

# Haploidentical Stem Cell Transplantation in Childhood

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**Abstract:** Allogeneic stem cell transplantation has become an important treatment option for many children with malignant and non malignant diseases during the past decades. However, this therapy was for a long time restricted to patients having an "HLA identical donor". In most recent years donor registries all over the world have extended and the numbers of registered volunteers have substantially increased during the last 20 years. In spite of this progress, there is still a substantial number of children lacking a well matched donor. Virtually all little patients have at least one "haploidentical" parent who could potentially serve as a stem cell donor. These donors are immediately available, are highly motivated and could be asked for a further time if the graft would have been rejected. In the post transplant course parents are repeatedly available for additional donor cell transfusions for preemptive immunotherapy. Moreover using haploidentical parents as donors can avoid the inauguration of new registries and banking expenditures in countries whose population is not very much represented in existing large donor registries. For long years, graft rejection, graft versus host disease (GVHD) and delayed recovery of the immune system used to be the limiting factors for haploidentical transplantation. Substantial progress has been made in the clinical application of haploidentical stem cell transplantation in children with leukemia as well as non malignant diseases in the last years. Recurrence of the underlying disease and delayed immune recovery, however, remained major cause for treatment failure yet to overcome to offer this procedure to a wider range of patients. Nevertheless, haploidentical stem cell transplantation has become a valuable alternative procedure for patients lacking an HLA identical donor. The development and recent advance is reviewed in the following.

## INTRODUCTION

During the past three decades allogeneic stem cell transplantation has become a valuable option with increasing success in treatment of pediatric malignant and nonmalignant diseases [1,2]. Human leukocyte antigen (HLA)-matched siblings are ideal donors, however, only available in 20-30% of cases and therefore attention has shifted to the use of matched unrelated donors enlisted in world wide registries [3]. At present, for 70-80% of Caucasians a matched unrelated donor can be identified but the odds is decreasing to only 10% for ethnic minorities [4]. Therefore, tremendous efforts have been taken to use alternative stem cell sources e.g. closely matched unrelated donors [5,6], cord blood grafts [7,8] and partially mismatched related donors [9,10].

A further drawback for a child who urgently needs a stem cell transplantation, is the time interval from initiating an unrelated donor search to the identification of an appropriate donor, also this has decreased from eight to four months during the last ten years [11]. Virtually all children have at least one parent sharing one HLA haplotype for HLA-A, B, C and DR. These donors are extremely motivated, immediately available and their availability is neither dependent on racial or ethnic roots. Moreover, the considerable costs of high resolution HLA typing, registry and banking expenditures can be avoided.

## EXPERIMENTAL AND CLINICAL BACKGROUND

When using T cell depleted grafts, rejection was the primary goal to be overcome in the experimental and clinical application of haploidentical stem cell transplantation. Resistance to engraftment is mainly mediated by anti donor cytotoxic T-cells surviving the conditioning [12]. In unmanipulated transplants these residual host T-cells are successfully eliminated by the presence of donor T-cells, thus allowing successful engraftment. T cell mediated engraftment was counterbalanced by severe and intractable graft versus host disease (GVHD).

The basis for clinical application of haploidentical transplantation has been laid by Reisner *et al.* by two major approaches using animal models with lethally and sublethally irradiated mice: Escalation of hematopoietic progenitor cell dose and the use of non-alloreactive T-cells [13-15]. Allogeneic chimeras generated by transplantation of large doses of Sca1+Lin- cells, permanently accepted allogeneic donor-type skin grafts. Non-alloreactive T-cells synergized with murine Sca1+Lin- cells and enabled engraftment of haploidentical transplants also in sub lethally conditioned recipients [16]. Henslee-Downey *et al.* performed sequential immunomodulation pre- and post-SCT using *ex vivo* T-cell depletion with the T10B9 monoclonal antibody and *in vivo* T-cell lysis with the immunotoxin H65-RTA after an intensive conditioning regimen. In adult and pediatric patients (n=72) an overall 88% engraftment probability and a 16% and 51% probability of acute and chronic GVHD was reported respectively [17]. In 1993 the Perugia group started a pilot trial using stem cell dose escalation by supplementing the graft with T-cell depleted granulocyte colony stimulating factor (G-CSF) mobilized peripheral blood progenitor cells (PBSCs). This al-

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lowed to increase the number of transplanted CD 34+ cells by 10 fold and it could be proven not only in mice but also in humans that megadoses ( $>10^6/\text{kgBW}$ ) of stem cells are a critical determinant for the engraftment of T-cell depleted incompatible marrow transplantations. A further prerequisite for successful clinical application of haploidentical transplantation was profound T-cell depletion for the prevention of GVHD. In their subsequent trial the Perugia group used grafts comprising of megadoses of purified CD 34+ cells and low numbers of T-cells, reduced to a mean of  $2 \times 10^4/\text{kg}$  – one log less than in the previous study. This level was reached by processing peripheral blood mononuclear cells by one round of E-rosetting followed by a positive immunoselection of the CD 34+ cells with a separate column. Following these experiences, it was our group in Tuebingen, which first introduced the method of CD 34+ enrichment using the MACS-Technology (Miltenyi Biotech, Bergisch Gladbach, Germany) for haploidentical stem cell transplantation [10,18-20]. In these initial trials it could be shown, that reliable engraftment and fast immune recovery could be achieved in the vast majority of patients using highly purified peripheral stem cell grafts.

The interpretation of outcome data of these initial trials is strictly confounded by the inherent advanced disease status among recipients of these initial studies [19,21,22], which needs to be considered when interpreting the transplant related morbidity and mortality as well as the high rate of relapse. At the beginning haploidentical transplantation was only performed in those patients who had no chance of survival with conventional therapy. As a result of these pioneering studies, data are now emerging to suggest that haploidentical transplantation may have a role in patients other than those in desperate straits. As graft rejection and GVHD may have largely be overcome, high relapse rates and delayed immune recovery remained as major obstacles yet to be improved.

## CLINICAL STUDIES

As haploidentical transplantation procedures are well established in the treatment of genetic diseases e.g. severe combined immunodeficiencies (SCID) its role in the treatment of malignant diseases is not yet fully defined. Haploidentical transplantations have been performed numerous in patients with SCID due to the immediate availability of the donors in children who otherwise could not receive appropriate treatment [23,24]. The outcome has improved impressively with a 75% probability for event free survival (EFS) during the past decade [25]. For other nonmalignant diseases where timing is less critical well matched unrelated donors were generally preferred as the results were similar to those obtained with HLA identical siblings [25,26]. With event-free survival of 75% haploidentical transplantation in these patients is inferior to the results using HLA identical donors [26]. However, since for many children of ethnic minorities in whom an HLA matched donor could not be found, this approach has become an important treatment option. During the past decades, haploidentical transplantations have been increasingly performed in children with nonmalignant diseases e.g. hemophagocytic lymphohistiocytosis [27-29]. There is lesser experience in thalassemia patients. In Pesaro,

28 children with homozygous  $\beta$ -thalassemia were transplanted with unmodified marrow from a haploidentical family donor, however, mismatched at zero HLA antigens (n=6), one HLA locus (n=15), two loci (n=5), and three loci (n=2) [30]. Major obstacle seems to be graft rejection, occurring in 16 out of 29 patients.

In contrast to heavy pretransplant transfusion protocols, HLA disparity seemed not to have any influence on graft rejection. Transplant related mortality was 34% and GVHD was a major contributing cause of death (50%), followed by infections (30%). Overall survival and thalassemia free survival was reported to be 65% and 21% respectively. More encouraging data in thalassemia patients are coming from the use of very closely matched unrelated donors using matching of extended haplotype [31]. For patients with severe aplastic anemia refractory to immunosuppressive treatment who do not have a matched donor, there are only few reports of haploidentical transplantations [10,26,32-35].

During the last 7 years, there are only a few studies published reporting data exclusively obtained in pediatric patients with malignant diseases. Studies reporting more than 10 pediatric patients are listed in Table 1. As mentioned above, the modern era of haploidentical transplantation started with clinical studies performed in Perugia, Italy, by Martelli and Aversa. These studies included children, realizing the “megadose” concept in a clinical setting. Fifteen children with high risk acute leukemia received stem cells from full haplotype mismatched family donors after a conditioning regimen that included single dose TBI, thiotepa, ATG and fludarabine [36]. The T-cell depletion of marrow cells was carried out by soybean agglutinin and E-rosetting, and of peripheral blood cells by E-rosetting followed by positive selection of the CD 34+ cells. No post transplant GVHD prophylaxis was given. In all patients complete donor type engraftment could be achieved. None of the evaluable patients developed either acute or chronic GVHD. Regimen related toxicity was minimal. Five patients were alive and event free at a median follow-up of 18 months (range: 13-28 months). All surviving patients have good quality of life. Seven patients died of relapse, three of transplant related causes. In a subsequent publication of the same group comprising larger patient series, including children these initial results were confirmed. In their most recent study Aversa *et al.* presented data on 67 patients with AML and 37 patients with ALL. Based on our own experience, published by Handgretinger *et al.* [19] they now used also CD 34+ enrichment using the CliniMACS separation technique. After conditioning with TBI, thiotepa, fludarabine and ATG sustained engraftment could be achieved in 100/101 patients. Acute GVHD occurred in eight out of 101, and chronic GVHD in five out of 70 assessable patients. Eight patients died of non leukemic causes. Relapse occurred in nine out of 66 patients receiving transplantation in remission and in 17 out of 38 patients receiving transplantation in relapse. Event free survival was 48% and 46% for the 42 AML and 24 ALL patients respectively, receiving their transplant in remission.

The CliniMACS technology enabled high purification and transplantation of megadoses of peripheral stem cells with only little contamination of residual T- and B-cells. This was first realized by Handgretinger *et al.* [10]. In this

Table 1.

Ref.	Number of Patients	Diagnosis n	Cell Dose CD 34 (x10 <sup>6</sup> /kg)	Cell Dose CD 3 (x10 <sup>4</sup> /kg)	Source and Graft Manipulation	Engraftment (%)	TRM (%)	GVHD (%)	Outcome and Median Follow-up
Hensley-Downey 1997	72 Adults and children	ALL (28), AML (19), CML/CLL (19) SAA/MDS (6)	BM: MNC= 4,5-6 x 10 <sup>8</sup>	7,5	BM: T-depletion with MoAb T10B9.1A-31	primary: 82 secondary: 90	29	aGVHD > grade II: 16	30% (20/59) at 21.5 months
Aversa 1998	15 Adults and children	ALL (13), AML (2)	12.0	3.0	BM: soybean, E-rosetting PBSC: E-rosetting, CellPro	100	20	None	33% (5/15) AW at 18 months
Aversa 1998	43 Adults and children	ALL (23), AML (20)	14.0 (PBSC) 10.6 (PBSC&BM)	2.7 (PBSC) 3.5 (PBSC&BM)	BM (28): soybean, E-rosetting PBSC (15): E-rosetting, CellPro	primary: 95 secondary: 100	40	None	28% (12/43) AW at 18 months
Handgretinger 2001	39 Children	ALL (16), AML (13), genetic (10)	20.7	1.6	PBSC: CD34 <sup>+</sup> selection (SuperMACS, CliniMACS)	primary: 83 secondary: 98	26	aGVHD: 16	38% (15/39) AW at 24 months
Peters 1999	14 (19 Tx) Children	M (9) genetic diseases (5)	21.5	4.7	PBSC: two-step CD34 <sup>+</sup> selection (Baxter Isolex)	primary: 71 secondary: 100	36	aGVHD > grade II: 7	57% (8/14) AW at 15 months
Sedlacek 2001	10 Children	ALL (4), AML (2), CML (2), NHL (1), MDS (1)	12.8	0.7	PBSC: CD34 <sup>+</sup> selection (Cell Pro 7 CliniMACS 3)	primary: 90 secondary: 100	40	aGVHD: 10 cGVHD: 30	20% (2/10) AW at 26 and 42 months
Ortin 2002	21 Children	M (16) genetic diseases (5)	11.13	7.01	PBSC: CD34 <sup>+</sup> selection (CliniMACS)	primary: 85 secondary: 95	5	aGVHD: 43 cGVHD: 19	76% (16/21) AW at 15 months
Klinge-biel 2004	27 Children	ALL	19.1	1.6	PBSC: CD34 <sup>+</sup> selection (CliniMACS)	primary: 96 secondary: 100	26	aGVHD: 13	37% (10/27) AW at 36 months
Lang 2004	63 Children	ALL (32), AML (13), MDS (4), NHL (4), genetic (10)	19.5	< 2.5	PBSC: CD34 <sup>+</sup> /CD133 <sup>+</sup> selection (SuperMACS, CliniMACS)	primary: 83 secondary: 98	29	aGVHD: 7 (grade II) cGVHD: 13	41% (26/63) AW at 4.1 years
Marks 2006	34 Children	AML (17), ALL (14), MDS (1), CML (1), biphenotypic AL (1)	13.8	0.7	PBSC: CD34 <sup>+</sup> selection (CliniMACS)	primary: 91 secondary: 94	35	aGVHD: 29 cGVHD: 12	24% (8/34) AW at 5.2 years

AW, alive and well; GVHD, graft versus host disease; M, malignancy; PBSC; peripheral blood stem cells; TRM, transplant related mortality; Tx, transplantation

pilot study using an average number of 14.2 x 10<sup>6</sup> CD 34 cells/kg, primary engraftment could be achieved in 80% of the 23 included children. Non-engraftment and rejection occurred in three and two patients, respectively. In four out of five patients a second transplant using purified CD34+ cells from the same donor after an immunological reconditioning resulted in a complete and sustained hematopoietic reconstitution [37]. GVHD was observed in only one patient after add-back of T-cells which was performed in some patients based on the recurrence of autologous cells. The speed of immunological recovery was dependent on the number of

transplanted CD 34+ cells and more rapid if this number was >20 x 10<sup>6</sup>/kg. The main cause of death was relapse.

At the same time Peters *et al.* from Vienna, Austria, reported the outcome of 19 transplantations from HLA two- or three antigen mismatched parental donors in 14 pediatric patients with malignancies. These patients received a median of 21.5 x 10<sup>6</sup> CD 34 cells/kg and only 4.7 x 10<sup>4</sup> CD 3 cells/kg. T cell depletion was performed using the Baxter Isolex facility (Baxter, Deerfield, IL, USA). Ten out of 14 patients presented with rapid myeloid engraftment. Four

patients rejected and were regrafted. In these patients the authors could also show that mixed chimerism by the recurrence or persistence of autologous T-cells put the patients at higher risk for graft rejection. Eight out of 14 patients were reported to survive after a median observation period of 15.6 months with complete donor chimerism in all hematological cell subsets. No acute organ GVHD and no chronic GVHD has occurred. Only one patient experienced relapse of leukemia [38]. Subsequently, the Prague group published their results in ten children with malignant disease (ALL n=4; AML n=2; CML n=2; NHL n=1; MDS n=1) who received their haploidentical grafts from either a parental (n=9) or from a sibling (n=1) donor. Primary engraftment was achieved in nine patients and one patient could be successfully regrafted. Primary acute GVHD was observed in one patient and induced by add back of T-cells in four patients. Three of these patients developed cGVHD. Four patients died due to veno-occlusive disease (n=1) or infections (n=3). Four patients relapsed and died. Two patients were reported to be alive 26 and 42 months post transplant at the time of publication [39]. 21 children were reported from the Birmingham Children's Hospital, UK, by Ortin *et al.* in 2002 [40]. These children received haploidentical transplantations for malignant (n=16) or non malignant (n=5) diseases after conditioning with TBI (14.4 Gy in eight fractions), cyclophosphamide (60 mg/kg/d for two consecutive days), fludarabine (25 mg/m<sup>2</sup>/d for 5 days) and anti lymphocyte globulin (ALG) 12.5 mg/kg/d from day -2 to +2. All patients received PBSCs after CD 34+ selection with CliniMACS (Miltenyi Biotech, Bergisch Gladbach, Germany). The mean CD 34+ cell dose was 11 x 10<sup>6</sup>/kg, with a mean of CD3+ T-cell dose of 7 x 10<sup>4</sup>/kg. Due to this relatively high T-cell dose all patients received CSA. Among 16 patients with malignant diseases, all engrafted, no TRM, occurred three patients have relapsed and died, and 13 out of 16 (81.3%) remained alive in complete remission after a median follow-up of 480 +/- 255 days. This study is remarkable for the high rate of engraftment and the lack of toxic deaths compared to the previous studies, reported by Handgretinger and Peters. In their studies the rate of graft rejection was 20% and 29% respectively, and the rate of TRM mainly related to infections was 13% and 36% respectively.

Successful engraftment and immune reconstitution after haploidentical transplantation is determined by different factors as mentioned above. Stem cell dose, T-cell content and intensity of the conditioning regimen were considered to be the most important factors; however, also pretransplant immunosuppression and the quality of remission were known to influence engraftment. While the median cell dose was lowest in the study of Ortin, T-cell numbers in the Birmingham study was higher. On behalf of the Tuebingen group Lang has reported the results of 63 children with malignant diseases. Sustained engraftment was achieved in 98% and acute and chronic graft versus host disease was only seen in 7% and 15% respectively after T cell administration based on increasing mixed chimerism. Peripheral stem cells were either selected using a CD 34<sup>+</sup> or a CD 133<sup>+</sup> enrichment technique with the CliniMACS system and a median number of 20 x 10<sup>6</sup>/kg were transplanted. Event free survival in children with acute lymphoblastic leukemia or non Hodg-

kin Lymphoma was 48% and in children with acute myelogenous leukemia 18%. Overall, 18% experienced lethal viral infections but newer strategies reduced this incidence to 8% [41]. In the most recent study the Royal Hospital For Sick Children in Bristol, UK reported their experience in the treatment of 34 high risk children with acute leukemia. Nine children with AML were not in remission at the time of transplant. Twenty-two patients with AML or ALL were in remission at the time of transplant and eight survived (36%). None of the children not in remission did survive. Ten out of 34 patients died of infection, with viral infection being a particular problem. Adenovirus infection was the most prominent cause of death in six patients [56].

These studies showed that haploidentical stem cell transplantation has become a valuable option for children in urgent need for transplantation but lacking an HLA identical donor. Overall survival rates for patients with acute leukemia with highest risk factors but being transplanted in remission were between 30% and 76%. This is equivalent to other treatment modalities e.g cord blood transplants. GVHD did not play a major role due to profound T-cell depletion. Although substantial T-cell depletion was performed transplantation of megadoses of stem cells allowed reliable final engraftment. Primary engraftment, immune reconstitution and anti leukemia activity remained to be improved.

## PERSPECTIVES

### Graft Versus Host Disease

As intractable GVHD was the limiting factor to perform haploidentical transplantations for long years, modern techniques have enabled graft processing with profound T cell depletion. Using these techniques, T cell depletion in the range of 4-5 logs, achieving a threshold of 2.5 x 10<sup>4</sup>/kg CD 3 positive cells has become possible, below which GVHD prophylaxis is not required [19]. Using these T cell depleted grafts GVHD has been largely overcome with very low GVHD rates in the largest studies [19,20,36,40,41].

### Engraftment and Graft Rejection

Mobilized peripheral stem cell grafts comprise a variety of different cell subpopulations as hematopoietic stem cells, granulocytes, monocytes, dendritic cells, NK-cells, regulatory T-cells and others. More recent data gave evidence that pluripotent CD 34 negative stem cells also occur in the peripheral blood after mobilization with cytokines [42]. These cells might contribute to facilitate engraftment, speed up immune reconstitution and support antileukemic effect of the graft. Using CD 34+ selection technique for graft manipulation has the consequence of discarding these cells. Especially the co-transplantation of haploidentical NK-cells did importantly contribute to rapid and sustained engraftment [43,44]. Ruggeri could show that mice, conditioned with nonlethal (<7 gy) TBI alone, rejected donor marrow grafts. Whereas mice conditioned with nonlethal irradiation and alloreactive NK-cells engrafted with durable donor type hematopoietic chimerism.

First Gordon and Handgretinger could show in large scale experiments, that profound T-cell depletion (>5 log) could be successfully performed using CD3 conjugated

magnetic microparticles using the MACS technology (Miltenyi Biotech, Bergisch Gladbach, Germany) [45]. This technique has been further improved, and meanwhile there are commercially available CD3/CD19 depletion kits available resulting in excellent CD 34+ yield and in a profound depletion of T- and B-cells. Using this enrichment technique the graft composition has substantially changed, and is comprised out of different cell subpopulations containing in particular large amounts of NK- but also of progenitor T-cells. Lang *et al.* could show in a pilot study in 11 children with malignant disease (ALL n=4; AML n=7) that primary engraftment rate was 91% compared to 85% in historical controls, although most of these patients received a reduced intensity conditioning regimen consisting of fludarabine 200 mg/m<sup>2</sup>, melphalan 140 mg/m<sup>2</sup>, thiopeta 10 mg/kg and OKT3 [46]. These results could be confirmed by our pilot trial in Frankfurt where we included 20 children and adolescents with malignant diseases. Using a similar reduced intensity conditioning regimen as described by Lang *et al.* with a shorter OKT3 transfusion duration of only 10 days, primary and sustained engraftment could be achieved in 20/20 transplanted patients [47]. Not only the engraftment rate but also immune reconstitution (discussed below in more detail) seemed to be improved by the latter graft processing technique.

In the haploidentical setting, the mismatched haplotype may originate from either of the parents, accordingly referred to as non-inherited paternal antigens (NIPA haplotype) or non-inherited maternal antigens (NIMA haplotype). Exposure to fetal cells during pregnancy might tolerance the mother's T-cells as well as maternal antigens might induce tolerance in the fetus [48]. Retrospective analysis showed, that NIMA mismatched transplants had similar rates of graft failure but lower rates of acute GVHD than NIPA transplantations [48]. Based on these findings several groups have recently reported successful cases of non T-cell-depleted hematopoietic stem cell transplantation from NIMA donors. Ichniohe *et al.* reported on 35 patients with advanced hematological malignancies who underwent HLA 2-antigen- or 3-antigen incompatible SCT from a microchimeric NIMA-mismatched donor. All patients attained successful engraftment with complete chimerism. Acute GVHD grade II/IV occurred in 19/34 patients (57%). Multivariate analysis demonstrated that NIMA mismatch in the GVH direction was associated with a lower risk of severe GVHD compared to NIPA transplants [49].

In the post transplant phase, regular characterization of hematopoietic chimerism might be helpful in maintaining engraftment [50,51]. Special interest should be focused on the analysis of different subpopulations. It could be shown that persisting or reappearance of autologous cells was connected with a high risk for graft rejection [26,38]. Low dose DLI is in principle feasible to prevent graft rejection in a cohort of these patients [26,52,53].

### Graft Versus Leukemia Effect

Graft versus leukemia effects were considered to be T-cell dependent in conventional bone marrow allografts from HLA identical donors, and patients receiving extensively T-cell depleted grafts were more likely to develop mixed chi-

merism and were therefore at higher risk of relapse [51]. In haploidentical transplantations the Perugia group reported especially for adult patients with AML a relatively low incidence of GVHD and a low relapse rate [22,36]. The work of Ruggeri *et al.* [43,54] suggested donor NK alloreactivity to play an important role in the haploidentical graft versus leukemia effect. Natural killer function is regulated by a family of membrane receptors specific for HLA class I alleles. NK cells are constantly activated to kill autologous targets through the engagement of several activating receptors. They are prevented from doing so by the coexpression of inhibitory receptors which recognize self-major histocompatibility complex class I antigens and which, upon engagement with their ligands, induce inhibitory signals to block activation of NK cell lysis. For review see Farag *et al.* [55]. After haploidentical transplantation a wave of regenerating donor derived NK-cells is emerging around day 21 to 30 post transplant. These NK-cell clones have demonstrated to have a potent activity against myeloid leukemia cells if donor and recipient were mismatch in graft versus host direction [54]. In adult patients transplanted from a haploidentical donor NK alloreactivity showed an impressive effect on subsequent relapse. None of 20 patients transplanted from a KIR mismatched donor developed subsequent relapse compared to 28 out of 37 patients who received their graft from a donor with no potential NK mismatch [43]. In the very recent report from the Bristol group by Marks *et al.* [56] an effect of potential NK cell alloreactivity could not be confirmed, although the numbers are small. Larger prospective studies in children are urgently needed to substantiate the important role of NK-cell alloreactivity. If these data could be confirmed, KIR mismatching between donor and recipient should become a major criteria for donor selection in haploidentical transplantation at least in myeloid malignancies.

Attempts have also been undertaken to use leukemia antigen specific T-cell therapies also in the setting of haploidentical stem cell transplantation. Different targets and strategies are currently under intense investigation and clinical trials in some of them have been started. Categories of antigens that might be targets of a GVL response can be grouped into leukemia specific antigens and normal proteins that are aberrantly or over expressed in the tumor cells and hematopoiesis [57]. Such targets are in particular the classical BCR-ABL fusion protein which can be recognized by specific CD4 T helper cells [58] and by CD 8 cytotoxic T-cells *in vitro* [59]. Other antigens are WT-1, a zinc finger transcription factor normally expressed in very few tissues, but highly expressed in many leukemias [60-63]. Also HLA minor antigens as HA 1 can serve as targets against antigen specific T-cells and have already been generated [64]. A very interesting approach was published recently by Amrolia *et al.* who performed a selective depletion of allo-reactive donor T-cells with preservation of CTL responses to myeloid tumor antigens [65]. In addition, many other concepts are studied but all these technologies are very labor intense and time consuming so that these therapies are not yet available for a broader cohort of patients.

### Immune Reconstitution

Immunological recovery after haploidentical stem cell transplantation has remained as the major obstacle to be

overcome, since the problems of engraftment and GVHD could largely be improved within the last years. Speed and quality of immune reconstitution is influenced by different mechanisms: (i) number of stem cells transfused, [10,19] (ii) profound T-cell depletion *ex-vivo* and ATG in the conditioning regimen [18] which may antagonize the expansion of residual T-cells, (iii) the degree of HLA disparity and (iv) decaying thymic function in adults [66-68]. The time to functional immune recovery is the most critical period in the post haplotransplant phase and patients are at highest risk to acquire viral, fungal and opportunistic infections [2]. It is well known, that it may take anywhere between 6 to 18 months to restore normal T- and B- cell numbers and to reconstitute efficient responsiveness of the immune system after CD 34+ selected transplantation [22,69].

The important observation that the use of post transplant G-CSF to speed up neutrophil recovery decreased IL-12 production by the antigen presenting cell led to discontinuation of its routine use [70]. Cessation of post transplant G-CSF did not negatively alter engraftment but resulted in the anticipated appearance of IL-12 producing dendritic cells 1-3 months post transplant. Moreover, CD4-positive cell counts increased significantly faster. Attention is focused in the adoptive immunotherapy using either allodepleted donor T-cells or disease specific non alloreactive T-cells. A very elegant approach was recently published by Amrolia *et al.* [71]. The group has used an anti-CD 25 immunotoxin approach to deplete alloreactive lymphocytes and have compared immune reconstitution after allodepleted donor T-cells when infused at two dose levels into recipients of haploidentical SCT. Eight patients received  $10^4$  cells /kg/dose and eight patients received  $10^5$  cells/kg/dose. Patients receiving the higher dose showed significantly improved T-cell recovery at three, four and five months post SCT compared to patients who received the lower dose. This approach did preserve *in vitro* T-cell response to CMV, EBV and adenovirus [65].

In recent years considerable progress has been made in generating non-alloreactive T-cells specific against different pathogens as EBV, CMV, adenovirus and aspergillus species. This approach has been successfully used in the prevention of CMV and EBV infection in the context of HLA identical transplants [72-75]. Feuchtinger *et al.* have used this approach also for the generation of adenovirus specific T-cells from adenovirus seropositive donors [76,77]. Virus-specific donor T-cells were isolated and infused into nine children with systemic adenovirus infection after SCT. Isolation was based on gamma-interferon (IFN-gamma) secretion after short *in vitro* stimulation with viral antigen, resulting in a combination of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells.  $1.2-50 \times 10^3$ /kg T-cells were infused for adoptive transfer. Isolated cells showed high specificity and markedly reduced alloreactivity *in vitro*. Adoptive transfer of adenovirus-specific immunity was successful in five of six evaluable patients, documented by a dose-independent and sustained *in vivo* expansion of adenospecific T-cells, associated with a durable clearance/decrease of viral copies. T-cell infusion was well tolerated in all nine patients, except one case with graft-versus-host disease grade II of the skin. This approach seems to be extremely important as adenovirus plays a utmost critical role in the haploidentical setting as could be seen most re-

cently in the study published by the Bristol group, reporting adenovirus as the cause of death in 6/10 children who died due to infection [56].

Since it is clear that T-lymphocytes provide a critical secondary defense against fungi, adoptive transfer of functionally active anti-aspergillus T-cells might be an option to restore adaptive immune effector mechanisms. Using the interferon (IFN)-gamma secretion assay, Beck *et al.* isolated human activated T-cells upon stimulation with a cellular extract of aspergillus fumigatus. Culturing this cell population for 14 days, they obtained an average of  $1.1 \times 10^7$  cells in seven out of seven healthy individuals. Within another 14 days, these cells were expanded to an average number of  $2.0 \times 10^8$  T-helper 1 (T(H)1) cells secreting IFN-gamma on stimulation with aspergillus antigens. Testing various fungal antigen extracts, similar proportions of IFN-gamma-producing CD3+/CD4+ cells were obtained upon activation with antigen extracts of *A. fumigatus*, *A. flavus*, *A. niger*, and *Penicillium chrysogenum*. CD4+ T-cell-mediated alloreactivity of generated anti- aspergillus T-cells was clearly reduced compared with that of the original cell population [78]. Although far away from being a routine procedure, the development of such adoptive therapy is clearly another promising clinical path to reduce the post transplant toxicity from infection.

## CONCLUSIONS

Successful haploidentical stem cell transplantation has a 20 years history. Early results in patients with acute leukemias were disappointing because of high incidence of severe GVHD in T-cells containing transplants and high rejection rates in T-cell depleted transplants. The breakthrough was achieved by the transplantation of megadoses of peripheral stem cells with only little T-cell contamination after conditioning with high intensity conditioning regimen. This approach was further improved by realizing the important role of NK-cells for engraftment and for the anti-tumor efficiency in adult patients with AML. Modification in graft processing, e.g. CD3/CD 19 depletion of peripheral stem cell grafts allows co- transplantation of immature T-progenitors together with other "facilitating" cells, thus favoring engraftment and immune regeneration. These factors have markedly contributed to reduce transplant related mortality. Today children with acute leukemias are transplanted with haploidentical grafts in less advanced stages of disease as 15 years before. Consequently, results of haploidentical transplantation have significantly improved, especially in children with ALL (Klingebl, manuscript in preparation). It is now no longer a treatment option for desperate straits, but has a clear and defined role in the different treatment protocols [20,56].

Children with ALL who are candidates for an allogeneic transplantation but who are lacking HLA identical donors may benefit from haploidentical transplantations. In contrast to adult patients children with myeloid leukemias who do not respond to initial chemotherapy will most likely also not be cured by haploidentical transplantation. The same seems to be true for children with ALL in relapse at the time of transplantation. Haploidentical transplantation is an alternative treatment option of children with nonmalignant diseases when lacking an HLA-identical donor.

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