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Matched Related and Unrelated Donor Hematopoietic Stem Cell Transplantation for DOCK8 Deficiency

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A B S T R A C T

We performed allogeneic hematopoietic stem cell transplantation in 6 patients with mutations in the dedicator-of-cytokinesis-8 (DOCK8) gene using a myeloablative conditioning regimen consisting of busulfan 3.2 mg/kg/day i.v. for 4 days and fludarabine 40 mg/m^2/day for 4 days. Three patients received allografts from matched related donors and 3 patients from matched unrelated donors. Two patients received peripheral blood stem cells and 4 patients bone marrow hematopoietic stem cells. Tacrolimus and short-course methotrexate on days 1, 3, 6, and 11 were used for graft-versus-host-disease (GVHD) prophylaxis. All 6 patients are alive at a median follow-up of 22.5 months (range, 14 to 35). All patients achieved rapid and high levels of donor engraftment and complete reversal of the clinical and immunologic phenotype. Adverse events consisted of acute skin GVHD in 2 patients and post-transplant pulmonary infiltrates in a patient with extensive bronchiectasis pretransplant. Thus, a uniform myeloablative conditioning regimen followed by allogeneic hematopoietic stem cell transplantation in DOCK8 deficiency results in reconstitution of immunologic function and reversal of the clinical phenotype with a low incidence of regimen-related toxicity.

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INTRODUCTION

The genetic defect in most autosomal recessive hyper-IgE syndrome cases was shown to be due to homozygous or compound heterozygous mutations in the dedicator-of-cytokinesis-8 (DOCK8) gene [1,2]. Consanguinity is highly correlated with homozygous mutations in DOCK8, whereas most instances of DOCK8 deficiency arising in the absence of a family history of the disease are compound heterozygotes with a different mutation in DOCK8 on each allele. DOCK8 is an atypical guanine nucleotide exchange factor that mediates guanosine triphosphate–guanosine diphosphate exchange on Cdc42, a member of the Rho family of GTPases [3]. DOCK8 deficiency results in a constellation of clinical signs that encompass allergic/atopic manifestations, infection, and malignancy, including severe cutaneous and sinopulmonary infections with bacterial organisms, severe mucocutaneous viral infections with herpes simplex (HSV), cutaneous and disseminated infections with varicella-zoster virus, severe and often debilitating Molluscum contagiosum and human papilloma virus infections, systemic infections with other herpes viruses such as Epstein-Barr virus (EBV), fungal

infections such as mucocutaneous candidiasis, disseminated histoplasmosis, *Pneumocystis jirovecii* pneumonia, and severe parasitic infections with *Cryptosporidium parvum* [1,2]. Most patients with DOCK8 deficiency display marked elevation in serum IgE levels and eosinophils with severe eczema and multiple food and drug allergies. Finally, these patients have the propensity to develop human papilloma virus–driven squamous cell carcinomas and lymphomas, including those driven by EBV [1,2]. Patients with DOCK8 deficiency typically present during childhood, and most die by their early twenties from infection, squamous cell carcinoma, or lymphoma.

Many primary immunodeficiency diseases (PIDs) such as DOCK8 deficiency are caused by intrinsic genetic defects of hematopoietic lineage-derived cells for which allogeneic hematopoietic stem cell transplantation (HSCT) represents an effective therapeutic approach. The life-threatening infections and malignant transformation arising from poor immune surveillance in DOCK8 deficiency and the high likelihood of death at a young age support a definitive therapeutic approach with allogeneic HSCT.

Allogeneic HSCT has been shown to reverse the phenotype in DOCK8 deficiency by reconstituting the normal host defense [4-11]. However, reports of HSCT in DOCK8 deficiency typically consist of case studies with heterogeneous conditioning regimens, including several in which DOCK8 deficiency was identified only retrospectively. Here we describe successful allogeneic HSCT for prospectively diagnosed patients with DOCK8 deficiency using matched related and unrelated donors and a uniform, reduced-toxicity, high-dose regimen of busulfan and fludarabine without serotherapy.

METHODS

Study Design and Procedures

We conducted a phase I pilot study to determine the efficacy and safety of myeloablative allogeneic HSCT for patients with DOCK8 deficiency. The primary objective of the study was to determine whether allogeneic HSCT reconstitutes T lymphocyte and B lymphocyte cells and myeloid cells with normal donor cells at 1 year post-transplant and reverses the clinical phenotype of severe recurrent infections in patients with DOCK8 deficiency. The secondary objective of the study was to determine the safety of this allogeneic transplant regimen in DOCK8 deficiency by assessing transplant-related toxicity, the incidence of acute and chronic graft-versus-host disease (GVHD), immune reconstitution, overall survival, and disease-free survival.

The study was approved by the Institutional Review Board of the National Cancer Institute and was independently monitored for safety and data accuracy. Written informed consent and assent was obtained for all patients and donors (ClinicalTrials.gov number, NCT01176006).

Inclusion criteria were as follows: (1) patient age of 8 to 40 years, (2) DOCK8 deficiency with clinical history of 1 or more episodes of life-threatening or severely disfiguring infection with opportunistic organisms, (3) homozygous or compound heterozygous mutations in the *DOCK8* gene performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, (4) 10/10 or 9/10 HLA-matched related or unrelated donor or a haploidentical related donor, (5) left ventricular ejection fraction > 40%, (6) pulmonary function tests with forced expiratory volume in 1 second > 20% of expected value, (7) adult patients < 2.0 mg/dL or creatinine clearance > 30 mL/min/1.73 m^2 and pediatric patients (<18 years old) creatinine < 1.5 mg/dL or a creatinine clearance > 30 mL/min/1.73 m^2, (8) serum total bilirubin < 2.5 mg/dL and serum alanine and aspartate aminotransferases ≤ 5 times upper limit of normal, (9) central venous access potential, and (10) written informed consent/assent obtained from patient/parent or legal guardian. Exclusion criteria consisted of (1) age < 8 years or >40 years, (2) HIV infection, (3) chronic active hepatitis B infection, (4) active central nervous system involvement by malignancy, (5) pregnant or lactating, (6) presence of active malignancy in another organ system other than the hematopoietic system (except when driven by viruses, in which case the immune reconstitution after transplant may control the malignancy), and (7) no available 10/10 or 9/10 HLA-matched related or unrelated donor or haploidentical donor.

Transplant Regimen

The pretransplant conditioning regimen consisted of fludarabine 40 mg/m^2/day i.v. on days −6, −5, −4, and −3 and busulfan 3.2 mg/kg on the same 4 days. Fludarabine was dosed based on actual body weight and adjusted for renal dysfunction. One patient (Patient 6) received dose-adjusted busulfan with the actual dose based on a test dose of .8 mg/kg of busulfan given before the start of the preparative regimen [12-14]. The purpose of the busulfan test dose was to obtain pharmacokinetic blood samples of busulfan for calculation of an area under the curve (AUC).

For the single patient who received the test dose of busulfan, the busulfan-conditioning dose was calculated to obtain a target AUC of 3600 to 4800 μM/min based on the discretion of the principle investigator to minimize toxicity in patients with an exceptionally high clearance. The busulfan test dose was given as a 2-hour infusion, and 4 busulfan blood samples were drawn after the administration of the test dose. The first specimen was drawn immediately after termination of the 2-hour i.v. infusion of .8 mg/kg busulfan. Additional specimens were drawn at 1, 2, and 4 hours after termination of infusion. All samples were sent to the Mayo Medical Laboratories.

Busulfan dose = target AUC of 3600 to 4800 μM/min × test dose (mg) divided by the

Test dose AUC (μM/min)

The calculated busulfan dose was administered by i.v. infusion over 3 hours once daily for 4 doses (days −6, −5, −4, and −3) by a controlled-rate infusion pump through a central venous catheter. Busulfan dosing (test dose and full conditioning regimen [days −6, −5, −4, and −3]) was based on the recipient's ideal body weight or actual body weight, whichever was lower.

Donors

Matched related and unrelated donor peripheral blood stem cells (PBSCs) or bone marrow cells were infused fresh on day 0. Matched related and unrelated donors providing PBSCs received 5 days of granulocyte colony-stimulating factor (10 μg/kg/day), followed by apheresis on day 5 with the goal of collecting at least 5×10^6 CD34$^+$ cells/kg of the recipient's body weight. The target dose of bone marrow was 2×10^8 total nucleated cells/kg recipient body weight. Mutation on 1 allele of *DOCK8* did not represent exclusion criteria.

Post-Transplant GVHD Prophylaxis

GVHD prophylaxis consisted of tacrolimus (target level of 5 to 10 ng/mL) starting on day −3 and short-course methotrexate 5 mg/m^2 i.v. on days +1, +3, +6, and +11. Immunosuppression was tapered at 6 months post-transplant if there was no evidence of GVHD.

CD4 and CD8 T Cells

CD4$^+$ and CD8$^+$ T lymphocytes, B cells, and natural killer (NK) cells were quantified by flow cytometry pretransplant and at designated intervals post-transplant.

Lymphocyte Subset Immunophenotyping

Lymphocyte subsets were analyzed before and after transplant by flow cytometry to identify CD4$^+$ and CD8$^+$ T cells, CD3$^+$/CD8$^+$/CD45RA$^+$/CCR7$^+$ (naïve CD8 T cells), CD3$^+$/CD8$^+$/CD45RA (memory CD8 T cells), CD3$^+$/CD8$^+$/CD45RA$^+$/CCR7$^-$ (T cell effector memory CD45RA$^+$ CD8 T cells), CD3$^+$/CD8$^+$/CD45RA$^-$/CD127$^+$ (CD8 memory T cells expressing IL-7Rα), CD3$^+$/CD8$^+$/CD38$^+$/CD57$^+$ (exhausted CD8 T cells), and CD3$^+$/CD8$^+$/CD45RA$^+$/CCR7$^+$/CD95$^+$ (naive CD8 T cells abnormally expressing CD95). B cell analysis consisted of CD19$^+$/CD27$^-$ (naive B cells), CD19$^+$/CD27$^+$/IgD$^+$ (unswitched memory B cells; marginal zone-like B cells), CD19$^+$/CD27$^+$/IgD$^-$ (switched memory B cells), and CD19$^+$/CD24hi/CD38hi (transitional B cells).

Analysis of Chimerism

Engraftment of donor cells was assessed using polymorphisms in regions known to contain short tandem repeats. Peripheral blood CD4$^+$ and CD8$^+$ T lymphocytes and CD19$^+$ and CD3$^-$/CD56$^+$ lymphocytes were selected by cell sorting using flow cytometry at the designated time points, and chimerism was assessed on these subpopulations. In addition, CD14$^+$/CD15$^+$ myeloid cells and CD3$^+$ T lymphocytes were selected using immunobeads, and chimerism was assessed on the selected cells. The lower limit of sensitivity for this method is 1% to 3% of donor-type polymorphic markers in the mixture; these sensitivities are based on studies using mixtures of known proportions of allogeneic DNA samples. Chimerism was also assessed on bone marrow aspirate at designated time points.

Supportive Care

We followed standard guidelines for supportive care established at the National Institutes of Health Clinical Research Center for patients undergoing allogeneic HSCT.

A paired *t*-test was used to compare the leukocyte subsets pretransplant and at 1 year post-transplant.

RESULTS

Characteristics of Patients

The clinical characteristics of the 6 patients with DOCK8 deficiency receiving allogeneic HSCT are summarized (Table 1). The median age of the recipients was 20.5 years (range, 10 to 27). DNA viral infections and recurrent bacterial infections were present in all patients. All 6 patients had a history of eczema, although the eczema in 2 patients improved early in life and was not present at the time of conditioning. Three patients also had significant food allergies. Pulmonary complications were also ubiquitous. Three patients had markedly elevated IgE levels, consistent with DOCK8 deficiency as an autosomal recessive hyper-IgE syndrome. Two patients were homozygous for *DOCK8* mutations due to consanguinity, and the other 4 patients were compound heterozygotes for *DOCK8* mutations.

These patients shared many clinical features; however, each patient had a unique constellation of clinical signs that led to transplant. Patient 1 had a refractory HSV gingivitis and periodontitis (Figure 1), severe refractory eczema (Figure 2A), and recurrent otitis and sinopulmonary infections with bronchiectasis and hearing loss. Patient 2 had severe, refractory eczema with recurrent bacterial skin infections and viral infections (Figure 2C), recurrent purulent otitis, and pneumonia. Patient 3 had severe primary varicella-zoster virus as a child and *Neisseria Neisseria meningitis* multifocal septic arthritis as an adolescent. Patient 4 had partial vision loss due to recurrent HSV keratitis, extensive warts (Figure 2B), squamous cell carcinoma in situ, and recurrent sinopulmonary infections. Patient 5 had severe bronchiectasis from recurrent pulmonary infections resulting in severe obstructive and restrictive ventilatory defects. She was oxygen dependent at the time of transplant and had been evaluated for a lung transplant. Patient 6 had chemotherapy-refractory, EBV-virus driven lymphoma.

Before transplantation, all infections were treated aggressively. To minimize infections during neutropenia related to abnormal skin barriers from eczema, Patients 1 and 2 received wet wrap therapy the week before conditioning. Wet wrap therapy was performed 3 times daily for several days, using triamcinolone .1% ointment for lesions on the body and desonide .05% ointment for lesions on the face, with substantial restoration of the skin barrier.

Transplantation Outcomes

The objectives of this study were to determine whether allogeneic HSCT reconstitutes T lymphocyte and B lymphocyte cells and myeloid cells with normal donor cells at 1 year post-transplant and reverses the clinical phenotype of severe recurrent infections in patients with DOCK8 deficiency. The secondary objective of the study was to determine the safety of this allogeneic transplant regimen in DOCK8 deficiency by assessing transplant-related toxicity, incidence of acute and chronic GVHD, immune reconstitution, overall survival, and disease-free survival. We were also interested in whether the donor source might influence engraftment or outcome.

Three patients received matched related donor transplants and 3 received matched unrelated donor transplants (Table 2). Bone marrow was the preferred donor source and was obtained in all 3 matched related donors and in 1 of 3 matched unrelated donors. Table 2 shows the composition of donor grafts. The median CD34$^+$ cells infused were 5.2×10^6/kg, and the median CD3$^+$ cells infused were 1.98×10^7/kg. Two of 3 matched related donors (Patients 1 and 2) were heterozygous for the mutant DOCK8 allele.

All patients engrafted, and the neutrophil engraftment (defined as a neutrophil count $> .5 \times 10^9$ cells/L for 3 consecutive days) was rapid in all patients, with a mean of 10.5 days (range, 10 to 20). The median duration of neutropenia was only 5.5 days (range, 4 to 15). The median time to platelet engraftment (defined as a platelet count $> 20 \times 10^9$ cells/L for 7 consecutive days without requiring platelet transfusion) was 18 days (range, 15 to 31). There were no instances of graft rejection. All patients were alive at a median follow-up of 22.5 months (range, 14 to 35).

All patients had a marked improvement or complete resolution of gingivitis (Figure 1), skin manifestations (Figure 2), and infection susceptibility by 100 days post-transplant. However, all patients had a transient worsening of their pretransplant bacterial infections (sinopulmonary) or warts between 1 and 3 months post-transplant. Eczema significantly improved or resolved in the patients presenting with eczema at baseline.

The patient with the chemotherapy-refractory, EBV-driven, diffuse large B cell lymphoma (Patient 6) had a complete response by positron emission tomography 100 days after unrelated donor PBSC transplant (Figure 3). However, because of the burden of disease pretransplant and because she had failed many cycles of chemotherapy before transplant, she received an infusion of donor-derived, EBV-specific cytotoxic T lymphocytes 4 months post-transplant at Baylor University [15]. At 1 year post-HSCT she had no detectable EBV viremia and showed complete resolution by positron emission/computed tomography.

All 6 patients are outpatients and return for their follow-up visits at 3- to 6-month intervals. Only the most recently transplanted patient remains on immunosuppression for skin GVHD, and he is tapering off tacrolimus.

Reconstitution of normal donor hematopoiesis was achieved in all 6 patients. All patients achieved 99% to 100% donor myeloid cells as well as 99% to 100% CD4$^+$ and CD8$^+$ T lymphocytes and CD19$^+$ B cells at 30 days and 1 year post HSCT (Table 3). In addition, all 6 patients had greater than 94% donor bone marrow chimerism at 1 year post-transplant (Table 3).

Correction of the cellular compartments that were deficient pretransplant represented a primary objective of this study. Statistically significant increases in CD4 T cells and CD19$^+$ B cells were observed; however, improvements in CD8 T cells and NK cells did not achieve statistical significance at 1 year, most likely due to continued immunosuppression in 2 patients (Figure 4). Two markers of the DOCK8 deficiency, elevated IgE levels and eosinophilia, improved post-transplant. The mean IgE level pretransplant was 4137 IU/mL (normal, 0 to 90) and 1 year post-transplant was 542 IU/mL (normal, 0 to 90). Similarly, eosinophil levels dropped from 316/μL (normal, 40 to 360) pretransplant to 181/μL (normal, 40 to 360) post-transplant. However, there was a very large standard deviation in both measurements due to the heterogeneity of the patients. Also, atopic manifestations persisted post-transplant in most patients, consistent with the IgE levels that remained above normal.

Detailed lymphocyte subset analyses before and 1 year after transplant for Patients 4 and 5 are shown (Supplemental Table 1). CD4$^+$ T cells significantly increased post-transplant, whereas CD8$^+$ T cells decreased in both. Naive CD8$^+$ T cells

Table 1
Baseline Characteristics of Patients with DOCK8 Deficiency Receiving HSCT

	Donor	Age at HSCT (yr)/Sex	Type of Infections		Eczema	Pulmonary Complications	Other	IgE	DOCK8 Mutation
			Viral	Other					
1	MRD	18/F	HSV, mucocutaneous refractory HPV, skin EBV viremia	Recurrent bacterial sinusitis/ pneumonias/otitis	Severe	Bronchiectasis Restrictive ventilatory defect	Hearing loss	8031	Homozygous Del exons 28-35
2	MRD	10/F	HPV, skin EBV viremia	Recurrent bacterial sinusitis/ pneumonias/otitis	Severe	None		5243	Homozygous c.2044 G>T (E682X)
3	MRD	23/M	HPV, skin M. contagiosum VZV, disseminated	Disseminated N. meningitides Recurrent bacterial sinusitis/ pneumonias	Moderate	Restrictive ventilatory defect		11,279	Compound heterozygous c.1153 G>T (E385X) Deletion of DOCK8
4	URD	27/M	HPV, skin, M. contagiosum HSV, keratitis recurrent EBV viremia	Recurrent bacterial sinusitis/ pneumonias	Moderate	Bronchiectasis	Partial vision loss	118	Compound heterozygous c.1266delC (p.Try423ThrfsX18) Del exons 2-47
5	URD	25/F	HPV, skin and genital M. contagiosum EBV viremia	Recurrent bacterial sinusitis/ pneumonias	Resolved pre-HSCT	Bronchiectasis, severe Obstructive/restrict ventilatory defects O_2 dependent		8.3	Compound heterozygous c.1266delC (p.Try423ThrfsX18) Del exons 2-47
6	URD	16/F	Skin HPV EBV malignancy	Recurrent bacterial sinusitis/ pneumonias	Mild	Obstructive/restrict ventilatory defects	EBV Lymphoma	143	Compound heterozygous c.1805G>A (p.W602X) c.4540delG (p.E1514KfsX8)

MRD indicates matched related donor; HPV, human papilloma virus; M. contagiosum, Molluscum contagiosum; VZV, varicella-zoster virus; N. meningitides, Neisseria meningitides; URD, unrelated donor.

Figure 1. Correction of the oral manifestations of DOCK8 deficiency. (A) Refractory HSV gingivitis and (B) periodontitis in Patient 1. (C and D) Post-transplant day +100 in the same patient.

increased post-transplant. Although effector/memory CD8$^+$ T cells decreased in only 1 of the 2 patients, terminally differentiated, "exhausted" CD8$^+$ effector memory CD45RA$^+$ T cells decreased in both patients. The latter was consistent with the interpretation that these patients had active inflammation pretransplant, leading to a relative depletion of the naive CD8$^+$ T cells pretransplant, and after transplant the immunologic system was achieving equilibrium. Aberrantly

differentiated naive T cells expressing CD95, which were previously reported in DOCK8-deficient patients, also normalized after transplant [16]. By contrast, the increased naive B cells and decreased memory B cells, consistent with that previously reported in DOCK8-deficient patients [17], were not corrected at 1 year after transplant, although the increased transitional B cells in 1 patient did improve. These results may reflect the fact that recovery of donor B cell

Figure 2. Cutaneous lesions in DOCK8 patients. (A) Severe persistent eczematous dermatitis on dorsal feet of Patient 1 before and after HSCT. (B) Extensive bulky, confluent verrucae on the antecubital fossa and knees of Patient 4 before and after HSCT. (C) Chronic wounds on hands and legs of Patient 2 before and after HSCT.

Table 2
Characteristics of Hematopoietic Stem Cell Grafts and Outcome of HSCT

	Donor	Cell Source	HLA Match	Composition of Donor Graft		Infections Post-HSCT	GVHD	Outcome/Follow-Up (mo)
				CD34$^+$ $\times 10^6$/kg	CD3$^+$ $\times 10^7$/kg			
1	MRD	BM	10/10	5.4	1.35	URI, otitis, pneumonia	Acute grade II skin	Alive/23
2	MRD	BM	10/10	7	1.86	URI, otitis	No	Alive/16
3	MRD	BM	10/10	3.5	2.1	HHV-6 viremia, URI	Acute grade III skin	Alive/14
4	URD	PBSC	10/10	5	17.6	BSI, C. difficile	No	Alive/35
5	URD	BM	10/10	5	5	Pneumonia	Possible BOS	Alive/25
6	URD	PBSC	10/10	7.5	1.45	Pneumonia, otitis	No	Alive/22

BM indicates bone marrow; URI, upper respiratory infection; HHV-6, human herpes virus 6; BSI, bloodstream infection; _C. difficile_, _Clostridium difficile_; BOS, bronchiolitis obliterans syndrome.

functions often is delayed relative to T cell functions, as has been observed in hematopoietic transplantation for other PIDs.

Adverse Events

We anticipated major complications with HSCT in this group of patients. Mucositis and toxic erythema of chemotherapy were the major side effects of the transplant-conditioning regimen. As mentioned previously, all patients had transient worsening of sinopulmonary inflammation after HSCT, but all responded to antimicrobial therapy, suctioning, sinus drainage, or the short-term use of bronchodilators. The patient with the EBV lymphoma (Patient 6) had an extensive lung mass and post-transplant developed a complete left lung collapse from a mucous plug. Bronchoscopy resulted in complete re-expansion of the collapsed lung. Acute GVHD of the skin developed in 2 patients: 1 responded to topical steroids and 1 required systemic steroids. The patient with the extensive bronchiectasis and severe lung dysfunction pre transplant (Patient 5) had improvement of her pulmonary defects post-transplant and was able to discontinue oxygen. However, shortly after her immunosuppression was stopped, her lung disease worsened, requiring reinitiating of immunosuppression. However, she is now 25 months post-transplant and off all immunosuppression.

DISCUSSION

Here we report that related and unrelated donor allogeneic HSCT with the combination of busulfan and fludarabine results in reliable engraftment and reversal of the clinical phenotype in patients with DOCK8 deficiency with a low regimen-related toxicity. DOCK8 deficiency, a form of autosomal recessive hyper-IgE syndrome, remains a lethal disease in which patients succumb to complications of infections or malignant transformation. HSCT represents the only curative therapy. There is controversy as to the ideal timing of HSCT in DOCK8 deficiency, but the development of life-threatening infections, end-organ damage, and malignancy are strong indicators that HSCT should be considered earlier in the clinical course, before these complications develop.

Over the preceding decades, HSCT has been shown to reverse the phenotype in a variety of PIDs. However, the optimal conditioning regimen for HSCT in PID has yet to be defined. In the most extensive use of reduced-intensity conditioning in patients with PID receiving unrelated donor HSCT, Veys et al. [18] demonstrated a 94% survival rate in 33 patients treated with a reduced-intensity regimen consisting of melphalan 140 mg/m^2, fludarabine 150 mg/m^2, and alemtuzumab (Campath 1H 1 mg/kg). They observed rapid engraftment in 32 of 33 patients with neutrophil recovery at 13 days and platelet recovery at 16 days. Of note, at 1 year post-transplant only 55% of patients had 100% donor chimerism. Possibly as a result of the low level of complete donor chimerism, only 9% of patients in the reduced-intensity conditioning group had acute GVHD more than grade II. However, there was also a very high rate of viral reactivation associated with the use of alemtuzumab.

In a study of 70 children with PID who received 42 g/m^2 or 36 g/m^2 treosulfan with cyclophosphamide 200 mg/kg (n = 30) or fludarabine 150 mg/m^2 (n = 40), along with alemtuzumab (in most patients), using different donor sources (8 matched sibling donor, 13 matched family donor, 4
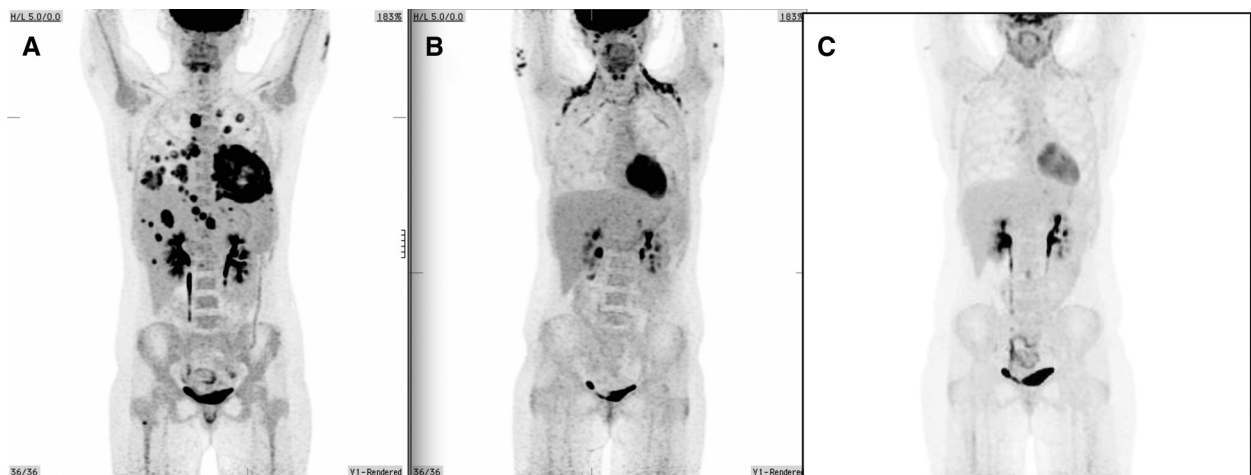

Figure 3. Positron emissions tomography scan of EBV-positive lymphoma in Patient 6, (A) pretransplant, (B) 100 days post-transplant, and (C) 1 year post-transplant.

Table 3
Percent Donor Chimerism at 30 Days and 1 Year after HSCT

	Donor	Peripheral Blood Day +30 (%)				Bone Marrow Day +100 (%)	Peripheral Blood 1 Year* (%)				Bone Marrow 1 Year* (%)
		Myeloid	CD3+	CD19+	NK		Myeloid	CD3+	CD19+	NK	
1	MRD	100	90	21	97	92	100	100	100	100	98
2	MRD	100	92	48	99	98	100	99	100	100	96
3	MRD	99	98	100	100	92	100	100	99	100	94
4	URD	100	94	100	100	97	100	100	100	100	94
5	URD	100	95	99	100	96	99	100	99	99	94
6	URD	100	100	ND	100	93	100	100	100	100	95

HSCT indicates hematopoietic stem cell transplant; MRD, matched related donor, URD, unrelated donor; ND, not done.

* Chimerism at 1 year.

haploidentical donor, and 45 unrelated donor), the overall survival rate was 81% [19]. Only 2 patients rejected, more than one-half had 100% donor chimerism by 1 year post-transplant, and the remaining patients had stable mixed chimerism.

In DOCK8 deficiency the aim of HSCT is to reverse the clinical phenotype of DNA viral infections and sinopulmonary infections and to correct the underlying immunologic defects. In this regard, the severe viral infections in patients with DOCK8 deficiency result from defects in T cell numbers and function [17,20]. DOCK8 deficiency results in a selective decrease in the number of circulating, naive CD8+ T cells [16]. CD8 T cell proliferation is reduced in most DOCK8 patients

[2]. In humans with DOCK8 deficiency, most CD8+ T cells display a CD45RA+/CCR7− phenotype, a pattern associated with cell exhaustion or replicative senescence [16]. This phenotype may reflect chronic viral infections.

The recurrent sinopulmonary infections in DOCK8 deficiency are typical of humoral immunodeficiency or defects in B cell activation. DOCK8 was shown to be involved in B cell proliferation and Ig production in which Toll-like receptor 9 responses were decreased in DOCK8-deficiency B cells. Loss of function mutations in DOCK8 in the mouse disrupts memory B cell development in the germinal centers [16,17].

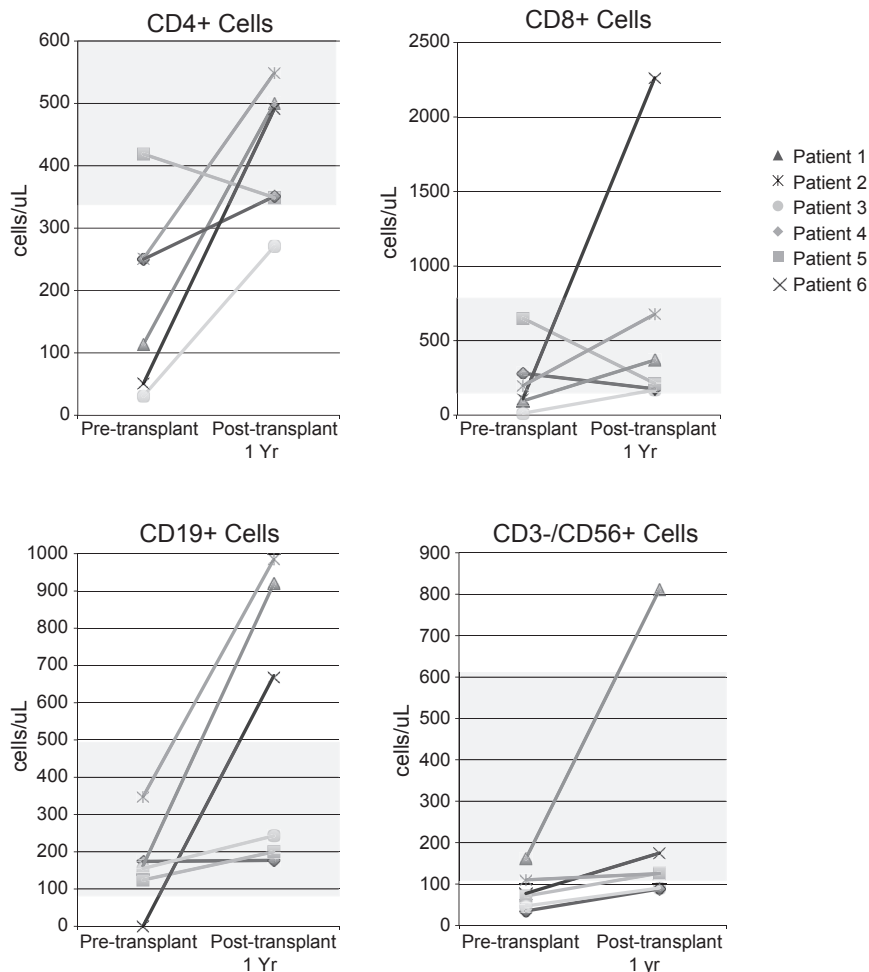Several groups have described allogeneic HSCT in DOCK8 deficiency using a variety of conditioning regimens with

Figure 4. Reconstitution of cellular compartments after HSCT. Leukocyte subsets are shown pretransplant and at 1 year post-transplant.

different donor sources [4-11]. Most successful regimens used a myeloablative dose of conditioning. Interestingly, Bittner et al. [8] described a 2 year-old girl who received 8/8 HLA-matched bone marrow from her father after conditioning with cyclophosphamide 60 mg/kg for 2 days, fludarabine 40 mg/m^2/day for 4 days, and 400 cGy total body irradiation. The patient had immunologic correction of the DOCK8 deficiency phenotype. However, 6 years later she remained with mixed donor chimerism, 98% donor T cells, 35% donor B cells, and only 6% donor neutrophils, suggesting a higher intensity regimen is required to achieve donor myeloid cell engraftment.

The most extensive review of HSCT in DOCK8 deficiency retrospectively collected data from 18 institutions worldwide: 32 patients were identified (some already published as single case reports). The conditioning regimen among institutions included no conditioning, myeloablative and nonmyeloablative conditioning, and different donor sources. The overall survival rate was 77%, and T cell chimerism varied between 50% and 100%. Correction of the phenotype was seen in 16 of 19 assessable patients (unpublished abstract from the 15th biennial meeting of the European Society for Immunodeficiencies (ESID), M. H. Albert, October 2012).

We used the combination of busulfan and fludarabine because this regimen has been shown to result in reliable engraftment with a low regimen-related toxicity in hematologic malignancies [21-24]. In our study, although only 1 patient received targeted busulfan, because of a decreasing age of our patients we are now routinely using dose-targeted busulfan with a .8-mg test dose administered 7 to 10 days before the start of conditioning.

In our study all 6 patients had rapid engraftment with high levels of donor chimerism with minimal regimen-related toxicity. All clinical manifestations of DOCK8 deficiency resolved post-transplant. The clinical correction correlated with laboratory evidence of decreased IgE levels and eosinophils, along with a lymphocyte profile consistent with a new immunologic equipoise.

The incidence of acute GVHD in this study was comparable with reported outcomes in HSCT series, with only 1 patient requiring systemic steroids. Only 1 patient (Patient 3) had definitive evidence of chronic GVHD. However, the patient with extensive bronchiectasis and severe lung dysfunction pretransplant (Patient 5) had initial improvement and then worsening of her lung disease due to suspected bronchiolitis obliterans syndrome. However, she is now off all immunosuppression 2 years post-transplant.

A caveat of this study that makes it problematic to compare with other transplant studies is that PIDs are a very heterogeneous group, and there is every reason to suspect that the T$^-$, B$^+$, NK$^-$ variants (ie, X-SCID) may respond to allogeneic transplant very differently from the T$^-$, B$^-$, NK$^+$ variants (Rag deficiency, Artemis), the T$^+$, B$^+$, NK$^+$ cohort (chronic granulomatous disease and leukocyte adhesion deficiency type 1), or even the T$^+$, B$^-$, NK$^-$ (GATA2 deficiency) patients. DOCK8 patients do not have the chemosensitivity seen in the T$^-$, B$^-$, NK$^+$ variants or the deep-seated fungal infections seen in the chronic granulomatous disease and leukocyte adhesion deficiency type 1 patients or the predisposition to acute myelogenous leukemia seen in the GATA2 patients. Moreover, we used a myeloablative conditioning regimen, only 10/10 matched donors, and bone marrow in 4 of 6 patients. Thus, the favorable outcomes we observed may be due to a unique constellation of factors, including the disease itself.

In summary, allogeneic HSCT in DOCK8 deficiency results in reconstitution of the deficient lymphocyte compartments that are present pretransplant and complete reversal of the clinical phenotype. The conditioning regimen had minimal regimen-related toxicity, despite the degree of infections in this cohort of patients, and resulted in reliable full donor engraftment. With genetic testing for DOCK8 deficiency now more widely available, we anticipate that earlier diagnosis will enable patients to be transplanted earlier in their clinical course, before significant organ damage or the development of viral-driven malignancies.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbmt.2015.01.022

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