Published in final edited form as:

Best Pract Res Clin Haematol. 2011 September ; 24(3): 359–368. doi:10.1016/j.beha.2011.05.001.

Treatment of hematological malignancies with nonmyeloablative, HLA-haploidentical bone marrow transplantation and high dose, post-transplantation cyclophosphamide

Ashley T. Munchel¹, Yvette L. Kasamon², and Ephraim J. Fuchs^{2,3}

¹Division of Pediatric Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

²Division of Hematologic Malignancies, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA

Abstract

Hematopoietic stem cell transplantation provides the only potential curative option in many patients with hematological malignancies. Finding a suitably matched donor in a timely manner is often difficult. However, most patients have a partially HLA-mismatched (HLA-haploidentical) first-degree relative readily available. Historically, HLA-haploidentical bone marrow transplantation (BMT) has been considered extremely high risk due to high rates of life-threatening graft-versus-host disease (GVHD) and non-relapse mortality (NRM). Modifications of the stem cell graft, such as T-cell depletion, have resulted in poor rates of engraftment. We have recently completed a phase II clinical trial of nonmyeloablative HLA-haploidentical hematopoietic BMT followed by post-transplantation high-cyclophosphamide. High-dose cyclophosphamide has been shown to create immunogenic tolerance by specifically killing activated mature T-cells. As a result, alloreactive T-cells in the donor graft are selectively destroyed thereby decreasing the incidence of severe GVHD. As well, host-versus-graft reactive T-cells are also selectively eliminated thereby increasing rates of engraftment. Among 210 patients with hematological malignancies receiving nonmyeloablative, HLA-haploidentical BMT with post-transplantation cyclophosphamide, the rate of sustained donor cell engraftment has been 87%. The cumulative incidence of grade 2–4 acute GVHD is 27%, grade 3–4 acute GVHD is 5% and chronic GVHD is 15%. Interestingly, increasing HLA disparity between donor and recipient was not associated with increasing incidence of GVHD or decreased event-free survival. Nonmyeloablative haploidentical stem cell transplantation with post-transplantation cyclophosphamide seems to be a promising, potentially curative, option for patients with hematological malignancies who either lack an HLA-matched related or unrelated donor, or in whom a myeloablative preparative regimen is contraindicated due to significant co-morbidities or history of extensive pre-treatment.

³Address correspondence to: Ephraim Fuchs, M.D., M.B.A., 288 Cancer Research Building, 1650 Orleans Street, Baltimore, MD 21287-0013, Phone: (410) 955-8143, fuchsep@jhmi.edu.

Conflict of Interest

The authors declare no conflicts of interest

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

stem cell transplantation; nonmyeloablative; HLA-haploidentical; hematological malignancies; cyclophosphamide

Introduction

Allogeneic stem cell transplantation, or alloSCT, is a potentially curative option for many patients with hematological malignancies, particularly those with cytogenetically standard or poor risk acute leukemia in remission [1]. Historically the curative potential of alloSCT was ascribed to the eradication of the malignancy by lethal doses of chemotherapy with or without radiation, and the bone marrow infusion “rescued” the patient from otherwise fatal marrow aplasia. However it is now quite apparent that an important mechanism of cure in alloSCT is the graft-versus-leukemia, or GVL, effect [2]. Donor T-cells contained in the graft are likely responsible for the GVL effect. Unfortunately, donor T-cells also play a significant role in graft-versus-host disease (GVHD), a major contributor to non-relapse mortality (NRM). The goal in alloSCT is to develop a way to maximize the graft-versus-tumor (GVT) effect while minimizing GVHD, graft failure, NRM, and relapse. We continue to search for the best combination of donor, conditioning regimen, and post-transplantation immunosuppression to achieve those goals.

Nonmyeloablative Conditioning Regimens

There has been increasing experience with the use of nonmyeloablative conditioning regimens prior to alloSCT. These regimens typically incorporate highly immunosuppressive but moderately myelosuppressive chemotherapy followed by high stem cell doses and post-transplantation pharmacologic immunosuppression. The principles underlying the use of nonmyeloablative conditioning [3] are: 1) immunosuppression should be sufficient to allow donor engraftment; 2) a high dose of donor stem cells increases the probability of engraftment; 3) compared to alloSCT after myeloablative conditioning, immunologic recovery may be faster and protects against opportunistic infections; and 4) alloSCT after nonmyeloablative conditioning results initially in a mixed chimeric state in the host, which is protective against GVHD by promoting immune tolerance. On the flip side, immune tolerance can also develop against the tumor and impede the desired GVL effect. As well, a mixed chimeric state, specifically low donor T-cell and NK cell chimerism at day 14, is associated with a higher incidence of graft failure [4,5]. Encouragingly, donor lymphocyte infusion (DLI) can quickly convert the host to full donor chimerism, restoring the GVL effect and preventing graft rejection. Although DLI increases risk of GVHD, the hope is that improved immune reconstitution will protect patients from developing severe GVHD in the event DLI is necessary [3,6].

Nonmyeloablative SCT provides a therapeutic alternative to patients with significant comorbidities and to elderly patients, who would be ineligible to receive myeloablative alloSCT due to high risk of NRM. Investigators at the Fred Hutchinson Cancer Center in Seattle published a retrospective analysis comparing recipients of nonmyeloablative versus myeloablative matched-related SCT for hematological malignancies [7]. Despite poorer pre-transplantation health status, patients treated on the nonmyeloablative protocol had significantly less grade 3–4 hematological, gastrointestinal, hemorrhagic, hepatic, infectious, metabolic and pulmonary toxicities. This translated into overall improvement of NRM at day 100 and day 365. A similar retrospective analysis was published comparing nonmyeloablative with myeloablative conditioning regimens in patients who received matched-unrelated donor BMT [8]. They reported similar results with decreased severe

hematological, gastrointestinal, hepatic, infectious and hemorrhagic toxicities in patients in the nonmyeloablative group. As well, they found a statistically significant decrease in grade 2–4 and grade 3–4 acute GVHD. In the study of matched unrelated donor SCT, there was a trend to improved NRM which became more pronounced when adjusted for pretransplantation comorbidities.

Nonmyeloablative alloSCT is a safe and effective alternative for patients who cannot undergo myeloablative alloSCT because of advanced age or significant comorbidities. It capitalizes on the GVL effect, which can be augmented in the case of mixed chimerism or relapse by giving donor lymphocyte infusion.

HLA-haploidentical stem cell transplantation

Most experience with alloSCT has been with matched sibling donors. However, only about 30% of patients have a matched sibling making it necessary to identify alternative donors. The National Marrow Donor Program (NMDP) was initiated in 1986 to create a network “to facilitate successful transplants of hematopoietic stem cells from volunteer unrelated donors as lifesaving therapy for patients of all racial and socioeconomic backgrounds.” Finding potential matched unrelated donors has proven to have its own obstacles. The average time to find a suitable match typically takes several months. Up to 20% of participants registered with the NMDP are unavailable once contacted to serve as donors. Minorities continue to be greatly underrepresented [9]. However, nearly all patients have an HLA-haploidentical first-degree relative readily available to serve as a donor.

In addition to easier donor availability, HLA-mismatched or haploidentical grafts may be associated with a more potent GVL effect, resulting in a reduced incidence of disease relapse [10,11]. The benefit of the GVL effect was first described in the 1970s when it was noted that patients who developed GVHD had lower rates of relapse [12,13]. More specifically patients who developed chronic GVHD had a lower probability of relapse and greater probability of survival than those with acute GVHD alone or no GVHD [5,12]. The theory has been further supported by the fact that there are higher rates of relapse in autologous versus allogeneic transplants for certain hematological malignancies [14–17], and donor lymphocyte infusion can induce durable remission in several diseases [15,18]. There is also evidence that the greater the degree of mismatch, the lower the incidence of relapse [10,19] suggesting there could be a greater GVL effect using an HLA-haploidentical donor compared to either a matched related or matched unrelated donor.

The major obstacle of using a partially HLA-mismatched related donor has been a higher incidence of grades II–IV acute GVHD [10,20]. As a result, grafts from haploidentical donors have routinely been T-cell depleted prior to transplantation. Depletion of T-cells is associated with an increased risk of graft failure, and limits the GVL effect [2,21–25]. Patients who receive T-cell depleted grafts also showed delayed immune reconstitution as evidenced by a delayed recovery of CD3⁺ cells, prolonged abnormality of CD4/CD8 ratio, decreased T-cell function and decreased T-cell receptor repertoire when compared to T-cell replete grafts [24,26–29]. Several institutions are working on ways to safely give unmanipulated haploidentical or HLA-mismatched grafts. We have shown that giving high dose cyclophosphamide after stem cell transplantation is a potential way to safely give a T-cell replete graft.

Drug-Induced Immunological Tolerance

The idea of giving post-transplantation cyclophosphamide came from studies that have shown that a properly timed, high dose of cyclophosphamide induces immunological tolerance. Drug-induced immunological tolerance is not a new concept. It was initially

reported in 1959 when it was shown that giving rabbits 6-mercaptopurine prevented their ability to produce antibodies against human serum albumin [30].

Through a progression of studies it was found that cyclophosphamide given one to three days after antigenic stimulation induces tolerance to tumor, skin grafts or other solid organ transplants in murine models [31–33]. The proposed mechanism has been clonal destruction of alloreactive T cells [34]. In other words, antigenic stimulation from the graft causes rapid proliferation of T-cell clones specific to the foreign antigen. When cyclophosphamide is given at the height of proliferation, 1–3 days after stimulation, it inhibits DNA replication and results in selective destruction of alloreactive T-cell clones. Evidence of clonal destruction is supported in studies in which immunosuppressives or anti-CD4 antibodies are given prior to or concurrently with antigen stimulation followed by cyclophosphamide administration [36–38]. Steroids and cyclosporine inhibit T-cell proliferation thereby preventing clonal expansion after antigenic stimulation. As a result, cyclophosphamide cannot eliminate the T-cells directed against the graft and there is greater risk of graft rejection. Conversely, anti-CD4 antibodies given prior to or concurrently with antigen enhance tolerance. The proposed mechanism is decreased competition from other mature T-cells since they have been depleted by anti-CD4 thereby allowing the cyclophosphamide to destroy a greater proportion of the graft specific T-cells. What keeps the host from producing new T-cells against the graft? It seems cyclophosphamide creates a degree of intrathymic mixed chimerism which limits production of anti-graft immune cells through intrathymic clonal deletion. There is also evidence that cyclophosphamide induces anergy and production of suppressor T-cells which serve to protect the graft [34,39,40].

In bone marrow transplantation, donor-derived mature T-cells in the marrow graft proliferate in response to alloantigens in the host. These T-cells are responsible for GVHD. Conversely, residual T-cells in the host proliferate in response to the graft. These T-cells contribute to graft failure. Based on the data in mouse models, we hypothesized that giving cyclophosphamide 2–3 days after transplantation will selectively destroy mature T-cells in the graft and the host thereby decreasing GVHD and graft failure. Because cyclophosphamide preferentially destroys mature T-cells, the stem cells in the graft will be unharmed and capable of engrafting in the host.

We showed in a mouse model that post-transplantation cyclophosphamide selectively depletes alloreactive graft T-cells without compromising engraftment [41]. We also showed that mice could achieve durable engraftment after conditioning with low dose total body irradiation when given post-transplantation cyclophosphamide suggesting that a myeloablative preparative regimen is not necessary to ensure engraftment [42].

Phase I Trial of nonmyeloablative, HLA-haploidentical BMT with high dose, post-transplantation cyclophosphamide

Based on the results of our mouse data we completed a phase I clinical trial to determine the minimal conditioning, including post-transplantation cyclophosphamide which permitted stable engraftment of partially HLA-mismatched marrow from first degree relatives [43]. All patients had high-risk hematological malignancies and were not eligible for standard alloSCT. The preparative regimen initially consisted of fludarabine and total body irradiation (TBI). GVHD prophylaxis consisted of high-dose cyclophosphamide on day 3 and 4, tacrolimus and mycophenolate mofetil (MMF). Two of three patients experienced graft failure. As a result, cohort 2 was modified to add cyclophosphamide to the preparative regimen. Of the 10 patients in cohort 2, 8/10 engrafted. At median follow-up of 191 days, 6/10 were alive and 5/6 were in complete remission [43]. Interestingly two of the five patients alive and in complete remission were the two patients that had graft failure. Both

had autologous marrow recovery confirming our regimen was truly non-myeloablative. It also suggests that patients can have an anti-tumor effect even in the absence of sustained engraftment.

Phase II trial of nonmyeloablative, HLA-haploidentical BMT with high dose post-transplantation cyclophosphamide

Because of the encouraging results of our phase I clinical trial we designed a phase II clinical trial. We provide here an update on data that have previously been reported [44,45]. As of September 14, 2010, 210 consecutive patients received nonmyeloablative, HLA-haploidentical transplantation with high-dose, post-transplantation cyclophosphamide and had at least one year of follow-up. Details concerning criteria for eligibility, HLA typing, transplantation procedure, and outcomes measures, and statistical analysis have been published previously [44,45].

Patients, Donors, and Graft Characteristics

Patients with hematological malignancies were enrolled in three similar clinical trials of non-myeloablative conditioning and transplantation of partially-HLA mismatched bone marrow at Johns Hopkins, Fred Hutchinson Cancer Research Center, or BMT Group of Georgia and Hahnemann University Hospital. Donors were first-degree relatives who were identical at one HLA-haplotype and mismatched at one or more loci of an unshared haplotype. Of the 210 patients, 149 were male. The median age of the patients at transplantation was 52 (range, 1–73). Eligible diagnoses included acute leukemia in 2nd or subsequent remission or in first complete remission with poor risk features; Hodgkin lymphoma (HL); Non-Hodgkin Lymphoma (NHL); chronic lymphocytic leukemia (CLL) with duration of remission < 6 months after chemotherapy or high risk features; multiple myeloma (MM) in resistant relapse or in relapse after autologous transplant; myelodysplastic syndrome (MDS); paroxysmal nocturnal hemoglobinuria (PNH); chronic myeloid leukemia (CML) beyond first chronic phase (CP1), or interferon- or imatinib resistant CML in CP1; and chronic myeloproliferative disorders other than CML. The two leading indications for transplantation were non-Hodgkin lymphoma (n=66) and acute myeloid leukemia (n=43).

Patients and their donors were heavily mismatched on the unshared HLA locus, with a median mismatch of four out of five of the HLA antigens that were typed.

The donor graft contained a median of 3.7×10^8 mononuclear cells, of which 10% were T cells and 1% expressed the CD34 antigen.

Transplantation procedure

All patients were intended to be treated as outpatients. Conditioning for transplantation (Figure 1) consisted of cyclophosphamide 14.5mg/kg/day on days –6 and –5, fludarabine 30mg/m²/day for five consecutive days starting on day –6, and 2 Gy total body irradiation given in a single fraction on day –1. Bone marrow was harvested from donors and infused into recipients on day 0. The graft was depleted of red-blood cells and plasma but there was no manipulation to deplete graft T-cells. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg IV, together with Mesna, each on days 3 and 4, mycophenolate mofetil 15 mg/kg po tid (maximum 3 g/day) from day 5–35, and tacrolimus from day 5–180. Tacrolimus levels were monitored at least weekly with a desired concentration from 5–15 ng/ml. Prophylactic antimicrobial therapy was started on day –6 and included norfloxacin, fluconazole, valacyclovir, and appropriate prophylaxis of *Pneumocystis carinii* pneumonia.

Engraftment and Donor Chimerism

Of the 210 patients transplanted, 204 were evaluable for donor cell engraftment. Twenty-seven patients (13%) failed to engraft. Nearly all patients with primary or secondary graft failure experienced recovery of autologous hematopoiesis. As reported previously, the median time to a neutrophil count of $\geq 500/\mu\text{l}$ was 15 days, and the median time to an unsupported platelet count of $\geq 20,000/\mu\text{l}$ was 24 days.

GVHD

Figure 2 shows the cumulative incidence of grade 2–4 aGVHD was 27%, grade 3–4 aGVHD was 5% and chronic GVHD was 13%. This coincides with the data previously reported in the 67 patients, which had shown a cumulative incidence of grade 2–4 aGVHD of 34%, grade 3–4 aGVHD of 6% [45].

Relapse and Nonrelapse Mortality

The cumulative incidences of relapse and nonrelapse mortality were 55% and 18%, respectively (Figure 3). One hundred thirteen patients have died. The causes of death are relapse (n=79), infection (n=15), pulmonary complications (n=7), GVHD (n=5), other (n=4), or unknown (n=3).

Overall and event-free survival

Three-year overall survival and event free survival are 41% and 32% respectively (Figure 4). Three year overall survival was 50% for patients transplanted for acute lymphocytic leukemia, 45% for patients transplanted for myelodysplastic syndrome or myeloproliferative disorder, and 35% for patients transplanted for acute myeloid leukemia (Figure 4B). Three year survival was 62% for 30 patients with Hodgkin lymphoma (22 of whom had undergone prior autologous SCT), 41% for patients with non-Hodgkin lymphoma, and only 22% for patients with chronic lymphocytic leukemia (Figure 4C).

Effect of HLA-disparity on outcome of HLA-haploidentical SCT with post-transplantation cyclophosphamide

Previous studies of T cell-replete, HLA-haploidentical BMT after myeloablative conditioning have shown that increasing HLA mismatch between donor and recipient was associated with worse survival due to an increased incidence of GVHD and NRM, which outweighed any potential reduction in the incidence of relapse [46–48]. Since our regimen incorporated nonmyeloablative conditioning and novel GVHD prophylaxis, we examined the impact of increasing HLA disparity on outcome in this context [44]. Interestingly, Figure 5 shows a trend to improved event-free survival with increasing HLA antigen disparity between donor and recipient (HR = 0.80; 95% confidence interval [CI] = 0.66–0.96; $P = .02$), with the hazard ratio of 0.8 indicating a 20% reduction in the risk of an event (death or relapse) for each increment of HLA mismatch. An HLA-DRB1 antigen mismatch in the graft-versus-host direction, and 2 or more HLA Class I (HLA-A, -B, and -Cw) mismatches in either direction were found to be associated with decreased incidences of relapse without an increased incidence of non-relapse mortality (data not shown). While an analysis of the effects of NK cell alloreactivity on outcome is ongoing, we have previously found that donor-recipient mismatches at genes for inhibitory Killer Immunoglobulin-like Receptors, or iKIRs, or KIR haplotypes, were associated with improved outcome of nonmyeloablative, HLA-haploidentical BMT with post-transplantation cyclophosphamide [49].

DISCUSSION

Our data suggest that nonmyeloablative, HLA-haploidentical bone marrow transplantation with high dose, post-transplantation cyclophosphamide is a safe alternative for patients with hematological malignancies without suitable donors or with co-morbidities that would preclude them from receiving a myeloablative transplant. It allows for transplantation to occur more quickly in patients in whom the disease is too advanced or aggressive to wait for a suitable matched unrelated donor to be found. This regimen has an acceptably low incidence of GVHD which has historically been the major limitation to using partially HLA-mismatched donors. The incidence of acute GVHD in our patients is similar to the incidence of GVHD reported in studies of patients undergoing HLA-matched related or unrelated donor SCT after myeloablative or nonmyeloablative conditioning [50].

In contrast to what other studies have shown, we have found that increasing HLA mismatch between donor and recipient is not harmful and may even improve event-free survival. Our results show a decreased incidence of relapse with increasing degree of mismatch with no effect on GVHD or nonrelapse mortality [44]. This result implies that the transplantation regimen in general, or post-transplantation cyclophosphamide in particular, differentially affects cells that induce GVHD versus GVL. We are currently testing this possibility in animal models of alloSCT. Because we can safely give a T-cell replete graft, we have found our rates of engraftment are equivalent to other studies using nonmyeloablative preparative regimens [4,51,52]. Most patients with graft failure experience autologous recovery of hematopoiesis, making graft failure less of an emergency than when it occurs in myeloablative transplants. Interestingly, we and others [53,54] have found evidence for anti-tumor effects even in patients who reject their donor's graft. Regardless of that finding, our goal would be to improve rates of engraftment.

A high incidence of relapse was the major cause of treatment failure among patients receiving nonmyeloablative, HLA-haploidentical BMT with post-transplantation cyclophosphamide. All patients enrolled onto the trial had poor prognosis hematological malignancies with a high risk of relapse. Many had failed extensive prior therapies, including autologous SCT in over one quarter of patients. The conditioning regimen may not have been sufficiently intense for patients with aggressive hematological malignancies. We are currently exploring reduced intensity conditioning with fludarabine and busulfan instead of conditioning with low dose TBI.

An area that is lacking in the literature is immune reconstitution post-stem cell transplantation. We are in the process of doing extensive qualitative and quantitative immune reconstitution studies on our patients enrolled in this trial. We are evaluating T-cell subset repopulation with flow cytometry, thymopoiesis with T-cell receptor excision circles, CMV reactivation and mixed lymphocyte cultures.

Patients with hematological malignancies who are referred for transplantation but lack an HLA-matched donor have two potential donor sources, either an HLA-mismatched relative or unrelated umbilical cord blood. It would be interesting to compare these two options in a randomized control trial which has never been done. The preliminary data from our nonmyeloablative haploidentical trial is exciting and suggests this regimen is a viable option for patients with hematological malignancies who lack a matched related or unrelated donor but have a first degree relative readily available to serve as a donor.

Acknowledgments

Supported by P01 CA15396 from the National Cancer Institute

Abbreviations

BMT	bone marrow transplantation
GVHD	graft versus host disease
NRM	nonrelapsed mortality
SCT	stem cell transplantation
GVT	graft versus tumor
GVL	graft versus leukemia
DLI	donor lymphocyte infusion
TBI	total body irradiation
MMF	mycophenolate mofetil
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
HL	Hodgkins lymphoma
NHL	non-Hodgkin lymphoma
CLL	chronic lymphocytic lymphoma
MM	multiple myeloma
MDS	myelodysplastic disorder
PNH	paroxysmal nocturnal hemoglobinuria
CML	chronic myeloid leukemia
MPD	myeloproliferative disease

Reference List

1. Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, et al. Allogeneic Stem Cell Transplantation for Acute Myeloid Leukemia in First Complete Remission: Systematic Review and Meta-analysis of Prospective Clinical Trials. *JAMA: The Journal of the American Medical Association*. 2009; 301(22):2349–2361. [PubMed: 19509382]
2. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. *Blood*. 1990; 75(3):555–562. [PubMed: 2297567]
3. Barrett J, Childs R. Non-myeloablative stem cell transplants. *British Journal of Haematology*. 2000; 111(1):6–17. [PubMed: 11091178]
4. Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood*. 2003; 102(6):2021–2030. [PubMed: 12791654]
5. Baron F, Maris MB, Sandmaier BM, Storer BE, Sorror M, Diaconescu R, et al. Graft-Versus-Tumor Effects After Allogeneic Hematopoietic Cell Transplantation With Nonmyeloablative Conditioning. *Journal of Clinical Oncology*. 2005; 23(9):1993–2003. [PubMed: 15774790]
6. Sandmaier BM, Mackinnon S, Childs RW. Reduced Intensity Conditioning for Allogeneic Hematopoietic Cell Transplantation: Current Perspectives. *Biol Blood Marrow Transplant*. 2007; 13(Supplement 1):87–97. [PubMed: 17222778]
7. Diaconescu R, Flowers CR, Storer B, Sorror ML, Maris MB, Maloney DG, et al. Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. *Blood*. 2004; 104(5):1550–1558. [PubMed: 15150081]

8. Sorror ML, Maris MB, Storer B, Sandmaier BM, Diaconescu R, Flowers C, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. *Blood*. 2004; 104(4):961–968. [PubMed: 15113759]
9. Confer DL. The National Marrow Donor Program. Meeting the needs of the medically underserved. *Cancer*. 2001; 91(1):274–278. [PubMed: 11148593]
10. Kanda Y, Chiba S, Hirai H, Sakamaki H, Iseki T, Kodera Y, et al. Allogeneic hematopoietic stem cell transplantation from family members other than HLA-identical siblings over the last decade (1991–2000). *Blood*. 2003; 102(4):1541–1547. [PubMed: 12714500]
11. Wang Y, Liu Dh, Xu Lp, Liu Ky, Chen H, Chen YH, et al. Superior Graft-versus-Leukemia Effect Associated with Transplantation of Haploidentical Compared with HLA-Identical Sibling Donor Grafts for High-Risk Acute Leukemia: An Historic Comparison. *Biol Blood Marrow Transplant*. In Press, Corrected Proof.
12. Weiden PL, Sullivan KM, Fournoy M, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med*. 1981; 304:1529–1533. [PubMed: 7015133]
13. Weiden PL, Flournoy N, Thomas ED, Prentice R, Fefer A, Buckner CD, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med*. 1979; 300(19):1068–1073. [PubMed: 34792]
14. Alyea E, Weller E, Schlossman R, Canning C, Mauch P, Ng A, et al. Outcome after autologous and allogeneic stem cell transplantation for patients with multiple myeloma: impact of graft-versus-myeloma effect. *Bone Marrow Transplant*. 2003; 32(12):1145–1151. [PubMed: 14647268]
15. Laport GG. Allogeneic hematopoietic cell transplantation for Hodgkin lymphoma: a concise review. *Leukemia & Lymphoma*. 2008; 49(10):1854–1859. [PubMed: 18949609]
16. Khouri IF. Reduced-Intensity Regimens in Allogeneic Stem-Cell Transplantation for Non-Hodgkin Lymphoma and Chronic Lymphocytic Leukemia. *Hematology*. 2006; 2006(1):390–397. [PubMed: 17124088]
17. Reynolds C, Ratanatharathorn V, Adams P, Braun T, Silver S, Ayash L, et al. Allogeneic stem cell transplantation reduces disease progression compared to autologous transplantation in patients with multiple myeloma. *Bone Marrow Transplantation*. 2001; 27(8):801–807. [PubMed: 11477436]
18. Bloor AJC, Thomson K, Chowdhry N, Verfuert S, Ings SJ, Chakraverty R, et al. High Response Rate to Donor Lymphocyte Infusion after Allogeneic Stem Cell Transplantation for Indolent Non-Hodgkin Lymphoma. *Biol Blood Marrow Transplant*. 2008; 14(1):50–58. [PubMed: 18158961]
19. Sierra J, Storer B, Hansen JA, Bjerke JW, Martin PJ, Petersdorf EW, et al. Transplantation of Marrow Cells From Unrelated Donors for Treatment of High-Risk Acute Leukemia: The Effect of Leukemic Burden, Donor HLA-Matching, and Marrow Cell Dose. *Blood*. 1997; 89(11):4226–4235. [PubMed: 9166868]
20. Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ, et al. Marrow transplantation from related donors other than HLA-identical siblings. *N Engl J Med*. 1985; 313(13):765–771. [PubMed: 3897863]
21. Aversa F, Tabilio A, Velardi A, Cunningham I, Terenzi A, Falzetti F, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype [see comments]. *N Engl J Med*. 1998; 339(17):1186–1193. [PubMed: 9780338]
22. Kernan NA, Flomenberg N, Dupont B, O'Reilly RJ. Graft rejection in recipients of T-cell-depleted HLA- nonidentical marrow transplants for leukemia. Identification of host-derived antidonor alloctotoxic T lymphocytes. *Transplantation*. 1987; 43(6):842–847. [PubMed: 3296349]
23. Anasetti C, Amos D, Beatty PG, Appelbaum FR, Bensinger W, Buckner CD, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med*. 1989; 320(4):197–204. [PubMed: 2643045]
24. Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. *Blood*. 2001; 98(12):3192–3204. [PubMed: 11719354]

25. Marmont AM, Horowitz MM, Gale RP, Sobocinski K, Ash RC, van Bekkum DW, et al. T-cell depletion of HLA-identical transplants in leukemia. *Blood*. 1991; 78(8):2120–2130. [PubMed: 1912589]
26. Keever CA, Small TN, Flomenberg N, Heller G, Pekle K, Black P, et al. Immune reconstitution following bone marrow transplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts. *Blood*. 1989; 73(5):1340–1350. [PubMed: 2649174]
27. Welte K, Keever CA, Levick J, Bonilla MA, Merluzzi VJ, Mertelsmann R, et al. Interleukin-2 production and response to interleukin-2 by peripheral blood mononuclear cells from patients after bone marrow transplantation: II. Patients receiving soybean lectin-separated and T cell-depleted bone marrow. *Blood*. 1987; 70(5):1595–1603. [PubMed: 3311208]
28. Soiffer RJ, Bosserman L, Murray C, Cochran K, Daley J, Ritz J. Reconstitution of T-cell function after CD6-depleted allogeneic bone marrow transplantation. *Blood*. 1990; 75(10):2076–2084. [PubMed: 1970938]
29. Roux E, Helg C, Dumont-Girard F, Chapuis B, Jeannet M, Roosnek E. Analysis of T-cell repopulation after allogeneic bone marrow transplantation: significant differences between recipients of T- cell depleted and unmanipulated grafts. *Blood*. 1996; 87(9):3984–3992. [PubMed: 8611731]
30. Schwartz R, Dameshek W. Drug-induced immunological tolerance. *Nature*. 1959; 183:1682–1683. [PubMed: 13666859]
31. Berenbaum MC. Prolongation of homograft survival in mice with single doses of cyclophosphamide. *Nature*. 1963; 200:84. Ref Type: Generic. [PubMed: 14074645]
32. Nirmul G, Severin C, Taub RN. Cyclophosphamide-induced immunologic tolerance to skin homografts. *Surg Forum*. 1971; 22:287–288. [PubMed: 4941317]
33. Mayumi H, Himeno K, Shin T, Nomoto K. Drug-induced tolerance to allografts in mice. VI. Tolerance induction in H-2-haplotype-identical strain combinations in mice. *Transplantation*. 1985; 40(2):188–194. [PubMed: 3895627]
34. Eto M, Mayumi H, Tomita Y, Yoshikai Y, Nishimura Y, Nomoto K. Sequential mechanisms of cyclophosphamide-induced skin allograft tolerance including the intrathymic clonal deletion followed by late breakdown of the clonal deletion. *J Immunol*. 1990; 145(5):1303–1310. [PubMed: 2143514]
35. Strauss G, Osen W, Debatin KM. Induction of apoptosis and modulation of activation and effector function in T cells by immunosuppressive drugs. *Clin Exp Immunol*. 2002; 128(2):255–266. [PubMed: 11985515]
36. Nomoto K, Eto M, Yanaga K, Nishimura Y, Maeda T. Interference with cyclophosphamide-induced skin allograft tolerance by cyclosporin A. *J Immunol*. 1992; 149(8):2668–2674. [PubMed: 1401906]
37. Dukor P, Dietrich FM. Prevention of cyclophosphamide-induced tolerance to erythrocytes by pretreatment with cortisone. *Proc Soc Exp Biol Med*. 1970; 133(1):280–285. [PubMed: 5412347]
38. Mayumi H, Good RA. Long-lasting skin allograft tolerance in adult mice induced across fully allogeneic (multimajor H-2 plus multiminor histocompatibility) antigen barriers by a tolerance-inducing method using cyclophosphamide. *J Exp Med*. 1989; 169(1):213–238. [PubMed: 2642528]
39. Eto M, Mayumi H, Tomita Y, Yoshikai Y, Nomoto K. Intrathymic clonal deletion of V beta 6+ T cells in cyclophosphamide-induced tolerance to H-2-compatible, Mls- disparate antigens. *J Exp Med*. 1990; 171(1):97–113. [PubMed: 2136907]
40. Eto M, Mayumi H, Tomita Y, Yoshikai Y, Nishimura Y, Nomoto K. The requirement of intrathymic mixed chimerism and clonal deletion for a long-lasting skin allograft tolerance in cyclophosphamide-induced tolerance. *Eur J Immunol*. 1990; 20(9):2005–2013. [PubMed: 2209702]
41. Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. *Blood*. 2001; 98(12):3456–3464. [PubMed: 11719388]

42. Luznik L, Engstrom LW, Iannone R, Fuchs EJ. Post-transplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation. *Biol Blood Marrow Transplant.* 2002 In press.
43. O'Donnell PV, Luznik L, Jones RJ, Vogelsang GB, Leffell MS, Phelps M, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant.* 2002; 8(7):377–386. [PubMed: 12171484]
44. Kasamon YL, Luznik L, Leffell MS, Kowalski J, Tsai HL, Bolaños-Meade J, et al. Nonmyeloablative HLA-Haploidentical Bone Marrow Transplantation with High-Dose Posttransplantation Cyclophosphamide: Effect of HLA Disparity on Outcome. *Biol Blood Marrow Transplant.* 2010; 16(4):482–489. [PubMed: 19925877]
45. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide. *Biol Blood Marrow Transplant.* 2008; 14(6):641–650. [PubMed: 18489989]
46. Anasetti C, Beatty PG, Storb R, Martin PJ, Mori M, Sanders JE, et al. Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma. *Hum Immunol.* 1990; 29(2):79–91. [PubMed: 2249952]
47. Szydlo R, Goldman JM, Klein JP, Gale RP, Ash RC, Bach FH, et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. *J Clin Oncol.* 1997; 15(5):1767–1777. [PubMed: 9164184]
48. Ash RC, Horowitz MM, Gale RP, van Bekkum DW, Casper JT, Gordon-Smith EC, et al. Bone marrow transplantation from related donors other than HLA- identical siblings: effect of T cell depletion. *Bone Marrow Transplant.* 1991; 7(6):443–452. [PubMed: 1873591]
49. Symons HJ, Leffell MS, Rossiter ND, Zahurak M, Jones RJ, Fuchs EJ. Improved Survival with Inhibitory Killer Immunoglobulin Receptor (KIR) Gene Mismatches and KIR Haplotype B Donors after Nonmyeloablative, HLA-Haploidentical Bone Marrow Transplantation. *Biol Blood Marrow Transplant.* 2010; 16(4):533–542. [PubMed: 19961944]
50. Mielcarek M, Martin PJ, Leisenring W, Flowers MED, Maloney DG, Sandmaier BM, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood.* 2003; 102(2):756–762. [PubMed: 12663454]
51. Koh LP, Chao NJ. Nonmyeloablative allogeneic hematopoietic stem cell transplant using mismatched/haploidentical donors: A review. *Blood Cells, Molecules, and Diseases.* 2008; 40(1): 20–24.
52. Banna GL, Aversa S, Sileni VC, Favaretto A, Ghiotto C, Monfardini S. Nonmyeloablative allogeneic stem cell transplantation (NST) after truly nonmyeloablative and reduced intensity conditioning regimens. *Critical Reviews in Oncology/Hematology.* 2004; 51(3):171–189. [PubMed: 15331077]
53. Dey BR, McAfee S, Colby C, Cieply K, Caron M, Saidman S, et al. Anti-tumour response despite loss of donor chimaerism in patients treated with non-myeloablative conditioning and allogeneic stem cell transplantation. *British Journal of Haematology.* 2005; 128(3):351–359. [PubMed: 15667537]
54. Colvin GA, Berz D, Ramanathan M, Winer ES, Fast L, Elfenbein GJ, et al. Nonengraftment Haploidentical Cellular Immunotherapy for Refractory Malignancies: Tumor Responses without Chimerism. *Biol Blood Marrow Transplant.* 2009; 15(4):421–431. [PubMed: 19285629]

Figure 1.

Treatment schema for nonmyeloablative conditioning regimen in HLA-haploidentical transplantation with post-transplantation cyclophosphamide. MMF=mycophenolate mofetil; TBI=total body irradiation; Cy=cyclophosphamide; G-CSF=granulocyte colony stimulating factor

Figure 2. Cumulative incidence of acute (**A**) and chronic (**B**) GVHD after nonmyeloablative haploidentical stem cell transplantation with post-transplantation cyclophosphamide.

Figure 3.
Cumulative incidence of relapse and nonrelapse mortality after nonmyeloablative haploidentical stem cell transplantation with post-transplantation cyclophosphamide

Figure 4.

Actuarial curves of **(A)** overall survival (OS) and event-free survival (EFS) in all patients undergoing nonmyeloablative haploidentical stem cell transplantation with post-transplantation cyclophosphamide; **(B)** overall survival in patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) or myeloproliferative disorder (MPD); **(C)** overall survival in patients with Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL) and chronic lymphocytic lymphoma (CLL).

Figure 5. Event-free survival (EFS) of patients undergoing nonmyeloablative haploidentical stem cell transplantation with post-transplantation cyclophosphamide according to number of mismatched HLA-antigens in any direction (GVH or HVG)