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Haploidentical Transplantation for Leukemia

Junya Kanda, MD¹, Nelson J. Chao, MD¹, and David A. Rizzieri, MD¹

¹Division of Cellular Therapy, Department of Medicine, Duke University Medical Center, Durham, NC

Abstract

Hematopoietic stem cell transplantation from human leukocyte antigen (HLA)-haploidentical family members offers a potential cure for patients in need of allogeneic immunotherapy who have no immediate access to an HLA-matched donor. The use of ex vivo T-cell-depleted stem cells combined with immuno-myeloablative conditioning has enabled durable donor engraftment with a low incidence of acute graft-versus-host disease despite the HLA disparity. Moreover, additional transplant techniques involving in vivo T-cell-depletion and reduced-intensity conditioning have further minimized the risks. However, a major drawback is delayed immune reconstitution leading to infections and high relapse rates, prompting significant research efforts focused on improving recovery in the post-transplant period. Infusions with donor lymphocytes are common, though newer manipulations with a focus on donor natural killer cells hold great promise as does other modified donor T-cell infusions. Success of these new procedures will make haploidentical transplants safer and more effective, further broadening its appeal.

Keywords

haploidentical transplantation; T-cell-depletion method; immune recovery; donor lymphocyte infusion; KIR ligand mismatch; NK cell infusion; interleukin-2

Introduction

Hematopoietic stem cell transplantation offers a potential cure for patients with leukemia or lymphoma at high risk of progression following conventional chemotherapeutic agents. Though a human leukocyte antigen (HLA)-identical sibling is the preferred donor because complications such as graft-versus-host disease (GVHD) and graft failure increase according to the number of HLA disparities between donor and recipient [1], in most developed countries, less than 30% of patients have an HLA-identical sibling. An HLA-matched unrelated donor is a good alternative [2], and though the number of volunteers in donor registries is increasing, their use is still limited by availability and the time delay required to arrange and actually receive a graft. Unrelated cord blood is a new promising stem cell source; however, the limited unit size, high incidence of post-transplant infections, and slow immune recovery are obstacles to its broader application [3,4].

To address current limitations in allogeneic transplant donor availability, HLA-mismatched, related donor has developed into an attractive alternative. In addition to potential sibling haplotypes matches, parents and children of patients share one HLA haplotype, thus a donor can be obtained for nearly all patients. Further, it allows for transplantation in rapid fashion

Corresponding author: David Rizzieri, MD, Associate Professor of Medicine, Division of Cellular Therapy, Department of Medicine, Duke University Medical Center, 2400 Pratt Street, suite 1100, Durham, NC 27705, USA.

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following successful induction of the patient and provides a foundation for additional cellular therapy after transplant recovery to improve long term outcomes.

A past report from Seattle evaluated the effect of HLA incompatibility on clinical outcomes after ablative, unmodified bone marrow transplantation from an HLA-matched or HLA-mismatched related donor [1]. Although the probability of survival for patients who underwent transplantation from an HLA 1-locus-mismatched family member was similar to that for patients who received a graft from an HLA-identical sibling, that for patients who underwent transplantation from an HLA 2- or 3-locus-mismatched family member was extremely poor, owing to the high incidence of graft failure and severe acute GVHD. In this article, we review more recent studies of haploidentical transplantation using various procedures that have improved upon prior results and discuss future prospects for improvements.

Myeloablative conditioning regimens

Transplant procedures using ex vivo T-cell-depletion methods

In the early 1980s, Reisner et al. [5] developed an effective method of depleting T cells by agglutination with soybean lectin followed by rosetting with sheep erythrocytes enabling a > 3 log reduction of T cells. This ex vivo T-cell-depletion method of haploidentical bone marrow cells without additional posttransplant immunosuppressive prophylaxis allowed durable engraftment rates over 75% with few major complications and an acceptable incidence of GVHD (36%) in patients with severe combined immunodeficiency [6]. However, its application to haploidentical transplants for leukemia were less encouraging due to the high incidence of graft failure (30%) and infectious complications (80%) [7]. During this same period, study groups from England developed a separate T-cell-depletion method using the anti-T-cell monoclonal antibody, OKT3 [8]. Although the incidence of grade II to IV acute GVHD (25%) was reduced by using this method [8], the high incidence of graft failure (21%) limited its utility [9].

In the 1990s, in attempts to overcome graft rejection as a host-versus-graft barrier as well as acute GVHD as a graft-versus-host barrier in haploidentical transplants, new methods were developed. One was the use of T cell depletion in combination with a “megadose” of stem cells after an immuno-myeloablative conditioning regimen (Table 1). In the first large reported pilot study from the University of Perugia, Aversa et al. [10] harvested ‘mega doses’ of stem cells from bone marrow and granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood, and transplanted T-cell-depleted stem cells following the soybean agglutination and E-rosetting technique, without any subsequent GVHD prophylaxis. More recently, this group has adopted other T-cell-depletion methods such as the E-rosetting technique followed by CD34-positive selection using Ceprate SC [11]; or positive selection of CD34 cells from peripheral blood by magnet beads using the CliniMACS (@Mitenyi) system [12]. These methods improve depletion of B-cells as well as T-cells, which provides a lower incidence of EB virus-associated lymphoproliferative disease, a serious potential complication of T-cell-depleted transplantation. In a phase II study in patients with acute leukemia at high risk of relapse who received haploidentical transplant after myeloablative conditioning utilizing total body irradiation, thiotepe, fludarabine, and antithymocyte globulin (ATG) and this method of T cell depletion [12], primary engraftment was obtained in 94/101 (93%) of assessable patients. Acute or chronic GVHD developed only in 8 of 100 patients and 5 of 70 assessable patients, respectively. This was encouraging data, though of concern a total of 38 patients died without relapse primarily due to infection (27 patients), and 26 patients had disease relapse at a median of only 6 months following transplantation. As an alternative approach, Henslee-Downey et al. [13] performed partial ex vivo T-cell depletion of bone marrow cells using a monoclonal

antibody (T10B9) and rabbit complement, followed by cyclosporine and ATG for additional in vivo T-cell depletion. This method resulted in approximately a 1.8 log reduction in infused T cells. While durable engraftment was obtained in 90%, this method when combined with intense conditioning of TBI, etoposide, cytosine arabinoside, cyclophosphamide, and methylprednisolone contributed to profound immunosuppression (Table 1). Mehta et al. [14] updated this experience with outcomes of 201 patients with acute leukemia who underwent haploidentical bone marrow transplantation using T10B9 or OKT3 for ex vivo T-cell depletion, followed by GVHD prophylaxis consisting of cyclosporine, methylprednisolone, and ATG. A median T-cell dose infused after ex vivo T-cell depletion was $5 \times 10^4/\text{kg}$. Neutrophil engraftment was obtained for nearly all evaluable patients (98%), and the cumulative incidence of acute GVHD was fairly low (13%). Relatively high treatment-related mortality (51% at 5 years) but acceptable relapse rate (31% at 5 years) translated to 34% and 14% of overall survival for standard and high-risk diseases, respectively. Although these depletion techniques offer attractive options for patients eligible for myeloablative transplantation, they need to be further improved to address the high relapse rate and treatment-related mortality rates.

Transplant procedures using in vivo T-cell-depletion methods

Lu et al. [15] from Peking University have investigated the combination of G-CSF-primed bone marrow and peripheral blood with intensive immunosuppression using ATG for in vivo T-cell depletion (Table 1). Other drugs in the conditioning regimen included cytosine arabinoside, busulfan, cyclophosphamide, and semustine, while GVHD prophylaxis included cyclosporine, mycophenolate mofetil, and short-course methotrexate. In the first report [15], they compared the clinical outcomes in HLA-haploidentical, related to HLA-matched sibling transplantation without ATG administration. The cumulative incidence of grade II to IV acute GVHD was 32% and 40% in matched sibling and haploidentical transplant, respectively ($p = \text{not significant}$). Surprisingly, treatment-related mortality was similar (14% vs 22%) as was relapse rate, and overall survival (13% vs 18%, and 72% vs 71% respectively). In an updated report, Huang et al. [16•] reported encouraging clinical outcomes in 250 patients who underwent HLA-mismatched/haploidentical transplantation; the 3-year probability of leukemia-free survival in standard and high risk AML groups was 70.7% and 55.9% respectively and 59.7% and 24.8% in ALL. Kanda et al. [17] from Tokyo University, Japan, has reported a small series utilizing alemtuzumab combined with conventional myeloablative therapy of TBI and cyclophosphamide for patients < 50 years old (Table 1). Engraftment was obtained for all 6 patients, and only 1 had grade II–IV acute GVHD.

T-cell anergy

Although non-selective T-cell-depletion methods have been effective in ensuring engraftment without severe GVHD, several obstacles remain, such as increased infectious complications and high relapse rates, potentially associated with delayed immune reconstitution and loss of pathogen- and tumor-specific T cells. To suppress alloreactive T cells specific to recipients, Guinan et al. [18] developed a new ex vivo depletion method in which they deplete only alloreactive T-cells or confer a T-cell anergic state, and keep the T-cell function of the remaining repertoire. Functional T-cell activation requires T-cell receptor signaling with co-stimulatory signals through interaction between B7 molecules on antigen-presenting cells and the CD28 molecule on the surface of T cells. T-cell receptor signaling without co-stimulatory signals induces T-cell anergy. Guinan et al. cultured bone marrow cells from a haploidentical donor with recipient peripheral blood cells inactivated by irradiation in the presence of CTLA4-Ig, an agent that inhibits B7:CD28-mediated co-stimulation, and transplanted bone marrow cells with an anergic state of recipient-specific alloreactive donor T cells. Conventional myeloablative radiation and cyclophosphamide

conditioning was given to all patients; additionally, cytosine arabinoside was given to the first 8 patients (Table 1). GVHD prophylaxis with cyclosporine and short-term methotrexate was used for all and they reported durable engraftment without severe GVHD. Davies et al. [19] updated clinical outcomes from 24 patients with hematologic malignancies who received haploidentical transplants by inducing T-cell anergy with CTLA-4 or monoclonal antibodies such as anti-B7.1 and anti-B7.2 and reported only 5 of 21 evaluable patients developed severe GVHD. No patient died due to viral infection, and 8 patients survived long term without disease recurrence. However, high treatment-related mortality in the early phase of transplantation, mainly due to sepsis and multiorgan failure, has limited the applicability of this transplant method.

Multicenter, retrospective summary data using haploidentical donors with ablative therapy

The European Blood and Marrow Transplant (EBMT) group [20••] surveyed 173 adult patients with AML and 93 with ALL who received myeloablative, haploidentical ex vivo T-cell-depleted grafts of peripheral blood stem cells in Europe (1995-2004). ATG-based in vivo T-cell depletion was used for the majority of patients. Leukemia-free survival rates at 2 years for patients with AML who received transplants in first complete remission (CR), more than first CR, and non-remission were 48%, 21%, and 1%, respectively, while those at 2 years for patients with ALL were 13%, 30%, and 7%, respectively. Multivariate analysis of treatment-related mortality and leukemia-free survival for patients in remission at transplant revealed that the donor's negative CMV serology to a patient's negative CMV serology was a significant factor in patients with ALL, whereas the donor of direct family member (parents or sibling) compared with other relatives was a favorable prognostic factor in patients with AML.

Reduced-Intensity Conditioning Regimens

Although the incidence of graft failure and GVHD have been reduced by using intensive T-cell depletion methods combined with immuno-myeloablative conditioning regimens, these procedures are still associated with high regimen-related toxicity and treatment-related mortality, mainly due to infectious complications, limiting haploidentical transplantation's applicability to the majority of patients. Therefore, the use of less intensive/non-myeloablative conditioning regimens (Table 1) combined with T-cell depletion has been tested in multiple small studies with encouraging results.

Transplant procedures using ex vivo T-cell-depletion methods

To deplete T cells but maintain progenitors, natural killer (NK), dendritic, and graft-facilitating cells, Bethge et al. [21] from the University of Tuebingen, Germany, demonstrated the feasibility of a new regimen using graft CD3/CD19 depletion with anti-CD3- and anti-CD19-coated microbeads on the CliniMACS device. The conditioning regimen consisted of fludarabine, thiotepa, melphalan, and OKT3, without subsequent GVHD prophylaxis (Table 1). In the updated report of 29 patients transplanted with this regimen for hematologic malignancies [22•], prompt engraftment was obtained for all but 1 (97%). The cumulative incidence of Grade II–IV and III–IV acute GVHD was 48% and 14%, respectively. A total of 20 out of 29 (69%) patients died mainly due to relapse (17%) and infections (24%). Although the incidence of acute GVHD was relatively high, this reduced-intensity conditioning with graft CD3/CD19 depletion enabled haploidentical transplantation with rapid engraftment and immune reconstitution for more infirmed or heavily pretreated patients and deserves further exploration.

Transplant procedures using in vivo T-cell-depletion methods

1) Alemtuzumab—Our group [23] has reported outcomes of reduced-intensity haploidentical transplants for 49 patients after conditioning with fludarabine, cyclophosphamide, and alemtuzumab; a humanized monoclonal antibody directed against human CD52 which is expressed on many T and B cells and some dendritic and NK cells (Table 1). Mycophenolate mofetil with or without cyclosporine was used as GVHD prophylaxis. This method successfully yielded a low cumulative incidence of acute GVHD (16% grades II–IV; 8% grades III–IV) and low treatment-related mortality (10.2%). Primary or secondary graft failure was observed in 6% and 8% of patients, respectively. The 1-year survival rates of 31% for all patients and 63% for patients in a standard risk group were encouraging considering the phase 1 nature of the study.

Kanda et al. [17] has also reported the clinical outcomes of haploidentical transplants with the use of alemtuzumab combined with fludarabine and busulfan for a small series with hematologic malignancies (Table 1). Engraftment was obtained for all 6 patients, and acute GVHD occurred in only 1 patient (grade II), though 2 patients with active disease at transplant died due to disease recurrence.

2) Post-transplant cyclophosphamide—In animal models, both graft rejection and GVHD after haploidentical bone marrow transplantation can be inhibited by posttransplant administration of high-dose cyclophosphamide, which is known to be highly toxic to lymphocytes proliferating in response to recent antigen stimulation [24]. Based on animal model results, O'Donnell et al. [25] from Johns Hopkins first demonstrated the feasibility of T-cell-replete bone marrow transplantation from a haploidentical related donor after reduced-intensity conditioning, followed by posttransplant cyclophosphamide. Extending their report, Luznik et al. [26•] reported the outcomes of 68 patients who received a haploidentical bone marrow transplant for hematologic diseases using high-dose, posttransplant cyclophosphamide at Johns Hopkins or Fred Hutchinson Cancer Research Center. Conditioning regimens consisted of fludarabine, cyclophosphamide, and TBI (Table 1). Posttransplant cyclophosphamide was administered at 50 mg/(kg·day) at day 3 or days 3 and 4 after transplantation, and mycophenolate mofetil and tacrolimus were used as GVHD prophylaxis. Although a relatively high reported incidence of graft failure (13%) and relapse rate at 1 year (51%) requires further improvement in this method, low cumulative incidence of acute GVHD (grades II–IV, 34%; grades III–IV, 6%) and low non-relapse mortality at 1 year (15%) were promising.

3) ATG and intensified GVHD prophylaxis—Ogawa et al. [27] from Osaka University, Japan, reported on 26 patients with a high risk hematologic malignancy who underwent reduced-intensity transplantation of peripheral blood stem cells from an HLA-haploidentical donor following ATG, fludarabine and busulfan (Table 1). GVHD prophylaxis consisted of tacrolimus and methylprednisolone (1 mg/(kg·day)). All but 1 engrafted and only 5 (19%) patients developed grade II acute GVHD. Sixteen of the 26 patients were alive in CR at the time of the report. Four died of TRM (15%), and 6 died of progressive disease (23%). These data suggest that reduced-intensity conditioning with GVHD prophylaxis consisting of ATG combined with tacrolimus and methylprednisolone allows stable engraftment and effectively suppresses GVHD in haploidentical transplants, providing an alternative approach to alemtuzumab or cyclophosphamide based regimens.

Improving Haploidentical transplantation

A variety of transplant procedures developed in the last decade have enabled sustained engraftment and a low incidence of GVHD in haploidentical transplants, however there is often a high incidence in relapse and opportunistic infections. Our program has reported

slow immune reconstitution after haploidentical transplantation as 1 potential cause [23]. Although patients without GVHD were noted to often have cellular recovery 3-6 months following transplantation, total lymphocyte numbers were still low, immunoscope analyses indicate significant deficits in the recovery of T cell families, and NK cellular function remains limited in the majority of patients. In fact, in our report, 49% died due to disease and 22% from infections, suggesting additional strategies to improve immune recovery are required [23].

To overcome this obstacle of poor immune function, much effort has been invested in furthering our understanding of mechanisms of immune recovery and to develop additional immunological modulation after haploidentical transplantation. This has led to better understanding of the role different donors may play in improving outcomes as well as novel post-transplant manipulations.

Importance of Donor Choice

Feto-maternal immunological tolerance—Bidirectional transmission of fetal or maternal hematopoietic cells during pregnancy can lead to persistent microchimerism associated with an antigen-specific suppression of T-cell responses. Immunological hyporesponsiveness against non-inherited maternal HLA antigens (NIMA) has been shown in experiments in mice [28] and human [29]. Van Rood et al. [30] from Leiden University Medical Center, the Netherlands, first showed that the incidence of acute GVHD was lower in patients who received non-T-cell-depleted grafts from a NIMA-mismatched sibling than those from a non-inherited paternal HLA antigen-mismatched (NIPA) sibling, suggesting the presence of immunological hyporesponsiveness against NIMA in haploidentical transplants. Ichinohe et al. [31] from Kyoto University, Japan, also showed that T-cell-replete HLA-haploidentical stem cell transplantation from a NIMA-mismatched family donor was feasible in selected patients with poor-risk hematologic malignancies under standard GVHD prophylaxis. Though, a relatively high incidence of acute GVHD, extensive chronic GVHD, and pulmonary complications, have been major problems in this T-cell-replete approach [32].

KIR/KIR ligand mismatching—In humans, immunoglobulin and lectin-like molecules called killer inhibitory receptors (KIR) are expressed on the surface of NK cells [33]. NK cell activity is regulated by the balance of activating and inhibitory NK-cell receptors and HLA class I molecules, or alternatives (Figure 1). Following a report on the promising role of KIR ligand mismatching in reducing relapse rate in stem cell transplant settings [34], several models of NK cell alloreactivity in stem cell transplantation have been proposed. One is the “ligand mismatch” or “ligand-ligand” model, in which donor NK cells attack target cells that do not have the same inhibitory KIR ligand group. In the analysis of 112 patients with high-risk AML who received haploidentical transplants, KIR ligand mismatched donors were associated with a significantly lower relapse rate in patients transplanted in complete remission and reduced risk of disease-associated death [35]. Another model is termed the “missing ligand” model, in which donor NK cells attack target cells that do not have KIR ligand corresponding to donor inhibitory KIR (inferred from KIR genotypes). On the basis of this model, several groups reported improved overall survival and reduced relapse-free survival in “missing ligand” transplantation [36,37]. A potential role of ‘activating’ KIR receptors are also purported, although ligands and functions are not well identified [38,39]. Our group has tested these three distinct models on our subjects who underwent T-cell depleted nonmyeloablative transplantation from haploidentical family members. Preliminarily, we have found reduced relapse related mortality in high-risk myeloid malignancies on the basis of the missing ligand model, but could not find any impact on clinical outcomes if other models were applied [40]. However, to evaluate which

model is appropriate to predict NK cell activity and clinical outcomes, larger studies analyzing the clinical data of transplantation with more homogenous diseases and conditioning regimens are required.

Early Clinical Results of Attempts to Improve Immune Recovery

Donor Lymphocyte Infusions—Unmodified donor T-cell infusions have been utilized to augment graft-versus-tumor effect and improve immune recovery. Various groups including ours have shown their safety if administered in limited numbers [41,42]. However, because clinical benefit has been limited, other methods have been investigated to improve immune recovery after transplantation.

Selected NK cell infusions—Findings of the association between KIR/KIR ligand mismatching and low relapse rate have stimulated interest in NK cell therapy in the haploidentical transplant setting. Several groups thus far have tested the feasibility of infusing NK cells in haploidentical transplants, collected either from separation following leukapheresis using ex vivo purification [43,44] or by ex vivo differentiation of selected CD34-positive cells [45]. Our group recently [44••] reported the feasibility of NK cell infusions in 14 matched and 16 mismatched transplanted patients. Single step NK cell enrichment by CD56+ selecting column yielded approximately 90% of cells being NK cells of interest, with a low level of CD4 or 8 cells to cause acute GVHD. NK cell enriched donor cell infusions resulted in minimal toxicity and long term survivors were noted to have improved immune recovery and outcomes, though it remains unproven if that was due to the NK enriched infusions and improved NK function directly. Yoon et al. [45] tested the feasibility of HLA haploidentical transplants for 14 patients with acute leukemia or myelodysplastic syndrome, combined with infusions of differentiated donor NK cells. In this instance, donor NK cells were generated from CD34-positive cells by ex vivo cell culture over 6 weeks and infused to recipients without additional GVHD prophylaxis. Although no acute toxicity was observed after infusion, their potential antitumor effects were not clear. Because feasibility and encouraging early results have been shown in several small cohort studies, a randomized study should be performed to evaluate the impact of NK cell infusions on infections, long-term survival, and relapse-free survival.

Selected T-cell subset infusions—As an alternative approach to unmodified donor T-cell infusions, several groups tested the feasibility of donor T-cell infusions that were depleted of alloreactive T cells [46,47], or that were introduced with a herpes simplex thymidine kinase suicide gene, allowing the alloreactive T cells to be killed in case of severe GVHD [48••]. In the haploidentical setting, Amrolia et al. [46] from Great Ormond Street Hospital for Children, London, used an anti-CD25 immunotoxin to deplete alloreactive lymphocytes and infused allodepleted donor T cells after ex vivo T-cell-depleted haploidentical transplants. Viral-specific responses were observed in 4 of 6 evaluable patients receiving higher doses of T cells with a low incidence of severe GVHD. Other promising technique is photodynamic purging. Efficient photodepletion of alloreactive T cells, which retained photosensitizer 4,5-dibromorhodamine 123 (TH9402) after a mixed donor and recipient lymphocyte reaction, was demonstrated in mice [49] and human [50]. On the other hand, pathogen-specific responses to pathogen antigens were retained [50]. Roy et al. [47] examined the feasibility of administering donor lymphocytes photodepleted of alloreactive T cells for 19 patients who received ex vivo T-cell-depleted haploidentical transplantation, and showed potential benefit of reducing treatment related mortality (0% at 2 years) and improving survival rates (82% at 2 years) without causing grade III to IV GVHD for 12 patients who received high CD3+ doses (3.2×10^5 to 5.0×10^6 cells/kg). Interestingly, the loss of HLA haplotype that differed from the donor's haplotype in leukemic cells was recently reported in patients who relapsed after haploidentical

transplantation and infusion of donor T cells, which implies that one mechanism underlying leukemia relapse is escape from donor alloreactive T-cell killing [51••]. Therefore, the status of mismatched HLA on relapsed leukemic cells may need to be examined before additional donor T-cell infusions are explored for utility. More recently, the Perugia group [52] have tested the infusion of freshly purified donor regulatory T cells, followed by infusion of mature T cells and positively selected CD34 cells in haploidentical transplants. In their study, the speed of immune recovery was enhanced as compared with their previous reports. Recovery of high frequencies of CD4 and CD8 T cells specific for opportunistic infections were also detected. Their innovative method of infusing regulatory T cells enabled the administration of larger amounts of mature T cells, which may lead to earlier immune reconstitution and improved outcomes.

Cytokine Stimulation—Interleukin-2 (IL-2) has been used for the treatment of some carcinomas, because IL-2 stimulates many types of cells in the immune system including T cells, B cells and NK cells [53,54]. We have reported the use of IL-2 after T-cell-depleted stem cell transplantation, noting safety and a potential improvement in immune function leading to encouraging survivals [55]. In a recent study investigating haploidentical NK cell infusions in combination with IL-2, Rubnitz, et al [56] have reported evidence of safe engraftment and expansion of donor KIR-mismatched NK cells after infusion. Thus, the use of IL-2 or other cytokines in combination with NK cell infusions after haploidentical transplantation deserves further exploration.

Conclusions

Newly developed transplant methods in the last decade have made it possible to perform haploidentical transplantation with sustained engraftment, lower early TRM, and acceptable rates of GVHD. However, poor immune recovery remains a major obstacle deserving of our focus. Improved understanding to optimize donor selection and novel post-transplant cellular therapy may allow further enhancements in outcomes. Currently, the most promising approaches for donor selection appear to involve those ideas based on KIR typing and NIMA matching. In the post transplant setting, NK cell boosts on the basis of KIR/KIR ligand mismatching and other populations of T cells, such as T cells without recipient-specific alloreactive T cells or regulatory T cells are likely to be fruitful areas of investigation. Continued progress will help ensure all patients who may benefit from allogeneic immunotherapy, but do not have an available HLA-matched related or unrelated donor, a chance for cure.

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Figure 1. NK cell activity is regulated by the balance of activating and inhibitory KIR and KIR ligands

(A) NK cell does not lyse target cell, because there is an inhibitory signal via inhibitory KIR/KIR ligand.

(B) Even if there is a signal via activating KIR/KIR ligand, activating signal is canceled by an inhibitory signal from inhibitory KIR.

(C) If activation via activating KIR/KIR ligand is not prevented by an inhibitory signal, the target cell is lysed.

Table 1
Various transplant procedures for haploidentical transplants

Category	Conditioning regimen	Myeloablative conditioning regimens	GVHD prophylaxis	Source
CD34-positive selection or CD34-positive selection + CD2-negative selection (12)	T-cell depletion (T10B9 or OKT) (14)	Ex vivo T-cell-depletion methods	None	PB
		TBI (8 Gy/d, d-9) + thiotepa (5 mg/kg/d, d-8, d-7) + FLU (40 mg/m ² /d, d-7~d-3) + ATG (5 mg/kg/d, d-5~d-2)		
		TBI (4.7 Gy/d, d-9~d-7) + etoposide (20 mg/kg/d, d-6) + Ara-C (4 g/m ² /d, d-5~d-3) + CPA (50 mg/kg/d, d-2, d-1) ± ATG (10 mg/kg, d-5~d-3)		
CTLA-4-Ig + irradiated recipient PB, or anti-B7.1, B7.2 antibody (19)		TBI (3.5 Gy/d, d-6~d-3) + CPA (1.8 g/m ² /d, d-2, d-1) ± Ara-C (6 g/m ² /d × 3 d)	CyA + MTX	BM
In vivo T-cell-depletion methods				
ATG (16)		Ara-C (4 mg/m ² /d, d-10, d-9) + BUS (4 mg/kg/d, d-8~d-6) + CPA (1.8 g/m ² /d, d-5, d-4) + Me-CCNU (250 mg/m ² /d, d-3) + ATG (2.5 mg/kg/d, d-5~d-2)	CyA + MTX + MMF	BM + PB
Alemtuzumab (17)		Alemtuzumab (0.2 mg/kg/d, d-8~d-3) + TBI (4 Gy/d, d-7~d-5) + CPA (60 mg/kg/d, d-3~d-2)	CyA + MTX	PB
Reduced-intensity conditioning regimens				
T- and B-cell depletion (anti-CD3/anti-CD19 and OKT3) (22)		Ex vivo T-cell-depletion methods	OKT3 ± MMF	PB
		FLU (150–200 mg/m ² /total) + thiotepa (10 mg/kg/total) + MEL (120 mg/m ² /total)		
		In vivo T-cell-depletion methods		
Alemtuzumab (23)		Alemtuzumab (20 mg/d, d-4~d0) + FLU (30 mg/m ² /d, d-5~d-2) + CPA (500 mg/m ² /d, d-5~d-2)	MMF ± CyA	PB
Alemtuzumab (17)		Alemtuzumab (0.2 mg/kg/d, d-8~d-3) + FLU (30 mg/kg/d, d-8~d-3) + BUS (4 mg/kg/d, d-6~d-5) + TBI (4 Gy/d, d-1)	CyA + MTX	PB
Posttransplant CPA (26)		CPA (14.5 mg/kg/d, d-6~d-5) + FLU (30 mg/m ² /d, d-6~d-2) + TBI (2 Gy/d, d-1)	CPA + MMF + FK	BM
ATG and mPSL for GVHD prophylaxis (27)		FLU (30 mg/m ² /d, d-10~d-5) + BUS (4 mg/kg/d, d-6, d-5) + ATG (2 mg/kg/d, d-4~d-1)	FK + mPSL	PB

TBI, total body irradiation; FLU, fludarabine; BM, bone marrow; PB, peripheral blood; CPA, cyclophosphamide; mPSL, methylprednisolone; ATG, antithymocyte globulin; CyA, cyclosporine; BUS, busulfan; Me-CCNU, semustine; MTX, methotrexate; MMF, mycophenolate mofetil; MEL, melphalan.