Adoptive immunotherapy with Tregs and Tcons hastens immune reconstitution without triggering GvHD in mismatched HSCT

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In AL patients with unfavorable prognosis an allogeneic transplant is usually required.

- Probability of having a bone marrow donor:
  - Matched sibling: 30%
  - No Donor: 70%

HSCT from an alternative donor is often the only option:
- Matched unrelated donor
- Unrelated cord blood unit
- Mismatched family donor

PROBABILITY OF HAVING A BONE MARROW DONOR
HLA-Haploidentical 2-3 Loci Mismatched HSCT

Obvious Advantages

a family donor for almost every patient
no undue delay

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

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.
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
Factors involved in engraftment of T-cell depleted Haploidentical HSCs

Conditioning
- sTBI
- Thiotepa
- Fludara
- ATG

No post-transplant immunosuppression

Graft
- Median Dose of CD34+ Cells: 12,8x10^6/kg b.w.
- Median Dose of CD3+ Cells: 1x10^4/kg b.w.
- Median Dose of CD20+ Cells: 4.1x10^4/kg b.w.

Primary engraftment: 93%
Acute GvHD: 8%
Chronic GvHD: 5%
Haplo-transplant in high-risk AML patients in any remission

Aversa F. et al., Blood 1994; 84:3948-3955
Aversa F. et al., J Clin Oncol. 2005;23:3447-3454

Probability of event-free survival

Cumul. incidence of relapse

KIR ligand matched (n=31)
P = 0.003
KIR ligand mismatched (n=30)

Probability of event-free survival

KIR ligand mismatched (n=30)
P = 0.02
KIR ligand matched (n=31)
Transplant Related Mortality due to:

- patient condition and disease stage
- slow post-transplant immune reconstitution
Mechanisms underlying post-transplant immunodeficiency in adults

- **after unmanipulated MUD transplant**
  
  the T cell repertoire is narrow; furthermore, expansion and functions of mature T cells are antagonized by GvHD prophylaxis and/or therapy; thymic damage induced by GvHD

- **after 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 *in vivo* T cell depletion of the graft and may antagonize homeostatic expansion of mature T-cells
Improving post-transplant immunity after HLA-haploidentical HSCT

**Adding back mature donor T-cells that are pathogen specific**

a) anti CMV or anti *Aspergillus* CD4+ clones after screening for cross-reactivity to host alloantigens

b) anti EBV

**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)
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

The impact of regulatory T cells on T-cell immunity following hematopoietic cell transplantation
Nguyen et al., Blood 2008, 111:945-953
Inflammation induced by irradiation and the allogeneic setting provided crucial stimuli for early Treg expansion and migration. Within the first 24 to 48 hours after transfer, donor luc+ Tregs localized to peripheral LNs and the spleen. Signal intensity increased and peaked on day 4, consistent with Treg migration to and proliferation within secondary lymphoid organs.

Inflammation induced by irradiation and the allogeneic setting provided crucial stimuli for early Treg expansion and migration.

Coinfusion of luc+Tregs at a 1:1 ratio with wt Tcons reduced GvHD incidence and severity, in parallel with a significant reduction of Tcon proliferation in both lymphoid and non lymphoid tissues. Tregs likely prevent early proliferation of effector T cells via interaction with APCs in priming sites.

When Tregs were given 2 days prior to Tcon transfer, a 10 fold reduction in Treg numbers was sufficient to reduce Tcon proliferation and protect from GvHD.

Long term survival of Tregs in vivo correlates with persistent protection against GvHD (i.e. the early expansion of Tregs was followed by a sustained population of functional Tregs that provided extended protection from alloreactive effector T cells for up to 3 weeks following HSCT).
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

TBI  TT  Cyclophosphamide  T regs  CD34+  Tcons

No post-transplant immunosuppression
Selection and Characterization of CD4+CD25+ Regulatory T Cells

1\textsuperscript{st} step: Depletion of CD8\textsuperscript{+}/CD19\textsuperscript{+} cells

2\textsuperscript{nd} step: Enrichment of CD25\textsuperscript{+} cells

Leukapheresis product

Immunomagnetic Selection of CD4\textsuperscript{+}CD25\textsuperscript{+} Cells

Gate on CD4CD25\textsuperscript{+}\textsuperscript{high}

Gate on CD4CD25\textsuperscript{+}

Gate on FoxP3

Starting fraction Final fraction

<table>
<thead>
<tr>
<th>Cells (x10\textsuperscript{9})</th>
<th>1060 (540-1370)</th>
<th>280 (202-390)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%CD4CD25</td>
<td>3.0 (1.5-7.45)</td>
<td>92.4 (90-97.1)</td>
</tr>
<tr>
<td>N° cells (x 10\textsuperscript{6})</td>
<td>330 (221-1020)</td>
<td>256 (185.6-365.4)</td>
</tr>
<tr>
<td>%CD4CD25\textsuperscript{high}</td>
<td>0.3 (0.12-0.89)</td>
<td>33.6 (14.4-39.6)</td>
</tr>
<tr>
<td>N° cells (x 10\textsuperscript{6})</td>
<td>36.12 (19.98-84)</td>
<td>68.6 (20.9-143)</td>
</tr>
</tbody>
</table>
Final Cell Fraction

- **CD4/CD25**
  - **CD62L**: 84.2%
  - **CD62L**
    - **CD39**: 64.9%
  - **CD49d**: 37.7%
  - **cyCD152**: 15.1%

- **CD4/CD25**
  - **CD49d**: 27%
  - **cyCD152**: 33.8%
Harvesting Tregs from peripheral blood before stem cell collection increases cell number in the starting fraction…

<table>
<thead>
<tr>
<th></th>
<th>Post HSC collection</th>
<th>Pre HSC collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells ($x10^9$)*</td>
<td>6.6 (3.6 - 16.8)</td>
<td>10.6 (5.4 - 13.7)</td>
</tr>
<tr>
<td>%CD4CD25</td>
<td>3.7 (2.1 - 4.2)</td>
<td>3.0 (1.5 - 7.45)</td>
</tr>
<tr>
<td>N° cells ($x10^6$)*</td>
<td>213.6 (75.3 - 621.6)</td>
<td>330 (221 - 1020)</td>
</tr>
<tr>
<td>%CD4CD25&lt;sub&gt;high&lt;/sub&gt;</td>
<td>0.4 (0.34 - 0.57)</td>
<td>0.3 (0.12 - 0.89)</td>
</tr>
<tr>
<td>N° cells ($x10^6$)*</td>
<td>28.5 (14.4 - 57.12)</td>
<td>36.12 (19.98 - 84)</td>
</tr>
</tbody>
</table>

N=8

N=15

*median+range
…and 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>Total n° Cells (x 10^6)</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&lt;sup&gt;high&lt;/sup&gt;</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)*</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.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T resp + Tregs 1:2</td>
<td>72.4</td>
<td>8.9</td>
</tr>
<tr>
<td>T resp + Tregs 1:1</td>
<td>60.8</td>
<td>20.5</td>
</tr>
<tr>
<td>T resp + Tregs 1:0.1</td>
<td>25.6</td>
<td>19.1</td>
</tr>
</tbody>
</table>
HLA identity between Tregs and Tcons is required for maximum suppression of alloresponses.

Tregs from a third party donor might not prevent GvHD after HSCT.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T resp allo + T regs</td>
<td>6.6</td>
<td>8.5</td>
</tr>
<tr>
<td>T resp auto + T regs</td>
<td>60.8</td>
<td>20.5</td>
</tr>
</tbody>
</table>
# Demographics

<table>
<thead>
<tr>
<th>No of patients</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years, range)</td>
<td>41 (21-60)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>11/17</td>
</tr>
</tbody>
</table>

## Disease and status at transplant

<table>
<thead>
<tr>
<th>Disease type</th>
<th>CR1</th>
<th>≥CR2</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myeloid leukemia</td>
<td>10</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Acute lymphoid leukemia</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High grade NHL in relapse</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Escalating doses (Kg/bw) of Tregs and Tcons infused into the recipient

<table>
<thead>
<tr>
<th></th>
<th>Group I (4 patients)</th>
<th>Group II (17 patients)</th>
<th>Group III (5 patients)</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
Adoptive transfer of Tregs and Tcons: what is the real ratio?

Tregs and Tcons Ratio in the Graft

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

Recipient Antigen Presenting Cell

Donor Tregs

FoxP3-

FoxP3+

T regs

Tcons
PRIMARY ENGRAFTMENT  
26/28 (93%)

- ANC/μl
  - Days post BMT: >500, >1000

- PLT/mm³
  - Days post BMT: >25000, >50000

GRAFT vs HOST DISEASE

Grade ≥II  2/26
Like all other TCRαβ-expressing T lymphocytes, Tregs are activated by host-type APCs. 

**In vitro** Treg antigen specificity is limited to the activation phase, while the Treg suppressor-effector function is non-antigen specific.

**In vivo** Tregs are activated in an antigen-specific fashion and also act during the effector phase in an antigen-specific manner.

Ljungman et al  BMT 35,737, 2005  
Auletta JJ et al  BMT 35, 835, 2005  
Joffer O et al  Blood 103, 4216, 2004  
Nguyen VH et al  Blood 109, 2649, 2007
Potential Dangers of Tregs Adoptive Immunotherapy (cont)

In murine models infusion of polyclonal Tregs and conventional T lymphocytes

- promotes lymphoid reconstitution
- improves immunity to MCMV (reduced viral load and increased survival)
- ensures long term immunity (needed for effective vaccination, adequate responses to recall antigens and the graft versus tumor effect)
Recovery of CD4+ and CD8+ T cell subpopulations

Spectratyping

Donors

Complexity score

Months after transplant

Days post BMT

CD4/µl

Days post BMT

CD8/µl

Spectratyping complexity score
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

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

Reconstitution of pathogen-specific T-cell repertoire

Limiting dilution analyses of pathogen-specific CD4+ and CD8+ cells
T cell response to CMV: cytokine profiles

<table>
<thead>
<tr>
<th></th>
<th>TNF-a</th>
<th>IL-2</th>
<th>IFN-y</th>
<th>IL-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sbj</td>
<td>VE-SE 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GI-MO 17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RO-DO 20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LE-AN 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA-SI 24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD4+ | CD8+
Evaluable Patients

Patients with CMV reactivation

Tregs Group

Control Group

CMV reactivation episodes

Days after transplant

Tregs Group

Control Group

Days after transplant

p<0.05
RECONSTITUTION OF B CELL REPERTOIRE

Days post BMT

CD20/μl

30 60 90 120

0 250 500 750 1000 1250

IgG

IgA

IgM

3 months

733 (80-1530) 31 (1-72) 71 (17-229)

6 months

692 (411-876) 44 (8-111) 63 (45-140)
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.

![Graph showing vaccination schedule]

- Day 0, 30, 60, and 240
- Visit 1, 2, 3, and 4
Hemoagglutinin Inhibition Assay (HI)

### Table: Hemoagglutinin Inhibition Assay Results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</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>

**Interpretation:**
- HI titer $\geq 1:40 = \text{protection against H1N1}$
- HI $\geq 4 \text{ fold} = \text{vaccination efficacy}$
Profile over time of Flu specific CD4\(^+\) after MF59-H1N1\textsubscript{california} vaccination

Pre immune
30 days post 1 dose
30 days post 2 dose
Regeneration of donor vs recipient alloreactive NK repertoires

“Standard” haplo
- (single) KIR2DL2/3+
- (single) KIR2DL1+
- (single) KIR3DL1+

Lysis of KIR ligand-mismatched targets
- C2 mismatch
- C1 mismatch
- Bw4 mismatch

“T-reg” haplo

Donor 1, 3, 6, 12 months post transplant

Donor vs recipient alloreactive NK repertoires
- (single) KIR2DL1+
- (single) KIR2DL2/3+
- (single) KIR3DL1+

C2 mismatch
C1 mismatch
Bw4 mismatch
**CONCLUSIONS**

**Tregs and stem cell “megadose” in HLA-haploidentical transplantation**

- Naturally occurring Tregs can be immuno-selected in a fully automated closed system which reliably yields 2-4\times10^6/kg Tregs.

- Infusion of freshly purified donor Tregs makes administration of high dose of conventional T cells feasible in haplo setting, with a low incidence of GvHD.

- In vitro priming of Tregs is not required for GvHD inhibition, since activation of alloantigen-specific Tregs occurs efficiently in vivo.

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

- Adoptive transfer of Tregs and Tcons hastens immune reconstitution and reduces the risk of CMV reactivation.
Tregs in HLA-haploidentical transplantation

WORK IN PROGRESS

- Improvement in Treg purification (FoxP3+ cell yield from 70% to 90%)

- Less regimen related toxicity

Alemtuzumab

TBI  TT

Tregs  CD 34+

No post-transplant immunosuppression

Alemtuzumab 30mg/day
8 Gy TBI in a single fraction at 16 cGy/m
Thiotepa 4 mg/kg/day
Fludarabine 40 mg/sqm/day

Fludarabine

Tcons

12 days
Translational Research in Full-Haplotype Mismatched Transplant

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Mauro Di Ianni
Paolo Sportoletti
Tiziana Zei
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Elisabetta Bonifacio
Beatrice Del Papa
Debora Cecchini
Alain Bell
PRIMARY ENGRAFTMENT
26/28 (93%)

GRAFT vs HOST DISEASE
Grade ≥II 2/26

NON-RELAPSE MORTALITY
10/27 (37%)
Adenoviral infection (1)
Bacterial sepsis (1)
Systemic toxoplasmosis (1)
Fungal pneumonia (2)
CNS aspergillosis (1)
VOD (3)
MOF (1)