Haploidentical hematopoietic transplantation: current status and future perspectives

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Abstract

For patients with hematological malignancies at high risk of relapse who do not have matched donors, a suitable alternative stem cell source is the HLA-haploidentical two or three-loci mismatched family donor who is readily available for nearly all patients. Transplantation across the major HLA barrier is associated with strong T cell alloreactions which were originally manifested as a high incidence of severe GvHD and graft rejection. The present review shows how these obstacles to successful transplantation were overcome in the last 15 years, making full haplotype mismatched transplantation a clinical reality that provides similar outcomes to transplantation from matched unrelated donors. The review also discusses the advantages and drawbacks of current options for full haplotype mismatched transplantation and highlights innovative approaches for re-building immunity post-transplant and improving survival.
Introduction

Despite advances in chemotherapy, allogeneic haematopoietic stem cell transplantation (HSCT) remains the best post-remission therapy for patients with acute leukaemia and unfavourable prognostic features at diagnosis, primary induction failure or in second and third complete remission. Even though HLA-identical siblings are the ideal source of haematopoietic stem cells (HSC) only 25% of patients have such donors. Therefore, an alternative HSC source is needed for majority of patients. Alternative options include matched unrelated donors (MUD), unrelated donor umbilical cord blood (UD-UCB) and full haplotype mismatched related donor.

Each alternative source has its own particular advantages and drawbacks. Suitable MUD are found for 60-80% of Caucasians but only for 10% of ethnic minorities\(^1\). Using molecular probes improves tissue typing and lessens the risk of graft-versus-host disease (GvHD) by very accurately matching unrelated donor-recipient pairs, but reduces the probability of finding a suitable donor. Furthermore, as months can pass while identifying the donor and harvesting the HSC, many acute leukemia patients relapse while awaiting transplantation.

UD-UCB offers the advantages of a shorter search time than MUD and acceptance of two of six antigens mismatch. In adults, however, the great discrepancy between body weight and the number of HSC in a standard cord blood unit, particularly if a two antigen mismatch is involved, delays hematopoietic reconstitution and increases the risk of graft failure. This drawback was partially overcome by using double cord blood units (dUCB)\(^2\).
The advantages of transplantation from a full haplotype mismatched family member include: availability for almost all patients, choice of best donor from a panel of candidate family members, no undue delay in obtaining the graft and finally, easy access to donor-derived cellular therapies if required after transplantation.

Major drawbacks are the very strong graft versus host and host versus graft alloresponses due to the high frequency of T cells that recognize major Class I or II HLA disparities between donor and recipient. Consequently, early attempts to use full haplotype mismatched donors in leukemia patients were not successful. Although ex-vivo T cell depletion of mismatched bone marrow with soybean agglutination and E-rosetting to reach the target of 2-4x10⁴ T cells/Kg recipient body weight, prevented GvHD in children with severe combined immunodeficiency, 30% of HLA-haploidentical grafts were rejected by leukemia patients due to anti-donor cytotoxic T lymphocyte precursors (CTLp) that survived supra-lethal conditioning.

From the 1980’s onwards attempts to overcome the HLA barrier focussed on strengthening myeloablation and immunosuppression in the conditioning regimen or on partially depleting the graft of T cells, by using ATG or the T10B9 monoclonal antibody in conjunction with in-vivo immunotoxin, and with post-transplant cyclosporine and steroids. A major advance came with a graft containing a mega-dose (on average >10x10⁶ CD34⁺ cells/kg body weight) of extensively T cell depleted granulocyte colony-stimulating factor (G-CSF) mobilized, peripheral blood haematopoietic progenitor cells, following a myeloablative conditioning regimen based on total body irradiation (TBI). This strategy ensured for the first time a high engraftment rate across the HLA barrier in the absence of GvHD.
In recent years, interest in T-cell replete full haplotype mismatched HSCT was reawakened by new transplant strategies for GvHD prophylaxis such as G-CSF primed grafts, post-transplant rapamycin or high-dose cyclophosphamide in combination with other immunosuppressive agents.

Each of these two current options for full haplotype mismatched HSCT presents an intrinsic challenge. In T cell depleted HSCT, the minimal residual T lymphocytes in the grafts successfully prevent lethal GvHD without any post-transplant immunosuppression, but at the same time the small number of T cells infused leads to delayed immune reconstitution. Thus, the present challenge is to accelerate immune reconstitution by adoptive transfer of non-alloreactive pathogen-specific or broad repertoire T lymphocytes, depleted of alloreactive clones.

T cell replete HSCT presents two major challenges. While the high T cell content of the graft potentially enhances the graft versus leukemia (GvL) effect, the same T cells induce significant GvHD-related morbidity and mortality. In other strategies for T cell replete HSCT, such as administering of high-dose cyclophosphamide post-transplant to prevent GvHD (performed mostly following reduced intensity protocols), a high incidence of leukaemia relapse has emerged as a major problem. Therefore, efforts are being made to reduce the incidence and severity of GvHD and to prevent leukaemia relapse.

This review outlines and evaluates these opposing modalities and discusses future directions in full-haplotype mismatched HSCT.

**T cell replete full-haplotype mismatched HSCT**

**G-CSF-primed graft**

According to the Huang group in Beijing, G-CSF-primed donor HSCs and robust
post-transplant GvHD prophylaxis reduced the risk of transplant-related mortality (TRM) and improved long-term survival\textsuperscript{10}. A follow-up study of 250 mismatched transplants in acute leukemia patients (89 at high-risk) reported nearly 100% full-donor engraftment, with 45.8% and 13.4% cumulative incidence of grade II-IV and III–IV acute GvHD (aGvHD), respectively. The cumulative incidence of chronic GvHD (cGvHD) was 53.9% (extensive in 22.6%). The 3-year probability of disease free survival (DFS) were 70.7% and 55.9% in standard and high-risk acute myeloid leukemia (AML) patients, and 59.7% and 24.8%, respectively in acute lymphoblastic leukemia (ALL)\textsuperscript{11}. Interestingly, survival in patients with acute leukemia was higher in haploidentical recipients than in matched sibling recipients (42% vs 20%, $P = .048$), presumably due to a stronger GvL effect\textsuperscript{12}.

The drop in the incidence and severity of GvHD deserves further investigation in Caucasian populations\textsuperscript{13}. Likewise, the mechanism of GvHD modulation, suggested by the authors to be mediated by G-CSF priming of T cells in the bone marrow is still obscure. In fact, ATG in the conditioning and the powerful post-transplant GvHD prophylaxis likely made the major contribution to controlling alloreactivity.

*Rapamycin-based GvHD prophylaxis:*

Since in-vitro evidence showed that rapamycin did not affect T regulatory (Treg) cells, Peccatori et al\textsuperscript{14} developed a protocol with rapamycin and mycophenolate mofetil (MMF) for GvHD prophylaxis following conditioning with treosulfan, fludarabine, ATG and a single dose of rituximab to treat 39 AML and 9 ALL advanced-stage patients (median age: 50 years). The cumulative incidence of aGvHD was 29% for grade II-IV and 13% for grade III-IV. 12/59 patients developed cGvHD. TRM and relapse were 25% and 44%, respectively, and projected 1 year overall
survival was 43%. Early immune reconstitution polarized towards central memory cells, with significantly higher peripheral blood Treg (CD4+CD25+CD127-Foxp3+) counts than in donors. Since rapamycin has never before been used in the clinical setting of full haplotype mismatched transplantation, this approach deserves particular attention as it explores the rapamycin effects on Treg induction and GvHD prevention. However, the short follow-up precludes any definitive conclusions.

**High-dose cyclophosphamide-based GvHD prophylaxis:**

In the 1970’s George Santos demonstrated in rodents that a short course of high-dose cyclophosphamide soon after BMT targeted activated donor or host alloreactive T cells\(^\text{15}\). This approach was abandoned when a randomized clinical trial demonstrated that a lower dose of cyclophosphamide was less efficacious than cyclosporine as prophylaxis against aGVHD after HLA-matched sibling alloBMT\(^\text{16}\). However, the observation that cyclophosphamide is non-toxic to hematopoietic stem cells because of their high expression of the detoxifying enzyme aldehyde dehydrogenase\(^\text{17}\), and the demonstration of Slavin group that, high dose cyclophosphamide administration can reduce GvHD and graft rejection in mice, without adverse effects on stem cell engraftment\(^\text{18}\), led to renewed clinical attempts using this approach.

Clinical trials by the John Hopkins and Fred Hutchinson Cancer Research Center groups, evaluated a non-myeloablative protocol of cyclophosphamide, fludarabine and 2Gy TBI, and post-transplant GvHD prophylaxis with cyclophosphamide (50mg/kg days +3, +4), MMF (days +5 to +35) and tacrolimus (days +5 to +180)\(^\text{19}\). Engraftment was sustained in 87% of 210 acute leukaemia patients treated by the Johns Hopkins group. Grade II-IV aGvHD occurred in 27% of patients, grade III-IV
in 5% and cGvHD in 15%. The cumulative incidences of relapse and non-relapse mortality were 55% and 18%, respectively. 113 patients died of relapse (79), infections (15), pulmonary complications (7), GvHD (5) or other causes (7). Three-year overall survival and event free survival (EFS) were 41% and 32% respectively\(^{20}\) (Fig.1).

Thus, the high relapse rate, which was probably due to poor disease debulking by the non-myeloablative conditioning and to lack of GvHD related GvL effect, dampened the advantage of a relatively low TRM\(^ {20}\)

Taken together, T cell replete full haplotype mismatched HSCT has developed in recent years and became a leading alternative option in several centers. However, higher rates and severity of aGvHD and cGvHD can significantly impair the quality of life of disease survivors, and could lead to higher relapse rates when applied following RIC or when using aggressive immune suppression.

**T cell depleted full-haplotype mismatched HSCT**

*Megadose HSCT after myeloablative TBI-based conditioning*

After myeloablative conditioning regimen, a high dose \((\geq 10 \times 10^6/\text{Kg})\) of purified CD34\(^+\) cells promotes engraftment in the majority of acute leukaemia patients. The first clinical trial was based on preclinical studies which showed that full-donor engraftment without GvHD could be achieved by escalating doses of T cell depleted bone marrow (BM) in a stringent mouse model for T cell mediated BM allograft rejection\(^ {21}\) or in sub-lethally irradiated mice\(^ {22}\). High-risk patients, most with acute leukaemia, received BM and G-CSF mobilized peripheral blood progenitor cells, which were depleted of T cells by soybean agglutination and E-rosetting. The
conditioning included TBI (8Gy in single fraction), thiotepa (10mg/kg), cyclophosphamide (100mg/kg) and rabbit ATG (25mg/kg). A high engraftment rate (16/17) was achieved, with 18% incidence of aGvHD even though no post-transplant immunosuppression was administered. In subsequent reports several modifications were made: fludarabine replaced cyclophosphamide in the conditioning protocol to reduce extra-haematological toxicity; graft processing was improved by positive immunoselection of CD34+ cells and post-transplant G-CSF administration was stopped after it was observed to impair IL-12 production by dendritic cells (DCs), leading to abnormal antigen presentation and T-cell activation. These strategies ensured primary sustained full donor type engraftment in 95% of 255 acute leukemia patients with an extremely low incidence of acute and chronic GvHD. The relapse rate was 18% in AML and 30% in ALL patients who were transplanted in any CR. The cumulative-incidence of TRM was 0.36 (95% C.I. 0.29-0.53) for 145 patients in any CR at transplant, rising to 0.58 (95% C.I. 0.40-0.65) for 110 patients transplanted in relapse. With a maximum follow-up of 17 years, LFS was respectively 43% and 30% in AML and ALL patients in any CR (Fig.2A). Although patients who were transplanted in chemoresistant relapse had poor outcomes, an 18% LFS for advanced AML patients is worth noting. All long-term survivors enjoy an excellent quality of life with no cGvHD. An EBMT retrospective study, analyzing the outcome of ‘mega-dose’ HLA-haploidentical transplantation in several European centres, reported similar results with 48% EFS in AML patients in first complete remission (Fig.2B). These results are comparable to reported outcome in a large observational study, based on data from the statistical center of the CIBMTR, summarizing the results of 584 patients in 151
centers, undergoing allogeneic transplantation for AML in first remission with unfavourable cytogenetics at diagnosis (Fig.2C).

These results highlight several aspects which deserve closer attention:

1) High numbers of CD34+ cells can overcome the HLA barrier (see below).

2) A threshold of $2 \times 10^4$ CD3+ T cells/Kg almost completely prevents GvHD, even in the absence of any post-transplant immunosuppression. It could be argued however, that ATG in the conditioning protocol, with its prolonged plasma half-life, induced additional in-vivo T cell depletion of the graft, contributing to reduce GvHD incidence and severity.

3) A low post transplant leukaemia relapse rate despite the lack of GvHD-related GvL effect. In our view several factors contributed to these crucial data, including the myeloablative strength of the conditioning, donor-vs-recipient natural killer (NK) cell alloreactivity (see below) and absence of post-transplant pharmacological immunosuppression.

4) A relatively high TRM rate due to slow post-transplant immune reconstitution (see below), patients’ clinical status and co-morbidities, and disease stage at time of transplant. Although most deaths were due to opportunistic infections (CMV, invasive aspergillosis), the risk of life-threatening episodes slowly declined and reached a plateau after approximately one year, confirming that complete immunological reconstitution is achieved by this stage, in transplant recipients who do not receive immunosuppression and do not have any chronic GvHD.

Encouraging results were also demonstrated in pediatric patients. A mean dose of $20.7 \times 10^6$/Kg purified CD34+, after myeloablative conditioning which included OKT3,
was administered to 39 children at the Tubingen University Hospital. Rapid primary engraftment was achieved in 36 with an extremely low incidence of GvHD. Relapse accounted for 13 deaths and TRM for 10. At a median follow-up of 2 years 15/39 patients were alive and well. In a later study from the same group, the DFS of 28 patients with ALL/NHL in remission, who received positively selected haploidentical grafts, was comparable to that of a similar historical control group receiving unmanipulated MUD transplant (48% vs. 38%; P = 0.6). Moreover, no significant difference was observed between the two groups in the 3-year probability of relapse (Fig.2D).

Similar results were also reported by the Locatelli group in Pavia. In contrast, a European multicenter analysis of 127 children with high-risk ALL, reported a 5-year DFS of 27% for patients in complete remission. However, multivariate analysis detected a trend toward a centre-related effect with better DFS in larger centers (39% vs 15%). An improved outcome, with less relapse was also described for patient's receiving a higher CD34+ cell dose, suggesting that a maximal CD34 dose should be infused whenever possible.

Thus, a megadose of T cell depleted full haplotype mismatched HSCT successfully prevents grafts rejection as well as GvHD, and results in survival rates similar to MUD transplants. Relatively high non-relapse mortality rates were mostly due to opportunistic infections. In the absence of GvHD and post-transplant immunosuppression, there is a window for adoptive T cell immunotherapy to improve immune reconstitution and outcomes.
How ‘mega dose’ transplants overcome the HLA barrier

Veto activity was defined in 1980 by Miller as the capacity to specifically suppress cytotoxic T cell precursors directed against antigens presented by the veto cells. Over the years different veto cell types, exerting their activity through distinct mechanisms, have been described. Thus, the term ‘veto’ represents an operational definition and not a specific cell subpopulation. Following the discovery that mega dose transplants can overcome T cell mediated rejection, Rachamim et al demonstrated in MLR that cells within the CD34+ fraction specifically suppressed CTLp directed against their own antigens, but not against 3rd-party antigens. This veto activity was shown to be mediated by TNF-α induced deletion of recognizing effector T cells, and was also exhibited by ex-vivo differentiated immature CD34+CD33+ and CD34-CD33+ myeloid cells. Likewise, immature dendritic cells previously shown to induce immune tolerance, exhibited marked veto activity on CD8+ T cells through a perforin based mechanism, while suppressing CD4+ T cells through an MHC independent mechanism mediated by the NO system. Thus, after transplantation of purified CD34+ cells, the likelihood of activation of anti-donor CTLp is proportional to the level of residual host T cells and is inversely correlated to the number of veto and other tolerizing cells. Veto activity could initially be exerted by infused CD34+ cells and their CD33+ progeny, as well as by CD11c+ immature dendritic cells. In addition, transplant from a HLA genotypes which allow the rapid generation of alloreactive NK cells (see below), can eradicate mature anti-donor CTLs escaping the veto cells. Thus, the 'mega-dose' haploidentical CD34 inoculum is responsible to veto anti-donor CTLp, and also to rapidly generate the second or third derivatives required to complete the eradication of host anti-donor T cells.
Selecting “good prognosis” donors: NK cell alloreactivity and the “mother donor effect”

Seminal work by Ruggeri et al. demonstrated that in the setting of haploidentical hematopoietic transplantation, NK cell alloreactivity is a powerful form of anti-leukemia immunotherapy 40.

Human NK cells possess clonally distributed, inhibitory receptors termed “Killer cell Immunoglobulin-like Receptors” (KIRs) that recognize allotypic determinants (“KIR ligands”) shared by certain HLA-class I allele groups (HLA-C “Group 2” alleles; HLA-C “Group 1” alleles; HLA-B alleles sharing the Bw4 specificity). Upon interaction with self HLA KIR ligands, NK cells become “licensed/educated” to exert alloreactivity against allogeneic targets which do not express self HLA KIR ligands. Thus, in full haplotype mismatched HSCT, donor-versus-recipient NK cell alloreactivity is effected by “licensed” NK cells of donor origin, which express as their only inhibitory receptor for self, a KIR whose ligand is absent on allogeneic targets. They, therefore, sense missing expression of the donor class-I KIR ligand on target cells and mediate alloreactivity (“missing self” recognition). Indeed, the engrafted stem cells give rise to an NK cell repertoire of donor origin which includes alloreactive clones that kill recipient leukemic cells. Evidently, donor NK cells mature in a bone marrow micro-environment where they are predominantly exposed to donor HLA (on hematopoietic cells) which shapes their repertoire to become fully functional and recipient-alloreactive.

Three KIR ligand-mismatches (in the graft-versus-host direction) trigger donor-versus-recipient NK cell alloreactivity: 1) HLA-C1 present in donor/missing in recipient; 2) HLA-C2 present in donor/missing in recipient; 3) HLA-Bw4 present in donor/missing in recipient (Figure 3).
Donor-versus-recipient natural killer (NK) cell alloreactivity improves outcomes of HLA haploidentical transplants by controlling leukemia relapse without causing GvHD. An updated analysis of 112 high-risk AML patients confirmed the presence of NK alloreactivity, reflected by significantly lower relapse rates and better event-free survival. Thus, in patients transplanted in complete remission, the cumulative incidence of relapse was significantly lower after transplantation from NK alloreactive donors (3% vs 47%; P < 0.003) and the probability of event-free survival was 18% vs 67% (P=0.02; Fig.4A). A high 34% event free survival rate was also observed in patients transplanted in relapse (Fig. 4B)41. Hematopoietic stem cell transplantation from haploidentical, NK alloreactive donors was also reported to lower the risk of relapse in children with ALL 33, 42, 43.

In addition to NK cell alloreactivity, maternal donors (as opposed to any other donor-recipient family relationship) provided much better protection from leukemia relapse. The effect was independent of, and additive to, the beneficial effects of NK alloreactivity (Fig. 4C)44. Better outcome of mother-to-child transplantation may be due to maternal immune system exposure to fetal antigens during pregnancy and ensuing memory T cell immunity against the child’s paternal HLA haplotype.

**T cell depleted full-haplotype mismatched HSCT after Reduced Intensity Conditioning**

Since high intensity conditioning regimens are not feasible for elderly, heavily pre-treated patients or those with significant co-morbidities, reduced intensity conditioning (RIC) regimens were investigated with diverse methods for achieving T cell depletion. **CD3/CD19 depletion**

In Tubingen and Memphis adults and children with acute leukemia were conditioned
with fludarabine (150-200mg/m²), thiotepa (10mg/Kg), melphalan (120mg/m²) and OKT-3 (5mg/day, day -5 to +14). The graft, depleted of T lymphocytes by anti-CD3 and anti-CD19 conjugated magnetic beads, contained high numbers of NK cells and monocytes. Although 28/29 high-risk adult patients with refractory disease or relapse engrafted with full donor chimerism, the incidence of acute GvHD (34% grade II; 14% grade III-IV) was higher than in transplants with positively selected CD34⁺ cells. Overall survival was 31% with a median follow-up of 241 days. Relapse accounted for 12/20 deaths, infections for 7 and GvHD for 1⁴⁵.

Very recently Klingebiel et al. reported their experience with 22 children with acute leukaemia, who were conditioned with fludarabine, melphalan and thiotepa, and received a CD3/CD19 depleted graft. Patients engrafted rapidly, with 10.7% TRM and a 68% probability of survival at 3 years for those in remission at transplantation⁴⁶. Using the same protocol in pediatric ALL patients, Gonzalez-Vicent et al showed most recently, significantly improved LFS, lower TRM and lower aGVHD rates following haploidentical transplantation, compared to UD-UCB transplantation performed during the same period (41±13% vs 26±9%, 25±9% vs 47±9% and 19±7% vs 44±10%, respectively)⁴⁷.

Although these data are very promising, the enhanced incidence of GvHD outcomes compared to that found following transplantation of positively selected CD34⁺ graft, could be due to the observed variability in the level of T cell depletion and could be addressed by a more rigorous procedure currently developed by Handgretinger group using magnetic beads coupled to anti-TCR αβ antibody⁴⁸.

**Alemtuzumab based T cell depletion**

A pilot study⁴⁹ with 12 high-risk patients showed that including in-vivo alemtuzumab in a myeloablative conditioning ensured full donor type engraftment of unmanipulated
haploidentical HSCT, with a low 9% incidence of grade II-IV acute GvHD. In a later study, 49 high-risk patients (median age 48 years) received non-myeloablative regimen (fludarabine, cyclophosphamide) and alemtuzumab for in-vivo and in-vitro T cell depletion. Engraftment was successful in 94% of patients with 10.2% TRM and 8% severe GvHD.

Although high rates of engraftment with acceptable risk of GvHD and TRM were achieved, high relapse rates, probably due to a weak disease debulking by the non-myeloablative protocol, markedly reduced overall survival to 31% at 1 year.

Table 1 summarizes some of the more common methodologies for achieving sustained engraftment of full haplotype mismatched HSCT.

Immune reconstitution

Since thymic function is poor in adults, post-transplant immune reconstitution depends for many months on peripheral expansion of mature T lymphocytes in the graft. Whether T cell depleted full haplotype mismatched HSCT follows myeloablative conditioning or RIC, it is associated with slow post-transplant immune recovery because of the very small number of T cells in the BM inoculum. Thus, recipients tend to remain susceptible to life-threatening opportunistic infections like CMV and aspergillus. Attempts to improve post-transplant immune reconstitution have, to date, focussed on adoptive transfer of T cells.
Adoptive immunotherapy with donor T cells

Specific Anti-Pathogen Immunotherapy

Non-alloreactive, donor origin, anti-pathogen specific CD4+ T cell clones were generated and successfully transferred to recipients of HLA-haploidentical HSCT51. None of the 34 recipients of up to 1x10^6/Kg anti-CMV or anti-Aspergillus T cells developed GvHD. Infusion of aspergillus-specific type-1 CD4+ clones controlled aspergillus antigenemia and helped clear invasive aspergillosis in 9/10 patients. Immunotherapy with anti-CMV CD4+ cell clones significantly reduced CMV reactivation rates and accelerated the development of anti-CMV CD8+ clones. In other studies, refractory CMV disease was successfully cured with CMV specific donor T lymphocytes52; anti human-adenovirus CD4+ clones were successfully used in the haploidentical setting53, and anti-EBV CTLs were effective as a salvage treatment for Post-Transplant Lymphoproliferative Disorder (PTLD) in six patients who failed to respond to rituximab treatment54.

Since laboratory procedures are cumbersome and time-consuming, all these forms of immune therapy are difficult to apply as routine prophylaxis or when urgently needed upon failure to respond to anti-viral therapy. Genetically modified APCs with triple viral antigens combined with a gas permeable culture device might markedly shorten these procedures and enable simultaneous production of CTLs against different viruses55.

To circumvent the need for individualized therapy, Haque et al. used EBV-specific CTLs that were generated in advance from 3rd-party EBV+ blood donors 56. 33 organ recipients who developed EBV+ PTLD and failed conventional treatment were treated. Complete or partial response rates were 64% at 5 weeks and 52% at 6 months.
with no adverse effects. Although 3rd-party CTLs are short lived in-vivo and require repeated infusions, use of ‘off-the-shelf’ 3rd-party CTLs could potentially treat other viral infections as well.

Using a panel of artificial APCs presenting different HLA determinants, the O’Reilly group generated, for almost every patient, a CTL line directed against the EBV or CMV peptide on at least one host HLA allele57. Although very interesting as an approach, no clinical data are as yet available.

Another approach to circumvent cell-culture-related problems is to sort out antigen specific T cells according to gamma-INF production. The Einsele group generated CMV-specific T cells by ex-vivo stimulation with pp65, and sorted out the antigen specific lymphocytes according to γINF production52. 18 HSCT recipients with refractory CMV disease and/or viremia were treated with 21x10³/kg CMV-specific T cells. The viral burden was cleared or significantly reduced in 83% of cases, including 2 patients with CMV encephalitis. Viral control was associated with in-vivo expansion of CMV-specific T lymphocytes in 12/16 patients. No acute side effects or GvHD were observed, even in 11 recipients of full haplotype mismatched transplants.

Further improvement of specific T cell selection could be afforded by using streptamers that present antigens in the desired HLA determinant. Unlike tetramers, streptamers are gradually stripped off following infusion and are therefore viewed more favorably by regulatory agencies. Proof of concept was achieved in 2 HSCT recipients with recurrent high CMV antigenemia. After a single infusion, the frequency of CMV-specific CD8+CD45RA-CCR7- effector T cells increased from 0.0% to 27.1% of all T cells. CMV antigenemia was cleared, allowing patients to discontinue antiviral drugs without toxicity or GvHD58.

Taken together, different methods for the generation of effective specific anti-
pathogen T cells free of GvHD risk, clearly offer a feasible clinical approach for mitigating post transplant infections.

**Broad repertoire Immunotherapy**

An alternative to pathogen-specific therapy is adoptive T cell immunotherapy which provides large numbers of wide repertoire cells, mirroring the physiological immune system. The key challenge is to infuse millions of T cells/Kg in full haplotype mismatched recipients without causing GvHD. Several strategies have been proposed:

1. **Ex-vivo depletion of alloreactive T cells**

T cell preparations are purged of alloreactive donor T cells upon activation in MLR against host stimulators, followed by depletion with immunotoxins, immunomagnetic selection or fluorescence-activated cell sorting, all of which exploit expression of cell surface activation markers (CD25, CD69, CD134, CD137, CD147, and HLA-DR)\(^ {59-63}\). Other methods include FasL mediated killing of activated T cells\(^ {64}\), apoptosis induction by heat shock protein 90 (HSP 90)\(^ {65}\) or anergy induction by co-stimulatory blockade\(^ {66}\).

The anti-CD25 antibody conjugated to ricin toxin was first used in pediatric patients with immune deficiencies\(^ {67}\). More recently\(^ {68}\), 8 children with hematological malignancies who were given \(10^5\) T cells/kg showed significantly improved T-cell recovery, with expansion of the effector memory population lasting for at least 3-5 months after HSCT. The incidence of severe GvHD was low. The authors later advocated using immunomagnetic selection of CD25/CD71\(^ +\) cells to remove more alloreactive cells and to increase the number of infused T cells\(^ {69}\).

Photodynamic allodepletion provided effective and extensive depletion of alloreactive
T cells, while retaining broad repertoire T cells for adoptive immunotherapy. Safety in haploidentical transplant recipients was demonstrated in a phase I-II study with infusion of 1-2x10^6/Kg photo-allodepleted T cells. An international multicentre study, designed to demonstrate efficacy, is currently under way.

2. **Suicide gene insertion into T cells**

Bonini et al. inserted the Herpes-Simplex thymidine kinase suicide gene (TK-cells) into T cells to achieve in-vivo susceptibility to ganciclovir. In a phase I-II study, 28 patients received ~10x10^6 TK-cells/Kg. Twenty-two patients exhibited immune reconstitution at a median of 23 days after infusion (range: 13-42). aGvHD (grade I-IV) in 10 patients and cGvHD in 1 were controlled by ganciclovir administration. Overall 3-year survival was 49% (95% CI 25-73%) for 19 patients who were in remission at transplant. The low incidence of GvHD indicated impaired functionality of the transduced T cells, which at a dose of 10x10^6/Kg, would have otherwise induced a high rate of lethal GvHD. Since long-term reconstitution was largely mediated by newly emerging T lymphocytes, the authors hypothesized that TK-cells triggered thymic function. A phase III clinical trial is now on-going.

3. **Combining donor T regulatory cells and conventional T lymphocytes**

Immunotherapy with either naturally occurring, freshly isolated donor T regulatory cells (Tregs), ex-vivo expanded polyclonal or recipient-type specific Tregs, was shown in mouse models to prevent the GvHD induced by co-infused conventional T cells (Tcons), and to promote post transplant immune reconstitution. In a pilot study by Brunstein et al on 23 patients, double unit UD-UCB transplantation was followed by infusion of 0.1-30.0x10^5/Kg 3rd-party unrelated cord blood Tregs, which
were ex-vivo expanded using anti-CD3/anti-CD28 antibody-coated Dynabeads. Patients received pharmacological immunosuppression after transplant. The incidence of Grade II-IV aGvHD was lower than in historical controls (43% vs 61%; p=0.05). No significant differences emerged in relapse or infection rates. In a study from Perugia\textsuperscript{79}, 28 patients with high-risk hematological malignancies received myeloablative conditioning, followed by $2 \times 10^6$/kg freshly isolated donor Tregs. Four days later, patients received $1 \times 10^6$ Tcons and $10 \times 10^6$ highly purified CD34$^+$ cells from full haplotype mismatched donors. Although no post-transplant immunosuppression was administered, the incidence of acute and chronic GvHD was extremely low. The pattern of post-transplant immune reconstitution was very different from standard T cell depleted full haplotype mismatched HSCT, with rapid recovery of T cell subpopulations, development of wide T cell repertoire and high frequencies of pathogen-specific CD4$^+$ and CD8$^+$ lymphocytes. There were significantly fewer CMV reactivation episodes, with no deaths from CMV disease. This pilot study demonstrated that naturally occurring polyclonal Tregs control the alloreactivity of up to $1-2 \times 10^6$/Kg Tcons immunotherapy and are not associated with bystander inhibition of general immunity that would compromise response to pathogens.

However, TRM did not improve, due to the clinical status of the patients at time of transplantation and to regimen related toxicity. Therefore, in an ongoing study, alemtuzumab replaced cyclophosphamide in the conditioning regimen. Preliminary results show the incidence of life threatening infections is greatly reduced and TRM has fallen to under 20% (Iani et al. unpublished results).

These pilot studies demonstrated that in-vitro priming of naturally occurring
CD4⁺CD25⁺FoxP3⁺ Tregs is not required for GvHD inhibition, since activation of alloantigen-specific Tregs occurs efficiently in-vivo. The number of naturally occurring Tregs that are harvested from healthy donors is sufficient to control the alloreactivity of up to 1-2x10⁶/Kg Tcons infused few days later, and favour immune reconstitution without bystander inhibition of general immunity that would compromise response to pathogens. Thus, ex-vivo expansion of donor Tregs is not a prerequisite for designing Treg-based cellular therapies

**Full haplotype mismatched HSCT as a platform for variety of clinical conditions:**

With its high engraftment rates, ability to prevent GvHD by T cell depletion and no need for post-transplant pharmacological immunosuppression, full haplotype mismatched transplantation might be used in the future for the following purposes:

1. **Haploidentical transplant as a platform for cell therapy with donor cells**

   Prevention of leukemia relapse by enhancing GvL effect without overt GvHD represents another major challenge that must be addressed, especially in patients transplanted in advanced stage disease and under RIC. Specific donor anti-neoplastic cells would be ideal for post-transplant adoptive immunotherapy. Already at present, different modalities can be used to generate anti-tumor cells from host T cells, either transfected with transgenes encoding tumor-specific T-cell receptors⁸⁰ or chimeric receptor–modified T cells, based on tumor-specific antibody-derived specificity (‘T-bodies’)⁸¹-⁸³. Prerequisites for any future treatment with donor-derived anti-leukemic T cells⁸⁴ or NK cells⁸⁵ are absence of GvHD and post-transplant immunosuppression.
Recently, anti 3rd-party CTLs with central memory phenotype were shown to promote BM allografting\textsuperscript{86}. Furthermore, these cells exhibited a novel TCR independent killing mechanism of human lymphoma cells, mediated by apoptosis induction following ligation to MHC-I on malignant cells. When tested in a murine model for B cell lymphoma, these CTLs eliminated residual disease and significantly prolonged survival\textsuperscript{87}. Thus, such cell therapy, combined with mega-dose CD34\textsuperscript{+} HSCT, could offer an attractive general platform for tolerance induction as a prelude for cell therapy or organ transplantation (see below) and, more specifically, for eradicating residual disease in patients with B cell malignancies who cannot tolerate intensive radio-chemotherapy.

2. \textit{Haploidentical transplants for non-malignant diseases}

Apart from transplantation in immune deficiencies (for recent review see\textsuperscript{88}), the use of haploidentical donors for other non-malignant diseases is in its infancy. Sodani et al recently described a series of 22 thalassemia patients undergoing haploidentical maternal stem cell graft\textsuperscript{89}. In order to prevent rejection, combination of mega-dose purified CD34\textsuperscript{+} cells and highly myeloablative conditioning regimen was used. Despite this, graft rejection was reported in 6 patients and 2 patients died of opportunistic infections. The delayed immune reconstitution observed was attributed to thalassemia related impaired thymic function and altered BM microenvironment with diminished IL7 secretion. Clearly, the use of RIC protocols in this setting is highly desirable. Very recently, a proof of concept for the use of RIC together with high-dose post transplant cyclophosphamide, was demonstrated in 3 patients with Paroxysmal Nocturnal Hemoglobinuria\textsuperscript{90}. 
3. Haploidentical transplant as a platform for organ transplantation

Owen\textsuperscript{91}, Medawer\textsuperscript{92} and others have established the concept that mixed hematopoietic chimerism can induce long lasting immune tolerance toward donor cells and tissues. This has led over the years to the development of strategies to co-transplant BM and solid organ from a single HLA-haploidentical family member. Kidney transplants, followed by conditioning with total lymphoid irradiation, ATG and transplantation of mismatched G-CSF mobilized progenitor cells from the same kidney donor, resulted in temporary mixed chimerism, and allowed for the discontinuation of immunosuppressive treatment for a limited time\textsuperscript{93}. Kawai et al.\textsuperscript{94} demonstrated that several months after combined BM and kidney transplants from HLA single-haplotype mismatched donors, all immunosuppressive therapy could be discontinued without significantly affecting transplant function. Even though only transient chimerism was observed with the non-myeloablative protocol used, it was sufficient to induce specific donor unresponsiveness. The mechanism of tolerance induction in their system was believed to be switching from central tolerance to peripheral mechanism that included Tregs. However, protocols which favour long term mixed chimerism may support a more stable tolerance towards donor tissues, preventing the reversible capillary leak syndrome observed in all patients, which might indicate minor rejection episodes.

Conclusions

When high-risk acute leukaemia patients do not have a HLA-matched donor, or urgently need transplantation, physicians are left with the perplexing question of what is the best alternative option, as no randomised or prospective observational studies have been conducted to date. A retrospective analysis compared the outcome of 1-2
loci mismatched UD-UCB or 8/8 HLA allele MUD transplantation in children (age<16). Survival rates overlapped while relapse rates were not significantly different. Consequently, in the absence of 8/8 HLA allele matched MUD, 4-6/6 HLA Ag-matched UBCT offers an attractive option for pediatric patients. The use of UCB transplants in adults is limited by the cell dose. Such limitation has been overcome by infusing two UCB units (dUCB) which improved engraftment and DFS rates.

Full haplotype mismatched T cell depleted megadose transplantation offers several advantages. In children, encouraging results have been achieved in European centres with experience in the field. However, a larger experience has been obtained in adults. Transplants in several European centers resulted in 43% disease free survival in AML and 30% in ALL patients who were transplanted in any CR. In the Perugia series the probability of EFS in AML increased to >65% with transplantation from an NK alloreactive donor, who can be found for almost 50% of patients. Since these results are in the range of best survival rates after well-matched MUD transplantation for patients with high-risk AML in first complete remission, they should encourage greater use of haploidentical transplants for patients who do not have a matched donor. In fact, with a follow-up of up to 17 years, long-term survivors of haploidentical T cell depleted transplants enjoy a normal life with no cGvHD.

Furthermore, when comparing full haplotype mismatched and MUD transplantation, one should note that virtually all patients receive the haploidentical graft with no undue delay, while for various reasons (inevitable time lapse of donor search and bone marrow harvest) many patients relapse and die while waiting for a MUD transplant.

Since approximately half the patients who start a donor registry search actually
undergo transplantation, omission of intention-to-treat from the calculation of final outcomes in most studies creates a marked bias in favor of MUD transplants. Therefore, intention-to-treat trials are needed to establish the most appropriate algorithm for alternative donor selection.

Attention at present, is focussing on the use of haploidentical T cell depleted HSCT without any post-transplant immunosuppression as a platform for adoptive T cell immunotherapy for hastening post-transplant immune reconstitution, reducing TRM and enhancing GvL effect. The availability of the haploidentical donor is therefore of even greater importance when considering the possibility of using the same donor for any developed cell therapy.

In parallel, attempts are ongoing to extend the use of haploidentical transplant for elderly and for patients with significant co-morbidities, by using reduced intensity conditioning together with either ex-vivo T cell depleted grafts, or T cell replete grafts and post-transplant cyclophosphamide to prevent GvHD.

In conclusion, this review has illustrated how full haplotype mismatched transplantation has evolved from a last-attempt option for end-stage patients, to an established form of treatment that must be considered for selected patients with acute leukemia in first remission and high risk of relapse.
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Authorship

Contribution: Y.R., D.H. and M.F.M. reviewed literature and wrote the paper.

Conflict of interest: The authors declare no conflict of interest to declare.
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34. Klingebiel T, Cornish J, Labopin M, et al. Results and factors influencing outcome after fully haploidential hematopoietic stem cell transplantation in children


Figure Legends

Figure 1: Non-myeloablative conditioning with post transplant high-dose cyclophosphamide. The figure summarizes the results of three similar clinical trials of non-myeloablative conditioning and transplantation of partially-HLA mismatched bone marrow at Johns Hopkins, Fred Hutchinson Cancer Research Center, or BMT Group of Georgia and Hahnemann University Hospital97. (A) Actuarial curves of overall survival (OS) and event-free survival (EFS) in 210 patients undergoing nonmyeloablative haploidentical stem cell transplantation with post-transplantation cyclophosphamide; (B) overall survival in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) or myeloproliferative disorder (MPD).

Figure 2: Representative results of haploidentical studies. (A) Results from Perugia describing long term EFS probability of AML patients transplanted in either remission or relapse28. (B) Results from the EBMT survey of 173 high risk adult leukemia patients undergoing fully haploidentical transplantation in 49 centers. The figure describes LFS of AML patients according to disease status at time of transplantation (25 in CR1 and 148 in CR≥2)30. (C) An observational study based on data from the statistical center of the CIBMTR, summarizing the results of 584 patients in 151 center undergoing allogeneic transplantation for AML in first remission and unfavorable cytogenetics at diagnosis. The figure describes adjusted overall survival probability based on stem cell origin98. As can be seen, the results presented are comparable and less favourable than the described for AML patients undergoing haploidentical hematopoietic transplantation in first complete remission. (D) Probability of DFS for ALL pediatric patients who received positive selected transplants from haploidentical donors (n = 28) or unmanipulated bone marrow transplants from matched unrelated donors (n = 18) while in remission32.

Figure 3. Selecting donor/recipient pairs with donor-versus-recipient NK alloreactivity. All individuals possess the KIR2DL2 and/or KIR2DL3 receptors for HLA-C group 1 alleles. If they have HLA-C group 1 allele(s) in their HLA type, they possess HLA-C1-specific NK cells which are alloreactive against cells from individuals who do not express HLA-C group 1 alleles (top panel). Ninety-seven percent of individuals possess the KIR2DL1 receptor for HLA-C group 2. If they possess HLA-C group 2 allele(s) in their HLA type, they have HLA-C2-specific NK cells which mediate alloreactions against cells from individuals who do not express HLA-C group 2 alleles (middle panel). In one study on a large cohort41, functional analyses detected alloreactivity when NK clones were tested against HLA-C group mismatched allogeneic targets. Frequencies of alloreactive NK clones were high, that is, 8 ± 6 cells in 100 (mean ± SD) for HLA-C group 2 mismatches; 5 ± 3 cells in 100 for group 1 mismatches. Finally (bottom panel), 90% of individuals possess the KIR3DL1 receptor for HLA-Bw4 alleles. When they have HLA-Bw4 allele(s) in their HLA type, they may have HLA-Bw4-specific NK cells that are alloreactive against Bw4-negative cells. When NK clones from HLA-Bw4-positive individuals who possessed the KIR3DL1 gene were tested against allogeneic HLA-Bw4-negative targets, alloreactive NK clones were detected in 2/3 of individuals41.
Figure 4: NK cell alloreactivity and the “mother donor effect”. (A-B) Transplantation from haploidentical NK alloreactive donors improves EFS. (A) EFS in patients transplanted in CR from NK alloreactive versus non-NK alloreactive donors. (B) EFS in patients transplanted in relapse from NK-alloreactive versus non-NK alloreactive donors.41. (C) Event-free survival of patients receiving parental donor haploidentical HSCT for acute leukemia. Stratified by both donor sex and NK alloreactivity (NK alloreactive mother donor transplantation, N=21; NK nonalloreactive mother donor transplantation, N=20; NK alloreactive father donor transplantation, N=19; NK nonalloreactive father donor transplantation, N=40).44.

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Figure 1 was reprinted from Best Practice & Research Clinical Haematology, Munchel AT, Kasamon YL and Fuchs EJ. Treatment of hematological malignancies with nonmyeloablative, HLA-haploidentical bone marrow transplantation and high dose, post-transplantation cyclophosphamide. July 2011. With permission from Elsevier.

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<table>
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<tr>
<th>T- Replete</th>
<th>Disease</th>
<th>Method of TCD</th>
<th>Conditioning</th>
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<td>Myeloablative</td>
<td>Huang <em>et al</em>, BBMT 2009</td>
<td>250</td>
<td>108-AML</td>
<td>No TCD</td>
<td>GCSF primed BM and PB, Ara-C, BU, Cy, Semustine, ATG</td>
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<tr>
<td>Liu <em>et al</em>, BBMT 2008</td>
<td>42</td>
<td>24-ALL</td>
<td>No TCD</td>
<td>GCSF primed BM and PB, Ara-C, BU, Cy, Semustine, ATG</td>
<td>CsA, MMF, MTX</td>
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<tr>
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<td>Non-Myeloablative</td>
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<td></td>
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<tr>
<td>Rizzi <em>et al</em>, JCO 2007</td>
<td>49</td>
<td>29-LL/Proch/MDS, 15-M1/MM, 2-AML, 3-MPD</td>
<td>No TCD</td>
<td>Altemuzumab, Flu, Cy</td>
<td>MMF ± CsA</td>
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<tr>
<td>Kurokawa <em>et al</em>, Int J Hematol 2010</td>
<td>66</td>
<td>32-High Risk AL, 10-MDS, 19-Ly, 2-CML, 3-MM</td>
<td>No TCD</td>
<td>(2Gy TBI or Flu) +ATG, BU, Mel</td>
<td>TAC ± mP</td>
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<td>Kasamon <em>et al</em>, BBMT2010</td>
<td>185</td>
<td>106-Lymphoid malignancy, 78-myeloid malignancies</td>
<td>No TCD</td>
<td>2Gy TBI, Cy, Flu</td>
<td>High dose Cy, TAC, MMF</td>
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<td>Mehta <em>et al</em>, BMT 2004</td>
<td>201</td>
<td>102-AML</td>
<td>T1089 or OKT3</td>
<td>TBI, Ara-C, Cy, VP-16, ATG, mP</td>
<td>Partial TCD with OKT3 or T1089 +mP, ATG, CsA</td>
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<td>Aversa <em>et al</em>, JCO 2005</td>
<td>104</td>
<td>67-AML</td>
<td>CD34 positive selection</td>
<td>8Gy TBI, Thiou, Flu, ATG</td>
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<td>Ciceni <em>et al</em>, for the IRMT, Blood 2008</td>
<td>266</td>
<td>173-AML</td>
<td>CD34 positive selection</td>
<td>TBI based myeloablution in 74% of AML and 92% of ALL, ATG in majority</td>
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<tr>
<td>Lang <em>et al</em>, Blood Cells Mol Dis. 2004</td>
<td>63</td>
<td>32-ALL, 13-AML/CML, 4-MDS, 4-Ly, 10 NM</td>
<td>CD34 or CD133 positive selection</td>
<td>BU or TBI +ATG/OKT3 +Cy-Thiou or Cy+Flu</td>
<td>None</td>
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<tr>
<td>Marks <em>et al</em>, BJH 2006</td>
<td>34</td>
<td>17-AL, 14-ALL, MDS/CML/Myelophenolastic IA-L</td>
<td>Campath in the bag (7), CD34 positive selection (27)</td>
<td>Cy+14-Aryl TBI+IV Campath or ATG</td>
<td>CsA in 7. None in 27.</td>
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<tr>
<td>Klingebiel <em>et al</em> for the EBMT, Blood 2010</td>
<td>127</td>
<td>Very high risk ALL</td>
<td>Mostly CD34 positive selection</td>
<td>Myeloablative, TBI in 76%</td>
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<td>Bethoe <em>et al</em>, Blood Cells, Mol Dis 2008</td>
<td>29</td>
<td>16-AML, 7-ALL, 3-NHL, 2-MM, 1-CML</td>
<td>CD3/CD19 depletion</td>
<td>Flu, Thiou, Mel, OKT-3</td>
<td>None</td>
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<tr>
<td>Chen <em>et al</em>, BJH 2010</td>
<td>22</td>
<td>9-AML, 9-ALL, 2-CML, 2-Ly</td>
<td>CD3 depletion</td>
<td>Flu, Thiou, Mel, OKT-3</td>
<td>MMF</td>
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<tr>
<td>Bader <em>et al</em>, Best Practice 2011</td>
<td>59</td>
<td>15-ALL, 14-AML, 2-MDS, 13-RMS, 5-solid tumors, 10 NM</td>
<td>CD3/CD19 depletion</td>
<td>Flu, Thiou, Mel</td>
<td>NA</td>
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<td>Gonzalez-Vicente <em>et al</em>, Euro J hearn 2011</td>
<td>29</td>
<td>18-ALL</td>
<td>CD3/CD19 depletion in 24; CD34 positive selection in 3</td>
<td>Flu, BU, Thiou, mP</td>
<td>CsA alone (6) or ATG alone (8) or CsA+MTX (15)</td>
</tr>
</tbody>
</table>

Table abbreviations: AML=Acute Myelocytic Leukemia; ALL=Acute Lymphocytic Leukemia; CML=Chronic Myelocytic Leukemia; HL=Hodgkin’s Lymphoma; Lymph=Acute Lymphocytic Lymphoma; MM=Multiple Myeloma; Leuk=Leukemia; MM=Non-Malignant; RMS=Rhabdomyosarcoma; TCD=T cell depletion; Ara-C=Cytosine Arabinoside; VP-16=Etoposide; TBI=Total Body Irradiation; Thiou=Thiopeta; Mel=methotrexate; Flu=Fludarabine; TAC=Tacrolimus; mP=methyLPrednisolone; BU=Busulfan; Cy=Cyclophosphamide; ATG=Anti Thymocytic Globulin; CsA=Cyclosporin A; MMF=Mycophenolate Mofetil; MTX=Methotrexate; LFS=Lymphoma Free Survival
Figure 1

(A) OS (%) 1 yr 2 yrs 3 yrs
  OS (%)  60  48  41
  EFS (%)  43  34  32

(B) Survival (%)

Days after transplantation

ALL (n=16) MDS, MPD (n=25) AML (n=43)
Figure 2

A. AML (n=147)

- CR1 (n=34) 0.50+/-.09
- CR2 and higher (n=49) 0.35+/-.07
- Relapse (n=64) 0.14+/-.04

P=0.0006

B. AML DFS

- CR1 48%
- CR≥2

C. Adjusted Prob of OS

- HLA-identical sibling
- URD, partially-matched
- URD, well-matched

D. Proportion Surviving

- Haplo group (ALL rem.)
- Control group (ALL rem.)

Years

Years from Transplantation
Figure 3

### Donors

<table>
<thead>
<tr>
<th>HLA</th>
<th>NK repertoire</th>
<th>HLA</th>
<th>Target</th>
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<td>KIR2DL2/3</td>
<td>Missing</td>
<td>Lysis</td>
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<tr>
<td>HLA-C group 2</td>
<td>KIR2DL1</td>
<td>Missing</td>
<td>Lysis</td>
</tr>
<tr>
<td>HLA-Bw4 group</td>
<td>KIR3DL1</td>
<td>Missing</td>
<td>Lysis</td>
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Haploidentical hematopoietic transplantation: current status and future perspectives

Yair Reisner, David Hagin and Massimo F. Martelli