Tregs and Tcons adoptive immunotherapy in T-cell depleted full-haplotype mismatched HSCT

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COSTEM

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Drawbacks in T-cell depleted full-haplotype mismatched HSCT in acute leukemias

- Graft rejection
- Graft *versus* host disease
  - Post-transplant leukemia relapse
  - Post-transplant immunodeficiency
Factors involved in engraftment of T-cell depleted Haploidentical HSCs

**Conditioning**
- sTBI
- Thiotepa
- Fludara
- ATG
- 8 Gy TBI in a single fraction at 16 cGy/m
- Thiotepa 4 mg/kg/day
- ATG
- Fludarabine 40 mg/sqm/day

**Graft**
- Median Dose of CD34+ Cells: $12.8 \times 10^6$/kg b.w.
- Median Dose of CD3+ Cells: $1 \times 10^4$/kg b.w.
- Median Dose of CD20+ Cells: $4.1 \times 10^4$/kg b.w.
- No post-transplant immunosuppression
Engraftment

- Primary: 92%
- Overall: 98%

GvHD

- Overall: 2%
- Acute: 3%

No of patients = 196

No post-transplant immunosuppression

Aversa F. et al., J Clin Oncol 2005;23:3447-3454
Post-transplant generation of alloreactive NK repertoire (Ruggeri L., Velardi A. Blood 1999)

High-intensity conditioning

The reconstituting NK cells have the same repertoire as the donor

A potentially NK alloreactive donor occurs in nearly 50% of transplant pairs
Relapse and Event-Free Survival

64 AML Patients (CR I #24, CR II #28, CR≥III #12)

Cumul. incidence of relapse

Probability of EFS

Ruggeri et al., Science 2002; Blood 2007; Stern et al., Blood 2008; updated 2011
Transplant Related Mortality due to:

• patient condition and disease stage

• slow post-transplant immune reconstitution

In T-cell-depleted mismatched transplant the number of T cells in the graft has to be extremely low to prevent GvHD, so T-cell repertoire is very narrow; ATG exerts an \textit{in vivo} T cell depletion of the graft and may antagonize T-cell homeostatic expansion.
Improving post-transplant immunity after HLA-haploidentical HSCT

Adding back mature donor T-cells with a broad repertoire

Clinical techniques to prevent graft versus host disease

a) suicide gene insertion (i.e. HSV-tk) into T-cells allows switch-off of GvHD

b) donor T cells ex vivo depleted of anti-host alloreactivity (i.e. photodynamic purging of alloreactive T cells; depletion of activated T cells with anti-CD25 bound to beads)
Rebuilding post-transplant immunity

Lessons from animal models

Adoptive transfer of naturally arising CD4+CD25+ regulatory T cells (Tregs), when coinfused with conventional T lymphocytes (Tcons), prevents GvHD, while favoring immune reconstitution

Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses

Donor type CD4+CD25+ regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation

CD4+CD25+ regulatory T cells preserve graft versus-tumor-activity while inhibiting graft-versus-host disease after bone marrow transplantation
Edinger et al., Nat Med 2003, 9:1144-1150

In vivo dynamics of regulatory T-cell trafficking and survival predict effective strategies to control graft-versus-host disease following allogeneic transplantation

The impact of regulatory T cells on T-cell immunity following hematopoietic cell transplantation
Nguyen et al., Blood 2008, 111:945-953

Immune reconstitution is preserved in hematopoietic stem cell transplantation coadministered with regulatory T cells for GvHD prevention
Gaidot et al. Blood 20010, 117:2975-2983
**DONOR**

- FOXP3
- GITR
- CTLA-4
- CD 62L
- CD 39

**RECIPENT**

- mismatched HSC transplant

- lymph node APCs

- activation of specific donor Tregs for recipient alloantigens

- expansion of alloantigen specific Tregs

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*Nguyen et al. Blood 109, 2646, 2007*
Alloantigen-specific Tregs control Tcon alloreactivity

First “check point” in lymph nodes

Alloantigen-specific Tregs act in an antigen-specific manner in vivo during their effector phase, thus …..

… they control activation and early proliferation of alloreactive donor T cells via interaction with APCs in priming sites. Conversely they do not cross inhibit pathogen-specific Tcon expansion and response.
Alloantigen-specific Tregs control Tcon alloreactivity

First “check point” in lymph nodes

Alloantigen-specific Tregs act in an antigen-specific manner in vivo during their effector phase, thus ..... 

Second “check point” in peripheral tissues (skin, gut, liver, lung)

... they control activation and early proliferation of alloreactive donor T cells via interaction with APCs in priming sites. Conversely they do not cross inhibit pathogen-specific Tcon expansion and response.
8 Gy TBI in a single fraction at 16 cGy/m
Thiotepa 4 mg/kg/day
Cyclophosphamide 35 mg/kg/day
Fludarabine 40 mg/sqm/day

Conditioning Regimen and Inoculum

8 Gy TBI in a single fraction at 16 cGy/m
Thiotepa 4 mg/kg/day
Cyclophosphamide 35 mg/kg/day
Fludarabine 40 mg/sqm/day

In vivo expansion of donor Tregs in the setting of HLA disparity

No post-transplant immunosuppression

- TBI
- TT
- Cyclophosphamide
- Thiotepa 4 mg/kg/day
- Cyclophosphamide 35 mg/kg/day
- Fludarabine 40 mg/sqm/day
- Fludarabine 40 mg/sqm/day
- 2x10^6/kg
- 10x10^6/kg
- 2x10^6/kg
- 1x10^6/kg
- Tregs
- CD34+
- Tcons
Selection and Characterization of CD4+CD25+ Regulatory T Cells

Leukapheresis product

Fully Automated Immunomagnetic Selection of CD4+CD25+Cells

1st step: Depletion of CD8+/CD19+cells

2nd step: Enrichment of CD25+ cells

Gate on CD4CD25+

Gate on CD4CD25+high

Starting fraction | Final fraction
--- | ---
Cells (x10^9) | 1060 (540-1370) | 280 (202-390)
%CD4CD25 | 3.0 (1.5-7.45) | 92.4 (90-97.1)
N° cells (x 10^6) | 330 (221-1020) | 256 (185.6-365.4)
%CD4CD25high | 0.3 (0.12-0.89) | 33.6 (14.4-39.6)
N° cells (x 10^6) | 36.12 (19.98-84) | 68.6 (20.9-143)

**Selection and Characterization of CD4+CD25+ Regulatory T Cells**

**Leukapheresis product**

*Fully Automated Immunomagnetic Selection of CD4+CD25+ Cells*

<table>
<thead>
<tr>
<th>1st step: Depletion of CD8+/CD19+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd step: Enrichment of CD25+ cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Final Cell Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced expression of CD62L, CD39, GITR, CTLA4</td>
</tr>
<tr>
<td>CD45RO was the predominant isoform while the CD45RA was around 10%.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Starting fraction</th>
<th>Final fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells (x10⁹)</td>
<td>1060 (540-1370)</td>
</tr>
<tr>
<td>%CD4CD25</td>
<td>3.0 (1.5-7.45)</td>
</tr>
<tr>
<td>Nº cells (x10⁶)</td>
<td>330 (221-1020)</td>
</tr>
<tr>
<td>%CD4CD25&lt;sub&gt;high&lt;/sub&gt;</td>
<td>0.3 (0.12-0.89)</td>
</tr>
<tr>
<td>Nº cells (x10⁶)</td>
<td>36.12 (19.98-84)</td>
</tr>
</tbody>
</table>
Harvesting Tregs from peripheral blood before stem cell collection increases cell number in starting fraction and significantly enhances the Treg count in the final fraction.

<table>
<thead>
<tr>
<th></th>
<th>Post HSC collection</th>
<th>Pre HSC collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total n° Cells (x 10^6)</strong></td>
<td>163.8 (58.2 - 387)</td>
<td>280 (202 - 390)</td>
</tr>
<tr>
<td>%CD4CD25</td>
<td>88.7 (76.2 – 91.7)</td>
<td>92.4 (90 – 97.1)</td>
</tr>
<tr>
<td>N° cells (x 10^6)</td>
<td>149.9 (57.2 – 321)</td>
<td>256 (185.6 – 365.4)</td>
</tr>
<tr>
<td>%CD4CD25^{high}</td>
<td>11.8 (0.9 – 25.5)**</td>
<td>33.6 (14.4 – 39.6)**</td>
</tr>
<tr>
<td>N° cells (x 10^6)^{high}</td>
<td>17.0 (0.7 - 41.8)**</td>
<td>68.6 (20.9 - 143)**</td>
</tr>
</tbody>
</table>

N=8                       N=15

* median+range

** p<0.05
Tregs inhibit MLR in a dose dependent way
HLA identity between Tregs and Tcons is required for maximum suppression of alloresponses.

Donor Tregs do not prevent graft rejection in mismatched transplant.

Tregs from a third party donor might not prevent GvHD after HSCT.
First clinical trial
September 2008-October 2009

No of patients 28
Median age (years, range) 41 (21-60)
Gender (male/female) 11/17

Disease and status at transplant

Acute myeloid leukemia 22
  CR1 10
  ≥CR2 10
  Relapse 2
Acute lymphoid leukemia 5
  CR1 4
  Relapse 1
High grade NHL in relapse 1

The two cases of GvHD were among the 5 patients who had received $4 \times 10^6$ Kg/bw Tregs and $2 \times 10^6$ Kg/bw Tcons.
Pattern of immunoreconstitution

Recovery of CD4\(^+\) and CD8\(^+\) T cell subpopulations

Days post BMT

CD4/\(\mu l\)

CD8/\(\mu l\)

Spectratyping

Complexity score

Donors

Months after transplant
Reconstitution of pathogen-specific T-cell repertoire

Limiting dilution analyses of pathogen-specific CD4+ and CD8+ cells

Proliferating CD4+ pathogen-specific T cells per 10^6 cells

INF-γ producing CD8+ pathogen-specific T cells per 10^6 cells

Months after transplant

Standard Haplo (n=150)  
Haplo with T-reg (n=26)

- ASP
- Cand
- CMV
- ADV
- HSV
- VZV
- Toxo
Evaluable Patients

Patients with CMV reactivation

CMV reactivation episodes

Tregs Group

Control Group

Infection-related deaths in the control group
N=38
CMV # 17 (45%)

No CMV-related deaths

Days after transplant

p<0.05
Reconstitution of peripheral blood B cell number, B cell repertoire and seric levels of immunoglobulins

![Graph showing CD20/µl levels over Days post BMT (30, 60, 90, 120).](image)

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>733 (80-1530)</td>
<td>31 (1-72)</td>
<td>71 (17-229)</td>
</tr>
<tr>
<td>6 months</td>
<td>692 (411-876)</td>
<td>44 (8-111)</td>
<td>63 (45-140)</td>
</tr>
</tbody>
</table>
Are these patients immunologically competent?

- In accordance with ISS guidelines
- 7 subjects (≥ 3 months after stem cell transplantation) were vaccinated against pandemic influenza with 2 doses of MF59-H1N1California. No vaccination for seasonal flu.

<table>
<thead>
<tr>
<th>Day</th>
<th>Visit</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>240</th>
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<td></td>
<td>visit</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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</table>

### Hemoagglutinin Inhibition Assay (HI)

<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>ID samples</td>
<td>VISIT 1</td>
<td>VISIT 2</td>
<td>VISIT 3</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>17</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>23</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>24</td>
<td>20</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

HI titer ≥1:40 = protection against H1N1
HI ≥ 4 fold = vaccination efficacy
Profile over time of Flu specific CD4$^+$ after MF59-H1N1$^{\text{California}}$ vaccination

- % Ctk$^+$ CD4$^+$ / CD4$^+$ T cells

- Patient No.: 6, 11, 17, 20, 23, 24

- pre immune
- 30 days post 1 dose
- 30 days post 2 dose
Naive and Memory CD4$^+$ and CD8$^+$ reconstitution

- CD4$^+$CD45RA$^+$: 0-250 cells/μl
- CD4$^+$CD45RO$^+$: 0-1500 cells/μl
- CD8$^+$CD45RA$^+$: 0-500 cells/μl
- CD8$^+$CD45RO$^+$: 0-2500 cells/μl

Months after transplant

0 1 3 6 9 12
Preventing Graft versus Host Disease in full haplotype mismatched transplant

Adoptive immunotherapy with Tregs controls alloreactivity of a significant number of Tcons.

In vitro priming of Tregs is not required for GvHD inhibition, since activation of alloantigen-specific Tregs occurs efficiently in vivo.
Adoptive Immunotherapy with naturally occurring polyclonal Tregs is not associated with inadvertent and bystander inhibition of general immunity with compromised responses to microbes and tumor following HSCT

- Alloantigen-specific Tregs do not cross-inhibit pathogen-specific Tcon responses

- Adoptive transfer of Tregs and Tcons ensures long term immunity, needed for effective vaccination and adequate responses to recall antigens
# Outcomes

<table>
<thead>
<tr>
<th>Non-relapse mortality</th>
<th>Disease-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>13/26 (50%)</td>
<td>12/26 (46%)</td>
</tr>
</tbody>
</table>

- Veno-occlusive disease (3)
- Multi-organ failure (1)
- Adenoviral infection (1)
- Adenoviral infection and GvHD (1)
- GvHD (1)
- Bacterial sepsis (1)
- Systemic toxoplasmosis (1)
- Fungal pneumonia (3)
- CNS aspergillosis (1)

Median follow-up 18.5 months (range 16.1-27.6)
Second Clinical Trial

*May 2010*

Less regimen related toxicity

- **Alemtuzumab**
- **TBI**
- **TT**
- **Tregs**
- **CD 34+**
- **Fludarabine**
- **Thiotepa 5 mg/kg/day**
- **Fludarabine 40 mg/sqm/day**

**Alemtuzumab 20mg/sqm**
8 Gy TBI in a single fraction at 16 cGy/m
Thiotepa 5 mg/kg/day
Fludarabine 40 mg/sqm/day

**16 days**

**Improvement in Treg purification**
FoxP3+ cell yield from 70% to 90%

**Doses (Kg/bw) of CD34+, Tregs and Tcons infused into the recipient**

- **CD34+ (x10^6)**: 8.9 (8.1-10.5)
- **Tregs (x10^6)**: 2.9 (1.6-4.8)
- **Tcons (x10^6)**: 0.9 (0.5-3)

**No post-transplant immunosuppression**
### Outcome of 18 Consecutive Patients in the 2nd Clinical Trial
(Alemtuzumab, TBI, TT, Fludara protocol)

<table>
<thead>
<tr>
<th>NAME</th>
<th>AGE</th>
<th>DISEASE and STATUS at BMT</th>
<th>RISK FACTORS</th>
<th>NK allo</th>
<th>ENGRAFTMENT</th>
<th>GVHD</th>
<th>RELAPSE</th>
<th>PRESENT STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>45</td>
<td>AML – 2°CR</td>
<td>Secondary to ALL</td>
<td>YES</td>
<td>+13</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+492)</td>
</tr>
<tr>
<td>SE</td>
<td>33</td>
<td>AML – 1°CR</td>
<td>FLT3-ITD+, t(6;9), PIF</td>
<td>NO</td>
<td>+28</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+461)</td>
</tr>
<tr>
<td>RC</td>
<td>22</td>
<td>AML – 2°CR</td>
<td>Relapse after consolidation</td>
<td>YES</td>
<td>+11</td>
<td>YES</td>
<td>NO</td>
<td>Alive and Well (+385)</td>
</tr>
<tr>
<td>GL</td>
<td>37</td>
<td>AML– PR</td>
<td>Partial remission</td>
<td>NO</td>
<td>+20</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+356)</td>
</tr>
<tr>
<td>PC</td>
<td>32</td>
<td>AML – 1°CR</td>
<td>PIF</td>
<td>NO</td>
<td>+12</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+349)</td>
</tr>
<tr>
<td>SC</td>
<td>45</td>
<td>AML – 2°CR</td>
<td>FLT3-ITD+, complex karyotype</td>
<td>NO</td>
<td>+12</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+322)</td>
</tr>
<tr>
<td>CS</td>
<td>21</td>
<td>AML – 1°CR (MRD positive)</td>
<td>t(16;16), -7, cytogenetic relapse</td>
<td>NO</td>
<td>+15</td>
<td>YES</td>
<td>NO</td>
<td>Alive and Well (+314)</td>
</tr>
<tr>
<td>VM</td>
<td>52</td>
<td>ALL – 1°CR</td>
<td>t(3;7;14), TCR+</td>
<td>NO</td>
<td>+12</td>
<td>NO</td>
<td>NO</td>
<td>Dead - Fulminant Hepatitis (+17)</td>
</tr>
<tr>
<td>PF</td>
<td>23</td>
<td>Biph. AL – 1°CR</td>
<td>Complex karyotype</td>
<td>NO</td>
<td>+15</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+229)</td>
</tr>
<tr>
<td>PM</td>
<td>46</td>
<td>AML – 1°CR (MRD positive)</td>
<td>FLT3-ITD+, NPMc+</td>
<td>NO</td>
<td>+10</td>
<td>YES</td>
<td>+176</td>
<td>Alive in relapse(+215)</td>
</tr>
<tr>
<td>SC</td>
<td>60</td>
<td>AML – 1°CR</td>
<td>Complex karyotype, PIF</td>
<td>YES</td>
<td>+12</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+202)</td>
</tr>
<tr>
<td>VM</td>
<td>35</td>
<td>AML – 1°CR</td>
<td>del11q</td>
<td>NO</td>
<td>+16</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+181)</td>
</tr>
<tr>
<td>NR</td>
<td>46</td>
<td>AML – 1°CR</td>
<td>FLT-ITD+, meningeal infiltration</td>
<td>NO</td>
<td>+10</td>
<td>YES</td>
<td>NO</td>
<td>Dead.GvHD+Infection (+119)</td>
</tr>
<tr>
<td>LD</td>
<td>40</td>
<td>AML – 1°CR</td>
<td>FLT-ITD+, NPMc+, PIF</td>
<td>NO</td>
<td>+11</td>
<td>YES</td>
<td>+77</td>
<td>Dead in relapse (+110)</td>
</tr>
<tr>
<td>PL</td>
<td>50</td>
<td>AML – 1°CR</td>
<td>FLT3-ITD-, NPMc-</td>
<td>NO</td>
<td>+13</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+139)</td>
</tr>
<tr>
<td>TR</td>
<td>31</td>
<td>AML – 1°CR</td>
<td>Secondary to MDS</td>
<td>YES</td>
<td>+13</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+132)</td>
</tr>
<tr>
<td>BC</td>
<td>53</td>
<td>AML – 1°CR</td>
<td>NPMc+, cutaneous relapse</td>
<td>YES</td>
<td>+11</td>
<td>NO</td>
<td>NO</td>
<td>Alive and Well (+118)</td>
</tr>
<tr>
<td>MA</td>
<td>61</td>
<td>ALL – 1°CR (MRD positive)</td>
<td>Ph+</td>
<td>YES</td>
<td>+9</td>
<td>NO</td>
<td>NO</td>
<td>Dead – Pneumonia (+14)</td>
</tr>
</tbody>
</table>

**Median Age:** 43 ys (range 23 - 61)
**Median FU:** 9 months (range 4–16)

*Updated 05-09-2011*
# CD4+ and CD8+ T cell recovery

<table>
<thead>
<tr>
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<th>Former Protocol</th>
<th></th>
<th>New Protocol</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>CD4/uL</td>
<td>average</td>
<td>SD</td>
<td>CD4/uL</td>
</tr>
<tr>
<td>&gt;50</td>
<td>50</td>
<td>35</td>
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<td>&gt;100</td>
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<td>52</td>
<td>28</td>
<td>&gt;100</td>
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<tr>
<td>&gt;200</td>
<td>200</td>
<td>77</td>
<td>31</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>CD8/uL</th>
<th>average</th>
<th>SD</th>
<th>CD8/uL</th>
<th>average</th>
<th>SD</th>
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<tr>
<td>&gt;50</td>
<td>50</td>
<td>31</td>
<td>18</td>
<td>&gt;50</td>
<td>50</td>
<td>32</td>
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<tr>
<td>&gt;100</td>
<td>100</td>
<td>46</td>
<td>24</td>
<td>&gt;100</td>
<td>100</td>
<td>51</td>
</tr>
<tr>
<td>&gt;200</td>
<td>200</td>
<td>54</td>
<td>24</td>
<td>&gt;200</td>
<td>200</td>
<td>51</td>
</tr>
</tbody>
</table>
Evaluable Patients

Patients with CMV reactivation

Days after transplant

CMV reactivation episodes

- <0: 19
- 0-30: 11
- 31-60: 14
- 61-90: 12
- 91-120: 10
- 121-150: 7
- 151-180: 7
- 181-365: 5
- >365: 1
Rebuilding immunity with natural CD4+CD25+FoxP3+ Treg and Tcon adoptive immunotherapy is associated with a low incidence of post-transplant infection related deaths

In vitro expansion of Tregs (polyclonal Tregs or recipient-type specific Tregs) is not an indispensable step for designing Treg-based cellular therapies
FOXP3+ Tregs are indispensable for the maintenance of dominant self tolerance and immune homeostasis. However, as a double edge sword, can also suppress antitumor immune responses and favour tumor progression.

Is graft *versus* leukemia effect maintained in patients who received adoptive immunotherapy with Tregs and Tcons?
Post-transplant leukaemia relapse in patients who were transplanted in hematological remission and who did not die because of transplant related causes

- 1st and 2nd clinical studies -

Demographics:
26 patients (NK allo 12/26):
- 23 AML (NK allo 11/23)
- 1 ALL
- 2 Biphenotypic AL

Relapse: 2/26
- 2/2 with FLT3-ITD+,
  NMPc+ AML
- 1/2 in molecular relapse at transplant
- 2/2 transplanted from a non-NK allo donor

Median follow up: 14 months (range 4 – 35)
GvL effect in absence of GvHD

- post-transplant generation of donor versus recipient alloreactive NK cell clones
Treg immunotherapy did not impair regeneration of donor vs recipient alloreactive NK cell repertoires.
GvL effect in absence of GvHD

- post-transplant generation of donor versus recipient alloreactive NK cell clones

- infusion of high number of T cells in the absence of any post-transplant immunosuppression
Translational Research in Full-Haplotype Mismatched Transplant

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Paolo Sportoletti
Tiziana Zei
Roberta Iacucci
Elisabetta Bonifacio
Beatrice Del Papa
Debora Cecchini
Alain Bell

Chimera of Arezzo  c.400 BC
A votive bronze dedicated to the supreme Etruscan God of day, *Tin* or *Tina*
High-risk AL patients

Diagnosis and HLA-typing

HLA-identical sibling

- HSCT

No HLA-identical sibling

Search for alternative donors

Well-matched URD available

- MUD HSCT

No well-matched URD available

- UCB HSCT
  - Haplo HSCT
Migration of alloantigen specific CD45RO+Tregs from lymph nodes to peripheral tissues

High expression of CLA, CCR4, CCR6

Integrin α4β7

CCR9

Skin

Gut

Lung

First “check point” in lymph nodes

Second “check point” in peripheral tissues (skin, gut, liver, lung)

The HSPC niche is an immune privileged site. FOXP3+Treg cells accumulate in the HSPC niche and may provide the HSPC niche with immune privilege mechanisms.


CD45RA+ Tregs are preferentially located in the bone marrow, associated with increased CXCR4 expression

CD45RO+ Tregs are preferentially located in the skin, associated with their increased expression of CLA and CCR4.

Booth NJ et al J Immunol 184, 4317, 2010

Alloantigen specific Tregs control Tcon alloreactivity

Donor Tregs

Recipient

Full haplotype mismatched HSCT

CD 45 RO+ 90-95%

CD 45 RA+ 5-10%

APCs

migration of alloantigen specific Tregs to peripheral tissues

CD45RO+ 100%

activation and expansion of alloantigen specific Tregs in lymph nodes
Migration of alloantigen specific CD45RO+ Tregs from lymph nodes

- **Skin**: High expression of CLA, CCR4, CCR6
- **Gut**: Integrin α4β7, CCR9
- **Lung**: Low expression of CXCR4
- **Bone marrow**: ?

Alloantigen specific Tregs control Tcon alloreactivity

- **First “check point” in lymph nodes**
- **Second check point in peripheral tissues** (skin, gut, liver, lung)

**Hypothesis**

After haplo HSCT, donor CD45RO+ Tregs may not migrate to bone marrow.

The bone marrow constitutes a less efficient “second check point” and the HSPC niche is not an immune privileged site anymore. Thus alloreactive Tcons might be free to lyse leukemic stem cells.

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**The HSPC niche is an immune privileged site.**

FOXP3+ Treg cells accumulate in the HSPC niche and may provide the HSPC niche with immune privilege mechanisms. *Fujisaki J et al  Nature 474, 216, 2011*

**CD45RA+ Tregs** are preferentially located in the bone marrow, associated with increased CXCR4 expression.

**CD45RO+** are preferentially located in the skin, and this is associated with their increased expression of CLA and CCR4. *Booth NJ et al  J Immunol 184, 4317, 2010*
Post-Transplant Leukemia Relapse

MUD in AML
ALWP Study (1992-2002)

Haplo in AML
Perugia Study (1993-2005)

Advanced (400) 60 ± 6%
CR1 (301) 33 ± 7%
CR2 (351) 29 ± 7%

Relapse (n=57) 0.34 (0.22-0.47)
CR (n=73) 0.18 (0.10-0.29)
P=0.0028

AML (n=130)

Advanced (400)
CR1 (301)
CR2 (351)
Relapse (n=57)
CR (n=73)

P=0.0028
Post-Transplant Leukemia Relapse (1993-2005)

**ALL (n=100)**

- CR1: 23
- CR2: 26
- CR>2: 9
- REL: 42

Cumulative Incidence
- CR (n=57): 0.30 (0.17-0.43)
- Relapse (n=43): 0.58 (0.41-0.71)

P=0.0000

**AML (n=130)**

- CR1: 28
- CR2: 32
- CR>2: 12
- REL: 58

Cumulative Incidence
- CR (n=73): 0.18 (0.10-0.29)
- Relapse (n=57): 0.34 (0.22-0.47)

P=0.0028
Secondary lymphoid organs have a critical role in determining lymphocyte compartmentalization with peripheral lymph nodes/DCs inducing CLA and CCR4 expression by lymphocytes, whereas mesenteric lymph nodes/DCs are associated with upregulation of α4β7 and CCR9.
UCBT versus UBMT in adults with AL

Transplant related mortality

UCBT 33%

UBMT 26%

UCBT 49±8%

UBMT 39±8%

P (Fine and Gray)=0.24

P (Fine and Gray)=0.17
CD 25
FOXP3
GITR
CTLA-4
CD 62L
CD 39

DONOR

Treg

mismatched HSC transplant

RECIPIENT

lymph node APCs

Tregs

CD 45 RO+ 90-95%
CD 45 RA+ 5-10%

activation of specific donor Tregs for recipient alloantigens

migration of alloantigen specific Tregs

CD45RO+ 100%

expansion of alloantigen specific Tregs

Nguyen et al. Blood 109, 2646, 2007

Skin

High expression of CLA, CCR4, CCR6

Integrin alpha 4 beta 7

CCR9

Low expression of CXCR4

Gut

Bone marrow

Lung

Skin, Gut, Lung, Bone marrow

FOXP3, GITR, CTLA-4, CD 62L, CD 39
Alloantigen-specific Tregs control Tcon alloreactivity

First “check point” in lymph nodes

Recipient dendritic cell

Alloantigen-specific Treg

Alloantigen-specific Tcon

... they control activation and early proliferation of alloreactive donor T cells via interaction with APCs in priming sites. Conversely they do not cross inhibit pathogen-specific Tcon expansion and response.

Second “check point” in peripheral tissues (skin, gut, liver, lung)
Alloantigen-specific Tregs control Tcon alloreactivity

First “check point” in lymph nodes

Alloantigen-specific Tregs control Tcon alloreactivity

Among various mechanisms of suppression, constitutive expression of CTLA-4, an inhibitory costimulatory molecule, and consumption of IL-2 by means of high expression of CD25 appear to have key roles for suppression.

Alloantigen-specific Tregs act in an antigen-specific manner in vivo during their effector phase, thus …..
Alloantigen APCs pre-incubation enhances Treg-mediated immunosuppression

Freshly selected polyclonal T-reg cells were co-cultured in the presence of allogeneic APCs for 48 hours.

Cells were plated under limiting dilution conditions and proliferating T cell clones counted.
Migration of alloantigen specific CD45RO+ Tregs from lymph nodes

Skin

High expression of CLA, CCR4, CCR6

Gut

Integrin α4β7
CCR9

Lung

Bone marrow

Low expression of CXCR4

Alloantigen specific Tregs control Tcon alloreactivity

First “check point" in lymph nodes

Second check point in peripheral tissues (skin, gut, liver, lung)

The HSPC niche is an immune privileged site. FOXP3+ Treg cells accumulate in the HSPC niche and may provide the HSPC niche with immune privilege mechanisms.


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Booth NJ et al J Immunol 184, 4317, 2010
### Escalating doses (Kg/bw) of Tregs and Tcons infused into the recipient

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tregs</strong></td>
<td>2x10^6</td>
<td>2x10^6</td>
<td>4x10^6</td>
</tr>
<tr>
<td><strong>Tcons</strong></td>
<td>0.5x10^6</td>
<td>1x10^6</td>
<td>2x10^6</td>
</tr>
</tbody>
</table>

*70% FoxP3*⁺* cells*

*NB 2 cases did not receive Tcons*
HLA-Haploidentical 2-3 Loci Mismatched HSCT

Obvious Advantages

2% or more of the total T-cells may be reactive with an allogeneic MHC determinant. Only one in 10,000 of the same T-cell pool is reactive with an exogenous protein.

In the setting of MHC disparity, GvH and HvG alloresponses are the strongest.

a family donor for almost every patient
no undue delay

Recipient
Antigen Presenting Cell

Donor T-lymphocyte
ONE HAPLOTYPE MISMATCHED HSCT

Obstacles

T-replete BMT

High incidence of severe GvHD*

*mediated by the high frequency of anti-host alloreactive T cells in unmanipulated grafts

T-depleted BMT

High incidence of rejection*

*mediated by residual anti-donor CTL-p’s which survive the conditioning

The basic dilemma was how to ensure engraftment of incompatible HSCT without causing GvHD
CD25
FOXP3
GITR
CTLA-4
CD62L
CD39

DONOR

Treg

CD4+ CD8-

IL 2

mismatched HSC transplant

activation of specific donor Tregs for recipient alloantigens

expansion and migration of alloantigen-specific Tregs

RECIPENT

APC

TCR αβ

TCR

Class II MHC

CD4+ CD8- TCRαβ

CD4+ CD8- TCRαβ

TCR

TCRαβ
AML: Post-Transplant Leukemia Relapse and Donor-vs-Host NK alloreactivity

AML IN RELAPSE (N=40)

- NO (n=18): 0.37 (0.20-0.55)
- YES (n=22): 0.32 (0.15-0.51)

P = 0.65

AML IN REMISSION (N=56)

- NO (n=26): 0.35 (0.18-0.52)
- YES (n=30): 0.03 (0.00-0.13)

P = 0.01
AML: Leukemia Relapse and Donor-vs-Host NK alloreactivity: 112 transplants from 1993 through 2006

Non-Relapse Mortality
60/150 (40%)

Infection
N=40

CNS, Others, Rejection, GvHD, I.P.

Fatal Infections

<table>
<thead>
<tr>
<th>Infection</th>
<th>Remission</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>(0.5-12)</td>
<td>(0.5-19)</td>
</tr>
</tbody>
</table>

Time to death in months:
median (range)

TOXO | 1 | 10 |
E.Coli | 1 | 2 |
STREPTO | 2 | 4 |
PSEUDOM | 0 | 16 |
CANDIDA | 1 | 0 |
ASPERG | 0 | 0 |
HHV6 | 1 | 0 |
EBV | 2 | 0 |
ADENO | 2 | 0 |
CMV | 0 | 0 |
Non-Relapse Mortality
60/150 (40%)

Patients (n=150)

Cumulative Incidence

Relapse (n=57) 0.58 (0.50-0.75)
Remission (n=93) 0.37 (0.29-0.53)
P=0.04

Table:

<table>
<thead>
<tr>
<th></th>
<th>Remission</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to death in months:</td>
<td>5 (0.5-12)</td>
<td>4 (0.5-19)</td>
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