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Progress in Haploidentical Stem Cell Transplantation

Ulas D. Bayraktar[1], **Richard E. Champlin**[1], **Stefan O. Ciurea**[1]

[1]Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX.

Abstract

Haploidentical stem cell transplantation is an attractive form of transplantation due to the immediate donor availability, ease of stem cell procurement and the possibility to further collect donor cells for cellular therapy. Historically, maintaining T-cells in the graft has been associated with very high rates of graft-versus-host-disease (GVHD), while T-cell depleted haploidentical transplantation has been limited by a higher incidence of graft rejection and non-relapse mortality related to infectious complications as a result of delayed immune reconstitution post-transplant. Recent approaches have attempted to eliminate the alloreactive T-cells to prevent GVHD post-transplant. Administration of high-dose cyclophosphamide early post transplantation in combination with tacrolimus and mycophenolate mofetil has produced engraftment and GVHD rates similar to HLA-matched sibling transplants, suggesting that the most important barriers against successful haploidentical transplantation can be overcome. Future directions should focus on optimizing conditioning regimens for different diseases and prevention of disease relapse post transplant.

INTRODUCTION

Hematopoietic stem cell transplantation is the treatment of choice for patients with high risk or advanced hematologic malignancies(1). Approximately 70% of patients do not have a matched related donor available for transplantation(2). For these patients, a matched unrelated donor (MUD) transplant produces similar transplant outcomes(3, 4). However, a matched donor can be identified for only 50% to 60% of patients and the donor search and acquisition process requires a median of 4 months. Patients are most likely to have an HLA match among individuals from their own racial and ethnic group. Therefore, the chance of finding such donor varies widely among different major ethnicities(5). A recent review of all 2117 MUD transplant recipients performed at the University of Texas MD Anderson Cancer Center past 25 years revealed that 1677 patients (79.2%) were Caucasian, 271 patients (12.8%) were Hispanics, 109 (5%) were African-Americans and 33 (1.5%) were Asians. A similar racial distribution was noted for patients who received a 9/10 MUD at our institution

Corresponding author: Stefan O. Ciurea, M.D., The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 423, Houston, TX, 77030, sciurea@mdanderson.org.

during the same period of time (N=122) (79.1% Caucasians, 12.2% Hispanics, 6.5% African-Americans, 2.4% Asians). Identification of a MUD is even more challenging for mixed race individuals. Interracial/interethnic marriages are at an all time high(6) and recent data from the 2010 US Census Bureau indicates that, approximately 3% of the US population identifies itself being of mixed race, and the percentage of mixed race individuals has increased by approximately 50% as compared with the year 2000(7).

Haploidentical stem cell transplantation (HaploSCT) is an alternative treatment option for such patients. Parents, children and half of siblings are haploidentical, so these donors are readily available for most patients. The use of haploidentical related donors for transplantation has the advantage of almost universal and immediate availability of donor stem cells for transplantation and maintains the possibility to further collect donor cells for cellular therapy, if needed. Here, we review the past experience and future directions in haploidentical transplantation.

Haploidentical transplants initially performed in late 70's were associated with severe GVHD and poor outcomes(8, 9). Of 105 patients who underwent HaploSCT without T-cell depletion at Fred Hutchinson Cancer Center, almost 20% had graft failure and 70% developed GVHD(10). Powles *et al.* described a syndrome of multiorgan failure (manifested as seizure, pulmonary edema, intravascular hemolysis and renal failure) leading to death after infusion of unmanipulated haploidentical stem cells likely related to alloreactive T-cells(11).

EX VIVO T-CELL DEPLETED HAPLOIDENTICAL TRANSPLANTATION

Depletion of T-cells effectively prevents GVHD in animal models(12–14). Human trials using T-cell depleted bone marrow transplantation has been extensively evaluated(15–17). Ex vivo T-cell depleted (TCD) HaploSCT was first performed successfully in an acute leukemic infant(18). This method proved useful in preventing GVHD and was effectively used in patients with severe combined immune deficiency who cannot build a significant host immune response against the transplanted donor cells. Unfortunately, extensive T-cell depletion of the BM graft results in an increased risk of graft rejection, occurring in up to 50% of cases(19). The risk of graft rejection could be reduced by intensifying the conditioning regimen(20, 21), in vivo T-cell depletion with antibodies(22), and increasing the BM inoculum (number of CD34+ cells infused)(12).

Aversa *et al.* reported successful use of "mega-dose" TCD HaploSCT using G-CSF mobilized peripheral blood stem cells (PBSCs) and positive selection of CD34+ cells as a T-cell depletion method, obtaining $>10 \times 10^6$ CD34+ cells/kg in the final product(23). The number of T-cells in the graft was reduced significantly by 3 to 3.5 logs, and conditioning regimen was intensified with the addition of thiotepa to total body radiation and cyclophosphamide. The Perugia group achieved primary engraftment in 96 of 104 patients with a revised protocol using positively selected CD34+ PBSCs(24). Although GVHD rates were low and relapse incidence was only 16% among those transplanted in remission, non-relapse mortality (NRM) rate approached 40% primarily due to opportunistic infections, likely related to the delayed immunologic reconstitution. Furthermore, a survey of European

Blood and Marrow Transplant Group reported a NRM approaching 50% at 2 years among 266 patients with high-risk acute leukemia who underwent fully HaploSCT with TCD PBSCs(25). More than half of these deaths were due to infections, again highlighting the need for new approaches to decrease treatment-related mortality and improve the immunologic reconstitution after HaploSCT.

Positive selection of CD34+ cells depletes T-cells as well as natural killer cells (NK cells), which could be exploited to improve efficacy and safety of HaploSCT. “Alloreactive” NK cells may help eradicate the remaining leukemia cells after the conditioning regimen and clear residual recipient lymphocytes and antigen-presenting cells (APCs), potentially preventing graft rejection and GVHD(26). Furthermore, NK cells are an important part of the antiviral immunity(27), potentially adding to the fight against viral infections which are the most common cause of infectious complications post HaploSCT(28). Consequently, new regimens involving negative depletion of T-cells by immunomagnetic beads were developed(29, 30). Bethge *et al.* later adapted this approach to adults utilizing negative depletion of CD3- and CD19-positive cells and reduced intensity conditioning(31). Twenty-nine patients with hematologic malignancies underwent HaploSCT with CD3/CD19 depleted peripheral blood grafts after a reduced-intensity conditioning including fludarabine, melphalan, and thiotepa. Median CD34 cell content of the grafts was considerably less than that given by the Perugia group after CD34 positive selection (7.6×10^6/kg vs. 13.8×10^6/kg). All but one patient engrafted with full donor chimerism. Although regimen was well tolerated, NRM in the first 100 days approached 20%. Incidence of grade II-IV GVHD was 48%. Twenty patients died, 12 due to relapse, 7 due to infections, and 1 due to GVHD. One-year OS remained 35%. Although this approach demonstrated that megadoses of stem cells higher than 10×10^6/kg and full myeloablative conditioning were not required for successful engraftment in HaploSCT, it was complicated with higher relapse rates, possibly due to reduced intensity of the conditioning and persistently delayed immune reconstitution.

IMPROVEMENTS IN EX VIVO T-CELL DEPLETED HAPLOIDENTICAL TRANSPLANTATION

Infusion of Regulatory T-cells

Regulatory T-cells (Tregs) suppress immune reactivity maintaining tolerance to self-antigens, and depletion of Tregs results in a spectrum of autoimmune diseases(32–35). In murine models of HLA-mismatched transplantation, Tregs suppressed lethal GVHD(36), and favored post-transplant immune reconstitution when coinfused with conventional T-cells(37). The Perugia group recently reported on a protocol using infusion of donor Tregs following a T-cell depleted haploidentical transplant as a means to further reduce the risk of GVHD(38). Donor Tregs were selected and infused after a myeloablative conditioning regimen followed, 4 days later, by infusion of TCD mega-dose PBSCs and donor conventional T-cells (Tcons). No post-transplant immunosuppression was administered. Of 28 patients treated, 26 achieved primary sustained engraftment while 2 patients developed aGVHD. No patient developed cGVHD. A wide T-cell repertoire developed rapidly. Thirteen patients died, 8 due to opportunistic infections. At a median follow-up of 12 months, 12 patients were alive and disease-free. This study demonstrated the feasibility of

adoptive immunotherapy with Tregs and their potential application to modify GVHD and enhance immune reconstitution after HSCT. However, the high treatment-related mortality of 50% in this group of patients remains a concern(38).

Infusion of Selectively Allodepleted T-cells

Although the use of Tregs with Tcons in this TCD transplant model may improve early immune reconstitution, Tregs may also have an inhibitory effect on desirable bystander T-cell responses(39, 40). Alternatively, infusion of T-cells depleted of cells alloreactive to recipient antigens may improve immune reconstitution while preserving graft-versus-tumor effect, without causing GVHD. Currently available alloreactive T-cell depletion methods rely on cocultures with recipients' cells to activate the alloreactive cells followed by either targeting the surface activation markers or using photoactive dyes which are preferentially retained in activated T-cells(41).

Amrolia *et al.* investigated the use of an anti-CD25 immunotoxin to deplete alloreactive lymphocytes in TCD HaploSCT patients. This group infused 10^4-10^5 cells/kg allodepleted lymphocytes on 30, 60, and 90 days post transplant in 16 patients (median age 9 years)(42). One patient developed graft failure and subsequently had autologous reconstitution. Two patients developed grade II and IV acute GVHD. Patients who received higher dose of lymphocytes exhibited more rapid recovery of T-cells. A higher polyclonal distribution of Vβ receptor gene was noted at 4 months after transplant compared with retrospective controls who did not receive T-cell add back. However, at a median follow-up of 33 months, 9 patients died (56%) due to relapse disease (5), infection (3), and interstitial pneumonitis (1)(42). Despite its small size, this study confirmed the safety of the addition of selectively allodepleted donor T-cells after HaploSCT. However, it should be noted that the allodepletion method based on CD25 expression also depletes Tregs. Further studies are needed to assess the efficacy of this approach.

Anti-HLA antibodies and Graft Rejection in TCD HaploSCT

To address the high toxicity of the myeloablative, TBI-based conditioning regimens used in the fore-mentioned trials, we studied the feasibility of a myeloablative yet reduced-intensity conditioning regimen consisting of fludarabine, melphalan, thiotepa (FMT) for patients with advanced hematological malignancies undergoing TCD HaploSCT(43). Of 28 patients enrolled in this phase II trial, 22 (79%) achieved primary engraftment while 5 achieved secondary engraftment either after a second transplant (n=4) or infusion of cryopreserved autologous cells (n=1). None of the patients developed grade III-IV aGVHD and 4 out of 21 patients developed cGVHD as seen in the European trials after TCD HaploSCT. NRM was 40% at 1 year and most of the deaths were related to infections, which made us to change our approach to using a TCR allograft to improve immune reconstitution post transplant and hopefully decrease the NRM associated with infectious complications(44). In addition, this study revealed a higher rate of graft failure in the TCD HaploSCT patients even if megadoses of CD34+ cells were used (median number of CD34+ cells: 10.2×10^6/kg). This prompted us to look for other causes of graft rejection in these patients and studied the relationship between donor-specific anti-HLA antibodies (DSA) identified using a solid-phase fluorescent assay and graft failure, based on the association found between anti-HLA

antibodies and graft rejection in solid organ transplantation(45, 46). Twenty-four patients were tested for the presence of DSA in pretransplant serum specimens. Three of 4 patients (75%) with DSA at the time of transplantation developed primary graft failure compared with only 1 of 20 patients (5%) who did not have DSA, suggesting that the presence of DSA is an important cause of graft rejection in patients undergoing TCD HaploSCT(47). Future studies should attempt to decrease antibody levels prior to infusion of CD34+ cells to prevent graft failure, if another donor is not available for such patients.

T-CELL REPLETE (TCR) HAPLOIDENTICAL TRANSPLANTATION

Due to delayed immunologic reconstitution and higher treatment-related mortality after TCD HaploSCT, alternative transplant options have been sought. Maintaining the T-cells in the graft while effectively preventing the development of GVHD post transplant could represent a viable alternative to TCD HaploSCT. Furthermore, preservation of the graft T-cell content and subsequent improvement in engraftment enables non-myeloablative conditioning regimens to be used in HaploSCT, expanding the pool of patients.

High-dose Post-transplant Cyclophosphamide for GVHD Prevention

Historical experience clearly showed that infusion of a TCR haploidentical graft without effective GVHD prevention was associated with unacceptable toxicity(10). Probably, one of the most promising ways of eliminating alloreactive T-cells responsible for both graft rejection and GVHD is using cyclophosphamide (Cy) in the immediate post-transplantation period, when the graft and host T-cells recognize each other as foreign and generate bidirectional alloreactivity. The use of post-transplant Cy was initially used in the 1960's by Barenbaum and Brown, who showed that it can prevent skin graft rejection when administered 2–3 days after allografting in a mouse model(48). Similarly, its use in the early post-transplant period has been shown to eliminate alloreactive T-cells and facilitate engraftment of donor cells as the hematopoietic stem cells are quiescent cells, resistant to cytotoxic chemotherapy due to their high levels of aldehyde dehydrogenase(49).

Luznik and colleagues subsequently showed that post-transplant Cy can attenuate lethal and non-lethal GVHD in mice and prolong their survival(50). O'Donnell *et al.* demonstrated the feasibility of using post-transplant Cy in a small cohort of patients with high-risk hematological malignancies treated with a non-myeloablative conditioning regimen, TCR haploidentical BM stem cells, and post-transplant Cy of 50 mg/kg on day 3 after transplant(51). Relatively low rates of graft failure and GVHD were noted among 13 patients treated(51). In a more recent update, Luznik *et al.* used Cy on post-transplant days 3 and 4 and intensified mycophenolate mofetil (MMF) dosing from twice to thrice daily, to further decrease the graft failure and GVHD rates(52). While graft rejection occurred in 9 of 66 evaluable patients, 8 of those experienced recovery of autologous hematopoiesis. Grade III-IV aGVHD incidence was 6%. Chronic GVHD (cGVHD) incidence was lower among those who received two doses of post-transplant Cy (5%) compared to those who received one dose (25%). Although NRM rate was relatively low at 15% at 1 year post-transplant, relapse incidence at 2 years was 58%(52).

Despite the success of using post-transplant Cy in reducing GVHD and graft failure rates without increased NRM rate, relapses arose as a major treatment failure which could be attributed primarily to the use of non-myeloablative conditioning, especially for patients with myeloid malignancies and acute leukemias. Recently, the Johns Hopkins group presented their findings in 17 patients after HaploSCT using myeloablative conditioning with busulfan, Cy, and total body irradiation; and post-transplant Cy(53). The cumulative incidence of NRM at 100 days was higher at 18% while GVHD rates were acceptable and none of the evaluable patients had graft rejection(53). However, data is not mature and further studies are needed to establish the safety and efficacy of post-transplant Cy after myeloablative conditioning.

More recently, Blood and Marrow Transplant Clinical Trials Network conducted two parallel multi-center phase II trials of double umbilical cord blood transplantation and TCR HaploSCT for individuals with lymphoma or leukemia(54). The conditioning regimen and GVHD prophylaxis in the HaploSCT trial were identical to those previously reported by Luznik *et al.*(52). One-year NRM and PFS were 7% and 48%, reproducing Johns Hopkins' results in a multi-center trial. Yet again, relapse was the primary cause of death, attributed primarily to the use of non-myeloablative conditioning for patients with leukemia, which represented more than half the patients treated on this trial.

We are investigating the use of post-transplant Cy in a phase II clinical trial ongoing at MD Anderson Cancer Center. To date more than 40 patients were treated and outcomes for the first 24 consecutive patients were recently reported in abstract format(44). Patients received the same conditioning regimen (FMT) previously reported by us in TCD HaploSCT, followed by post-transplant Cy on days +3 and +4, tacrolimus and MMF. Median age was 47 years (range, 24–65 years) and 66% were ethnic minority patients. All 23 evaluable patients engrafted with 100% donor cells after a median of 19 days. Day 100 NRM was 14% for first transplants, and no patient < 50 years of age died due to treatment-related mortality. Grade II-IV aGVHD occurred in only 4 patients, all immediately after the MMF was abruptly discontinued on day 35 post-transplant. We are now continuing MMF until day 100 post-transplant and taper weekly thereafter. After a median follow-up of 6 months for these patients (range 3–22 months), overall survival was 71% for first transplants and progression-free survival was 80% for patients in remission at the time of transplant. No patient died of NRM after 6 months in this group. These early results suggest that outcomes with TCR HaploSCT are better compared with our previous experience with TCD HaploSCT primarily due to improved immune recovery post-transplant. Longer follow-up is necessary to confirm these findings(44).

Alloanergized HaploSCT after *ex vivo* Costimulatory Blockade

T-cell activation requires 2 signals from antigen presenting cells (APCs): Displayment of an immunogenic peptide on major histocompatibility complex (MHC) to T-cell receptor and a costimulatory signal, most commonly through CD80/86 on APCs to CD28 receptor on T-cells. Blockade of the latter may result in induction of anergy(55) and could allow successful transplantation of histoincompatible allografts(56). Guinan *et al.* demonstrated 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-Ig(57). CTLA-4 is a counterreceptor for CD80/86 and has a much higher affinity for it than CD28. Of 12 patients transplanted, 1 died early post-transplant, 11 patients achieved sustained engraftment, while 3 had aGVHD. No deaths due to GVHD occurred in this group. In a recent update, Davies *et al.* reported their experience in 24 patients with high-risk hematological malignancies or BM failure(58). Five patients developed severe aGVHD and 12 patients died within 200 days of transplantation (5 due to infection). 8 patients were alive and free of disease with a median follow-up of 7 years. Of concern, none of the patients older than 18 years survived the first 200 days. A similar protocol revised to minimize the early transplant related mortality using reduced intensity conditioning and megadoses CD34+ HSCT is currently in trials.

The Combination of G-CSF Primed Bone Marrow and Mobilized PBSCs

G-CSF can induce T-cell hyporesponsiveness and skewing towards a T_{H2} phenotype through an increase in plasmacytoid dendritic cells and downregulation of CD28-CD80/86 signaling(59, 60). Based on these findings, Chinese researchers developed a HaploSCT protocol utilizing myeloablative conditioning, intensified immunologic suppression with Anti-thymocyte globulin (ATG), and donor graft composed of G-CSF primed bone marrow and PBSCs(61). In their most recent update including 250 acute leukemic patients(62), of whom 149 (60%) were transplanted while in CR1 with standard-risk genetics, donors were treated with G-CSF 5 mg/kg/day subcutaneously and BM cells were harvested on 4^{th} day of G-CSF followed by collection of PBSCs on 5^{th} day. GVHD prophylaxis included cyclosporine, MMF (both initiated on transplant day −9), ATG 2.5 mg/kg from days −5 to −2, and methotrexate on days +3, +6, and +11. Early post-transplant mortality rate approached 13% and cumulative incidence of grade 2–4 aGVHD was relatively high at 45.8%. The cumulative incidence of cGVHD was 53.9% at 3 years, which comes in sharp contrast with cGVHD rates obtained with post-transplant Cy. Overall, the 3-year cumulative incidence of relapse was less than 20% and leukemia-free survival approached 70% among AML patients with standard risk disease (CR1 or CR2 without Philadelphia chromosome) (62). Even though a higher disease-free survival was achieved – partly due to inclusion of standard- and good-risk patients, the concern remains that a higher incidence of GVHD is associated usually with a higher treatment-related mortality and higher cost of care for these patients.

In Vivo Depletion of T-cells

The anti-CD52 antibody, alemtuzumab (Campath®), has been used for in vivo depletion of host and donor T-cells to increase engraftment and decrease GVHD rates in transplants from matched sibling or unrelated donors(63–65). Rizzieri *et al.* treated 49 patients with hematological malignancies utilizing nonmyeloablative conditioning and alemtuzumab(66). Preparative regimen included fludarabine and Cy on days −5 to −2, and alemtuzumab 20 mg/day on days −4 to 0. Further GVHD prophylaxis included MMF 2 g/day for 45 days, with or without cyclosporine. Three and four patients experienced primary and secondary graft failure. Twenty-four (49%) and 11 (22%) patients died of progressive disease and infections, respectively, while 2 (4%) patients died of post-transplantation lymphoproliferative disease. One-year OS was 31%. The relatively high relapse rate observed was attributed partly due to the reduced intensity of the conditioning. However,

disease relapse rate may be further increased by the use of alemtuzumab, as recently reported in a Center for International Blood and Marrow Transplant Research registry analysis(67).

Natural Killer Cells in HaploSCT

As previously detailed, NK cell alloreactivity may be exploited to improve the efficacy and safety of HaploSCT. It is thought that NK cells recognize their targets through both inhibitory and activating receptors. Various algorithms explaining NK cell alloreactivity have been proposed(26, 68–70). 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(26, 71). NK cells attack primarily hematopoietic cells sparing the solid organs, rendering them almost incapable of causing GVHD(72). Therefore, if the recipient cells lack the HLA class I alleles specific to the donor KIRs, donor NK cells may decrease the risk of GVHD and disease relapse by killing the residual recipient APCs and leukemia cells. Furthermore, following stem cell transplantation, including TCD HaploSCT, NK cells are the first lymphoid cells to recover by rapid differentiation from engrafted stem cells(73).

Several studies evaluated the feasibility of natural killer cell infusions after HaploSCT to utilize innate immunity against different tumors(74–76). Recently, Yoon *et al.* reported on a series of 14 patients with acute leukemia or myelodysplastic syndromes in which patients were infused with donor NK cells derived from CD34+ hematopoietic cells, 6–7 weeks after TCR HaploSCT(77). There were no acute side effects with 4 patients developing cGVHD. Four patients were alive and disease-free 18–21 months post-transplant. Two patients who received NK cell infusion during active leukemia did not have a response(77). Prospective studies are needed to explore the use of NK cells post HaploSCT.

DONOR SELECTION

Most patients have more than one potential haploidentical donor and various factors have been implicated in selection of the most suitable donor for HaploSCT. We provide a summary of the most relevant studies which involve various factors considered in the decision to use one haploidentical donor versus another.

KIR Mismatch

KIR mismatch between recipient and donor has been associated with improved outcomes after HaploSCT in several studies(78, 79). Ruggeri *et al.* reported improved graft rejection, GVHD, and disease relapse rates among patients with AML who received stem cells from donors with KIR mismatch in the graft-versus-host direction compared to those without(78). More recently, Symons *et al.* reported similar results in a cohort of 86 patients with various hematological malignancies who underwent TCR HaploSCT with non-myeloablative conditioning and post-transplant Cy with improved NRM, OS, and EFS among those transplanted with KIR mismatch donors compared to those without(79). Conversely, Huang *et al.* found KIR mismatch to be an independent risk factor for aGVHD, relapse, and decreased OS in a cohort of 116 patients after TCR Haplo SCT using myeloablative

conditioning(80). The conflicting results may be partly due to differences in stem cell sources, treated diseases, type of conditioning and variations in the definition of KIR mismatch. Although NK cell alloreactivity is likely to play a role in the success of HaploSCT, further studies are needed to better define the role of KIR mismatch in donor selection and exploit the NK alloractivity to improve outcomes post transplant.

Mismatched Maternal HLA antigens

Several clinical observations suggested that the development of immunological tolerance between mother and fetus during pregnancy(81, 82) could impact the transplant outcomes due to a lifelong downregulation of immune responses if the mismatched haplotype is of maternal origin, as happens in transplants from a mother to her sibling, or between siblings mismatched for non-inherited maternal HLA antigens (NIMA) as compared with non-inherited paternal antigens (NIPA). Accordingly, patients with maternal donors were found to have longer overall survival after HaploSCT compared to those with paternal donors in a Japanese registry study(83). Subsequently, van Rood et al. demonstrated lower acute and chronic GVHD rates and lower treatment-related mortality in T-cell replete haploidentical transplant recipients NIMA compared with NIPA mismatched(84). Separate studies later confirmed these findings in patients transplanted from NIMA compared with NIPA mismatch donors after both myeloablative and non-myeloablative regimens(85, 86).

Number of HLA Mismatches between the Donor and Recipient

Historically, increasing degrees of HLA mismatch have been associated with shorter survival and higher GVHD rates after Haplo SCT(10, 87, 88). Recently, the Johns Hopkins group reported that greater HLA disparity was not associated with worse outcomes after TCR HaploSCT with post-transplant Cy(89). In this retrospective analysis of 185 patients with various hematological malignancies, having 3 or 4 total antigen or allele mismatches was not associated with increased risk of grade II-IV aGVHD as compared with fewer mismatches. Moreover, in multivariate analysis, the event-free survival of patients having 3 or 4 total antigen or allele mismatches appeared to be better compared to those with fewer mismatches due to a lower relapse rate(89). Although limited by its retrospective nature, this study suggests that, by using post-transplant Cy, the higher treatment-related mortality rates associated usually with more mismatches can be eliminated and improved outcomes could be potentially achieved compared with matched transplantation, as recently showed by the Chinese group(90).

A multivariate analysis in a large study is needed to further elucidate the role of these factors in donor selection for HaploSCT.

FUTURE DIRECTIONS

Due to the universal and immediate availability of haploidentical related donors for almost all patients including those from minority groups or with mixed race, and lower cost of HaploSCT as compared with unrelated donor transplantation, improvement in this form of transplantation is warranted. Although various methods have been used to overcome the significant HLA-barriers in HaploSCT, so far none has excelled over another. However, we

are encouraged by the use of post-transplant Cy as it provides a straight forward, effective way to control GVHD post-transplant without affecting engraftment. This approach limits treatment-related mortality due to GVHD and possible infectious complications which in our experience occur more frequently in TCD HaploSCT. However, relapse after HaploSCT remains an issue as depletion of alloreactive T cells eliminates graft-versus-leukemia effect, regardless of the method used. Future directions will likely include improvement in conditioning regimens tailored to myeloid and lymphoid diseases, the use of cellular therapy post-transplant in an attempt to decrease disease relapse and possible replacement of cyclophosphamide with other drugs to selectively deplete alloreactive T-cells post transplant in the future.

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