

Advances in haploidentical stem cell transplantation

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Hematopoietic stem cell transplantation from haploidentical donors is an attractive method of transplantation due to the immediate donor availability, ease of stem cell procurement and the possibility to collect additional donor cells for cellular therapy, if needed. Historically, maintaining T-cells in the graft has been associated with very high rates of graft-versus-host disease, while T-cell depleted haploidentical transplantation has been limited by a higher incidence of graft rejection and delayed immune reconstitution post-transplant. Recent approaches attempt to maintain the T-cells in the graft while effectively preventing the development of graft-versus-host disease post-transplant. Selective depletion of alloreactive T-cells post-transplant using high-dose post-transplant cyclophosphamide is under investigation as a promising alternative in haploidentical transplantation. While engraftment has improved and graft-versus-host disease is controlled with this approach, future directions should focus on optimizing conditioning regimens and the prevention of disease relapse post-transplant.

Keywords: Hematopoietic stem cell transplantation; Hematologic neoplasms; Bone marrow transplantation; T-lymphocytes/immunology

Introduction

Hematopoietic stem cell transplantation is the treatment of choice for patients with high risk or advanced hematologic malignancies.⁽¹⁾ However, approximately 70% of patients do not have a matched related donor available for transplantation.⁽²⁾ For these patients, a matched unrelated donor (MUD) transplant is preferred, due to similar transplant outcomes.^(3,4)

MUD transplants, on the other hand, are limited by the fact that a matched donor can be identified for only 50% to 60% of patients who need them and the donor search and acquisition of an unrelated donor hematopoietic stem cell product takes much longer than the use of a related donor transplant (approximately three months). Probably the most important problem is that the chance of finding a MUD for a non-Caucasian individual varies widely among different ethnic minority groups. A recent review of all 2117 MUD transplants performed at M.D. Anderson Cancer Center (MDACC) during the 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 (n = 122) during the same period of time (79.1% Caucasians, 12.2% Hispanics, 6.5% African-Americans and 2.4% Asians).

Finding a MUD becomes even more challenging for mixed race individuals for whom the chance to find a matched donor in the registries is low. Interracial/interethnic marriages are at an all-time high in the U.S.⁽⁵⁾ Recent data from the 2010 U.S. Census Bureau indicates that approximately 3% of the U.S. population identifies itself as being of mixed race, and the percentage of mixed race individuals has increased by approximately 50% compared to the year 2000.

Haploidentical stem cell transplantation (HaploSCT) is an alternative treatment option for such patients. Most individuals will have a relative (parents, offspring, or siblings) that matches in at least one human leukocyte antigen (HLA) haplotype, unless they were adopted. The demographic changes at least in the U.S. clearly emphasize the need to further develop this form of transplant.

The use of mismatched related donors for transplantation has the advantage of almost universal and immediate availability of donor stem cells for transplantation and maintains the possibility to collect additional donor cells for cellular therapy, if needed. Here, we review the past experience and future directions in haploidentical transplantation.

T-cell depleted haploidentical transplantation

The first haploidentical transplants performed in the late 1970s led to severe graft-versus-host disease (GvHD) in most patients.^(6,7) Of 105 patients who underwent HaploSCT

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without T-cell depletion at Fred Hutchinson Cancer Center, almost 20% had graft failure and 70% developed GvHD.⁽⁸⁾ Several new complications were noted. Powles described a syndrome of multiorgan failure (seizure, pulmonary edema, intravascular hemolysis and renal failure) leading to death after infusion of unmanipulated haploidentical stem cells which was probably related to alloreactive T-cells.⁽⁹⁾

To prevent GvHD after HaploSCT *in vitro* T-cell depletion of the bone marrow (BM) inoculum was first performed successfully in an acute leukemic infant.⁽¹⁰⁾ This method proved useful in preventing GvHD and was effectively used in patients with severe combined immune deficiency who could not build a significant host immune response against the transplanted donor cells. Unfortunately, extensive T-cell depletion of the BM graft in leukemic patients resulted in graft rejection in up to 50% of cases.⁽¹¹⁾ Various experiments in mouse models subsequently revealed that the graft rejection could be overcome by intensifying the conditioning regimen,^(12,13) *in vivo* T-cell depletion with antibodies⁽¹⁴⁾ and increasing the BM inoculum (number of CD34⁺ cells infused).⁽¹⁵⁾

Aversa et al. were the first to clinically use an increased number of CD34⁺ cells ("mega-doses") in T-cell depleted (TCD) HaploSCT by adding G-CSF mobilized peripheral blood stem cells (PBSCs) to the BM inoculum, thus obtaining $> 10 \times 10^6$ CD34⁺ cells/kg in the final product.⁽¹⁶⁾ In addition to increasing the number of CD34⁺ cells infused, the number of T-cells in the graft was reduced significantly by 3 to 3.5 logs, and the conditioning regimen was intensified with the addition of thiotepa. As a result, 16 of the 17 leukemic patients achieved sustained engraftment with only one patient developing acute GvHD (aGvHD). In a follow-up report, the Perugia group achieved primary engraftment in 96 of 104 patients with a revised protocol using positively selected CD34⁺ PBSCs without BM cells.⁽¹⁷⁾ Although GvHD rates were low and relapse incidence was only 16% among those who were in remission at the time of transplant, the non-relapse mortality (NRM) rate approached 40% primarily due to opportunistic infections, a direct consequence of T-cell depletion and delayed immune reconstitution. Furthermore, a survey of the European Blood and Marrow Transplant Group demonstrated a NRM approaching 50% at two years among 266 patients with high-risk acute leukemia who underwent fully HaploSCT with *ex vivo* TCD PBSCs. 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 TCD HaploSCT.

In order to improve the immunologic reconstitution post-transplant, the Perugia group recently reported on a protocol using donor regulatory T-cells (Tregs).⁽¹⁸⁾ Tregs suppress immune function maintaining tolerance to self-antigens, while the depletion of Tregs results in a spectrum of autoimmune diseases. In murine models of hematopoietic stem cell transplant (HSCT) across HLA barriers, Tregs suppressed lethal GvHD⁽¹⁹⁾ and favored post-transplant immune reconstitution when co-infused with other T-cell

subsets.⁽²⁰⁾ In the Perugia protocol, donor Tregs were selected and infused after a myeloablative conditioning regimen followed four days later by an mega-dose infusion of TCD PBSCs and conventional donor T-cells. No post-transplant immunosuppression was given. Of 28 patients treated, 26 achieved primary sustained engraftment while two patients developed aGvHD. No patient developed chronic GvHD (cGvHD). A wide T-cell repertoire developed rapidly. Thirteen patients died, eight due to opportunistic infections. At a median follow-up of 12 months, 12 patients were alive and disease-free. This study demonstrated that adopting immunotherapy with Tregs counteracted the GvHD potential of conventional T-cells in HaploSCT, and could potentially be used to fasten immune reconstitution after HSCT without significantly increasing the incidence of GvHD.

To address the high toxicity of the myeloablative total body irradiation (TBI)-based conditioning regimens used in the aforementioned trials, we studied the feasibility of a myeloablative yet reduced-intensity conditioning regimen consisting of fludarabine, melphalan and thiotepa (FMT) for patients with advanced hematological malignancies undergoing TCD HaploSCT.⁽²¹⁾ Of 28 patients enrolled in this phase II trial, 22 (79%) achieved primary engraftment while five 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 four out of 21 patients developed cGvHD confirming the low rate of GvHD seen in the European trials after TCD HaploSCT. Twelve patients relapsed and all relapses occurred in those with active disease at the time of transplant. NRM was 40% at one year and most of the deaths were related to infections. This study demonstrated the feasibility of the FMT regimen and confirmed the high NRM from infectious complications associated with TCD HaploSCT.

Based on the association found between solid organ rejection and the presence of anti-HLA antibodies,^(22,23) we also evaluated the impact of donor specific anti-HLA antibodies (DSA) in the development of graft failure after HaploSCT.⁽²⁴⁾ Twenty-four patients were tested for the presence of DSA in pre-transplant serum specimens. Three of four patients (75%) with DSA at the time of transplantation developed primary graft failure (PGF) compared with only one of twenty patients (5%) who did not have DSA. Furthermore, two patients who had a significant decrease in DSA levels after being treated with rituximab/plasma exchange achieved primary engraftment. These results suggest that the presence of DSA is associated with a high rate of graft rejection in patients undergoing TCD HaploSCT.⁽²⁴⁾

T-cell replete haploidentical transplantation

Due to high incidence of opportunistic infections and NRM after TCD HaploSCT, alternative transplant options have been sought. Maintaining the T-cells in the graft while effectively preventing GvHD could represent a

viable alternative. To date, three notable approaches emerged using T-cell replete (TCR) stem cell products in HaploSCT.

High-dose post-transplant cyclophosphamide for GvHD prevention

Historical experience clearly shows that infusion of a TCR haploidentical graft without effective GvHD prevention is associated with unacceptable toxicity. Cyclophosphamide (Cy) appears to deplete alloreactive T-cells responsible for both graft rejection and GvHD when used in the immediate post-transplantation period. The use of post-transplant Cy was proposed in the 1960s by Barenbaum and Brown, who showed that high-dose post-transplant cyclophosphamide (HDPTCy) can prevent skin graft rejection when administered 2-4 days after allografting.⁽²⁵⁾ The drug eliminates alloreactive T-cells and facilitates donor cell engraftment, while preserving hematopoietic stem cells that are resistant to cytotoxic chemotherapy due to high levels of expression of aldehyde dehydrogenase.⁽²⁶⁾

Luznik et al. subsequently showed that HDPTCy can attenuate lethal and non-lethal GvHD in mice and prolong their survival.⁽²⁷⁾ O'Donnell et al. showed the feasibility of this approach in humans, with relatively low rates of graft failure and GvHD in 13 patients with high-risk hematological malignancies treated with TCR haploidentical BM stem cells, using a non-myeloablative conditioning regimen and HDPTCy (50 mg/kg on day 3 after transplant).⁽²⁸⁾ In a more recent update, Luznik et al. used Cy on post-transplant days 3 and 4 with mycophenolate mofetil (MMF) thrice daily.⁽²⁹⁾ While graft rejection occurred in nine of 66 evaluable patients, eight of those experienced recovery of autologous hematopoiesis. The incidence of grade III-IV aGvHD was 6%. The incidence of cGVHD was lower among those who received two doses of post-transplant Cy (5%) compared to those who received one dose (25%). Although the NRM rate was relatively low at 15% at one year post-transplant, relapse incidence at two years was 58%.

Despite the success of using HDPTCy in reducing GvHD and graft failure rates without increasing the NRM rate, high relapse rates were attributed primarily to the non-myeloablative conditioning used. Recently, the Johns Hopkins group presented their findings of 17 patients treated with HaploSCT using myeloablative conditioning and HDPTCy.⁽³⁰⁾ The early mortality rate was 18%, while GvHD rates were acceptable. None of the evaluable patients rejected the graft. However, data is not mature and further studies are needed to establish the safety and efficacy of HDPTCy after myeloablative regimens.

We are investigating the use of HDPTCy in an ongoing phase II clinical trial at MD Anderson Cancer Center. To date more than forty patients were treated and outcomes for the first 24 consecutive patients were recently reported.⁽³¹⁾ Patients received the FMT regimen followed by HDPTCy on days +3 and +4, in addition to tacrolimus and MMF. Median

age was 47 years (range: 24 - 65 years) and 66% were members of ethnic minorities. All evaluable 23 patients engrafted neutrophils 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. Grade II-IV aGvHD occurred in only four patients. After a median follow-up of six months for survivors (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. These preliminary results suggest that outcomes with TCR HaploSCT are better than our previous experience with TCD HaploSCT; however, a longer follow-up is necessary to confirm these findings.⁽³¹⁾

The combination of G-CSF primed bone marrow and mobilized PBSCs

Granulocyte colony stimulating factor (G-CSF) can induce T-cell hyporesponsiveness and skew towards a TH2 phenotype through an increase in plasmacytoid dendritic cells and downregulation of CD28-CD80/86 signals.^(32,33) Based on this assumption, Chinese researchers developed the GIAC protocol which stands for donor treatment with G-CSF, Intensified immunologic suppression, Anti-thymocyte (ATG), and Combination of PBSCs and G-BM.⁽³⁴⁾ The most recent update included 250 acute leukemia patients⁽³⁵⁾ of whom 149 (60%) were transplanted while in first remission with standard-risk genetics. Donors were treated with G-CSF 5 mg/kg/day subcutaneously and BM cells were harvested on the 4th day of G-CSF, while PBSCs were collected on the 5th day. BM and PBSCs were infused fresh and unmanipulated. Patients received a myeloablative conditioning regimen and GvHD prophylaxis included cyclosporine, MMF (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 three years. 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. These results obtained in a better risk patient population would suggest that survival rates similar to those achieved with matched related transplants are possible with HaploSCT. The higher incidence of GvHD observed here is a source of concern, however.

Alloenergized HaploSCT after *ex vivo* costimulatory blockade

T-cell activation requires two signals from antigen presenting cells (APCs): presentation of an immunogenic peptide on the major histocompatibility complex (MHC) to the T-cell receptor and a costimulatory signal, most commonly through CD80/86 on APCs to CD28 on T-cells. Blockade of the latter results in anergy induction⁽³⁶⁾ and permits successful

transplantation of incompatible allografts.⁽³⁷⁾ Guinan et al. demonstrated the feasibility of HaploSCT using BM graft of which donor T-cells were anergized through incubation with the recipient's mononuclear cells and cytotoxic T lymphocyte antigen 4 (CTLA-4-Ig).⁽³⁸⁾ CTLA-4 is a counter receptor for CD80/86 and has a much higher affinity for it than CD28. Of 12 patients transplanted, one died early post-transplant, 11 patients achieved sustained engraftment, while three had aGvHD. No deaths occurred due to GvHD. In a recent update, Davies et al. reported their experience in 24 patients with high-risk hematological malignancies or BM failure.⁽³⁹⁾ Five patients developed severe aGvHD and 12 patients died within 200 days of transplantation (five due to infection). Eight patients were alive disease free with a median follow-up of seven years. Of concern, none of the patients older than 18 years survived the first 200 days. A similar protocol revised to minimize early NRM using reduced intensity conditioning and mega-doses of CD34⁺ cells is currently under investigation.

Natural killer cell therapy

Natural killer (NK) cells kill cells lacking MHC class I molecules specific to the inhibitory receptors (killer immunoglobulin-like receptors, KIRs) on the NK cells. Furthermore, NK cells spare solid organs while attacking primarily hematopoietic cells, rendering them almost incapable of causing GvHD.⁽⁴⁰⁾ After HaploSCT, if donor NK cells express KIRs that are not engaged by any of the class I MHC molecules on the recipient cells, these "alloreactive" NK cells may help eradicate remaining leukemia cells, and to clear residual lymphocytes and APCs, potentially preventing graft rejection and GvHD.⁽⁴¹⁾

Donor/recipient KIR mismatch has been associated with improved outcomes after HaploSCT.^(42,43) Consequently, the feasibility of NK cell infusions after HaploSCT has been evaluated. A pediatric case series of CD3-CD56⁺ selected donor NK cell infusions demonstrated promising results.^(44,45) Recently, Yoon et al. reported 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 transplant.⁽⁴⁶⁾ There were no acute side effects with four patients developing cGvHD. Four patients were alive and disease-free 18-21 months post-transplant. Two patients who received NK cell infusions during active leukemia did not have a response. We are currently exploring the use of NK cells in a phase I/II clinical trial for patients with advanced hematologic malignancies aiming at preventing disease relapse post-transplant.

Future directions

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 HDPTCy as it provides a straight forward,

simple and effective way to control GvHD without affecting engraftment. This approach limits NRM associated with GvHD. 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 Cy with other drugs to selectively deplete alloreactive T-cells post-transplant.

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