The question is not whether or not to deplete T-cells, but how to deplete which T-cells

- CD34+ positive selection
- Negative Depletion of:
  - CD3/CD19
  - TcRαβ/CD19
T-cell depletion: positive selection versus negative depletion

**CD34+ positive selection**

**CD3/19 depletion**


CD34+ positive selection


BM + G-PBSC’s: E-rosetting + CD34+selection (CellPro)
Tübingen Pilot Study (children)

Handgretinger et al.; Bone Marrow Transplant. 2001; 27: 778-83.

Perugia (adults)

Aversa et al., J Clin Oncol 2005; 23: 3447-54.
GvHD after myeloablative conditioning (TBI/Busulfan/ATG/OKT-3 based)

**no GvHD prophylaxis**

*T-cells*: 10 000/kg (range 5000-31000)

<table>
<thead>
<tr>
<th>grade</th>
<th>0-I</th>
<th>II</th>
<th>III-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>91%</td>
<td>7%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Critical threshold for T-cells: **25 000/kg** (without GvHD prophylaxis)
Reconstitution of CD3+ T-cells after haploidentical transplant with CD34+ positively selected stem cells

Handgretinger et al.; Bone Marrow Transplant. 2001; 27: 778-83.

% of patients with CD3 > 0.1 x 10^9/L

Cumulative Incidence

- lethal viral infections (ADV, CMV, HSV)
- lethal viral infections (only ADV)

Days after transplantation

Years

> 20 x 10^6/kg

< 20 x 10^6/kg
EFS after haploidentical transplantation in patients with **AML** with refractory disease or remission at time of Tx


![Graphs showing survival rates](image-url)
Risk of relapse in pediatric patients with ALL (n=19) after haploidentical transplantation of CD34+ stem cells from a NK alloreactive or NK nonalloreactive donor

Journal of Immunology 172, 644-650, 2004

Days after transplantation

Probability of relapse

Non-alloreactive

alloreactive

P=0.01
Thiotepa (10 mg/kg)

CD3/19-depleted haploidentical PBSC’s

Infusion of stem cells

Fludarabine (160 mg/m²) or Clofarabine (200 mg/m²)

Thiotepa (10 mg/kg)

Melphalan (140 mg/m²)

OKT-3

Tuebingen

Donor: G-CSF PBSC’s

+10

MMF (if T-cells > 25,000 /kg BW)
Graft composition (/kg BW)

- **Stem cells**: $16.2 \times 10^6$
- **T-cells**: $57.8 \times 10^3$
- **B-cells**: $32.1 \times 10^3$
- **NK-cells**: $107.3 \times 10^6$
- **Myeloid**: $533.7 \times 10^6$

GvHD Prophylaxis

MMF (1200mg/m²) if residual T-cells > 25000/kg
Recovery of Platelets (>20,000/µl)

- **CD3/CD19 depleted: 9 days**
- **CD34+ 23 days**

---

**Neutrophils**

- Median: day 11 (9-17)
- Engraftment rate: 89.7%
Incidence of GvHD (grade 1-3)

- °1; 36.7%
- °2; 23.5%
- °3, 4.4%

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

Days 100 TRM: 0

Percent death

years from transplantation

CD3/19 depletion
CD34+ selection

p<0.05
EFS acute leukemias/MDS
(influence of remission status)

Years from Trp

Portion

CR 1-3

Refractory disease

0.0

0.1

0.2

0.3

0.4

0.5

0.6

0.7

0.8

0.9

1.0

0

1

2

3

4

5

6

7

(all T-cell depleted)
Negative depletion strategy of αβ+ T-cells


Biotin-anti-αβ (BMA031) + anti-biotin mAb

Magnet

Waste

CD34+ and CD34- progenitors

NK cells

Dendritic/myeloid cells

γδ T-cells

Graft
Comparative analysis of the T-cell depletion efficacy of the different methods
TcRαβ/CD19- depleted haploidentical PBSC’s Conditioning regimen

Conditioning regimen:
- Thiotepa
- Melphalan
- Clofarabine
- Anti-CD3 (OKT-3)

Donor: G-CSF

Infusion of stem cells

No post-transplant immunosuppression

Donor: G-CSF
TCRαβ/CD19 Depletion: Graft composition (/kg BW)

- CD34+stem cells: 16.2 x 10^6 cells/µl
- TCRγδ: 15 x 10^6 cells/µl
- TCRαβ: 14 x 10^3 cells/µl
- NK-cells: 100 x 10^6 cells
- Myeloid: 600 x 10^6 cells
TcRαβ/CD19 Depletion: Engraftment

Engraftment rate vs. days after SCT

- Blue line: Thrombo > 20x10³/µl, n=11
- Red line: ANC > 500/µl, n=11
TCR $\alpha\beta$/CD19 Depletion: residual $\gamma\delta$ T-cells do not cause severe GvHD

<table>
<thead>
<tr>
<th>GvHD grade</th>
<th>n=</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>no GvHD</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>$^\circ1$ (only skin)</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>$^\circ2$ (only skin)</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>$^\circ3-4$</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Rather than inhibiting the effector cells by GvHD prophylaxis or GvHD therapy, we need to exploit their anti-leukemic potentials

Cytokines

Antibodies

Antibody constructs
Which cytokines augment NK activity best against ALL blasts in patients post transplant
Use of low dose s.c. IL 2 in vivo after Haplo trp.: Temporal development of NK-cell activity against K562
ADCC 3 weeks after haploTx with TcRαβ/CD19-depleted stem cells against the Patient's own cALL- blasts.

**ADCC overrides KIR mediated inhibition**


Anti-CD19 antibody provided by Prof. Jung, Tübingen.
Single-chain bispecific antibody MT103
(Micromet AG, Munich, Germany)

Tumor Regression in Cancer Patients by Very Low Doses of a T Cell–Engaging Antibody

Ralf Bargou, Eugen Leo, Gerhard Zugmaier, Matthias Klinger, Marielle Goebeler, Stefan Knop, Richard Noppeney, Andreas Viardot, Georg Hess, Martin Schuler, Hermann Einsele, Christian Brandl, Andreas Wolf, Petra Kirchinger, Petra Klappers, Margit Schmidt, Gert Riethmüller, Carsten Reinhardt, Patrick A. Baeuerle, Peter Kufer

Previous attempts have shown the potential of T cells in immunotherapy of cancer. Here, we report on the clinical activity of a bispecific antibody construct called blinatumomab, which has the potential to engage all cytotoxic T cells in patients for lysis of cancer cells. Doses as low as 0.005 milligrams per square meter per day in non-Hodgkin’s lymphoma patients led to an elimination of target cells in blood. Partial and complete tumor regressions were first observed at a dose level of 0.015 milligrams, and all seven patients treated at a dose level of 0.06 milligrams experienced a tumor regression. Blinatumomab also led to clearance of tumor cells from bone marrow and liver. T cell–engaging antibodies appear to have therapeutic potential for the treatment of malignant diseases.

Science 2008; 321: 974 -976
Treatment of a patient with chemorefractory ALL relapse post Tx
Handgretinger et al., Leukemia 2010

Prior MT103

After MT103

MT103 infusion
Conclusion:

• T-cell depletion prevents severe GvHD
• The immune recovery depends on the T-cell depletion method
• Omission of GvHD prophylaxis allows a platform on which further in vitro or in vivo manipulation of the graft (adoptive transfer of donor cells, in vivo IL-2, IL-15, antibodies, antibody constructs etc.) can be envisioned.

Yes !