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Is in vitro T-cell depletion necessary for Haploidentical Transplantation

Disclosure of Interest: Nothing to Disclose
How to avoid GvHD?
In vitro T-cell depletion is the most effective way to reliably avoid GvHD independent of the HLA disparity

<table>
<thead>
<tr>
<th>Table 1 HLA genotype of the recipient and respective donors.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Patient</td>
</tr>
<tr>
<td>Cord blood 1</td>
</tr>
<tr>
<td>Cord blood 2</td>
</tr>
<tr>
<td>Cord blood 3</td>
</tr>
<tr>
<td>Third party CD34^+ cells</td>
</tr>
<tr>
<td>Anti-CMV T cells</td>
</tr>
</tbody>
</table>
CD34 selection: 
BM + G-PBSC’s: E-rosetting + CD34 selection (CellPro)


Delayed immune reconstitution, high incidence of infections
Reconstitution of CD3+ T-cells after haploidentical with CD34+ positively selected stem cells

Cumulative Incidence of lethal viral infections (ADV, CMV, HSV) over time after transplantation.

- CD34+ cells
- Days after transplantation
- % of patients with CD3 > 0.1 x 10^9/L

CD34+ stem cell reconstitution and viral infection risk.
The important role of NK alloreactivity

The role of NK alloreactivity in pediatric acute Lymphoblastic Leukemia


![Graph showing probability of relapse over days after transplantation for NK nonalloreactive and NK alloreactive cases.](image)

P = 0.01

Lowe EJ et al.
T-cell depletion: positive selection versus negative depletion

CD34+ positive selection

CD3/19 depletion

Gordon et al.:
A large-scale method for T cell Depletion: towards graft engineering of mobilized peripheral Blood stem cells.
Bone Marrow Transplant 2002; 30:69-74.

Barfield et al.:
A one-step large-scale Method for T-and B-cell depletion of Mobilized PBSC for allogeneic transplantation.

NK cells
Monocytes/myeloid cells
Committed precursors
Dendritic cells

Determination of residual T-and B-cells:
Incidence of GvHD (grade 1-3) (Children)

- °1; 36.7%
- °2; 23.5%
- °3, 4.4%

35.3% without GvHD
Comparison of TRM:
Positive selection vs. CD3/19 depletion
(Children)

p<0.05

Day 100 TRM: 0

Percent death

years from transplantation

CD3/19 depletion
CD34+ selection
CD3/19 depletion in adults

Federman B et al.
Haematologica 2012; 97: 1523

<table>
<thead>
<tr>
<th>Category</th>
<th>Cause/Grade</th>
<th>Days from haplo-HSCT to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections (n=16)</td>
<td>Meningitis</td>
<td>595</td>
</tr>
<tr>
<td></td>
<td>Cerebral toxoplasmosis</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>CMV pneumonia</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>CMV pneumonia</td>
<td>186</td>
</tr>
<tr>
<td></td>
<td>CMV pneumonia</td>
<td>299</td>
</tr>
<tr>
<td></td>
<td>Pneumonia</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Pneumonia</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Viral pneumonia</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Viral pneumonia</td>
<td>375</td>
</tr>
<tr>
<td></td>
<td>Viral pneumonia</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Bacterial pneumonia</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Fungal infection</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Fungal infection</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>Sepsis, MOF</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td>Sepsis, MOF</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Sepsis, MOF</td>
<td>2</td>
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<tr>
<td>GVHD (n=4)</td>
<td>Acute GVHD IV</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Acute GVHD IV</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Acute GVHD IV</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Chronic GVHD liver</td>
<td>268</td>
</tr>
<tr>
<td>Other (n=6)</td>
<td>Progressive multifocal leukencephalo</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Progressive multifocal leukencephalo</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>Idiopathic pneumonia syndrome</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Cardiac failure</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Cardiac failure</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>Tetraparesis of unknown origin</td>
<td>74</td>
</tr>
</tbody>
</table>
The potential role of gamma/delta T-cells

Godder et al., Long term disease-free survival in acute leukemic patients Recovering with increased g/d T cells after partially mismatched related Donor bone marrow transplantation. BMT 2007; 39,751-757.

Vantourout P.

Lysis of infected or stressed cells
Cytokine and chemokine production
Regulation of stromal cell function via growth factor production
B cell help and IgE production
Dendritic cell maturation
Priming of αβ T cells via antigen presentation

Otto M. .. Handgretinger R.
Human g/d T cells From G-CSF mobilized donors retain Strong tumoricidal activity and produce immunostimulatory cytokines after clinical scale isolation. J Immunotherapy 2005: 28: 73
Negative depletion strategy of $\alpha\beta^+$ T-cells


Biotin-anti-$\alpha\beta$ (BMA031) + anti-biotin mAb

Graft

Waste

CD34+ and CD34- progenitors

NK cells

Dendritic/myeloid cells

$\gamma\delta$ T-cells
Efficacy of TcRαβ T-cell depletion

Schumm M et al., Cytotherapy 15; 1253-8: 2013
Cumulative incidence of grade I-II skin-only acute GvHD in children

Unpublished data kindly provided by Franco Locatelli and Alicia Bertaina

50 patients with hematological malignancies

Chronic limited (skin): 2

aGvHD I-II = 26%
# TcRαβ/CD19 depletion in adults

## No. of Patients

<table>
<thead>
<tr>
<th></th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Engraftment</td>
<td></td>
</tr>
<tr>
<td>Overall Engraftment</td>
<td></td>
</tr>
<tr>
<td>Median (range) days to:</td>
<td></td>
</tr>
<tr>
<td>Neutrophils ≥ 0.5 x 10⁹/L</td>
<td>11 (9-15)</td>
</tr>
<tr>
<td>Platelets ≥ 25 x 10⁹/L</td>
<td>10 (5-13)</td>
</tr>
<tr>
<td>Acute GvHD</td>
<td></td>
</tr>
<tr>
<td>grade 0- I</td>
<td>12 (skin)</td>
</tr>
<tr>
<td>grade II</td>
<td>1 (skin)</td>
</tr>
<tr>
<td>grade III-IV</td>
<td>1 (skin, gut) (αβ &gt; 10 x 5/kg)</td>
</tr>
<tr>
<td>Chronic GvHD moderate/severe</td>
<td>0</td>
</tr>
</tbody>
</table>

**TRM:** 2/14

Data kindly provided by Franco Aversa
Posttransplant T lymphocyte recovery

Cell/μl

Days since transplant

CD3
CD4
CD8

HSCT Program
University of Parma
47 y-old man
CML-BT
Relapse after MUD
Refractory to TK inhibitors
CR, 4 months after Haplo

Posttransplant immunological recovery

CD4
CD8
CD19
NK
αβ CD3+
γδ CD3+
NK cells
CD4+
CD19+

HSCT Program
University of Parma
Comparison of CD3+ recovery at day +30 (children)

CD3/19 vs TcRab day 30 CD3+

p<0.0001

CD3/19 n = 45  TcRab n = 19
Haploidentical Tx as platform for further immunotherapy

Prep. regimen

- Anti-CD19 (ongoing)
- Anti-GD2 (ongoing study)
- Bispecific antibodies
- CAR T-or NK –cells (CD19, GD2)
- NK cells/CIK cells
- Virus-specific T-cells

No GvHD prophylaxis, no GvHD

TcRαβ-depleted stem cells
ADCC overrides KIR mediated inhibition: CD16 is the strongest activatory signal for NK cells

![Diagram showing inhibitory and activatory signals](image)

Second HaploTx in NR and post-transplant treatment with anti-CD19 antibody 4G7 (Prof. G. Jung, Tübingen)
(20 mg as a 3 hour infusion weekly or every other week)

PCR-MRD FACS-MRD

2nd haplo SCT

> 2 year
Phase II feasibility study using ch14.18/CHO antibody and subcutaneous Interleukin 2 after haploidentical stem cell transplantation in children with relapsed neuroblastoma

Eudra CT 2009-015936-14

- **Haplo Trp.**
  - ca. $200 \times 10^6$/kg NK

- **anti GD2 mAb (CH14.18/CHO)**
  - 8 hour infusion
  - day 1-5

- **MIBG**

- **Interleukin 2 s.c.**
  - 1 Mio U day 6, 8, 10

- **6-9 cycles**
  - Evaluation after cycles 3, 6

- **20mg/m^2**
  - day 0, 30, 60, 90, 120
Bispecific T-cell engaging antibodies (BiTE) induce posttransplant donor-derived T-cell proliferation w/o causing GvHD
Recovery and log Depletion after TcRab Depletion with the fully automated Prodigy

<table>
<thead>
<tr>
<th></th>
<th>Recovery</th>
<th>Log Depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+</td>
<td>80 %</td>
<td></td>
</tr>
<tr>
<td>CD56+</td>
<td>74 %</td>
<td></td>
</tr>
<tr>
<td>TcRgd+</td>
<td>51%</td>
<td></td>
</tr>
<tr>
<td>TcRab+</td>
<td></td>
<td>5.05 %</td>
</tr>
<tr>
<td>CD19</td>
<td></td>
<td>4.98 %</td>
</tr>
<tr>
<td>CD3</td>
<td></td>
<td>1.63%</td>
</tr>
</tbody>
</table>

n = 1
Fill out the gaps:

- Tumor-specific T-cells?
- Vaccination?
- ?
- ?
- ?
- ?

Prep.regimen

No GvHD prophylaxis, no GvHD

T-cell depleted stem cells
University Children´s Hospital Tübingen:

Peter Lang
Tobias Feuchtinger
Michael Schumm
Heiko-Manuel Teltschik
Matthias Pfeiffer
Martin Ebinger
Patrick Schlegel
Karin Schillbach

MiltenyiBiotec