NEW TRENDS IN HEMATOPOIETIC CELL TRANSPLANTATION IN CHILDREN

Department of Pediatric Oncology, Hematology and Transplantology

TOPICS

- HSCT FROM UNRELATED DONORS
- UNRELATED CORD BLOOD TRANSPLANTATION
- HAPLOIDENTICAL HSCT
- HSCT FROM MISMATCHED UNRELATED DONORS
- REDUCED INTENSITY CONDITIONING FOR ALLOGENEIC HSCT
- TRESULFAN-BASED CONDITIONING
- ALLOGENEIC HSCT FOR CHILDHOOD SOLID TUMORS
- PROGRESS IN TREATMENT OF RESISTANT GvHD

HSCT FROM UNRELATED DONORS (UD-HSCT)

- 12 500 000 registered volunteer unrelated stem cell donors
- 60-70% of probability to find matched unrelated donor
- Matching of unrelated donor at allelic level
- Optimization of preparative regimen for UD-HSCT and GvHD prophylaxis after UD-HSCT (T-cell depletion in vivo)
- Finding of optimal hematopoietic stem cell dose for UD-HSCT according to HSC source and degree of compatibility at allelic level
- More effective methods of prophylaxis, diagnosis and treatment of toxic, infectious and immunological complications related to UD-HSCT

Factors Supporting Progress in Hematopoietic Stem Cell Transplantation from Unrelated Donors (UD-HSCT)

- Proportion of patients aged 18 years or younger: in 1989 - 11.8 mln (31%); in 2000 - 9.9 mln (25.3%); in 2006 - 8.0 mln (23%)

YEARLY NUMBER OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTS PERFORMED IN POLISH PEDIATRIC TRANSPLANT CENTERS AND DONOR TYPE 1989-2008

- MUD
- MMFD
- MSD
- Total
- Population of 18 years of age: in 1990 - 11.8 mln (31% of whole nation); in 2000 - 9.9 mln (25.3%); in 2006 - 8.0 mln (23%).
HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN IN EASTERN EUROPEAN COUNTRIES 1985-2004: DEVELOPMENT, RECENT ACTIVITY AND ROLE OF THE EBMT/ESH OUTREACH PROGRAMME

J Wachowiak, M Labopin, M Miano et al. Bone Marrow Transplant 2008; 41 (suppl. 2): S112-S117

- Matched sibling donor: 14% ± 3% vs 7% ± 1%
- Mismatched related donor: 45% ± 12% vs 21% ± 3%
- Matched unrelated donor: 34% ± 7% vs 15% ± 2%

Comparable long-term survival after unrelated and HLA-matched sibling donor hematopoietic stem cell transplantations for acute leukemia in children younger than 18 months

M Eapen, Rubinstein P, Zhang MJ et al. on behalf of CIBMTR
J Clin Oncol 2006; 24 (1): 145-151

MSD-HSCT: N = 101, ALL, n = 49 (49%); AML, n = 52 (51%);
1.CR, n = 81 (80%); ≥ 2.CR, n = 9 (9%), relapse, n = 11 (10%)

UD-HSCT: N = 166, ALL, n = 97 (58%); AML, n = 69 (42%);
1.CR, n = 81 (49%); ≥ 2.CR, n = 53 (32%); relapse, n = 32 (19%)

pEFS in AML 1CR MSD vs MUD

0 6 12 18 24 30 36
TIME (months)

pEFS

MSD

MUD

Log rank p=NS

0.64

0.66

Results of allogeneic HSCT in children with high risk AML in CR1 – report of the Polish Pediatric Group for Hematopoietic Stem Cell Transplantation
Dawid Szpecht, Joanna Owoc-Lempach, Katarzyna Drabkó, Jan Styczeński, Michał Leda, Mariusz Wysocki, Jerry R. Kowalczyk, Alicja Chybicka, Jacek Wachowiak
Acta Haematol Pol 2009; 40 (2); 129 / SIOP 2009

Recent improvement in UD-HSCT in SAA

Recent improvement in UD-HSCT in SAA

> 1998

0.57

≤ 1998

0.32

Cum. survival

Years after SCT

UD-HSCT:
- graft failure (26% vs 11%)
- aGvHD (37% vs 28%)
- cGvHD (38% vs 22%)

Better HLA match results in better survival in UD-HSCT in SAA

Log rank p=NS

0.63

0.64

Viollier et al., BMT 2008

Deeg et al., Biol Blood Marrow Transpl 2001

Hematopoietic stem cell transplantation trends in children over the last three decades: a survey by the Paediatric Diseases Working Party of the EBMT
Bone Marrow Transplant 2007; 39: 89-99

100-DAY TRM PROBABILITY BY YEAR AND BY TYPE OF TRANSPLANT

MSD  SCT

VUD  SCT

PMFD  SCT

Auto  SCT

Day +100 transplant related mortality after allogeneic HSCT in relation to donor type before and after 01.01.1999

ALLOGENICZNA HSCT

≤ 1998 1999 - 2004

Matched sibling donor 14% ± 3% 7% ± 1%
Mismatched related donor 45% ± 12% 21% ± 3%
Matched unrelated donor 34% ± 7% 15% ± 2%

Lower rejection rate and better survival in children < 14 years conditioned for UD-HSCT with Fluda+LD-Cy+ATG

Less graft failure after UD-HSCT in children < 14 years: 5% vs. 32% (p = 0.03)

p = 0.2

Bacigalupo et al., BMT 2005

Lower rejection rate and better survival in children < 14 years conditioned for UD-HSCT with Fluda+LD-Cy+ATG

Alternative donor HSCT for Primary Immunodeficiencies

Wolfram Ebell

6th Meeting of the EBMT Paediatric Diseases WP and 1st Meeting of the EBMT Paediatric Nurses Poznan, 2-4 June 2008

Results (N = 321)


TRM

CCR


Improvement of alternative donor transplants

✓ Early diagnosis
✓ Avoidance of critical infections before HSCT
✓ High resolution molecular HLA typing
✓ Donor pool
✓ Graft-versus-host management
✓ Pre-emptive anti-infectious treatment
✓ Competent follow-up

Alternative donor HSCT for Primary Immunodeficiencies

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UNRELATED DONOR CORD BLOOD TRANSPLANTATION (UD-CBT)

Gluckman E, Blume KG, Auerbach AD et al.

Hematopoietic reconstitution in a patient with Fanconi anemia by means of umbilical cord blood from an HLA-identical sibling.


• AGE / SEX :  5 years / boy
• DIAGNOSIS : Fanconi anemia
• CB-DONOR : HLA-id. sister (chromosomal breakage within the normal range)
• PREPARATIVE REGIMEN : Cy 4 x 5 mg/kg (day -6 to -3) + TA 5 Gy (day -1)
• SOURCE OF HSC : cryopreserved cord blood
• CB CELL DOSE : 4 x 10^8 NC/kg
• DATE OF CB-SCT : October 6th, 1988
• GVHD PROPHYLAXIS : CsA (from day -1)
• RETICULOCYTES 1% : day +29
• ANC >0,5 x 10^12/L : day +36
• PLATELETS >50,0 x 10^12/L : day +62
• ACUTE GVHD : 0 (from day +15)
• HEMATOPOIETIC CHIMERISM : complete donor, stable
• SURVIVAL : 20 years (Gluckman E. History of cord blood transplantation. Bone Marrow Transplant 2009; 44 (10): 621-626)
Haematopoietic stem cell transplantation trends in children over the last three decades: a survey by the Paediatric Diseases Working Party of the EBMT
Bone Marrow Transplant 2007; 39: 89-99

Cord blood transplants (CBT) in Europe:
- in 1997 r. N = 86
- in 2006 r. N = 544, including
  - 90.5% CBT from unrelated donor (UCBT)
  - 9.0% CBT from matched sibling donor (MSD-CBT)
  - 0.5% CBT from partially mismatched donor

Number of unrelated CBT reported to Eurocord Registry according to diagnosis and recipient age 1999 - 2000

Cairo MS, Rocha V, Gluckman E, Hale G, Wagner J.
Bone Marrow Transplant 2008; 41 (1): S4-53

Cairo MS, Rocha V, Gluckman E, Hale G, Wagner J.
Bone Marrow Transplant 2008; 41 (1): S4-53
The EBMT activity survey 2006 on hematopoietic stem cell transplantation: focus on the use of cord blood products
Gratwohl A, Baldomero H, Frauendorfer K, Rocha V, Apperley J, Niederwieser D; Joint Accreditation Committee of the International Society for Cellular Therapy (ISCT) & European Group for Blood and Marrow Transplantation (EBMT) (JACIE)
Bone Marrow Transplant 2008; 41 (8): 687-705

- CBTs in Europe in 2006:
  - n = 544
  - only 5% out of all allo-HSCTs
  - 27% allo-HSCTs for metabolic diseases
  - 16% allo-HSCTs for congenital bone marrow failures
  - 15% allo-HSCTs for primary immunodeficiencies

Outcomes of transplantation of unrelated umbilical cord blood and bone marrow in children with acute leukemia: a comparison study.
Eapen M, Rubinstein P, Zhang M et al.

Cairo MS, Rocha V, Gluckman E, Hale S, Wagner J
Bone Marrow Transplant 2008; 41 (Suppl 1): 44-53

Overall survival in 259 patients with non-malignant disorders according to cell doses infused (x10^7/kg) and HLA differences

Advantages of cord blood transplantation
Cairo MS et. al. Biol Blood Marrow Transplant 2008; 14: 44-53;
Gluckman E et al. Bone Marrow Transplant 2008; 41 (suppl. 2): S80-S82;
Locatelli et al. Bone Marrow Transplant 2008; 41 (suppl. 2): S3-S5

- Rapid identification of compatible and immediate availability of matched CB unit;
- Extension of donor pool, because of tolerance of 1-2 HLA mismatches out of 6 in loci A, B & DR;
- Unlimited of transplant date choice;
- Low risk of latent viruses transmission (CMV, EBV);
- Better long-term immune recovery;
- Lower rate of severe acute and chronic GvHD without increased risk of leukemia relapse;
- Long term results, both in children with malignant disorders as well as in children with non-malignant one, comparable with results of of BMT and PBSCT irrespective of the donor type, i.e. matched sibling or unrelated donor. Therefore, CBT should be considered for children who need an HSCT and unable to find a matched donor;
- Small cryopreserved volume;
- No risk to the donor;

Major disadvantages of cord blood transplantation
Cairo MS et. al. Biol Blood Marrow Transplant 2008; 14: 44-53;
Gluckman E et al. Bone Marrow Transplant 2008; 41 (suppl. 2): S80-S82;
Locatelli et al. Bone Marrow Transplant 2008; 41 (suppl. 2): S3-S5

- Fixed numbers of CB stem cells available per CB unit with only a median of 1x10^9 total NC, i.e. CB unit contains number of NC appropriate for recipients with body weight below 30 kg; According to EUROCORD recommendations units containing less than 1-1.5x10^7 NC/kg should not be utilized;
- Increased risk of graft failure, if CB-NC dose amounts less than 3x10^7/kg;
- Delayed reconstitution of granulo- and megakaryopoiesis;
- Delayed the early immunological reconstitution due to lack of the transfer of antigen-experienced, memory T cells;
- Increased risk day +100 TRM due to higher risk infectious complications (the risk decreases if CB-NC dose amounts above 3x10^7/kg);
- Lack of HSC for second transplantation and/or lack of donor lymphocytes for adoptive immunotherapy (DLI);
- Risk of inherited disorders transmission;

Criteria of donor choice - Eurocord recommendations

1. First look at the number of cells:
   - ≥ 3x10^7 TNC/kg or ≥ 2x10^5 CD34+/kg
2. Second look at HLA matches:
   - D-1 mm better than 2, avoid 3-4mm
   - prefer class I mismatches than class II
   - if no choice increase the number of cells
3. Then adapt to graft indication:
   - Malignant diseases: cell dose is the best prognostic factor, because HLA differences reduces relapse risk (GVL)
   - non-malignant diseases: find the best HLA match and increase cell dose
HAPLOIDENTICAL HSCT (HAPLO-HSCT)

MAJOR ADVANTAGES OF HAPLOIDENTICAL HSCT

• Haploidentical donor:
  - available for each child in need of HSCT
  - highly motivated;
  - readily available throughout the transplant process for initial and subsequent HSC donation;
  - rapidly evaluated for product donation in recipient’s transplant center without loss of time for an unrelated donor search and problems related to cooperation with another donor center or cord blood bank;

MAJOR DISADVANTAGES OF HAPLOIDENTICAL HSCT

• Graft failure;
• GvHD;
• Posttransplant lymphoproliferative diseases (PTLPD);
• Prolonged immunodeficiency with high risk of life-threatening infections, either viral or fungal;
• Leukemia relapse;

Haploidentical SCT in children: an update and future perspectives
Lang P, Handgretinger R.
Bone Marrow Transplant 2008; 42 (Suppl 2): S54-S55.

• Haploidentical transplantation of megadose CD34+ (>10 x 10^6/kg) purified stem cells demonstrated:
  - high rate of engraftment (>90%)
  - low risk of significant GvHD
  - important role of alloreactive NK cells,
  - delayed immunoreconstitution with high risk of infections
  - significant relapse rate

Prediction of relapse in 36 patients after haploidential transplantation with CD34+ positive selection
W. Leung et al., J Immunol 2004; 172: 644

Receptor-Ligand Model (Donor-KIR versus Recipient-HLA)

Adenoviral infections after transplantation of positive selected stem cells from haploidentical donors in children: an update

Cumulative incidence

lethal viral infections (ADV, CMV, HSV)
lethal viral infections (only ADV)
Haploidentical SCT in children: an update and future perspectives
Lang P, Handgretinger R.
Bone Marrow Transplant 2008; 42 (Suppl 2): S54-S59.

Optimal composition of the graft for haploidentical transplantation should demonstrate:

- High number of CD34+ cells (>10 x 10⁶/kg);
- High number of alloreactive NK cells (CD56+) to assure engraftment and significant GvL effect;
- Safe threshold of T lymphocytes in the graft (<2.5x10⁴ CD3/kg) to avoid risk of severe GvHD;
- Appropriate level of depletion of mature B lymphocytes harboring the EBV to reduce risk EBV-associated lymphoproliferative disease;
- Presence of dendritic cells and monocytes able, along with NK cells, to generate antitumor, antiviral and/or graft-facilitating effects;

CD3/CD19 negative depletion strategy of mobilized PBSC’s
CD3 depletion: Gordon i wsp., Bone Marrow Transplant 2002; 30: 69-74
CD3/19 depletion: Barfield i wsp., Cytotherapy 2004; 6: 1-6

Feasibility and outcome of reduced-intensity conditioning in haploidentical transplantation
Handgretinger R, Cifon X, Pfeiffer M, Mueller T, Feuchtinger T, Hale GA, Lang P.
Ann N Y Acad Sci. 2007;1106: 279-289

Tübingen
(CD3/CD19 Depletion)

- Fludarabine (160 mg/m²)
- Thiotepa (10 mg/kg)
- Melphalan (140 mg/m²)
- OKT-3
- MMF

Infusion of stem cells +10

Recovery of Platelets (>20 000/µl)

CD3/CD19 depleted (9 days)
Positive selected (CD34+) (23 days)
p<0.001

CD3/CD19 Depletion
Cumulative incidence

0.0 0.2 0.4 0.6 0.8 1.0

Years from Transplantation

Handgretinger R, 2006

Cause of Death:
Relapse/Progression in 16/32 Patients

Transplant related toxicity:
Liver / Kidneys
Cardiac / Neurological Infections
WHO grade 0-2

Transplant related mortality:
none (up to now)

Outcome

Handgretinger R, 2006
Allo-SCT in children with high-risk leukemia using unmanipulated grafts from alternative donors
Sedlacek P, Mejstrikova E, Formankova R, Keslova P, Dobrovolna M, Vrana M, Stary J.
Bone Marrow Transplant 2008; 42 (suppl. 2): S10-S15

• Study period: I/01-XII/07
• Number of patients: 87
• Age: 1.0-20.5 years (median 12.2)
• Diagnosis: 39 x ALL; 12 x AML, 2 x AH; 8 x sAL/MDS; 15 x MDS; 9x CML; 3 x NHL;
• Transplant material: unmanipulated allogeneic graft
• Donors: 56 x MUD (9-10/10); 21 x MMUD (7-8/10); 10 x MMCB (4-5/6)
• Source of HSC: 42 x PBSC; 15 x BM; 10 x CB
• Conditioning regimen: 48 x FTBI; 36 x Busulfan-based; 3 x RIC;
• GvHD prophylaxis: ATG + CsA + MTX;
• Engraftment: 100%, no graft failure, no graft rejection;
• TRM: day + 100 4.5%; total 13.8%;
• Relapse incidence: 23.2%

EFS according to HLA match (CB excluded)
(HLA match 7-8/10 vs. 9-10/10; n=77)

<table>
<thead>
<tr>
<th>Year</th>
<th>0.0</th>
<th>0.2</th>
<th>0.4</th>
<th>0.6</th>
<th>0.8</th>
<th>1.0</th>
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<tbody>
<tr>
<td>EFS</td>
<td>Rem (ALL, AML, Non-mal)</td>
<td>Non-Rem (ALL, AML, MDS-RAEB-T)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>0.0</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

p = 0.25198

FEASIBILITY AND RESULTS OF BONE MARROW TRANSPLANTATION FROM HLA-MISMATCHED UNRELATED DONOR FOR CHILDREN AND YOUNG ADULTS WITH ACQUIRED SEVERE APLASTIC ANEMIA
Yagasaki H et al., Int J Hematol 2007; 85 (5): 437-442

• N = 11
• Age: 11 (3-20)
• Conditioning: TBI 5 Gy, Cy 4 x 50 mg/kg + ATG 10 mg/kg
• GvHD-prophylaxis: Tacrolimus + MTX
• Engraftment: 10/11
• aGvHD II – IV: 2/11; cGvHD lim.: 3/11 ext. 1/11
• Results: 11/11 survive 3-56 months (median: 33)
RIC FOR ALLOGENEIC HSCT IN CHILDREN WITH NON-MALIGNANT DISORDERS

- Mixed chimerism will cure majority of non-malignant diseases being indication for allogeneic HSCT;
- Majority of children with non-malignant diseases demonstrate specific predisposition to organ toxicity related to conventional myeloablative preparative regimens (MAT).

RIC/MIC survival by HLA match/mismatch

Non-malignant diseases

Reduced intensity conditioning and allogeneic stem cell transplantation in childhood malignant and nonmalignant diseases.

Satwani P, Cooper N, Rao K, Veys P, Amrolia P.
Bone Marrow Transplant 2008; 41: 173-182

RIC FOR ALLOGENEIC HSCT IN CHILDREN WITH NON-MALIGNANT DISORDERS

- In children there was no randomized study to compare results of RIC-HSCT and MAT-HSCT;
- There is already sufficient evidence to consider RIC as routine method of conditioning in all children with primary immunodeficiency and hemophagocytic lymphohistiocytosis;
- RIC is also recommended in children with Langerhans cell histiocytosis, Glanzmann thrombasthenia, and some hereditary and acquired bone marrow failures, i.e. when mixed or lineage-specific chimerism may be sufficient to cure;

RIC BEFORE ALLOGENEIC HSCT IN CHILDREN WITH HEMATOLOGICAL MALIGNANCIES

- Majority of children after RIC-HSCT for hematological malignancies demonstrate engraftment with stable mixed chimerism, which creates excellent platform for adoptive immunotherapy in prophylaxis or treatment of post-transplant leukemia relapse or PTLPD;
- However, due to still scanty data, RIC-HSCT in children with hematological malignancies should be restricted to patients with contraindications for MAT related to co-morbidities or relapsed after previous MAT-HSCT;
- To improve anti-leukemic effect of RIC-HSCT it is recommended to reduce T cell depletion in vivo and to withdraw early the GvHD prophylaxis with subsequent administration of DLI and cytokines (IL-2, INF-α);
- There is a pressing need for prospective studies to evaluate the late effects of different variants RIC on growth, fertility, chronic GvHD and secondary malignancies occurrence in children undergoing RIC-HSCT;
Treosulfan in pediatric hematopoietic stem cell transplantation: overview of results in children with malignant and non-malignant disorders
Jacek Wachowiak on behalf of the EBMT Pediatric Disease WP
40th Congress of SIOP, Berlin, 3-6.10.2008

MYELOABLATIVE, LOW-ORGAN TOXICITY TREOSULFAN-BASED PREPARATIVE REGIMEN IN CHILDREN

Treosulfan in pediatric hematopoietic stem cell transplantation: overview of results in children with malignant and non-malignant disorders
Jacek Wachowiak on behalf of the EBMT Pediatric Disease WP
40th Congress of SIOP, Berlin, 3-6.10.2008

Non-malignant disorders (N = 30)

ALLOGENEIC HSCT FOR SOLID TUMOR TREATMENT IN CHILDREN
WHY ALLOGENEIC HSCT FOR CHILDHOOD SOLID TUMORS?

- Results of megachemotherapy with autologous HSCT in children with high-risk, refractory or relapsed neuroblastoma, Ewing sarcoma and rhabdomyosarcoma remain unsatisfactory;

- Therefore, new concept of allogeneic HSCT for solid tumors do not rely on further escalation of chemotherapy intensity, but rather on graft versus tumor effect (GvT) after allogeneic HSCT preceded with RIC or treosulfan-based preparative regimen in combination with immunotherapy and/or targeted therapy;

- The number of children treated according such strategy remains still rather low, but is growing quickly, and results achieved until now seem to confirm that this strategy is in the right;

- It was demonstrated that GvT effect after haploidentical HSCT is mainly related to the presence of inhibitory KIR-HLA mismatch;

- GvT effect in the allogeneic setting remains questionable;

Graft-versus-tumor effect in a patient with advanced neuroblastoma who received HLA haploidentical bone marrow transplantation – a case report.

Inoue Mi in. Bone Marrow Transplant 2003; 32 (1), 103-106

- Age: 5 years
- Sex: boy
- Diagnosis: refractory advanced neuroblastoma with periaortaly lymphnodes involvement and multiple metastatic bone involvement;
- Donor: father;
- Preparative regimen: myeloablative (FTBI+thiotepa+etoposide);
- HSC source: bone marrow;
- GvHD: acute – grade I; chronic – not observed;
- Other complications: not observed;
- Results: CR (within 2 years residual tumor disapperared completely);

Haploidentical hematopoietic stem cell transplantation in children using CD3/CD19 depletion
Lang P, 2008

Diagnoses (n = 64)

<table>
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<tr>
<th>Diagnosis</th>
<th>CR 1</th>
<th>CR 2</th>
<th>PR/NR</th>
<th>NR</th>
<th>2nd SCT</th>
<th>Total</th>
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<tbody>
<tr>
<td>AML</td>
<td>14</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ALL</td>
<td>18</td>
<td>1.3</td>
<td>12</td>
<td>6</td>
<td>2</td>
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<td>CML</td>
<td>1</td>
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<td>MDS</td>
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<td>RAEB-T</td>
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<td>Neuroblastoma</td>
<td>11</td>
<td>2</td>
<td>1 PR/NR</td>
<td>10</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Ewing s./RMS/others</td>
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<td>3</td>
<td>2</td>
<td></td>
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<td>4</td>
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<td>SAA/PNH</td>
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<tr>
<td>Immune deficiency</td>
<td>2</td>
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</table>

Haploidentical hematopoietic stem cell transplantation in children using CD3/CD19 depletion
Lang P, 2008

Conditioning regimen

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<thead>
<tr>
<th>Melphalan (140 mg/m²)+ Fludarabin/Clofarabin + TT + OKT3</th>
<th>TBI/VP16 /Flud /ATG</th>
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<tr>
<td>n=53</td>
<td>n=6</td>
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<table>
<thead>
<tr>
<th>Busulfan /Cy /Flud or Bu /Cy /Mel + OKT3</th>
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<tr>
<td>n=5</td>
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Graft composition (/kg BW)

<table>
<thead>
<tr>
<th>Zellen x10⁶/µl</th>
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<tr>
<td>stem cells</td>
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<tr>
<td>16.2x10⁶</td>
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<tr>
<td>T-cells</td>
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<tr>
<td>57.8x10³</td>
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<tr>
<td>B-cells</td>
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<td>32.1x10³</td>
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<tr>
<td>NK-cells</td>
</tr>
<tr>
<td>107.3 x10⁶</td>
</tr>
<tr>
<td>myeloic</td>
</tr>
<tr>
<td>533.7 x10⁶</td>
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</tbody>
</table>

Haploidentical hematopoietic stem cell transplantation in children using CD3/CD19 depletion
Lang P, 2008

Aim: to improve antileukemic/antitumor effects

- increase NK activity of CD3/19 depleted cells by ex vivo stimulation with cytokines
Haploidentical hematopoietic stem cell transplantation in children using CD3/CD19 depletion

Lang P, 2008

Current immunotherapeutic approach

**Results**

<table>
<thead>
<tr>
<th>Patient (relapse after HSCT)</th>
<th>Side effects</th>
<th>Outcome (days after NK cell infusion)</th>
</tr>
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<tbody>
<tr>
<td>1 (AML)</td>
<td>no</td>
<td>† d 350</td>
</tr>
<tr>
<td>2 (ALL)</td>
<td>no</td>
<td>† d 304</td>
</tr>
<tr>
<td>3 (NB)</td>
<td>no</td>
<td>alive d 200</td>
</tr>
<tr>
<td>4 (ALL)</td>
<td>no</td>
<td>† d 120</td>
</tr>
<tr>
<td>5 (ALL)</td>
<td>no</td>
<td>alive d 64</td>
</tr>
<tr>
<td>6 (ALL)</td>
<td>no</td>
<td>alive d 30</td>
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</table>


Jacek Wachowiak et al., Poznan, Gliwice, Poland
Bone marrow Transplantation 2008; 42 (suppl. 2): S119

**EXTRACORPOREAL PHOTOCHEMOTHERAPY (ECP)**

- ECP is based on the immunomodulating action of UV-A radiation (320-400 nm) on blood mononuclear cells collected by apheresis and photosensitized by 8-methoxypsoralen (8-MOP), which are subsequently infused back to the patient;
- Mechanism of action:
  - reduction of CD80 i CD86 expression on DCs;
    (Gorgun i wsp., Blood 2002; 1000: 941-947);
  - IL-10 release by DCs after ECP
    (Di Renzo i wsp., Clin Exp Immunol 2008; 151: 407-413);
- Minimal side effects;
- No systemic immunosuppressive effects;
- Main problems:
  - lack of randomized studies;
  - lack of the standarization of the procedure;

**PROGRESS IN TREATMENT OF STEROID RESISTANT OR DEPENDEND GvHD IN CHILDREN**

Calore E, Calo A, Tridello G et al., Bone Marrow Transplant 2008; 42: 421-425
MESENCHYMAL STROMAL CELLS (MSC)

Characteristics of MSCs:
- Can be isolated from several human tissues, including bone marrow, and expanded for clinical use;
- Migrate to sites of tissue injury and/or inflammation;
- Promote repair of damaged tissues;
- Inhibit inflammation development;
- Demonstrate immunomodulatory properties, mainly immunosuppressive, including inhibition of GvHD effector cell activity and downregulation of events responsible for GvHD development, which provide explanation of the efficacy of MSCs in the treatment of GvHD, even refractory to its conventional therapy;

Generating MSCs:
1. Collect bone marrow from the donor (150 ml)
2. Wash, ficoll separate
3. Cell count > 0.5 x 10^6/kg
4. MSC culture medium and plate in flasks
5. Incubate

Expansion of MSCs:
- > 90 confluence - trypsinize,
- Re-plate
- Passage
- Final cell count > 1 x 10^6/kg recipient weight
- Cryopreserve or infuse

BM Chimerism:
MSC donor/recipient/MSC origin
- 3 m, 4 m, 5 m, 6 m, 12 m

Overviews study endpoints
- Grade II-IV a GvHD
- Start Steroids
- MSC harvest and infuse
- Immune recovery
- Assess
- Assess
- Assess
- Assess
- Assess
- Assess
- Assess
- Assess
- Assess
- Assess
- Assess

Skin biopsy

Response to treatment of steroid resistant acute GvHD in 25 pediatric patients:
- Complete regression of all symptoms 16
- Improvement, partial response 4
- Stable GvHD 3
- Progression of GvHD 2
- Overall response 20/25 (80%)

Mesenchymal stem cells for treatment of steroid-resistant, severe acute graft versus host disease: a phase II study
Katarina Le Blanc, Francesco Frassoni, Lynne Balli wsp.
Lancet 2008; 371: 1579-1586

Grade of steroid-resistant acute GvHD in 55 patients treated with MSCs:
- Grade II, n = 5
- Grade III, n = 26
- Grade IV, n = 24

Immunomodulatory properties of mesenchymal stromal cells
Nauta AL, Fibbe WE
Blood 2007; 110: 3499-3506

Mesenchymal stem cells for treatment of steroid-resistant, severe acute graft versus host disease: a phase II study
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THANK YOU!