Haploidentical Stem Cell Transplantation using T-cell depleted grafts

Peter Bader
Frankfurt, Germany
Agenda

■ History

■ Developments

■ Current used strategies for T-cell depletion
  - T-depletion, positive selection of CD34+ cells, myeloablative: Perugia and Tübingen
  - T-depletion, positive selection of CD34+ cells, myeloablative: EBMT Survey
  - T-depletion, negative selection of $\alpha\beta^+$ T-cells, nonmyeloablative: Tübingen, Rome
Haploidentical Stem Cell Transplantation
Things are different

Engraftment

GVHD

T cell depletion and NK alloreactivity

Rejection

Non Engraftment
Haploidentical Stem Cell Transplantation
The mouse model

MHC- identical
9 Gy
BM

Haploidentical
BM

Spleen
anti T-Z -AB

KM

- T-C
+ SC↑

Megadose Concept
Haploidentical Stem Cell Transplantation

Successful Engraftment of T-Cell–Depleted Haploidentical “Three-Loci” Incompatible Transplants in Leukemia Patients by Addition of Recombinant Human Granulocyte Colony-Stimulating Factor–Mobilized Peripheral Blood Progenitor Cells to Bone Marrow Inoculum

By Franco Aversa, Antonio Tabilio, Adelmo Terenzi, Andrea Velardi, Franca Falzetti, Claudia Giannoni, Roberta Iacucci, Tiziana Zei, Maria Paola Martelli, Cesare Gambelunghe, Massimo Rossetti, Pierfranco Caputo, Paolo Latini, Cynthia Aristei, Carlo Raymond, Yair Reisner, and Massimo F. Martelli


The New England Journal of Medicine

TREATMENT OF HIGH-RISK ACUTE LEUKEMIA WITH T-CELL–DEPLETED STEM CELLS FROM RELATED DONORS WITH ONE FULLY MISMATCHED HLA HAPLOTYPE

FRANCO AVERSA, M.D., ANTONIO TABILIO, M.D., ANDREA VELARDI, M.D., ISABEL CUNNINGHAM, M.D., ADELMO TERENCE, M.D., FRANCA FALZETTI, M.D., LOREDANA RUGGERI, M.D., GIULIANA BARBADETOLA, M.D., CYNTHIA ARISTEI, M.D., PAOLO LATINI, M.D., YAIR REISNER, PH.D., AND MASSIMO F. MARTELLI, M.D.
Pre Requisites for Haploidentical Stem Cell Transplantation

- Aggressive and severe immune depleting conditioning regimen
- Megadosis of highly purified peripheral stem cells
  - No T- Cells
  - No B- Cells
## T-Deplete Strategies

Perugia Group

**Table 2**
The ideal CD34⁺ selected haploidentical transplantation.

<table>
<thead>
<tr>
<th>Disease, disease status:</th>
<th>Any CR, AML in relapse if donor NK alloreactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>No donor-negative into recipient-negative</td>
</tr>
<tr>
<td>CMV serology</td>
<td>Mother</td>
</tr>
<tr>
<td>Family member</td>
<td>Donor vs recipient</td>
</tr>
<tr>
<td>NK cell alloreactivity</td>
<td></td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td>Single (8 Gy) or fractionated (12 Gy)</td>
</tr>
<tr>
<td>TBI</td>
<td>4 Gy sTBI/9 Gy fTBI</td>
</tr>
<tr>
<td>Lung shielding</td>
<td>Fludarabine, Thiotepa</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Fresenius 25 mg/kg or Thymoglobulin 6 mg/kg</td>
</tr>
<tr>
<td>ATG</td>
<td></td>
</tr>
<tr>
<td>Graft preparation</td>
<td>Extensive (CD3 ≤ 3 x 10⁴/kg)</td>
</tr>
<tr>
<td>T and B cell depletion</td>
<td>Megadose (≥10 × 10⁶/kg)</td>
</tr>
<tr>
<td>CD34⁺ cell dose</td>
<td></td>
</tr>
<tr>
<td>Post-transplant treatments</td>
<td></td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>No</td>
</tr>
<tr>
<td>G-CSF</td>
<td>No</td>
</tr>
<tr>
<td>Anti-CMV, anti-aspergillus prophylaxis</td>
<td>Yes</td>
</tr>
</tbody>
</table>
T-Deplete Strategies
Perugia Group

AML (n=147)

- CR1 (n=34): 0.50 +/- 0.09
- CR2 and higher (n=49): 0.35 +/- 0.07
- Relapse (n=64): 0.14 +/- 0.04

P = 0.0006

Haploidentical Stem Cell Transplantation
Children with ALL

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>Relapse</th>
<th>TRM</th>
<th>alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CR2</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>CR3</td>
<td>4</td>
<td>3</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NR</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>11 (41%)</td>
<td>7 (26%)</td>
<td>9 (33%)</td>
</tr>
</tbody>
</table>

Survival

0.42 ± 0.11 n=21 in Remission
n=6 not in Remission

Engraftment

CD34
n=52
85% pri. E.
98% Sek. E.

Klingebiel, Blood Reviews, 2004
European Survey
Event Free Survival

## European Survey

### Univariate Analysis

<table>
<thead>
<tr>
<th>Center-related factor</th>
<th>No. of alloHSCTs performed</th>
<th>15 ± 6</th>
<th>41 ± 7</th>
<th>44 ± 7</th>
<th>39 ± 7</th>
<th>31 ± 6</th>
<th>30 ± 6</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fewer than 231</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.005*</td>
</tr>
<tr>
<td>231 or more</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.26</td>
</tr>
</tbody>
</table>

Interaction between NK-cell-Receptor and HLA-Molecule

NK-cell-Receptor: KIR2DL2
HLA-Molecule: HLA-Cw3

Ergänzende Farbe
grau:
R = 100
G = 100
B = 100
NK Cell- Activation

Interaction of multiple activating and inhibitory signals

Cytokines, chemocines and their receptos are missing although relevant for regulation of NK cell function

Vivier, Nature Immunol., 2008, 9, 503
Natural Killer Cells ('Missing Self') Ljunggren und Karre

**Activation**

[Diagram: Interaction between APC and Natural Killer Cells with arrows indicating activation and inhibition]

**Lyse**

[Diagram: Interaction with an additional step showing lyse]
Haplo Transplantation
Improvements

- Importance of NK Cells for Engraftment:

Ruggeri et al., Science 2002
Graft Composition

**CD34+ enrichment**

- CD34+ PE
- CD3 PE

**CD3/19 depletion**

- CD3 PE
- CD34 PE

Graft Comparison

<table>
<thead>
<tr>
<th></th>
<th>CD34+</th>
<th>CD3</th>
<th>CD56+</th>
<th>CD14+</th>
<th>CD19+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pat: AA 19 kg</td>
<td>29x10^6/kg</td>
<td>7x10^6/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13x10^3/kg</td>
<td>18x10^3/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0x10^3/kg</td>
<td>180x10^6/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2x10^3/kg</td>
<td>110x10^6/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0x10^3/kg</td>
<td>0.3x10^3/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pat: SB 39 kg

- CD34+ 7x10^6/kg
- CD3 18x10^3/kg
- CD56+ 180x10^6/kg
- CD14+ 110x10^6/kg
- CD19+ 0.3x10^3/kg
Frankfurt Prospective Trial
Objectives

- Primary:
  - Assessment of engraftment at day 28

- Secondary:
  - Evaluation of speed and quality of immune regeneration
  - Evaluation of viral and fungal reactivation, infections and infections related mortality
  - Assessment of incidence and severity of GVHD
  - Survival
Conditioning
Myeloablative versus reduced toxicity

Impact on toxicity, GVHD and immune reconstitution!

(1) Toxicity
- IL1, IL6, TNFa
- IFNg
- IL2
- APC
- MHC ↑
- CTL
- Target-Cell

(2) GvHD

(3) Immune reconstitution
# Frankfurt Study

<table>
<thead>
<tr>
<th>Criterion</th>
</tr>
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<tbody>
<tr>
<td><strong>Stem Cell Apheresis</strong></td>
</tr>
<tr>
<td>Stimulation for 5 Days with G-CSF</td>
</tr>
<tr>
<td><strong>Graft Engineering</strong></td>
</tr>
<tr>
<td>Depletion with CD 3/19 Microbeads (Miltenyi; Clinimacs&lt;sup&gt;®&lt;/sup&gt;)</td>
</tr>
<tr>
<td><strong>Graft Composition</strong></td>
</tr>
<tr>
<td>CD 34&lt;sup&gt;+&lt;/sup&gt; 7x10&lt;sup&gt;6&lt;/sup&gt;/kg</td>
</tr>
<tr>
<td>CD 3&lt;sup&gt;+&lt;/sup&gt; ≤1x10&lt;sup&gt;5&lt;/sup&gt;/kg</td>
</tr>
<tr>
<td>No Stimulation post transplant</td>
</tr>
<tr>
<td><strong>Immune Suppression</strong></td>
</tr>
<tr>
<td>MMF</td>
</tr>
<tr>
<td><strong>Viral and fungal prophylaxis</strong></td>
</tr>
<tr>
<td>Ribavirin, Voriconazole</td>
</tr>
</tbody>
</table>
Frankfurt Study
Conditioning Regimen

- **Infusion of stem cells**
  - Thiotepa (10 mg/kg)
  - Fludarabine mg/m²: 200 later: 150
  - Melphalan (140 mg/m²)*
  - OKT-3
  - MMF

*Melphalan dosage is indicated for bone marrow transplantation.*
## Patient Characteristics
### Demography

<table>
<thead>
<tr>
<th>Recruitment Period</th>
<th>01/2005 – 03/2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time point analysis</td>
<td>As of 15/04/2014</td>
</tr>
<tr>
<td><strong>Age (Range) [Years]</strong></td>
<td><strong>12.9 [0.6-27.2]</strong></td>
</tr>
<tr>
<td>Follow up surviving patients, median. [Months]</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>[1 - 92]</td>
</tr>
</tbody>
</table>
## Patient Characteristics

### Leukemia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total</th>
<th>CR1</th>
<th>CR2</th>
<th>CR3</th>
<th>CR4</th>
<th>NR</th>
<th>Of these: 2nd transpl</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>21</td>
<td>7</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AML</td>
<td>21</td>
<td>2</td>
<td>12</td>
<td>1</td>
<td></td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>CML</td>
<td>2</td>
<td></td>
<td>CP 2</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>9</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
## Transplantations – Grafts

<table>
<thead>
<tr>
<th>Number</th>
<th>44</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Cellularity Transplant/kg</th>
<th>CD34$^+$</th>
<th>CD 56$^+$</th>
<th>CD14/15$^+$</th>
<th>CD3$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[median] /kg</td>
<td>7.44x10⁶</td>
<td>140.05x10⁶</td>
<td>414x10⁶</td>
<td>2.45x10⁴</td>
</tr>
<tr>
<td>[mean] /kg</td>
<td>9.95x10⁶</td>
<td>160.29x10⁶</td>
<td>475x10⁶</td>
<td>3.98x10⁴</td>
</tr>
<tr>
<td>Range/kg: from to</td>
<td>5.93x10⁶</td>
<td>8.20x10⁶</td>
<td>64x10⁶</td>
<td>0.00x10⁴</td>
</tr>
<tr>
<td></td>
<td>20.74x10⁶</td>
<td>400.71x10⁶</td>
<td>1009x10⁶</td>
<td>1.40x10⁵</td>
</tr>
</tbody>
</table>
## Engraftment

<table>
<thead>
<tr>
<th>Number</th>
<th>44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engraftment (median) [Days]</td>
<td>N</td>
</tr>
<tr>
<td>73 (100%)</td>
<td>12 (7-27)</td>
</tr>
</tbody>
</table>
Engraftment

Leukocytes

Thrombocytes

Time [Days]

04/14
Frankfurt Study

Transfusions

Number of Transfusions

All Patients – including non malignant diseases

Erythro

Thrombo

Median

5

4
Immunreconstitution

CD3⁺ T- and CD3⁻CD56⁺ NK cells

cells/µl

days post Tx

T cells
NK cells
Cumulative Incidence
All Patients with Leukemia

TRM @2 years: 0.17; n=44; events n=6
Relapse: 0.44; n=44; events n=16
Cumulative Incidences
Patients in Remission

TRM @2 years: 0.14; n=33; events n=4
Relapse: 0.33; n=33; events n=9
Cumulative Incidence a GVHD
Patients with leukemia

Grad I: CI 3 years: 0.54; n=44; events n=18
Grad II: CI 3 years: 0.24; n=44; events n=4
Grad III: CI 3 years: 0.08; n=44; events n=2
All Patients with Leukemia
ALL und AML (n=42) and CML in CP2 (n=2)

pEFS 3 years: 0.45; n=44; events n=22
Event Free Survival
ALL, AML and CML according to Remission

rem:  pEFS 3 years: 0.58; n=33; events n=13
non rem: pEFS 1 years: 0.11; n=11; events n=9
Event Free Survival
Patients with Leukemia

ALL (n=21)  
AML (n=21), CML (n=2)

Remission  
PFS: 0.53; n=16; ev. n=7
Non Remission  
PFS: 0.00; n=5; ev. n=1

Remission  
PFS: 0.63; n=17; ev. n=6
Non Remission  
PFS: 0.17; n=6; ev. n=1
Importance of Chimerism and Intervention by DLI

Chimerism-PCR

- PB
- CD3
- CD19
- CD56

Donor signal [%]

Days post SCT

Immune Recovery

Zeit [µl]

Zellen/µl

- CD3+
- CD3+CD4+
- CD3+CD8+
- CD3-CD56+
- CD19+

0

100

200

300

400

500

600

700

800

900

1000

Datum

9.7.06
10.7.06
11.7.06
12.7.06
13.7.06
14.7.06
15.7.06
16.7.06
17.7.06
18.7.06
19.7.06
20.7.06
21.7.06
22.7.06
23.7.06
24.7.06
25.7.06
26.7.06
27.7.06
28.7.06
29.7.06
30.7.06
31.7.06

Immune Recovery

Ergänzende Farbe
grau:
R = 100
G = 100
B = 100

Willasch, BMT 2009
Conclusions I

- Transplantation with CD3/CD19 depleted stem cells allows
  - Excellent and reliable engraftment
  - Reduced acute toxicity
    - Second transplantation after 1\textsuperscript{st} allogeneic SCT
    - Preservation of fertility?
  - Prevention of GVHD
- Early immune regeneration
  - Treatment of virus reactivation and viral diseases
- Reduced TRM
- Further improvement by specific cellular therapies?
Best Donor for Haploidentical Transplantation?

![Graph showing survival rates for different donors over time. The x-axis represents years, and the y-axis represents pEFS. The graph compares survival rates between father and mother donors. The survival rate is significantly higher for the mother donor (p<0.0001).]

Best Donor for Haploidentical Transplantation?

![Graph showing survival rates for different donors]  
- NK-allo mother donor
- NK non-allo mother donor
- NK allo father donor
- NK non-allo father donor

EFS vs. Years

p<0.0001

T-Deplete Strategies
Summary

- High numbers of CD34\(^+\) cells can overcome the HLA barrier
- A threshold of 2\(\times\)10\(^4\) CD3\(^+\) T-cells/kg almost completely prevents GVHD
- A low posttransplantation leukemia relapse rate
- A relatively high TRM rate because of slow posttransplantation immune reconstitution

Agenda

- Different Strategies in Haploidentical SCT
  - T-depletion, positive selection of CD34+ cells, myeloablative: Perugia and Tübingen
  - T-depletion, positive selection of CD34+ cells, myeloablative: EBMT Survey
  - T-depletion, negative selection of $\alpha\beta^+$ T-cells, nonmyeloablative: Tübingen
Developments in Graft-engineering techniques for T-cell depletion of mobilised PBSC’s for haploidentical transplantation

1995
CD34+ Selection of purified stem cells

2003
CD3/19 Depletion Stem cells + effectors (NKs)

2011
TCRαβ/CD19 Depletion stem cells + effectors (NKs + γδT-cells)

Courtesy: P. Lang
T-Cell Depletion Strategies

**CD3/CD19-depletion**
*Negative-selection*

**TCRαβ/CD19-depletion**
*Negative-selection*
Patients and Methods

Depletion of TCRαβ-T cells with:
- biotinylated anti-αβ antibody
- followed by an anti-biotin antibody conjugated to magnetic microbeads

Depletion of B cells with:
- CD19 conjugated microbeads
- device: CliniMACS® system.

Conditioning regimen
(n=7)
Melphalan (2x70mg/m²), Fludarabine (4x40mg/m²) or Clofarabine (4x50mg/m²), Thiotepa (10mg/kg) and OKT3 (0.1mg/kg) day -8 to day -1

(n=28)
Melphalan (2x70mg/m²), Fludarabine (4x40mg/m²) or Clofarabine (4x50mg/m²), Thiotepa (10mg/kg) and ATG-Fresenius (15mg/kg) given at start of the regimen in order not to impair NK and γδ+ T-cells of the grafts (1 mg/kg d -12, 4 mg/kg d -11, 5 mg/kg d -10 and -9)

Pharmacological GvHD prophylaxis
MMF until d 30-60

Growth factors
G-CSF (5μ/kg) was given in most patients

Donors
Full haplotype mismatched parents (n=35)

Patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n=</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>20</td>
</tr>
<tr>
<td>AML/MDS/JMML</td>
<td>9</td>
</tr>
<tr>
<td>Nonmalignant</td>
<td>4</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease status</th>
<th>n=</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR2-CR6</td>
<td>17 (55%)</td>
</tr>
<tr>
<td>NR/active disease</td>
<td>11 (45%)</td>
</tr>
<tr>
<td>2nd/3rd SCT</td>
<td>23 (65%)</td>
</tr>
</tbody>
</table>
Results

Graft composition

The overall depletion of αβ+ T-cells was highly effective with 4.6 log. Patients received a median number of only 19 x 10⁹/kg residual αβ+ T-cells. Recovery of CD34+ stem cells was 72%, and the median number of infused CD34+ stem cells was 12x10⁹/kg. Additionally, the patients received potential antileukemic effector cells: 107x10⁹/kg CD56+ NK cells and 11x10⁹/kg γδ+ T-cells.

<table>
<thead>
<tr>
<th></th>
<th>CD34+ x10⁹/kg</th>
<th>CD3+ x10⁶/kg</th>
<th>CD19+ x10⁶/kg</th>
<th>CD56+ x10⁹/kg</th>
<th>CD14+ x10⁹/kg</th>
<th>αβ TcR+ x10⁷/kg</th>
<th>γδ TcR+ x10⁷/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>5</td>
<td>4.6</td>
<td>48</td>
<td>35</td>
<td>351</td>
<td>1.6</td>
<td>5</td>
</tr>
<tr>
<td>max</td>
<td>38</td>
<td>41.8</td>
<td>528</td>
<td>192.2</td>
<td>811</td>
<td>46.4</td>
<td>30</td>
</tr>
<tr>
<td>median</td>
<td>12</td>
<td>13.6</td>
<td>110</td>
<td>107.4</td>
<td>618</td>
<td>18.9</td>
<td>11</td>
</tr>
</tbody>
</table>

OKT3 Conditioning

![Graph showing CD3, TcRab, and TcRgd cells/µl before and after OKT3 conditioning.]

ATG Conditioning

![Graph showing CD3, TcRab, and TcRgd cells/µl before and after ATG conditioning.]

P. Lang, EBMT Meeting Milano 2014
Current results with transplantation of TcRαβ/CD19 depleted stem cells from haploidentical donors in children

P. Lang, T. Feuchtinger, HM. Teltschik, M. Schumm, P. Schlegel, M. Pfeiffer, M. Ebinger, CP. Schwarze, R. Handgretinger
Children’s University Hospital, Tübingen, Germany

TRM (22%)

CR (37%) vs. NR (9%)

% TRM

% EFS

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5

years post SCT

0 10 20 30 40 50 60 70 80 90 100

0 10 20 30 40 50 60 70 80 90 100

years post SCT

P. Lang, EBMT Meeting Milano 2014
T-Deplete Strategies

Summary

- High numbers of CD34+ cells can overcome the HLA barrier
- A threshold of $2 \times 10^4$ CD3+ T-cells/kg almost completely prevents GVHD
- A low posttransplantation leukemia relapse rate
  - Might be improved by additional cellular therapies (CIK cells/CAR T-cells)
  - Antibodies (e.g. Blinatumomab)
- A relatively high TRM rate because of slow posttransplantation immune reconstitution
  - This might be overcome by different T cell depletion strategies
    - CD3/CD19; or alpha/beta depletion
    - Reduced intensity regimens
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Jeder einzeln zählt

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