

Non-myeloablative Hematopoietic Cell Transplant for Treatment of Nonmalignant Disorders in Children

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Abstract

Hematopoietic stem cell transplantation (HCT) may offer the only curative therapy for certain life-threatening immune deficiency disorders. Conventional HCT poses a risk to patients for severe morbidity, mortality, and late sequelae resulting from myeloablative preparative regimens. This review summarizes the development of nonmyeloablative regimens that have the potential to reduce both short- and long-term risks of HCT. Results of NM-HCT in a small number of patients indicate that this procedure may play an important role in treatment of life-threatening immune deficiencies.

1. Principles of Hematopoietic Cell Transplant

The primitive hematopoietic stem cell (HSC) has the capability for self-renewal and differentiation. These characteristics allow transplantation of small numbers of HSC sufficient for complete restoration of the hematopoietic system of another individual. Transplanted HSC ultimately differentiate into multiple lineages, including erythrocyte, monocyte/macrophage, granulocyte, megakaryocyte, and lymphoid cells. Thus HSC transplantation (HCT) has the potential to cure a variety of disorders resulting from defects in the pluripotent progenitor cells as well as defects in single hematopoietic lineages. The establishment of multilineage donor chimerism is required for successful treatment of bone marrow failure syndromes or disorders involving multiple cell lineages, such as Wiskott-Aldrich syndrome. For disorders that affect a single lineage, such as immunodeficiency diseases or hemaglobinopathies, the goal is to restore sufficient numbers of normal cells of the affected lineage; reconstitution of an unaffected lineage is not required for cure of the disease.

The graft-vs.-host (GVH) and host-vs.-graft (HVG) responses form primary and opposing immunologic bar-

riers to successful allogeneic HCT. The extent of this bi-directional barrier is determined by the differences in major or minor histocompatibility antigens (MHC) between donor and recipient. The HVG barrier is determined by the strength of the recipient alloreactive immune response, which is contingent upon the antigenic stimulation provided by donor cells and the capacity of host immune cells to generate a response. A contributing factor is postulated to be host cell occupancy of a hematopoietic cell compartment that presumably functions as a space-occupying barrier to engraftment. The GVH barrier is determined by the alloreactive T cell response directed toward disparate MHC or minor histocompatibility antigens. Emerging evidence suggests that donor alloreactivity may be modulated by the proportion of co-transplanted pro-inflammatory or anti-inflammatory accessory cells [1].

The conventional strategy to overcome this bi-directional barrier relies upon three elements. First, a conditioning regimen is delivered, with the dual purposes of immunoablation and myeloablation. Supralethal doses of irradiation or chemotherapy are used to surmount the immunologic barrier to engraftment, as well as to create marrow space. Second, donor HSCs are given to rescue

Table 1.

Toxicity Associated with Conventional Hematopoietic Cell Transplant Regimens.

Early Toxicities	Late Effects
Anemia	Cataracts
Neutropenia	Growth hormone deficiency
Thrombocytopenia	Thyroid hormone deficiency
Hemorrhage	Sex hormone deficiency
Infection	Infertility
Bacterial sepsis	Intellectual impairment
Invasive fungal infection	Dental caries
Disseminated viral infection	Aseptic necrosis of bone
Mucositis	Chronic pulmonary disease
Diarrhea	Second malignancy
Veno-occlusive disease of liver	
Renal insufficiency/ renal failure	
Hemorrhagic cystitis	
Interstitial pneumonitis	
Cardiomyopathy or arrhythmia	
Transfusion-transmitted infection	

sion or by elimination of T cells from the donor graft.

Supralethal doses of irradiation or chemotherapy commonly used in transplant regimens are associated with significant toxicities (Table 1). The substantial risks for transplant-related mortality from toxicity or severe GVHD have precluded transplants for all but healthy, younger patients. Unfortunately, many patients with life-threatening hematologic or immunologic disorders have associated conditions such as infections or organ dysfunction that greatly increase the risk for mortality and prohibit use of potentially life-saving procedure of HCT.

2. Development of Nonmyeloablative Hematopoietic Cell Transplants

Conditioning regimens that do not employ agents at doses resulting in permanent marrow aplasia are considered nonmyeloablative. Until recently, nonmyeloablative hematopoietic cell transplants (NM-HCT) have been used routinely for only two conditions: severe aplastic anemia and severe combined immune deficiency (SCID) (Figure 1). Regimens for aplastic anemia have included immunosuppressive agents alone to overcome the alloimmune rejection responses, since these patients have unoccupied marrow space. Consequently, these reduced intensity regimens have resulted in markedly lower incidence of both early and late complications [2,3]. In general, pretransplant conditioning regimens have not been used for SCID patients, because these patients have no immune system capable of rejecting the graft [4]. Furthermore, myeloablation has not been required, because there exists an unoccupied lymphoid niche for engraftment. While the lymphoid graft can be established without a conditioning regimen in SCID patients, engraftment of other cell lineages has been uncommon.

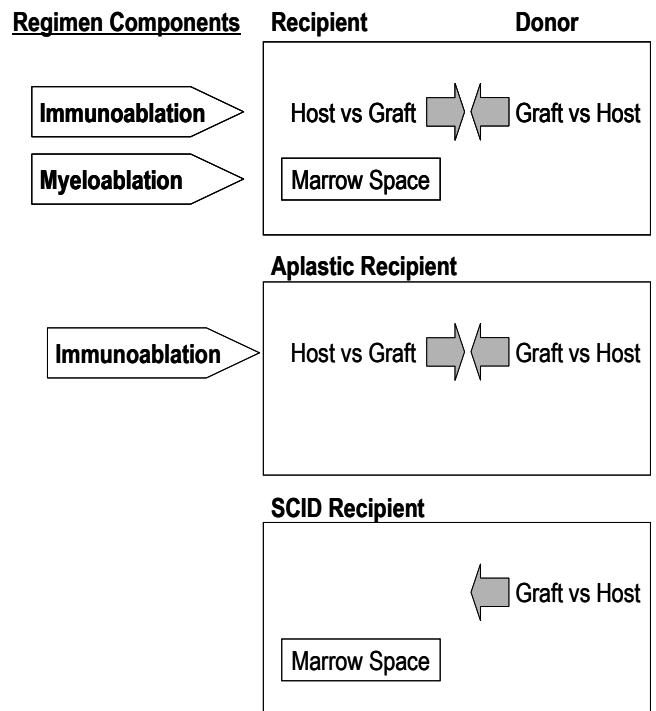


Figure 1. Conventional conditioning regimens used for HLA-related marrow transplants for leukemia, severe aplastic anemia, and severe combined immune deficiency. A bi-directional barrier to engraftment is set by the degree of HLA disparity and the strength of the recipient alloimmune response. Conventional regimens used for patients with leukemia include both immunoablative and myeloablative agents to overcome recipient barriers to engraftment (top). Immunoablative agents are directed toward elimination of host-vs.-graft immunity, while myeloablative agents are used to eradicate leukemic cells and creates marrow space. The marrow space is unoccupied in severe aplastic anemia (middle), therefore only the host immune system must be overcome to achieve donor engraftment. The recipient alloimmune response is absent in severe combined immune deficiency (bottom), and occupied marrow space does not prevent engraftment of lymphocytes; therefore no conditioning regimen is needed.

HCT for treatment of hematologic malignancies and most other non-malignant conditions conventionally have employed a myeloablative conditioning regimen. As the importance of the graft-vs.-leukemia (GVL) effect became evident in the late 1970s and early 1980s, studies subsequently found that donor lymphocyte infusions (DLI) could be used to treat leukemic relapse after HCT [5,6]. The success of DLI set the stage for the introduction of NM-HCT, based on the hypothesis that the graft itself participated in creation of its own niche through a subclinical GVH reaction directed toward recipient hematopoietic cells. Using concepts learned from animal models and armed with new potent immunosuppressive agents such as 2-CDA and fludarabine, investigators in Texas, Israel, Seattle, Boston, and Washington, DC pioneered regimens that have resulted in significantly less toxicity while allowing partial or full chimerism in most patients. These studies have

Table 2.

Comparison of Therapeutic Strategies Used in Conventional and Nonmyeloablative Hematopoietic Cell Transplant (HCT).

	Conventional HCT	Nonmyeloablative HCT	
		Reduced-Toxicity Regimens	Minimal-Toxicity Regimens
Control recipient alloimmune response	Supralethal chemo/radiotherapy	Pre-transplant immune suppression	Pre- and post-transplant immune suppression
Create marrow space	Supralethal chemo/radiotherapy	Pre-transplant myelosuppression plus graft-vs.-marrow reaction	Graft-vs.-marrow reaction
Eradicate malignancy	Supralethal chemo/radiotherapy	Graft-vs.-leukemia +/- pre-transplant chemotherapy	Graft-vs. leukemia
Restore hematopoiesis	Hematopoietic cell transplant	Hematopoietic cell transplant	Hematopoietic cell transplant
Control graft-vs.-host response	Post-transplant immune suppression or T cell depleted graft	Post-transplant immune suppression or T cell depleted graft	Post-transplant immune suppression

shown that intense immunosuppression can be sufficient to establish full or partial donor chimerism and that myeloablation is not required for creation of marrow space.

Two general approaches have been used to develop NM-HCT regimens (Table 2). First, regimens with *reduced toxicity* have been developed by replacing myeloablative agents with those that have more immunosuppressive and less myelosuppressive properties. Most successful have been studies from M.D. Anderson Cancer Center in Houston and the Hadassah University in Jerusalem, which have incorporated fludarabine, a drug with intensive T-cell immunosuppressive properties, as a replacement for traditional myeloablative agents [7-10]. Second, regimens with *minimal toxicity*, developed initially in animal models, have used low-dose irradiation to induce a degree of immune suppression pre-transplant, followed by post-transplant immunosuppression given to control residual host, as well as newly infused donor, alloreactive T cells. Using a dog model, investigators in Seattle showed 200 cGy total body irradiation (TBI) followed by synergistic post-transplant immunosuppression with mycophenolate mofetil and cyclosporine could establish chimerism with little or no neutropenia or thrombocytopenia [11,12]. This approach was used successfully to establish donor chimerism in human patients as well, and subsequently the addition of fludarabine to the regimen has reduced the incidence of rejection to <3% following HLA-matched related grafts [13]. Investigators in Boston found that thymic irradiation to 700cGy plus high-dose cyclophosphamide and anti-thymocyte globulin (ATG) also resulted in partial or full donor engraftment [14].

The development of donor chimerism following NM-HCT depends on multiple factors, including the degree of intensity of the preparative regimen (reduced vs. minimal toxicity), the source of hematopoietic cells (marrow vs. PBSC), the degree of HLA-matching, and the extent of T cell depletion. *Reduced-toxicity* regimens report high rates of stable donor engraftment, although

at the expense of higher rates of transplant-related mortality compared to minimal toxicity regimens [7-10]. *Minimal-toxicity* regimens such as low-dose TBI, low dose fludarabine/cyclophosphamide, or cyclophosphamide/ATG plus thymic radiation report stable donor grafts in 69-90% of patients [13]. Stable engraftment after minimal toxicity regimens may be improved by use of peripheral blood stem cells, presumably due to both the 10-fold increase in donor T cells and the higher number of hematopoietic stem cells infused. Stable engraftment has been more difficult to achieve with unrelated or HLA disparate grafts [15,16].

Fortunately, graft rejection following NH-HCT generally has been associated with persistence of autologous hematopoiesis, rendering the patient neither better nor worse than before transplant [13]. Some investigators have advocated use of NM-HCT as a first-step, followed by a myeloablative transplant for those patients with graft rejection. In support of this approach has been our anecdotal experience of myeloablative HCT for establishing grafts following NH-HCT, although these second transplants have been complicated by toxicities as would be expected for such high risk patients.⁷⁰ Further studies must be done to confirm that a second-step myeloablative HCT can overcome rejection following NM-HCT, since presumably the prior exposure will have sensitized the patient, increasing the risk for graft rejection.

Minimal-toxicity regimens have resulted in a dramatic improvement in early morbidity and resource utilization [13]. The Seattle consortium reported that 57% of patients given low-dose TBI, with or without fludarabine, were treated entirely in the outpatient department. Among patients admitted to hospital, the median number of inpatient days was reduced to 8 compared to 31 days for conventional HCT. These patients did not experience any of the complications typically associated with conventional HCT, including mucositis, severe nausea, pulmonary or cardiac toxicity, hemorrhagic cystitis or alopecia. Pancytopenia was uncommon and the

number of blood products transfused was reduced significantly compared to historic controls [17]. More intensive *reduced-toxicity* regimens have reported a higher incidence of severe toxicities, although still lower than observed with myeloablative HCT [18,19]. Deaths from infectious causes have been reported in 12-38% of patients treated with regimens that combine intensive immunosuppression with nonmyeloablative chemotherapy [7-10].

Comparison of the risk for GVHD following NM-HCT with conventional HCT has been made difficult because NM-HCT regimens were designed to enhance the graft-vs-tumor effect, and these studies have been conducted mainly in older patients who are theoretically at high risk for GVHD. In theory, some factors associated with NM-HCT regimens potentially could mitigate the risk for GVHD: 1) regimens that result in minimal tissue damage may limit release of cytokines implicated in incitement of GVHD; 2) intensive post-transplant immune suppression used to control HVG reactions may concurrently reduce GVH reactions. Furthermore, while donor anti-host T cell reactivity has been thought to be the basis for success of the very-low dose regimens, reduction of the incidence of GVHD may also be expected in those cases where T-cell depleted grafts have been established [20]. The reported incidence of grades II-IV acute GVHD has ranged from 20-51% among patients treated with NM-HCT regimens, essentially equivalent to the historical incidence after conventional HCT [7-14]. However, the reported incidence of chronic GVHD has ranged from 50-74%, somewhat higher than expected for conventional HCT, presumably related to purposeful induction of the GVL effect. Data to show the risk for acute GVHD in pediatric patients or those with nonmalignant diseases have been limited, however studies with small numbers of patients have reported a range from 18-75% [20-23].

There are several reasons to consider developing a nontoxic, nonmyeloablative regimen for establishment of mixed chimerism in patients with nonmalignant disorders. One of the main obstacles to successful transplants for non-malignant diseases is the relatively high risk for mortality due to infection and toxicities resulting from the ablative regimen. Thus conventional HCT has been reserved for children with life-threatening disorders. In addition, the transplant procedure often will be postponed until the development of significant disease complications, in an effort to avoid a high-risk procedure in a less-affected child. Moreover, the long-term side effects associated with conventional regimens, including infertility, hormonal dysfunction, growth failure, and secondary malignancies, deter patients and families from seeking a curative treatment.

The potential for reversal of disease symptoms with partial chimerism has been demonstrated in a number of studies [24-26]. Persistent mixed chimerism has been reported after conventional transplants, and has been associated with full or partial immune reconstitution. Effectiveness of partial chimerism also can be inferred from studies of carriers of X-linked disorders, in which

women rarely have symptomatic disease even when Lyonization results in low numbers of unaffected cells.

3. Nonmyeloablative Hematopoietic Cell Transplants for Treatment of Immune Deficiencies

NM-HCT has been studied for treatment of non-malignant disorders in limited numbers of patients. Most of the experience has been in the treatment of immunodeficiency syndromes. *Reduced-toxicity* regimens have been studied by a number of investigators. Amrolia and colleagues reported results using fludarabine, melphalan, and anti-lymphocyte globulin for 2 SCID patients and 6 patients with other immune deficiencies given transplants from HLA-matched related or unrelated donors [23]. The regimen was marrow suppressive, with a median duration of neutropenia of 13 days, and the patients were hospitalized a median of 52 days. Severe hepatotoxicity occurred in 3 patients, otherwise toxicities were reduced, and neither acute GVHD grades II-IV nor clinical extensive chronic GVHD was observed. Seven of the 8 patients were reported to survive from 8 to 17 months after transplant. One patient died, after developing hemophagocytic lymphohistiocytosis and *pseudomonas* septicemia. The regimen established donor chimerism in all 8 patients, 6 maintained stable high-level donor chimerism, while 2 had mixed donor-host chimerism was persistent for 9 and 12 months follow-up, respectively. Donor chimerism correlated with improvement in T cell number and function in all patients evaluable.

Horowitz and colleagues have reported a strategy using a highly immunosuppressive, but nonmyeloablative, regimen to establish T-cell depleted matched-related allografts, followed by delayed infusion of donor lymphocytes to improve the level of donor chimerism [20]. The approach was studied in 10 patients with chronic granulomatous disease (CGD) conditioned with cyclophosphamide, fludarabine, and antithymocyte globulin and given HLA-matched related peripheral blood stem cells that were enriched for CD34+ cells and contained approximately 1×10^5 CD3+ cells per kg recipient weight. The regimen was marrow suppressive, with a median duration of neutropenia of 10 days. The only significant adverse event reported to have been associated with the regimen was interstitial pneumonitis that occurred in 1 patient. Acute GVHD of grades II-IV was observed in 3 patients, with onset after receipt of donor lymphocyte infusions, and 1 of these developed clinical extensive chronic GVHD. One patient died from GVHD at 8 months after transplant. Donor chimerism was established in 9 of 10 patients, and 1 patient experienced late graft rejection with reconstitution of autologous hematopoiesis. The infusion of donor lymphocytes was reported to improve the level of donor chimerism in 4 of the patients. Donor chimerism was associated with presence of oxidase-positive neutrophils in 8 of the patients at a median of 17 months followup. Overall, 7 patients have survived from 16 to 26 months, there

were 2 deaths related to the procedure from pneumococcal pneumonia and GVHD, respectively, and a third patient who rejected the graft died after a second transplant. A similar approach was reported by Nagler that resulted in full chimerism with no GVHD in a child with CGD [27].

In contrast to the reduced toxicity regimens, the minimal toxicity regimen uses significantly less pre-transplant conditioning, and relies more heavily on post-transplant immune suppression to control both HVG and GVH reactions. The Seattle group has studied post-transplant immune suppression in 2 patients with SCID and 7 with other immunodeficiency syndromes who were given related or unrelated marrow grafts [21,22]. All of these patients had high risk factors for conventional transplant, including older age and/or active opportunistic infections. One SCID and one non-SCID patient received only post-transplant mycophenolate mofetil and cyclosporine, while the remaining patients were given 200 cGy TBI delivered at a low dose rate of 7 cGy per minute with or without fludarabine (total dose 90 mg/m²) depending on the extent of T cell dysfunction. The regimen induced minimal suppression of the peripheral blood counts, and no significant toxicities related to the regimen were observed. GVHD grades II-III has developed in 5 patients, and clinical extensive chronic GVHD in 4. Donor chimerism was achieved in all patients, ranging from 5-100% donor T cells, B cells, and granulocytes. Evidence for T and B cell reconstitution has been shown for 6 of 7 evaluable patients, including an X-SCID patient with donor B cell engraftment following an unrelated graft. Among patients with >180 days of followup, 5 of 7 have survived with stable donor chimerism from 9-38 months after transplant. One patient died at day 214 for infection, and 1 patient was taken off study for a second transplant using an ablative regimen, performed to improve low-level donor chimerism.

4. Nonmyeloablative Hematopoietic Cell Transplants for Treatment of Congenital Bone Marrow Failure Syndromes

The evolution of transplantation in the treatment of Fanconi anemia (FA) exemplifies the evolution of the nonmyeloablative approach. Early investigators found an unusually high degree of toxic deaths associated with conventional regimens, and subsequent studies demonstrated that engraftment could be achieved with successively lower doses of cyclophosphamide alone. The Curitiba and Seattle group have established that engraftment can be achieved reliably with a total dose of 60 mg cyclophosphamide, with low toxicity and survival of 100% [M.E. Flowers, personal communication]. It has been more difficult to develop a regimen with lower toxicity and improved survival for patients with alternative donors. Lower doses of TBI combined with lower doses of cyclophosphamide did not significantly improve survival for patients receiving unrelated grafts. *Reduced-toxicity* regimens that have incorporated fluda-

rabine have shown early promise. MacMillan and colleagues reported 83% engraftment and improved survival with a regimen containing fludarabine, cyclophosphamide, and ATG [28]. While the addition of fludarabine to TBI based regimens have increased engraftment, infection continues to be a problem.

Patients with Schwachman-Diamond syndrome (SDS) also are considered at relatively high risk for mortality with conventional HCT. The Seattle consortium has used the *minimal-toxicity* regimen of fludarabine and low-dose TBI to treat 1 patient with SDS who received matched related PBSC and 1 with FA who received matched unrelated PBSC, with promising results. High-level donor chimerism was achieved without significant toxicity, and both patients survive >1 year following NM-HCT [Kurre P, Seattle, WA, unpublished data]. A second patient with SDS who had transformed to myelodysplastic syndrome received unrelated PBSC following fludarabine and low-dose TBI, however donor engraftment was transient. These results suggest that the *minimal-toxicity* regimen may be very useful for FA and other bone marrow failure syndromes, if the transplant is performed in early phases of the disease before malignant transformation.

5. Nonmyeloablative Hematopoietic Cell Transplants for Treatment of Other Nonmalignant Disorders

5.1. Metabolic Storage Disorders

Marrow transplantation has been shown to slow or stop neurologic deterioration, among other physiologic improvements, in certain lysosomal acid hydrolase deficiencies and leukodystrophies [29,30]. Although a less toxic approach is desired, engraftment and achievement of adequate donor enzyme levels are likely to be more challenging in these disorders, given the not insignificant rates of graft failure reported with conventional regimens. While animal models have shown promise, there have been few reports of clinical studies. *Reduced-toxicity regimens* have been studied in limited number of cases. Slavin reported 100% donor chimerism and no GVHD using fludarabine, busulfan, and ATG for treatment of Gauchers syndrome [31], and Ketznel and colleagues in Chicago successfully engrafted a child with Sandhoff Syndrome using the same regimen (personal communication, 2001). The *minimal-toxicity* regimen of fludarabine and low-dose TBI was used in Seattle, Salt Lake City, and Leipzig for unrelated PBSC transplants in 1 patient with metachromatic leukodystrophy (MLD) and 2 with MPS-1. Full donor chimerism, normal enzyme levels, and disease improvement has been observed in the MLD patient with >1 year of follow-up. Among patients with MPS-1, 1 rejected the graft, and 1 developed 50% donor chimerism and normal enzyme levels early after transplant. As experience is gained in NM-HCT, the question arises as to what level of donor chimerism is needed to adequately treat the underlying abnormality. The answer

will likely be complex, since many of these disorders rely on enzyme secretion from tissue macrophages, making the direct measurement of myeloid chimerism important.

5.2. Sickle Cell Disease and Thalassemia

There have been tremendous advancements over the past decade in use of HCT for treatment of children with sickle cell disease and thalassemia. Matched sibling marrow transplantation for severe SSD, including patients with history of stroke or acute chest syndrome, has resulted in long-term overall survival and event-free survival of 94% and 84%, respectively [32]. Similar results have been obtained for patients with thalassemia [33]. Certain factors place patients at higher risk for mortality, including age, and for thalassemic patients, liver abnormalities. Moreover, the sickle cell studies have shown that infertility and hormonal deficiencies were common late effects, reducing the attractiveness of conventional HCT for some patients [32]. The rationale for studying NM-HCT in these disorders is based upon reports of stable mixed chimerism following conventional transplantation that has been associated with disease amelioration, suggesting a biologic advantage for normal erythroid cells in these settings [34]. However, there remains a theoretical argument against reduced dose regimens, as patients have been alloimmunized through multiple transfusions, resulting in strengthened HVG responses. There have been limited number of clinical trials of NM-HCT in the treatment of hemoglobinopathies, including several reports of *reduced-intensity* regimens. Krishnamurti and colleagues demonstrated engraftment of matched related graft in a patient with sickle cell disease using fludarabine, busulfan, ATG, and total lymphoid irradiation, although a second patient rejected an unrelated graft [35]. Kletzel and colleagues treated 3 patients with sickle cell disease using fludarabine, busulfan, and ATG and matched related HCT (personal communication, 2001). One developed cGVHD, one rejected, and one is well and with donor chimerism. Results from *minimal-toxicity* regimens have been less satisfactory (Mark Walters, Oakland, CA, personal communication). Although not associated with toxicity, stable long-term engraftment has been difficult to achieve, likely because patients have been highly alloimmunized. Currently, NM-HCT should be considered experimental and perhaps reserved for patients with risk factors associated with higher mortality, since the results of conventional HCT have been excellent.

6. Conclusions

Studies to date indicate that NM-HCT may have an important role to play in the treatment of life-threatening nonmalignant diseases. *Minimal toxicity* regimens have been used without severe toxicity in very ill patients, and therefore may provide the only acceptable transplant alternative to older patients or those with opportunistic infections or severe lung or pulmonary

disease. Both *minimal toxicity* and *reduced toxicity* regimens offer the potential of achieving donor engraftment with less morbidity than standard regimens, an important consideration for patients who currently may consider the risk of conventional transplant unacceptably high. In the future, NM-HCT may be considered as a first-step toward establishing allogeneic grafts. Patients who reject the initial graft will return to baseline their condition and may be given a second transplant using a myeloablative regimen, or may continue to receive standard medical therapies. For those situations in which low-level donor chimerism is established, donor lymphocyte infusions, additional immune suppression, or second infusions of donor stem cells are being investigated as methods to increase donor chimerism.

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