First clinical results with
αβ+ T-cell depleted
haploidentical stem cells in children

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Haploidentical donors: T cell depletion mandatory

1. CD34+ Selection „pure stem cells“
2. CD3/19 Depletion Stem cells + effectors (NK cells)
3. TCRαβ/CD19 Depletion Stem cells + effectors (NK cells + γδ T cells)
4. Antigen-specific T cells
The anti-leukemic activity of gamma/delta T-cells

Godder et al., Long term disease-free survival in acute leukemic patients recovering with increased γδT cells after partially mismatched related donor bone marrow transplantation. BMT 2007; 39,751-757.

Otto M. et al:
Strategy for depletion of $\alpha\beta^+$ T-cells

Chaleff S. et al.: A large scale method for the selective Depletion of $\alpha\beta$ T-lymphocytes from PBSC for allogeneic Transplantation. Cytotherapy, 2007

1. biotin-anti-$\alpha\beta$ mAb
2. microbeads with anti-biotin mAb

waste
($\alpha\beta$ T cells)

graft
$\gamma\delta$T-cells
CD34+ and CD34-progenitors
NK cells
dendritic cells
**TCRαβ depletion: Efficacy of procedure**

<table>
<thead>
<tr>
<th>n = 9</th>
<th>Before</th>
<th>After</th>
<th>Log Depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent TCRαβ</td>
<td>29.2 ± 6.4</td>
<td>0.0015 ± 0.0008</td>
<td></td>
</tr>
<tr>
<td>Absolute TCR αβ+</td>
<td>1.4 ± 0.52 x10^{10}</td>
<td>0.38 ± 0.22 x10^{6}</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Absolute CD3+</td>
<td>1.4 ± 0.44 x10^{10}</td>
<td>3.5 ± 1.8 x10^{8}</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Absolute CD19+</td>
<td>2.7 ± 1.4 x10^{9}</td>
<td>5.3 ± 5.8 x10^{6}</td>
<td>3.9 ± 0.6</td>
</tr>
</tbody>
</table>

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![Flow cytometry graphs showing TCRαβ FITC-A and TCR GD PE-A](image)
Comparative analysis of the efficacy of T-cell depletion
CD34+ selection vs CD3- vs TcRαβ-depletion

$n=49$

4.6 log
Graft composition after TCR$\alpha\beta$ /CD19 depletion (n=24 patients)

<table>
<thead>
<tr>
<th></th>
<th>CD34+ x10^6/kg</th>
<th>CD3+ x10^6/kg</th>
<th>CD19+ x10^3/kg</th>
<th>CD56+ x10^6/kg</th>
<th>CD14+ x10^6/kg</th>
<th>a$\beta$TcR+ x10^3/kg</th>
<th>$\gamma$$\delta$TcR+ x10^6/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>5</td>
<td>4.6</td>
<td>46</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>
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</tbody>
</table>
Patients (pediatric, n=24)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n=</th>
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<tbody>
<tr>
<td>ALL</td>
<td>12</td>
</tr>
<tr>
<td>AML/MDS/JMML</td>
<td>7</td>
</tr>
<tr>
<td>Nonmalignant</td>
<td>4</td>
</tr>
<tr>
<td>Solid tumors</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease status</th>
<th>n=</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR2-CR6</td>
<td>8 (35%)</td>
</tr>
<tr>
<td>NR/active disease</td>
<td>11 (48%)</td>
</tr>
<tr>
<td>2nd/3rd SCT</td>
<td>16 (70%)</td>
</tr>
</tbody>
</table>
ATG-F (15 mg/kg)

1 mg | 4 mg | 5 mg | 5 mg

Fludarabin (mg/m²) or Clofarabin

40 mg | 40 mg | 40 mg | 40 mg

Thiotepa (mg/kg)

10 mg

Melphalan (mg/m²)

70 mg | 70 mg

TCRαβ/CD19 depleted cells

steroids

days from transplantation

-12 11 10 9 8 7 6 5 4 3 2 1 0 +1

Conditioning regimen (2) n=12

MMF, if >25,000 residual abT cells
TCR $\alpha\beta$/CD19 Depletion: Engraftment

ANC>500/µl and independence from platelet substitution

- ANC>500 (median: 10 days)
- Independence from platelets (median: 11 days)
**TCRαβ/CD19 Depletion:**
residual γδT-cells do not cause GvHD  
(22/24 patients without pharmacological immune suppression)

<table>
<thead>
<tr>
<th>acute GvHD grade</th>
<th>n=</th>
<th>%</th>
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<tbody>
<tr>
<td>no GvHD</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
TCRαβ/CD19 Depletion: Outcome

- Alive: 17 out of 24 patients (70%)
- Median Follow up: 0.5 (0.06-1.4) years
Comparison of CD3+ recovery: CD3/19 vs TCRαβ/CD19 Depletion

![Graph showing CD3+ recovery comparison between CD3/19 and TCRαβ/CD19 depletion processes over time post transplant.]
Comparison of CD3+ recovery at day +30

CD3/19 vs TcRab day 30 CD3+

cell count/µl

CD3/19 n = 45   TcRab n = 19

p<0.0001

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Comparison of CD3+ recovery: CD3/19 vs TCRαβ/CD19 Depletion

Time to CD3 > 100/µl

Percent with CD3 > 100/µl

Time to CD3 > 100

- CD3 depl
- TcRab depl n=21

p<0.01
T cell recovery after TCRαβ Depletion

TcRab Depletion and OKT3 Conditioning

TcRab Depletion and ATG-F Conditioning
T cell recovery after TCRαβ Depletion
Conclusions

- TCR\(\alpha\beta\)-Depletion results in grafts with high numbers of effector cells (\(\gamma\delta\)T cells, NK cells)
- Robust engraftment
- Profound depletion of \(\alpha\beta\)T cells: prevents GvHD in HLA mismatched trp
- Immune recovery seems to be significantly faster than after other graft manipulation procedures
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