SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.12.563.

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Unrelated Donor Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Hemoglobinopathies Using a Reduced-Intensity Conditioning Regimen and Third-Party Mesenchymal Stromal Cells

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Allogeneic hematopoietic stem cell transplantation for patients with a hemoglobinopathy can be curative but is limited by donor availability. Although positive results are frequently observed in those with an HLA-matched sibling donor, use of unrelated donors has been complicated by poor engraftment, excessive regimen-related toxicity, and graft-versus-host disease (GVHD). As a potential strategy to address these obstacles, a pilot study was designed that incorporated both a reduced-intensity conditioning and mesenchymal stromal cells (MSCs). Six patients were enrolled, including 4 with high-risk sickle cell disease (SCD) and 2 with transfusion-dependent thalassemia major. Conditioning consisted of fludarabine (150 mg/m^2), melphalan (140 mg/m^2), and alemtuzumab (60 mg for patients weighing $>$ 30 kg and .9 mg/kg for patients weighing $<$30 kg). Two patients received HLA 7/8 allele matched bone marrow and 4 received 4-5/6 HLA

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Mesenchymal stromal cells
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matched umbilical cord blood as the source of HSCs. MSCs were of bone marrow origin and derived from a parent in 1 patient and from an unrelated third-party donor in the remaining 5 patients. GVHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil. One patient had neutropenic graft failure, 2 had autologous hematopoietic recovery, and 3 had hematopoietic recovery with complete chimerism. The 2 SCD patients with autologous hematopoietic recovery are alive. The remaining 4 died either from opportunistic infection, GVHD, or intracranial hemorrhage. Although no infusion-related toxicity was seen, the cotransplantation of MSCs was not sufficient for reliable engraftment in patients with advanced hemoglobinopathy. Although poor engraftment has been observed in nearly all such trials to date in this patient population, there was no evidence to suggest that MSCs had any positive impact on engraftment. Because of the lack of improved engraftment and unacceptably high transplant-related mortality, the study was prematurely terminated. Further investigations into understanding the mechanisms of graft resistance and development of strategies to overcome this barrier are needed to move this field forward.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for sickle cell disease (SCD) and thalassemia [1,2]. However, most experience with this treatment modality is in patients who have an HLA-matched sibling donor after myeloablative conditioning, such as busulfan, cyclophosphamide, and antithymocyte globulin [3]. These studies established the proof of concept that ablation of host hematopoiesis and immunity could allow durable engraftment. In those with matched sibling donors, the overall probability of cure has been consistently in the 80% to 85% range, with less than 10% treatment-related mortality [2,4–12]. Importantly, stabilization or reversal of organ damage from SCD has been documented [7]. However, most patients do not have an HLA-matched sibling donor and are rarely considered for allogeneic HSCT because of the difficulty in finding a suitably HLA-matched unrelated donor. Moreover, conventional myeloablative conditioning regimens are often associated with a high risk of early death from regimen-related side effects as well as from late effects, such as infertility [13]. Use of partially HLA-matched donors is associated with increased risks of graft failure, acute and chronic graft-versus-host disease (GVHD), and slow immune recovery with consequent high risks of opportunistic infection.

One approach to decrease transplant-related mortality is to decrease the intensity of the conditioning regimen. Although effective in patients with malignant disease, nonmyeloablative conditioning regimens have typically failed to ensure a high rate of engraftment in patients with a hemoglobinopathy [14]. Higher dose but reduced-intensity conditioning (RIC) regimens have met with limited success [15–18], at least in the context of HLA-matched marrow or mobilized peripheral blood from unrelated donors.

The lack of a suitable HLA-matched donor has been a major limitation in the field because most patients with hemoglobinopathies are of non-European decent. For this reason, HLA-mismatched umbilical cord blood (UCB) has been explored as a graft source. Although there appears to be lower stringency for the degree of HLA match, UCB is associated with a higher rate of graft failure, particularly in patients with nonmalignant diseases [19,20]. Although the use of haploidentical donors is emerging, early reports also suggest it is associated with a high rate of graft failure/autologous recovery in this setting [21–23].

In an attempt to address the engraftment barrier, we proposed the cotransplantation of mesenchymal stromal cells (MSCs) to facilitate engraftment of unrelated donor HSCs. MSCs are multipotential, nonhematopoietic progenitor cells capable of differentiating into various lineages [24–28], but they also support the expansion of HSC progenitors in vitro and engraftment in murine models in vivo. Therefore, we hypothesized that MSCs might enhance hematopoietic recovery after RIC in patients with hemoglobinopathy [29–31]. Herein, we report the results of a prospective pilot study designed to explore the use of bone marrow–derived MSCs as a strategy to enhance the engraftment of unrelated donor marrow–or UCB-derived HSCs in the context of a RIC regimen in patients with hemoglobinopathies lacking a HLA matched sibling donor.

METHODS

Patient Characteristics

Patients were enrolled at 3 institutions, Stanford University, University of Minnesota, and University of Alabama at Birmingham. The institutional review boards of each institution approved the study. The Institutional Review Board at the National Marrow Donor Program also approved the study. The trial was registered in the clinical trials network as NCT00957931.

All patients underwent allogeneic HSCT between 2009 and 2011. Four patients had an underlying diagnosis of homozygous SCD, and 2 patients had transfusion-dependent thalassemia major. None of the patients had an HLA-matched sibling donor available. Median patient age was 10 years (range, 8 to 18 years). Indications for HSCT are shown in Table 1, and patient characteristics are shown in Table 2.

Conditioning Regimen and GVHD Prophylaxis

Conditioning was a reduced intensity regimen that consisted of alemtuzumab administered on days −21 through −19, fludarabine on days −7 through −3, and melphalan on days −2 and −1. Alemtuzumab was given as 3 daily doses of 10, 20, and 30 mg to patients weighing >30 kg and at a dose of .3 mg/kg for patients weighing <30 kg. Fludarabine was administered at 30 mg/m^2 per dose for a total of 150 mg/m^2, and melphalan was administered at 70 mg/m^2 per dose for a total dose of 140 mg/m^2. GVHD prophylaxis consisted of cyclosporine A and mycophenolate mofetil. Both drugs were started on day −2 with plan to continue cyclosporine A for 6 months and mycophenolate mofetil for 100 days before initiating taper.

Stem Cell Sources

The HSC source was bone marrow in 2 patients, a single UCB graft in 1 patient, and a double UCB graft in 3 patients. UCB units were 4-5/6 HLA matched to the recipients at the antigen level for HLA-A and -B and allele level for HLA-DRB1, whereas marrow donors were 7/8 HLA matched at allele level for HLA-A, -B, -C, and -DRB1. The criteria for selecting UCB units changed to the following after patient 1, who received a single 4/6 HLA-matched UCB, failed to engraft. Only 5-6/6 HLA-matched single units were allowed and only if they met the following cell dose criteria: $\geq 5 \times 10^7$/kg total nucleated cells for 5/6 matched UCB and $\geq 4 \times 10^7$/kg total nucleated cells for 6/6 matched UCB units. If no single UCB unit with the above-mentioned cell dose and HLA-matching criteria was available, 2 UCB units were selected for transplant. Criteria for double UCB units included units that were 4-6/6 HLA matched to the patient as well as to each other irrespective of the locus of mismatch. Cell dose criteria for double UCB units included a minimum combined total dose of at least 4×10^7/kg total nucleated cells with each unit having a minimum dose of 1.5×10^7/kg total nucleated cells.

Table 1
Indications for HSCT

Patient No.	Disease	Indication for HSCT	No. of Known Transfusions before HSCT
1	SCD	Recurrent episodes of acute chest syndrome, lung disease with oxygen requirement at night, failure of hydroxyurea therapy	20
2	SCD	Recurrent episodes of severe acute chest syndrome, 2-3 episodes of priapism/year, multiple vaso-occlusive crises, restrictive lung disease with small airway obstruction, 3 episodes of splenic sequestration	21
3	β-Thalassemia	Transfusion dependent, intolerance to chelator therapy; iron overload in liver with 7.1 mg iron/g dry liver tissue by liver MRI	84
4	SCD	Major cerebrovascular accident with neurologic sequelae, abnormal MRA of brain with significant intracranial arterial disease, most severely involving the left MCA territory, with high-grade arterial stenosis and occlusions; mild restrictive defects in PFTs; iron overload with 8 mg/g iron in liver detected by liver MRI	74
5	β-Thalassemia	Transfusion dependent, severe iron overload in multiple organs (t2* scan), on chelation with 2 agents, cardiac dysfunction secondary to iron overload	220
6	SCD	Multiple vaso-occlusive crises with frequent hospitalizations, abnormal transcranial Doppler with increased right-sided velocities	4

MRI indicates magnetic resonance imaging; MRA, magnetic resonance angiogram; MCA, middle cerebral artery; PFT, pulmonary function test.

MSC manufacturing was performed under investigational new drug status following US Food and Drug Administration review. For the first patient enrolled on the study, MSCs were derived from a haploidentical parent; all other patients received MSCs from a healthy, unmatched third-party donor. MSC donors (third party and haploidentical) were cytomegalovirus (CMV) seronegative. The University of Minnesota Molecular and Cellular Therapeutics Facility performed cell processing under the National Heart, Lung and Blood Institute's Production Assistance for Cell Therapy Program (Principle Investigator, J.E.W.). Briefly, after enrichment of the marrow mononuclear cells by Ficoll density gradient (Ficoll Hypaque, GE Healthcare, Pittsburgh, PA), cells were seeded at 1.0 to 1.5 $\times$ 10^5 cells/cm^2 at a media depth of 1.6 mm in an appropriately sized T-flask and placed in a 5% CO_2/37°C incubator. Media consisted of alpha-MEM (Life Technologies, Grand Island, NY), 16.5% FBS (Hyclone/Thermo Scientific, Rockford, IL), and GlutaMax (1% 200 mM; Life Technologies). On days 1 and 2, nonadherent cells were removed. Media exchanges took place every 2 to 4 days until 70% to 80% confluence (7 to 10 days). Cells were washed, detached, and inoculated into a cell factory at 40 to 50 cells/cm^2. Media was exchanged every 2 to 4 days over the next 7 to 12 days. At $\geq$90% confluence, cells were harvested, washed, resuspended in 5% human serum albumin (Buminate, Baxter, Deerfield, IL) at 2 to 20 $\times$ 10^6 cells/mL, and cryopreserved using Plasmalyte A (Baxter), DMSO (final concentration 10%; Bioniche Pharma, Morgantown, WV), and human serum albumin (10%; Baxter).

Quality control and lot release testing included infectious disease markers for relevant communicable diseases, sterility, mycoplasma, endotoxin, karyotype (G-banding), flow cytometry (immunophenotype and viability) [32], and trilineage differentiation [32]. As previously published, MSC lot release included CD105 and CD90 $\geq$ 85% [32], CD45 and HLA-DR $\leq$ 15%, viability (prefreeze) $\geq$ 70% by 7-amino-actinomycin, endotoxin $<$ 5.0 EU/mL, sterility cultures $=$ no growth, cytogenetics without clonal abnormality, and *Mycoplasma* points to consider negative. For our patients, MSCs were 95% CD105 and 98% CD90 positive and were 1% CD45 and HLA-DR; prefreeze viability was 90% by 7-amino-actinomycin staining, endotoxin levels were $<$1.0 EU/mL, and aerobic/anaerobic/fungal cultures showed no growth. *Mycoplasma* testing (Points to Consider) was negative, and cytogenetics (G-banding) showed normal female karyotype. MSC had trilineage potential in vitro based on special stains for oil red O (adipose tissue), von Kossa (osteogenic tissue), and toluidine blue (chondrogenic tissue).

On days 0 (4 hours after HSC infusion) and 2, MSCs were thawed at the bedside for immediate administration and infused. Patients were premedicated with 15 mg/kg acetaminophen and .5 to 1 mg/kg diphenhydramine orally. Vital signs were checked 1 hour and 15 minutes before MSC infusion and 15 minutes, 30 minutes, 60 minutes, 2 hours, and 4 hours after infusion. O_2 saturation was monitored for the duration of the infusion and until 9 hours after infusion.

Supportive Care

Supportive care guidelines followed institutional standards. All UCB patients received granulocyte colony-stimulating factor in the immediate post-HSCT period. All patients were monitored for infections as per institutional supportive care guidelines. Antimicrobial prophylaxis included acyclovir with weekly viral surveillance, including monitoring for CMV and human herpesvirus 6 (HHV-6), and trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis jiroveci* pneumonia prophylaxis as per institutional guidelines. For patient 5, who was seropositive for toxoplasma before transplant, a weekly monitoring by PCR was put in place with the plan to resume trimethoprim-sulfamethoxazole for prophylaxis after engraftment. Transfusion parameters were 10 g/dL for hemoglobin and 50,000 for platelets for SCD patients and 8 g/dL for hemoglobin and 10,000 for platelets for thalassemic patients. Additionally, SCD patients received antiseizure prophylaxis with phenytoin or levetiracetam.

Endpoints/Statistical Evaluation

The primary endpoint of the study was attainment of stable engraftment. Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $>$ 500/μL, and platelet recovery was defined as the first of 7 consecutive days of a platelet count $\geq$50,000/μL without transfusion. In addition, donor engraftment was determined by demonstrating chimerism by short tandem repeat analysis in patients' bone marrow and/or peripheral blood. Lineage-specific chimerism analysis was done by using CD3 for T cell, CD15 for myeloid, CD19 for B cell, and CD34 for

Table 2
Patient Characteristics

Patient No.	Age/Sex	Diagnosis	Stem Cell Donor	HLA Match	HSC Graft Characteristics (dose/kg body weight)			MSC Graft Characteristics (dose/kg body weight)	
					TNC ($\times10^7$)	CD34+ ($\times10^6$)	CD3+ ($\times10^7$)	Day 0 ($\times10^6$)	Day 2 ($\times10^6$)
1	8 y/M	SCD	Single UCB	4/6	4.8	.5	2.5	2.0	2.0
2	12 y/M	SCD	Bone marrow	7/8 (B allele mm)	58.6	4.4	4.5	1.72	1.9
3	10 y/M	Thalassemia major	Double UCB	4/6 (both)	Unit 1: 4.6 Unit 2: 2.9	Unit 1: .36 Unit 2: .31	Unit 1: 1.8 Unit 2: 1.1	2.0	2.0
4	10 y/M	SCD	Bone marrow	7/8 (A allele mm)	52.4	7.2	n/a	2.0	2.0
5	18 y/M	Thalassemia major	Double UCB	4/6 and 5/6	Unit 1: 2.7 Unit 2: 2.4	Unit 1: .15 Unit 2: .29	Unit 1: .6 Unit 2: .48	1.8	1.5
6	8 y/F	SCD	Double UCB	4/6 (both)	Unit 1: 3.9 Unit 2: 2.25	Unit 1: .19 Unit 2: .14	Unit 1: .34 Unit 2: .49	2.97	2.97

TNC indicates total nucleated cells.

Table 3
Results of Neutrophil and Platelet Engraftment, Outcomes, Complications, and Causes of Death

Patient No.	Age/ Sex	Diagnosis	Stem Cell Donor	HLA Match	Day of Recovery		Severity of Acute GVHD	Outcome	Complications	Cause of Death
					ANC >500/μL	Platelets >50,000/μL				
1	8 y/M	SCD	UCB	4/6	Day +15	Day +37	None	Alive, with autoreconstitution	HHV-6 viremia Graft failure with autologous recovery	n/a
2	12 y/M	SCD	BM	7/8	Day +10	Day +25	Grade III	Death, day +141	PRES Intracranial hemorrhage Grade III acute GVHD EBV-PTLD	Steroid-refractory GVHD
3	10 y/M	Thalassemia major	dUCB	4/6 and 4/6	Not achieved	Not achieved	Grade II	Death, day +24	Intracranial bleeding Grade II GVHD CMV viremia	Intracranial bleed
4	10 y/M	SCD	BM	7/8	Day +9	Not achieved	None	Death, day +43	CMV pneumonitis	CMV pneumonitis
5	18 y/M	Thalassemia major	dUCB	4/6 and 5/6	Day +33	Not achieved	None	Death, day +59	Gram-negative septic shock Klebsiella and enterobacter SOS CMV reactivation Disseminated toxoplasmosis	Disseminated toxoplasmosis
6	8 y/F	SCD	dUCB	4/6 and 4/6	Day +34	Day +56	None	Alive, with autoreconstitution	CMV viremia Adenovirus reactivation BK virus reactivation Klebsiella bacteremia Graft failure with autologous recovery	n/a

BM indicates blood marrow; ANC, absolute neutrophil count; PRES, posterior reversible encephalopathy syndrome; PTLD, post-transplant lymphoproliferative disease; EBV, Epstein-Barr virus; dUCB, double UCB; SOS, sinusoidal obstruction syndrome; BK, BK virus; n/a, not applicable.
In patient 3, peripheral blood chimerism showed 100% donor engraftment but ANC >500 was not achieved in blood.

stem cell chimerism. Because MSCs were derived from third-party donors, short tandem repeat analysis was used to determine MSC chimerism as well.

Simon's optimal 2-stage design was used for statistical considerations of this pilot study [33]. The planned enrollment for the first stage of the study was 9. Stopping rules of the study included an unacceptable engraftment rate of 6 or fewer engraftments in the first stage and a ≥20% incidence of unexpected grade 3 or higher toxicities or ≥30% treatment-related mortality from expected or unexpected causes in the first 100 days after HSCT.

RESULTS

Engraftment/Chimerism

Table 3 depicts engraftment results. Three of 6 patients achieved an absolute neutrophil count ≥500 on days 10 (patient 2), 9 (patient 4), and 33 (patient 5). Patient 3 demonstrated complete donor chimerism but without neutrophil recovery at the time of his death on day +24. His WBC count on the day of death was 500/μL. The remaining 2 patients (patients 1 and 6) with SCD had autologous hematopoietic recovery. Patient 1 had autologous neutrophil recovery on day 15 with HHV-6 viremia with high fevers documented on day 18. Patient 6 developed CMV viremia with high fever and rash at day 10 after HSCT with autologous reconstitution at day 34. Both patients had received UCB grafts, including 1 who had received a double UCB graft (patient 6).

Platelet engraftment was not attained in 3 patients before death. MSC engraftment was not demonstrated in marrow aspirates in any of the 6 patients. The presence of donor-specific antibodies was reviewed in this patient population and was found in 3 patients, all of whom were double UCB recipients. Two of these patients had engrafted, and in patient 6, who did not engraft, a weak donor-specific antibody with a mean fluorescence index of 503 was found against an HLA-C locus of 1 of the cord blood units. The HLA type of the third-party MSC donor was also reviewed, and no donor-specific antibodies were found against the HLA antigens of the MSC donor in the nonengrafting patients.

Graft-versus-Host Disease

Acute GVHD was observed in 2 of 3 engrafted patients. Patient 3 developed grade II GVHD that responded to systemic and topical steroids. Patient 2 developed grade III GVHD of the gastrointestinal tract, which was steroid resistant. He was treated with infliximab as a second-line agent as well as additional available doses of third-party MSCs with doses ranging from 1.79 to 2.44 $\times$ 10^6/kg on days 23, 44, 47, and 74 to treat GVHD, but without significant response.

Regimen-Related Toxicities

There were no infusion-related toxicities associated with the MSCs. However, significant regimen-related toxicities unrelated to the MSC infusion were observed (Table 3). Patient 5, who had a history of severe iron overload, developed severe sinusoidal obstruction syndrome and was successfully treated with defibrotide. Patient 3 with thalassemia had grade 5 intracranial hemorrhage on day +11. All 6 patients developed opportunistic infections (Table 3). CMV reactivation occurred in 4 patients (patients 3, 4, 5, and 6), patient 6 developed reactivation of BK virus and adenovirus in addition to CMV, and patients 1 and 2 developed reactivation of HHV-6 and Epstein-Barr virus, respectively. Patient 2 developed post-transplant Epstein-Barr virus lymphoproliferative disease. CMV pneumonitis and disseminated toxoplasmosis were the causes of death in 2 patients.

Survival

Only 2 of 6 patients survived, both with SCD and autologous hematopoietic recovery. Because of the lack of consistent donor-derived engraftment and excessive mortality, the study was terminated.

DISCUSSION

These results suggest that the strategy of cotransplantation of allogeneic MSCs did not enhance the engraftment of

HLA-mismatched HSCs in patients with high-risk hemoglobinopathy in the context of the RIC regimen used in this study. Although it is possible that a fully myeloablative conditioning is required, particularly in patients who do not have an HLA-matched donor, previous alloimmunization secondary to prior transfusions is likely a formidable barrier to successful engraftment. Although the impact of donor-specific anti-HLA antibodies was not known at the time of this study, it is possible that the presence of such antibodies had a deleterious effect on engraftment, particularly in the setting of UCB transplantation. Although potentially effective in controlling T cell responses, MSCs do not seem to eliminate the deleterious effect of existing donor-specific anti-HLA antibodies.

The decision to attempt the use of MSCs in this setting was based on several reports that demonstrated intravenous infusion of MSCs is safe and well tolerated [30,34–36]. In addition, cotransplantation of autologous MSCs was associated with rapid hematopoietic recovery in patients with breast cancer who underwent autologous SCT [29]. In a prospective study of 46 patients with hematologic malignancies, MSCs were cotransplanted with HSCs from matched sibling donors, and rapid engraftment of neutrophils and platelets was reported [30]. Several studies have been undertaken to investigate the effect of MSC cotransplantation on HSC engraftment with mixed results [29,31,37,38]. Whereas most of these studies were done in adult patients with malignancies, Macmillan et al. [31] described their experience in pediatric patients undergoing UCB transplantation for malignant disorders. In this small cohort of high-risk leukemia patients, all assessable patients had neutrophil engraftment at median of 19 days, whereas the probability of platelet engraftment was 75% with a median of 53 days. No adverse effects of infusion of MSCs were noted and no increase in the risk of infectious complications seen. Additionally, no long-term effects of MSC infusion were apparent at a median of 6.8 years of follow-up. Although some studies have shown engraftment of MSCs in the bone marrow [39,40], others did not find engraftment of MSCs in the marrow [35,41]. In our cohort of 6 patients, MSC engraftment was not found in the bone marrow by short tandem repeat analysis.

In addition, the immunosuppressive properties of MSCs [42–46] have been exploited for treatment of GVHD [47–49]. Although not the primary reason for their use in this study, a secondary aim of the MSC infusion was to reduce the incidence and severity of GVHD in such high-risk patients. Two patients in this study developed grades II and III acute GVHD, with the latter having steroid-refractory GVHD that failed to respond to additional MSC infusions as well. Still, the numbers of patients are too small to make any conclusion on the effectiveness of MSC on the prevention of GVHD.

Novel strategies are needed to overcome the engraftment barrier that all studies have thus far observed in patients with SCD. Moving forward, the presence of anti-HLA antibodies should be taken into consideration to select appropriate HSC donors whether an unrelated adult donor or UCB unit. However, the limited choice of donors in this particular patient population makes this approach of selecting donors difficult and will pose another layer of challenge in finding suitable donors. The presence of anti-HLA antibodies might also help select which MSC “off the shelf” product to use because it is possible that antibodies might eliminate MSCs shortly after administration. Certainly, the development of novel strategies for overcoming the engraftment barrier are generally needed but especially for this most challenging patient population with SCD.

In addition to lack of consistent donor-derived hematopoietic recovery, viral reactivations and infections were particularly problematic. In a recent report, patients treated with MSCs for acute GVHD were shown to have high CMV viral load and CMV-associated disease [50]. Certainly new strategies are needed to speed immune recovery. However, in patients treated with alemtuzumab, more intensive prophylactic strategies and monitoring must be considered. One such strategy includes use of intensive monitoring and prophylaxis against CMV, such as the use of ganciclovir during conditioning regimen followed by high-dose acyclovir [51]. Additionally, cytotoxic T lymphocytes with specificities toward adenovirus, Epstein-Barr virus, and CMV have shown promise and are already being studied in clinical trials [52,53]; cytotoxic T lymphocytes with specificity toward HHV-6 are currently under investigation as well [54]. More generally, new strategies to enhance immune recovery are needed for all patients undergoing HLA-mismatched transplantation.

At this point, it is premature to believe that RIC or a nonmyeloablative conditioning approach can provide reliable engraftment, particularly in the setting of a mismatched transplant. Although combining low-dose conditioning with any HSC source is the ultimate goal, perhaps a stepwise approach should be considered, segregating the competency of the graft from the conditioning regimen. In the interim, RIC might best be limited to those patients who have matched or closely matched grafts from an adult volunteer donor from whom large numbers of HSCs can be collected, with the use of more mismatched UCB with limited numbers of HSCs limited to those who can tolerate fully myeloablative conditioning.

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