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“No Donor”? Consider a Haploidentical Transplant

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Abstract

Haploidentical stem cell transplantation (HaploSCT) is an attractive option for patients requiring a hematopoietic stem cell transplant who do not have an HLA-matched donor, because it is cheaper, can be performed faster, and may extend transplantation to virtually all patients in need. Significant advances have been made in the recent decade with dramatic improvement in treatment outcomes. Historically, overcoming the HLA-incompatibility barrier has been a significant limitation to the expansion of this form of transplant. While *ex vivo* T-cell depletion effectively prevented the development of acute GVHD, it was associated with a higher treatment-related mortality, in excess of 40% in some series, due to a significant delay in recovery of the adaptive immune system. Newer methods have successfully maintained the memory T cells in the graft and/or selectively depleted alloreactive T cells, and are associated with improved treatment outcomes. Post-transplant cyclophosphamide for GVHD prevention has proven very effective in controlling GVHD with lower incidence of infectious complications and treatment-related mortality – as low as 7% at one year-, and has become the new standard in how this transplant is performed. Here, we reviewed the current experience with this approach and various other strategies employed to control alloreactivity in this setting, including selective depletion of T cells from the graft, as well as we discuss post-transplantation therapy to prevent disease relapse and improve immunologic reconstitution.

Keywords

Haploidentical Transplantation; post-transplantation cyclophosphamide; alpha-beta T cell depletion; cellular therapy; graft engineering

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Conflict of Interest

None.

INTRODUCTION

Haploidentical hematopoietic stem cell transplantation (HaploSCT), with progenitor cells from HLA-half-matched first degree related donors (siblings, children and parents), could revolutionize hematopoietic stem cell transplantation as it expands the this form of treatment to approximately 40% of patients who do not have an HLA-matched donor¹. This need is particularly acute in developing countries, which usually do not have an unrelated donor registry and/or cost is a major issue in acquiring unrelated donor progenitor cells.

Advantages to HaploSCT include almost universal (more than 95% of patients will have a half-matched related donor) and immediate availability of donor progenitor cells, the opportunity to select the best donor among family members to minimize treatment-related mortality, decrease relapse rate and improve outcomes², and the possibility to collect donor cells for cellular therapy post-transplantation, with the goal to enhance the anti-tumor effects of the graft. Despite its potential advantages, until recently, high donor-recipient HLA-histoincompatibility has proven very difficult to overcome.

Haploidentical transplants initially performed with conventional GVHD prophylaxis in late 70's led to a strong bidirectional alloreactivity, manifested by both high incidence of primary graft failure as well as the development of a syndrome suggestive of hyperacute GVHD (manifested with seizures, renal failure, respiratory failure in the majority of patients) and very poor outcomes^{3, 4}. To prevent GVHD after HaploSCT, *ex vivo* T-cell depletion (TCD) was used successfully in the 80's⁵; however, this approach resulted in a high incidence of graft rejection in up to 50% of cases⁶. This high incidence of graft failure, thought to be primarily related to the remaining T cells in the recipients system and lack of donor T cells in the graft to support engraftment, was improved in the 90's by intensifying the conditioning regimens, combining *ex vivo* and *in vivo* T-cell depletion, and increasing the donor graft inoculum using "mega-doses" of CD34+ cells⁷. Primary engraftment was achieved in >90% patients with a low GVHD rate⁸. Subsequently, we have shown that not only T cells can mediate rejection of donor cells, but also B cells via anti-HLA antibodies against donor's HLA antigens, now acknowledged as playing a major role in the development of primary graft failure in these patients⁹. Moreover, we and others have shown that extensive T-cell depletion of the haploidentical graft was associated with a high non-relapse mortality (NRM) rate in excess of 40%, primarily due to slow post-transplant immune recovery leading to many opportunistic infections, and likely decreased graft-versus-leukemia effect^{8, 10, 11} (Table 1).

In the past decade, significant progress has been made as researchers from around the world have tried to overcome the fore-mentioned barriers in HaploSCT by using T-cell replete grafts with intensified GVHD prophylaxis, or by the use of methods to selectively deplete T cells from the haploidentical graft¹². In addition, the development of post-transplant cellular therapy to prevent or treat disease relapse and infectious complications after transplant has found an ideal applicability in related donor transplantation, including haploidentical transplants. Here, we present the current and foreseeable new approaches to HaploSCT and graft manipulation, which have already revolutionized this field and will likely extend this form of transplantation word wide (Table 2).

T-CELL REplete (TCR) HAPLOIDENTICAL TRANSPLANTATION

Without extensive T cell depletion of the haploidentical graft, highly effective GVHD prevention strategies become necessary to overcome the intense bidirectional alloreactivity (in the graft-versus-host and host-versus-graft directions) associated with this type of transplant. Based on initial experiments on murine mouse models¹³, the Johns Hopkins group has used high-dose cyclophosphamide early post-transplant (PTCy) to control GVHD by eliminating rapidly dividing donor T cells generated by the major HLA mismatch graft. PTCy has successfully maintained the quiescent progenitor cells and memory T cells in the graft, which are not susceptible to cytotoxic chemotherapy, in part due to high levels of aldehyde dehydrogenase^{14, 15}. This approach has been initially developed using minimally intense, non-myeloablative (NMA) conditioning and bone marrow (BM) grafts with a lower T-cell content compared to peripheral blood (PB)^{15, 16}. Assessing the feasibility of this approach, a multi-center BMT CTN 0603 trial demonstrated an acceptable incidence of GVHD (32% acute grade II-IV and 13% chronic GVHD) and very low NRM. A disappointing high relapse incidence (45%) at one year in these patients¹⁷ was primarily attributed to the use of NMA conditioning for patients with acute leukemias. On the other hand, this approach has been particularly successful in patients with lymphoma. A retrospective analysis of 151 consecutive patients with poor-risk or advanced lymphoma who underwent HaploSCT with post-Cy revealed a progression-free survival of 40% at 3 years¹⁸, while patient with Hodgkin's disease had similar outcomes with matched transplants¹⁹.

Early results with the PTCy approach and an intensified conditioning regimen was reported by the same group in a pediatric and young adult population with acceptable GVHD and engraftment rates²⁰. Recently, Raiola et al. also reported encouraging results in 50 patients with high-risk hematological malignancies who underwent HaploSCT with post-CY and busulfan or TBI-based myeloablative conditioning²¹. Successful engraftment was achieved in 90% of patients and grade II-III acute GVHD incidence was only 12%. After a median follow-up of 333 days, NRM was 18% and disease-free survival at 22 months was 68% for patients in remission at the time of transplant. Our experience with PTCy approach using a myeloablative yet reduced-intensity conditioning with fludarabine, melphalan +/- thiopeta (subsequently changed to 2Gy TBI) has been very good, with NRM and progression-free survival of 21% and 53% after a median follow-up of 14 months in 57 patients with advanced hematological malignancies²². Updated results for our first 100 patients treated showed a 3-year PFS of 56% for patients with AML in CR1/CR2 or chronic-phase CML, 62% for patients with lymphoid malignancies and 44% for patients with advanced acute lymphoblastic leukemia²², results comparable with matched transplants.

With a higher T cell content, peripheral blood (PB) grafts may shorten the period of neutropenia, improve engraftment and potentially influence post-transplant immune recovery and relapse incidence. Early results suggest that the incidence of grade II-IV aGVHD appears to be twice as much as with a BM graft, albeit the incidence of severe, grade III-IV aGVHD may not be much higher than using a BM graft. It remains to be seen if outcomes with a PB graft are as good as with a BM graft in this setting. It turns out that the

higher incidence of aGVHD has a negative impact on outcomes, an optimized peripheral blood graft will likely be needed²³.

Antigen presentation by dendritic cells to donor T cells is an important step in the development of GVHD and is NF- κ B pathway dependent, which may be blocked by bortezomib. Therefore, a combination of cyclophosphamide and bortezomib post-transplant may further decrease GVHD incidence after HaploSCT as supported by *in vitro* studies²⁴. Early phase clinical trials are exploring this hypothesis.

Overall, the PTCy approach is associated with low incidence of acute and chronic GVHD and NRM, with outcomes comparable with matched transplantation. Recently, Bashey et al. demonstrated similar outcomes after TCR HaploSCT with PTCy when retrospectively compared them with transplant outcomes using matched related and matched unrelated donors, with probabilities of DFS of 60%, 53%, and 52%, respectively²⁵. We have recently compared outcomes of a uniform cohort of 227 AML/MDS patients treated with the same conditioning regimen (fludarabine and melphalan) and found similar results. The 3-year DFS for patient in CR using a matched sibling, unrelated donor and haploidentical transplants were 51%, 45% and 41%, respectively (p=0.4) with similar immune reconstitution between the 3 groups (Di Stasi A, et al. *manuscript in press*).

The Chinese investigators developed a different approach to control GVHD after haploidentical transplantation. They used a myeloablative conditioning regimen, an intensified GVHD prophylaxis using multiple immunosuppressive medications, along with a G-CSF-primed BM graft with PB collected progenitor cells²⁶. In 250 acute leukemia patients, incidences of GVHD were higher than those seen with post-transplantation cyclophosphamide (46% grade II-IV aGVHD and 54% cGVHD), while almost all patients had successful engraftment and good outcomes. Di Bartolomeo et al. obtained similar results in Europe, except reported a lower GVHD incidence using different myeloablative regimens and only a BM graft²⁷.

GRAFT ENGINEERING

While T cells in the donor graft are the primary actor in the development of GVHD, they also facilitate engraftment, play a significant role in post-transplant immune reconstitution, and eliminate residual disease through the HLA-incompatibility with the recipient malignant cells. However, specific T cell subsets may contribute more to the development of GVHD, while memory T cells are known to contribute to immune reconstitution post-transplant. NK cells have been shown to contribute to anti-tumor effects; therefore, enhanced alloreactivity of these cells may decrease relapse rates post-transplant. Consequently, engineering the graft to optimize the immune cell content may improve graft-versus-tumor effect and immunologic reconstitution without generating more GVHD.

Co-infusion of Regulatory and Conventional T cells

Tregs modulate the immune system and maintain tolerance to self-antigens. In murine models of mismatched transplantation, Tregs suppressed lethal GVHD²⁸, and favored post-transplant immune reconstitution when coinfused with conventional T-cells (Tcon)²⁹.

Although the role of Tregs on GVL is still debated, co-infusion of Tregs and Tcons protected mice from GVHD while preserving GVL effect in mismatched transplant models³⁰. To improve GVL effect and immunologic reconstitution with Tcons while preventing GVHD with Tregs, the Perugia group infused donor Tregs before the infusion of mega-doses of TCD PB progenitor cells and donor Tcons without any post-transplant immunosuppression. Only 2 of 28 patients developed aGVHD and none developed cGVHD. Although a wide T-cell repertoire developed rapidly, 8 patients still died of opportunistic infections. This study suggested that adoptive immunotherapy with Tregs counteracted the GVHD potential of conventional T-cells in HaploSCT, however, the high incidence of opportunistic infections and treatment-related mortality remains a concern³¹. Long-term results of the study was recently presented in abstract format³². Of 45 patients with high-risk leukemia, 43 achieved primary engraftment and 6 patients developed grade 2 acute GVHD. At a median follow-up of 46 months, disease-free survival and transplant-related mortality rates were 56% and 37%. Further studies may be needed to assess the optimal ratio of Tregs to Tcons.

Allodepletion

Selective depletion of donor T cells alloreactive to recipient antigens may prevent GVHD, improve immune reconstitution by maintaining memory T cells, and preserve GVL effects of the graft due to retention of NK cells. Current allodepletion methods rely on, first, generating an alloresponse by co-culture of donor T cells and recipient cells, second, labeling of the activated donor T cells with antibodies against surface activation markers or photoactive dyes which are preferentially retained in activated T cells, and finally, depleting the activated donor T cells³³.

Amrolia et al. used an anti-CD25 immunotoxin to deplete alloreactive lymphocytes and infused 10^4 - 10^5 cells/kg allodepleted lymphocytes on 30, 60, and 90 days post-transplant in 16 patients (median age 9 years)³⁴. Only 2 patients developed grade II-IV acute GVHD. A wider TCR repertoire was developed 4 months after transplant compared with retrospective controls who did not receive T-cell add back. However, 9 patients (56%) died due to relapse disease (5), infection (3), and interstitial pneumonitis (1).

One caveat with allodepletion using anti-CD25 antibodies is that Tregs also do express CD25 on their surface. An alternate method using photodepletion with TH9402, a phototoxic dye that accumulates in activated T cells due to their inability to efflux rhodamide-like drugs, was first reported in 2002^{35, 36} and further improved at the NCI³³. Recently, Bastien et al. showed that photodepletion using TH9402 in transplanted patients with resistant chronic GVHD eradicated proliferating T cells while sparing Tregs³⁷. Early results from a clinical trial using this approach are very promising.

Alloanergization

Conventional T-cell activation requires 2 signals from antigen presenting cells (APCs). First, an immunogenic peptide on major histocompatibility complex (MHC) activates the T-cell receptor. Secondly, a costimulatory signal from CD80/86 or an inhibitory signal from CTLA-4 on APCs to the CD28 on T-cells induces development of Tcons and Tregs,

respectively. Therefore, an inhibitory signal from CTLA-4 may result in induction of anergy³⁸ allowing transplantation of histoincompatible allografts³⁹. Guinan *et al.* showed the feasibility of HaploSCT using a BM graft of which donor T-cells were anergized through incubation with recipient's mononuclear cells and CTLA-4-Ig⁴⁰. In a follow-up study, 5 of 24 transplanted patients were reported to develop severe aGVHD and 12 patients died within 200 days of transplantation (5 due to infection)⁴¹. A similar protocol revised to minimize the early transplant related mortality using reduced intensity conditioning and mega-doses CD34+ cells is being investigated.

Alpha-beta T cell depletion

Selection of T cells by T cell receptor (TCR) phenotype has proven useful in discriminating T cells capable of eliciting GVHD from others. $\gamma\delta$ T cells, with TCRs made up of one γ (gamma) and one δ (delta) chain, are a unique population of lymphocytes possessing properties of both innate and adaptive immune system with rearranged TCRs producing diversity and rapid, innate-like responses⁴². Importantly, it has been suggested that $\gamma\delta$ T cells do not require antigen processing and HLA presentation of antigens rendering them unlikely to generate GVHD⁴³. Moreover, a faster recovery of $\gamma\delta$ T cells after SCT has been associated with longer disease-free survival⁴⁴. Accordingly, methods to deplete $\alpha\beta$ T cells preserving $\gamma\delta$ T cells have been developed⁴⁵. Recently, Bertaina et al. reported their results in 45 children (median age of 10 years) with acute leukemia who underwent HaploSCT with TCR- $\alpha\beta$ and CD19 depleted PB grafts⁴⁶. Pre-transplant anti-thymocyte globulin was the only pharmacologic GVHD prophylaxis used. Primary engraftment was achieved in 44 patients and only observed acute GVHD were grade I-II skin-only in 13 children. Two patients died of infectious complications. With a median follow-up of 11 months, the 2-year leukemia-free survival was 75%. Using a similar protocol but with the addition of short-course post-transplant mycophenolate mofetil for GVHD prophylaxis, the Tuebingen group observed a low incidence of grade II-IV aGVHD in 29% of patients with a transplant-related mortality rate of 20% at one year⁴⁷. Longer follow-up is needed to better assess outcomes of these patients.

The underlying strong rationale and promising initial results warrant further studies to be performed with selective depletion based on T cell subsets.

CD45RA depletion

T cells differ in their functional activity and various classification schemes exist according to their cell surface phenotype⁴⁸⁻⁵⁰. Majority of T cells that can respond to minor H antigens and cause GVHD are thought to be naïve (T_N , never exposed to their cognate antigen) with a CD45RA⁺CD62L⁺ surface phenotype⁵¹. Several in vitro and mouse studies support this hypothesis⁵²⁻⁵⁶. Consequently, depletion of CD45RA⁺ naïve T cells has been explored using CliniMACS magnetic bead separation system^{57, 58}. Because a subset of CD34+ hematopoietic progenitor cells express CD45RA⁵⁹, Bleakley et al. devised a 2-step procedure in which first donor pheresed PB is selected for CD34+ cells and then CD34-negative fraction was depleted for CD45RA to preserve all CD34+ cell subsets⁵⁸. Recently, investigators at St. Jude reported their experience in small number of patients (ages 8-19) who underwent HaploSCT using a parent donor with myeloablative conditioning, CD3/

CD45RA depleted PB graft (CD3 depletion for the first day apheresis preserving all CD34+ cell subsets and CD45RA depletion for the second day), and a 28-day course of sirolimus for GVHD prophylaxis (Leung W, *personal communication*). A 4.5 log depletion in T_N cells were detected in final product to be infused. On post-transplant day 30, almost all T cells were negative for CD45RA. Complete engraftment was achieved in all patients and no acute GVHD was observed. After a median follow-up of 171 days, none of the patients died of infectious complications.

POST-TRANSPLANT CELLULAR THERAPY

Unmodified donor lymphocyte infusion (DLI)

Ready availability of the related donors may be exploited to prevent or treat disease relapse and improve immunologic reconstitution after HaploSCT. Donor lymphocyte infusion (DLI) is an accepted treatment option for relapsed disease after transplant; however, it is associated with a significant risk of GVHD. There is limited data on efficacy and GVHD inducing potential of DLI after HaploSCT. Recently, the Johns Hopkins group demonstrated feasibility of unmodified haploidentical DLI (haploDLI) with relatively modest activity in 40 patients who received HaploSCT with post-Cy approach for early disease relapse⁶⁰. Grade II-IV aGVHD occurred in 10 patients (25%) while 6 developed severe aGVHD (grade III-IV). Twelve patients (30%) achieved a complete remission after DLI, of whom 11 had received cytoreductive therapy prior to DLI and 8 remained in CR at last follow-up. The proposed starting dose was $1 \times 10^6/\text{kg}$. This experience suggested that a haploDLI should be administered in conjunction with either chemotherapy or hypomethylating agents and starting at a higher dose, as no significantly more GVHD was observed than with DLI administered in matched transplants⁶⁰.

Engineered donor lymphocytes with a safety switch

Engineered T cells with safety switches to control GVHD may help develop a safer DLI with an improved GVL effect and immune reconstitution post-transplant. Accordingly, Ciceri et al. infused donor lymphocytes engineered to express herpes simplex virus-thymidine kinase suicide gene - which can be triggered by the use of ganciclovir - (TK-cells) monthly for 4 times post-transplant⁶¹. Of 28 patients who underwent HaploSCT with TCD PB grafts and received engineered donor lymphocytes, 22 obtained engraftment of TK-cells. Immune responses against CMV and EBV improved after TK-cell infusions. Without any GVHD prophylaxis, 10 patients developed acute GVHD and required ganciclovir resulting in abrogation of GVHD in all. There were no GVHD related deaths or long-term complications⁶¹. Ganciclovir is a commonly used drug to treat CMV in transplantation thus using this drug for this purpose may not be optimal.

An alternative approach was developed by the Baylor group using donor lymphocytes engineered to express an inducible caspase-9 transgene (iC9), activated by a bio-inert molecule, AP1903⁶². Of 10 pediatric patients (age 3-17) who underwent HaploSCT with TCD grafts and were infused iC9-T cells between 30 and 90 days after transplantation, all achieved engraftment of iC9-T cells⁶³. In 5 patients who developed GVHD, iC9-T cells were >90% eliminated within 2 hours of AP1903 administration and GVHD was rapidly

reversed. AP1903 did not affect T-cell immune reconstitution in these patients. Viral reactivation or disease resolved within 4 weeks of iC9-T cell infusion in all patients who had evidence of viral replication. Furthermore, AP1903 administration did not significantly affect anti-viral immune reconstitution in patients with active viral disease⁶³. Clinical trials using this approach are ongoing. Moreover, a safer DLI using iC9 modified T cells could conceivably be used in the future to control severe GVHD associated with unselected T cell infusions.

T cells with chimeric antigen receptors (CAR)

Although engineering donor lymphocytes to express suicide genes has a security system against development of severe GVHD, it provides a non-targeted, yet broad antitumor effect. On the other hand, T cells engineered to express CARs (CAR T cells) - fusion proteins with an extracellular antigen recognition moiety and intracellular T-cell activation domain may have significantly higher anti-tumor efficacy for B cell hematological malignancies without added risk for the development of GVHD. Recently, Kochenderfer et al. reported their findings in 10 patients who received anti-CD19 CAR T cells for patients with relapsed B cell malignancies after transplantation from matched related or unrelated donors⁶⁴. All patients had received standard DLIs prior to CAR T cells with only 2 achieving a response. Two patients achieved response lasting >3 and >9 months after CAR T cell infusion, while 6 patients achieved stable disease lasting between 1 to more than 11 months. None of the patients developed GVHD after infusion. Extending the use of CAR T cells after HaploSCT is also feasible, with cells generated from the same donors as progenitor cells. An ongoing study using CAR T cells obtained using the Sleeping Beauty system is ongoing at MDACC. We have treated 3 ALL patients with a HaploSCT followed by CAR T cells (all with relapsed/refractory disease, one relapsed after a cord blood transplant). Two received the CAR T cells as preemptive therapy and one as treatment for relapsed disease after the HaploSCT. All patients tolerated the infusions well with no significant GVHD. The 2 patients that received the CAR T cells as preemptive therapy are alive in remission more than 6 months post-transplant while, the other patient died of disease relapse. These are the first haploidentical transplant patients treated with CAR T cells. Although very limited experience, prevention of disease relapse post-transplant for high-risk ALL patients appears to be the most important therapeutic benefit at the present time.

Natural Killer Cells

Natural killer (NK) cells are involved in innate immune system⁶⁵. According to the widely used “missing self” model, a NK cell recognizes a cell as foreign when the particular cell lacks one or more HLA class I alleles specific to the inhibitory receptors (killer immunoglobulin-like receptors, KIRs) on the NK cell^{66, 67}. NK cells attack primarily hematopoietic cells sparing the solid organs, rendering them almost incapable of causing GVHD⁶⁸. Recently KIR genotyping has been shown to be important in decreasing relapse rate post-transplant for patients with myeloid malignancies^{69, 70}. Patients with KIR-Bx genotype were associated with lower relapse rates and should be the preferred donors, if available.

NK cell infusions after HaploSCT have been utilized to exploit innate immunity against a variety of tumors including myeloid malignancies⁷¹⁻⁷³. Yoon et al. reported no acute side effects in 14 patients who were infused with donor NK cells 6-7 weeks after TCR HaploSCT using a RIC conditioning with fludarabine and busulfan⁷⁴. Two patients who received NK cell infusion during active leukemia did not have a response and 4 patients developed cGVHD. Four patients were alive and disease-free 18-21 months post-transplant. Further studies are needed to explore the use of NK cells post HaploSCT. More recently, the same group reported no acute toxicity after NK cell infusions up to 1×10^8 cells/kg. A significant reduction leukemia relapse rate was suggested when retrospectively compared to a similar cohort of patients who underwent HaploSCT without NK cell infusion⁷⁵.

A phase 1 clinical trial for haploidentical transplant patients with advanced hematologic malignancies was recently started at MD Anderson using *ex vivo* expanded NK cells using the mbIL-21 method⁷⁶ with the goal to decrease the rate of disease relapse post-transplant. First 3 patients (2 with CML and one with refractory AML) who received *ex vivo* expanded NK cells using this method at doses 1×10^4 /kg and 1×10^5 /kg engrafted and experienced no infusion-related toxicities.

CONCLUSIONS AND FUTURE DIRECTIONS

Outcomes of haploidentical transplants have improved dramatically past several years, now approaching outcomes of matched transplantation. The use of haploidentical donors has extended safe transplantation to virtually all patients in need, thus lack of an HLA matched donor is not a limitation against a successful transplant anymore, and should not preclude patients in need of this procedure to benefit from an allogeneic stem cell transplant. While more studies are needed to compare different sources of progenitor cells, it is now clear that post-transplantation cyclophosphamide for GVHD prevention is associated with low NRM and improved outcomes, and has established as new standard in haploidentical transplantation. Novel methods of performing haploidentical transplants will have to be eventually compared with this approach.

While this field is expanding in the direction of graft manipulation and post-transplantation cellular therapy, haploidentical transplantation maintains an edge due to lower cost, easy accessibility of donor cells, and with the use of post-transplantation cyclophosphamide, has the potential to be the preferred source of progenitor cells for patients without HLA matched donors world-wide, especially in developing countries where cost of developing and maintaining unrelated donor registries or acquiring progenitor cells from the international registries might be prohibitive.

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Practice Points

- Advances in haploidentical transplantation rendered this form of transplant a viable approach for patients without HLA-compatible donors
- The two major strategies used in haploidentical transplantation are post-transplantation high dose cyclophosphamide and *ex vivo* T cell depletion
- Early results with haploidentical transplantation using post-transplantation cyclophosphamide are similar with HLA-matched unrelated donor transplants
- Selective depletion of T cells may lead to control GVHD without post-transplantation immunosuppression and may lower transplant related mortality compared to unselective *ex vivo* T cell depletion

Research Agenda

- Developing alternative approaches to haploidentical transplantation using modified peripheral blood grafts
- Investigating approaches to improve immunologic reconstitution post-transplant
- Evaluate cell therapy to decrease rate of disease relapse post transplant

Table 1

The rationale and potential shortcomings of the current approaches in haploidentical stem cell transplantation.

General approach/Modifications	Mechanism and rationale	Potential shortcomings
Complete/partial <i>ex vivo</i> T cell depletion	Most efficacious GVHD preventive method	↑ graft rejection ↑ NRM and possible ↑ RI due to delayed immune reconstitution
“Mega-dose” stem cells, ATG, conditioning intensification	To prevent graft rejection by increasing inoculum and eliminating residual recipient immune cells with ATG and intensified conditioning	Immune reconstitution still delayed
T _{reg} and T _{con} co-infusion	Addition of Tcons to promote immune reconstitution while preventing GVHD with Tregs	T _{reg} may decrease GvL effect T _{reg} / T _{con} ratio needs to be optimized
Allodepletion using anti-CD25 antibodies	<i>ex vivo</i> depletion of alloreactive T cells by targeting activation marker CD25 after incubation with recipients APCs	T _{reg} also express CD25 Clinical efficacy not proven Possible effect on GvL response
Allodepletion with phototoxic dye	<i>ex vivo</i> depletion of alloreactive T cells with TH9402 that accumulates in activated T cells	Clinical efficacy not proven Possible effect on GvL response
Selective αβ T cell depletion	Preservation of γδ T cells (unlikely to induce GVHD while effective against infections with an innate-like response) while eliminating αβ T cells most responsive for aGVHD Potential to avoid post-transplant immunosuppression	Clinical efficacy not proven; however promising early data Possible effect on GvL response
Selective CD45RA ⁺ T cell depletion	Elimination of CD45RA ⁺ naïve T cells (capable of precipitating GVHD) while preserving memory T cells (active against infections) Potential to avoid post-transplant immunosuppression	Clinical efficacy not proven Possible effect on GvL response
Alloanergization	Alloreactive T cells are anergized by blocking co-stimulatory CD80/86 signal	T cells are not depleted ↑ GVHD rate
High-dose post-transplantation cyclophosphamide RIC/NMA conditioning	Eliminating the allo-activated T cells early after transplant without affecting stem cells. T cell preservation allows lower intensity conditioning extending transplantation to elderly patients Low incidence of cGVHD	Low cost GvHD incidence higher than after <i>ex vivo</i> T cell depletion; however similar with matched transplantation Higher leukemia relapse incidence after NMA conditioning
Myeloablative conditioning	To decrease relapse incidence in leukemia patients	↑ in NRM and possibly in GvHD
Peripheral blood as stem cell source	To decrease relapse incidence and possible improve immune reconstitution through higher T cell content in PB	↑ in acute GvHD potential

General approach/Modifications	Mechanism and rationale	Potential shortcomings
Intensified immune suppression	To demeliorate immune reaction both ways G-CSF priming of BM and PB graft to induce T cell hyporesponsiveness	Higher aGVHD and cGVHD incidence
Post-transplant lymphocyte infusion after <i>ex vivo</i> T cell depleted transplantation	To treat disease relapse through GvL effect	Limited efficacy GVHD precipitation
Engineered donor lymphocytes with a safety suicide switch	To prevent/treat disease relapse and improve immune reconstitution post-transplant. Safety switch allowing T cell suicide in case of GVHD precipitation => Higher T cell doses are possible	T cells are not targeted => While immune reconstitutive effect is demonstrated, GvL effect not yet clear
T cells with chimeric antigen receptors	T cells engineered to recognize specific antigens (CD19) provides GvL effect without GVHD	Clinical efficacy after HaploSCT not shown yet

Legend: GVHD – graft-versus-host disease, NRM – non-relapse mortality, RI – relapse incidence, ATG – anti-thymocyte globulin, T_{reg} – regulatory T cells, T_{con} – conventional T cells, APCs – antigen presenting cells, GvL – graft versus leukemia effect, RIC – reduced-intensity conditioning, NMA – non-myeloablative conditioning, HaploSCT – haploidentical transplantation.

Table 2

Major studies in haploidentical stem cell transplantation.

Reference	Graft	Conditioning	Immune suppression	Patient characteristics	Engraftment and GVHD	Survival
T cell depleted/modified grafts						
Aversa F, 2005 ⁷⁷	Mega dose of CD34 selected PB	Ablative	ATG	n=104 Median age: 33 (9-64) 67 AML (19 in CR1) 37 ALL (14 in CR1)	94 pts (91%) engrafted Grade II-IV aGVHD in 8 pts (8%) cGVHD in 5 pts (7%)	TRM: 37% for pts in remission; 44% in pts with active disease 27 pts (26%) died of infections RI: 16% for pts in remission; 51% in pts with active disease EFS @ 3yrs: 48% for pts in remission; 4% for pts with active disease
Amrolia PJ, 2006 ³⁴	Mega dose of CD34 selected PB Allodepleted T cell addback (anti-CD25)	Ablative/RIC	Alemtuzumab	n=16 Median age: 9 (2-58) 7 AML (1 in CR1) 2 ALL 1 HL 1 CML 1 MDS 3 BMF	All engrafted Grade II-IV aGVHD in 2 pts (both after donor T cell infusion) cGVHD in 2 pts	5 pts alive @ median follow-up of 33 mos
Davies JK, 2008 ⁴¹	BM after alloanergy induction through CTLA4-Ig	RIC	Mtx, CsA	n=24 Age range: 0.5-50 21 high-risk heme malignancy (none in CR1, 14 with PD) 3 with bone marrow failure	2 (8%) graft failure Grade B-D aGVHD in 8 pts (38%) cGVHD CI 8%	TRM incidence 50% EFS and OS: 33% at 10 years
Di Ianni, M, 2011 ³¹	Mega dose of CD34 selected PB Tcon addback preceded by Treg infusion	Ablative	None	n=28 High-risk heme malignancies	26 pts (93%) engrafted Grade II-IV aGVHD in 2 pts No cGVHD	TRM 13 pts (50%) 8 pts (31%) died of infection 1 pt relapsed OS @ 1 yr: 46%
Federmann B, 2012 ¹¹	CD3/CD19 depleted PB	RIC	OKT-3 ± MMF	n=61 38 AML 8 ALL 6 NHL 4 MM 3 CML 1 MDS 1 CLL	3 primary graft failure Grade II-IV GVHD CI 46% cGVHD CI 18%	NRM @ 2 yrs: 42% 18 pts (30%) died of infections RI @ 2 yrs: 31% EFS @ 2 yrs: 25% OS @ 2 yrs: 28%
Bertaina A, 2013 ⁴⁶	TCR-αβ and CD19 depleted PB	Ablative	ATG	n=45 Median age: 10 (0.9-18) AML/ALL	1 primary graft failure Grade I-II skin-only aGVHD in 13 pts	TRM in 2 pts LFS @ 2yrs: 75%

Reference	Graft	Conditioning	Immune suppression	Patient characteristics	Engraftment and GVHD	Survival
					Skin-limited cGVHD in 2 pts	
Ciceri F, 2009 ⁶¹	Mega dose of CD34 selected PB HSV-TK expressing T cells (TK cells) monthly x4 post-transplant	Ablative	None	n=28 (Of initial 50, 22 did not received HSV-TK T cells) High risk heme malignancy	22 obtained TK cell engraftment Grade I-IV aGVHD in 10 pts (all resolved with GCV) extensive cGVHD in 1 pt	NRM @ 3yrs among intent-to-treat 50 pts: 40%
Zhou X, 2014 ⁶³	Mega dose of CD34 selected PB HSV-TK expressing inducible Caspase-9 gene (iC9-T cells) post-transplant	Ablative/RIC	None	n=10 Median age: 8 (3-17)	All pts obtained iC9-T cell engraftment aGVHD in 5 pts (all resolved with AP1903)	NRM in 1 pt Relapse in 4 pts
Unmanipulated grafts						
Luznik L, 2008 ¹⁶	BM	RIC	Post-SCT Cy, Tacrol, MMF	n = 68 Median age: 46 (1-71) 27 AML (12 in CR1) 4 ALL (2 in CR1) 1 MDS 6 CML/CMML 3 CLL 13 HL (refractory) 10 NHL (refractory) 3 MM (refractory) 1 PNH	Graft rejection in 9 pts (13%) Grade II-IV aGVHD 34% cGVHD CI 5% (two doses of post-SCT Cy) and 25% (one dose of post-SCT Cy)	NRM @ 1 yr: 15% EFS @ 2 yrs: 26% OS @ 2 yrs: 36% EFS longer in lymphoid vs. myeloid malignancies (p=0.02)
Brunstein CG, 2011 ¹⁷ BMTCTN 0603	BM	RIC	Post-SCT Cy, Tacrol, MMF	n=50 Median age: 48 (7-70) 22 AML 9 ALL 12 NHL 7 HL	1 pt had primary graft failure Grade II-IV aGVHD CI 32% cGVHD CI 13%	NRM @ 1 yr: 7% RI @ 1 yr: 45% PFS @ 1 yr: 48% OS @ 1 yr: 62%
Ciurea SO, 2012 ¹⁰	BM	Ablative	Post-SCT Cy, Tacrol, MMF	n=32 Median age: 45 (20-63) 16 AML/MDS 4 ALL 5 CML 5 lymphoma	94% engraftment Grade II-IV aGVHD: 20% cGVHD CI @ 1 yr: 7%	NRM @ 1yr: 16% RI @ 1yr: 34% PFS @ 1yr: 50% OS @ 1yr: 64%
Raiola AM, 2013 ²¹	BM	Ablative	Post-SCT Cy, CsA, MMF	n=50 Median age: 42 (18-66) 25 AML (9 in CR1) 12 ALL (2 in CR1) 5 lymphoma (chemorefractory) 4 MF (leukemic transformation) 4 MPD (blast crisis)	2 (4%) graft failure Grade II-IV aGVHD in 6 pts (12%) cGVHD CI 26%	6-month TRM: 18% RI: 22% (33% in pts with active disease at SCT) 18-month DFS: 51% 18-month OS: 62%
Raj K, 2014 ²³	PB	RIC	Post-SCT Cy, Tacrol, MMF	n=55 Median age: 49 (14-69) 21 AML/MDS	2 (4%) graft failure Grade II aGVHD CI: 53% Grade III aGVHD: 8%	1-yr NRM: 17%

Reference	Graft	Conditioning	Immune suppression	Patient characteristics	Engraftment and GVHD	Survival
				2 ALL 12 NHL 9 HL	cGVHD CI @ 2 yrs: 18%	Relapse in 12 pts 2-yr EFS: 51% 2-yr OS: 48%
Lee KH, 2011 ⁷⁸	PB	RIC	ATG, CsA, Mtx	n = 83 Median age: 40 (16-70) 52 AML (12 in CR1) 16 ALL (3 in CR1) 15 MDS	No primary graft failure but early PD in 4 pts Grade II-IV aGVHD in 16 pts (20%) cGVHD CI 34%	TRM CI 18% RI: 27-32% in pts with acute leukemia in CR; 79% in pts with refractory leukemia OS: 41-60% in pts with leukemia in CR; 9% in pts with refractory leukemia
Huang XJ, 2009 ²⁶	G-CSF primed BM/PB	Ablative	ATG, CsA, MMF, Mtx	n=250 Median age: 25 (2-56) 108 AML (67 in CR1) 142 ALL (82 in CR1)	249 (99%) engrafted Grade II-IV aGVHD in 115 pts (46%) Limited cGVHD in 61 (28%), extensive cGVHD in 31 (14%) pts	3-year TRM: 29% and 51% in high-risk AML and ALL 3-year RI: 20% and 49% in high-risk AML and ALL 3-year LFS: 55% and 25% in high-risk AML and ALL
Di Bartolomeo P, 2012 ²⁷	G-CSF primed BM	Ablative/RIC	ATG, CsA, Mtx, MMF, basiliximab	n=80 Median age: 37 (5-71) 45 AML (21 in CR1) 15 ALL (8 in CR1) 5 HL 5 CML 3 MDS 2 NHL 2 MF 3 MM	1 pt had primary graft failure Grade II-IV aGVHD CI 24% cGVHD CI 17%	TRM CI @ 1 yr: 36% 11 pts (14%) died on infections RI @ 3 yrs: 26-28% OS @ 3 yrs: 45% DFS @ 3 yrs: 38%