start; target 6 - 8</td>
</tr>
<tr>
<td><strong>rATG (mg/kg)</strong></td>
<td>3 - 4.5 (non-ICU)</td>
</tr>
<tr>
<td><strong>EVR (µg/L)</strong></td>
<td>Start; target 6 - 8</td>
</tr>
<tr>
<td><strong>Tregs (x10⁶)</strong></td>
<td>0,↑doses</td>
</tr>
</tbody>
</table>

**Todo Treg Trial**

“Treg” made by mixing donor cells with recipient lymphocytes AND recipient splenocytes with costimulation blockade. **No purification of cells at any point**

**Todo Treg Trial-LDLT**

![Todo S et. al Hepatology](image.png)
Todo Treg Trial

<table>
<thead>
<tr>
<th>Case</th>
<th>POD</th>
<th>Drug Free (Month)</th>
<th>Liver function (U/mL)</th>
<th>AST/ALT-r-GTP</th>
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<tbody>
<tr>
<td>1</td>
<td>1,620</td>
<td>Off (33 mo)</td>
<td>19/12/14</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1,543</td>
<td>Off (31 mo)</td>
<td>26/26/14</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1,615</td>
<td>Off (32 mo)</td>
<td>23/21/79</td>
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</tr>
<tr>
<td>4</td>
<td>1,410</td>
<td>Off (29 mo)</td>
<td>23/4/10</td>
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<tr>
<td>5</td>
<td>1,326</td>
<td>Tac (4 mg, 1/d)</td>
<td>17/12/25</td>
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<tr>
<td>6</td>
<td>1,284</td>
<td>MMF (500 mg/d)</td>
<td>27/21/25</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1,263</td>
<td>Off (23 mo)</td>
<td>33/39/24</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1,186</td>
<td>Off (18 mo)</td>
<td>18/13/20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1,123</td>
<td>Tac (4 mg, 1/d)</td>
<td>20/13/18</td>
<td></td>
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<tr>
<td>10</td>
<td>1,018</td>
<td>Off (16 mo)</td>
<td>18/13/16</td>
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</table>

Todo Treg Trial-Considerations

- 7 of 10 patients were successfully taken off immunosuppression by 18 months post tx
  - Follow-up out to >40 months for some patients
  - Did not work for autoimmune diseases
- Simple manufacturing is attractive
- Demonstrates feasibility of using cyclophosphamide as depleting agent
- However, the composition of cells is not consistent—may be problematic especially with regulatory agencies
- Requires splenectomy of recipient
  - Approximately 10% portal vein thrombosis rate
- Overall is an exciting proof of concept and is a great stimulus for further trials

The ONE Study Consortium

to Study Cellular Therapy in Renal Tx

One Study:
Treg Therapy in Transplantation: Bench to Bedside

The ONE Study Clinical Trials

- Tr1
- Treg Ag-specific
- Treg poly
- tolDC
- M reg

Living donor kidney transplant recipients

» All cells tested using the same clinical trial design/immune monitoring program

DART: One Study@UCSF

- Donor-alloantigen-reactive Tregs (darTreg) manufactured with donor B cells
- 3 patients @ 300 x 10^6 cells total (~4 x 10^6/Kg)
- 5 patients @ 900 x 10^6 cells total (~12 x 10^6/Kg)
- Tracking infused Tregs using deuterium label and TCR sequencing
  - How long do infused Treg last?
  - Do they divide?

Results to date – Cell Therapy Trials

Patient Recruitment & Treatment Status: August 2016
- 58 patients enrolled
- 33 patients treated (confirmed)

Safety conclusions thus far after 32 patients

So far in The ONE Study:
- No major events suggesting general safety concerns with cell therapy
- The relative number of serious adverse events is not elevated
- Events possibly related to cell therapy injection have been rare and resolvable
- Rejection rate in cell therapy treated is not higher than controls (so far)
- No signs of excessive immunosuppression due to cell therapy
- TAC mono therapy has been successful in all patients where attempted
- Early evidence of reduced infectious complications
Treg Adoptive therapy for Subclinical inflammation in Kidney transplantation (TASK)

Subclinical inflammation in protocol biopsy

- Re-biopsy in 14d
- Infused Tregs in circulation
- Biomarkers

Vincenti & Chandran et al unpublished data

Treg Therapy in Transplantation: Bench to Bedside

Treg Therapy program at UCSF

<table>
<thead>
<tr>
<th>Trial</th>
<th>PI</th>
<th>Indication</th>
<th>Treg type</th>
<th># of Pt</th>
<th>Enrollment</th>
<th>Infusion</th>
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</thead>
<tbody>
<tr>
<td>T1D-I</td>
<td>Gitelman/Herold</td>
<td>T1D</td>
<td>Poly</td>
<td>14</td>
<td>complete</td>
<td>complete</td>
</tr>
<tr>
<td>TILT</td>
<td>Gitelman/Herold</td>
<td>T1D</td>
<td>Poly</td>
<td>12</td>
<td>enrolling</td>
<td>0</td>
</tr>
<tr>
<td>SLE</td>
<td>Wofsy</td>
<td>Cutaneous lupus</td>
<td>Poly</td>
<td>12-18</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>DART</td>
<td>Kang</td>
<td>LD kidney 3d</td>
<td>Alloreactive</td>
<td>8</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>TASKp</td>
<td>Vincenti/C Chandran</td>
<td>LD kidney 6m</td>
<td>Poly</td>
<td>3</td>
<td>complete</td>
<td>complete</td>
</tr>
<tr>
<td>TASK</td>
<td>Vincenti/C Chandran</td>
<td>LD kidney 6m</td>
<td>Poly</td>
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<td>enrolling</td>
<td>0</td>
</tr>
<tr>
<td>deLTA</td>
<td>Feng/Kang</td>
<td>Liver Tx 3m</td>
<td>Alloreactive</td>
<td>12-18</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Artemis</td>
<td>Feng</td>
<td>LD liver Tx 2-6yr</td>
<td>Alloreactive</td>
<td>9</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

Summary of Treg cell therapy in humans

- **Feasibility**: billions of polyclonal or donor-specific cells can be made and distributed even from immunosuppressed patients
- **Safety**: in 130+ patients thus far, well tolerated, MTD not reached at 2.6 billion total dose or 100 million/Kg
- **Pharmacokinetics**: 2.6 billion cells reached 15% of circulating pool at peak
  - Some of the cells are long-lived and
  - Phenotype stable, even in patients with chronic inflammation or on immunosuppression
- **Efficacy**:  
  - Control of GvHD  
  - Tolerance in liver transplantation  
  - Suppression of inflammation

TASKp pt 1 – Biopsies

Index bx: i1/t1/i1/t1/a1  
2w post-Treg: i0/t0/i1/a1  
6m post-Treg: i0/t1/i0/a1

Treg Therapy in Transplantation: Bench to Bedside
Key conclusions

- Antigen-specific Tregs work better than polyclonal Treg
- “De-bulking” large alloreactive T cell pool is critical
- Treg supportive immunosuppression are likely to support Tregs and may enhance efficacy of Treg therapy
- Cell therapy is complex and will require further development before large scale application
  - Numerous manufacturing issues, storage, etc.
- We should know the efficacy of Treg within 5-10 years
  - We will likely see efficacy in liver well before kidney tx

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