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Haploidentical Related Donor Hematopoietic Stem Cell Transplantation for Dedicator-of-Cytokinesis 8 Deficiency Using Post-Transplantation Cyclophosphamide

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A B S T R A C T

Dedicator-of-cytokinesis 8 (DOCK8) deficiency, a primary immunodeficiency disease, can be reversed by allogeneic hematopoietic stem cell transplantation (HSCT); however, there are few reports describing the use of alternative donor sources for HSCT in DOCK8 deficiency. We describe HSCT for patients with DOCK8 deficiency who lack a matched related or unrelated donor using bone marrow from haploidentical related donors and post-transplantation cyclophosphamide (PT/Cy) for graft-versus-host disease (GVHD) prophylaxis. Seven patients with DOCK8 deficiency (median age, 20 years; range, 7 to 25 years) received a haploidentical related donor HSCT. The conditioning regimen included 2 days of low-dose cyclophosphamide, 5 days of fludarabine, 3 days of busulfan, and 200 cGy total body irradiation. GVHD prophylaxis consisted of PT/Cy 50 mg/kg/day on days +3 and +4 and tacrolimus and mycophenolate mofetil starting at day +5. The median times to neutrophil and platelet engraftment were 15 and 19 days, respectively. All patients attained >90% donor engraftment by day +30. Four subjects developed acute GVHD (1 with maximum grade 3). No patient developed chronic GVHD. With a median follow-up time of 20.6 months (range, 9.5 to 31.7 months), 6 of 7 patients are alive and disease free. Haploidentical related donor HSCT with PT/Cy represents an effective therapeutic approach for patients with DOCK8 deficiency who lack a matched related or unrelated donor.

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INTRODUCTION

Dedicator-of-cytokinesis 8 (DOCK8) deficiency is a combined primary immunodeficiency disease initially described as autosomal recessive hyper-IgE syndrome [1,2] and characterized by allergic/atopic manifestations, DNA viral infections, central nervous system (CNS) events, autoimmunity, vasculopathy, and malignancy [3-5]. DOCK8 deficiency is associated with a high degree of morbidity and mortality—including life-threatening infections and virus-driven malignancies—with an estimated overall survival of 50% at 20 years [3]. Allogeneic hematopoietic stem cell transplantation (HSCT) represents a curative therapy for DOCK8 deficiency [6-14].

We previously reported our experience with allogeneic HSCT in patients with DOCK8 deficiency using matched related or unrelated donors and a high-dose fludarabine/busulfan-based conditioning regimen [13]. All 6 patients had prompt engraftment with reconstitution of the deficient lymphocyte compartments and reversal of the clinical phenotype with minimal regimen-related toxicity.

For patients with DOCK8 deficiency who lack an HLA-matched sibling donor, and for whom a full phenotypic

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HLA-matched unrelated donor is unavailable, haploidentical related donor transplantation represents a potential option. However, there are only a few reports of transplantations using haploidentical related donors in DOCK8 deficiency [9,14,15]. The success of haploidentical transplantation in other primary immunodeficiency syndromes suggested the feasibility of this approach in patients with DOCK8 deficiency [16-19]. Paramount to the success of haploidentical HSCT is the ability to prevent graft-versus-host disease (GVHD). This can be addressed using in vitro techniques with T cell depletion by $CD34^+$ selection or selective T cell depletion (eg, depletion of T cell receptor, depletion of $TCRab^+$ (T cell receptor alpha beta) T cells, associated with depletion of $CD19^+$ B lymphocytes) [19,20]. Alternately, in vivo depletion of T cells can be accomplished by pretransplantation serotherapy [21,22] or by using post-transplantation cyclophosphamide (PT/Cy) [16,23].

We describe the results for 7 patients with DOCK8 deficiency who received a busulfan/fludarabine-based haploidentical related donor HSCT utilizing T cell–replete bone marrow harvest grafts with PT/Cy for GVHD prophylaxis. Our results indicate that haploidentical HSCT represents an effective therapeutic approach for patients with DOCK8 deficiency who lack a matched related or unrelated donor.

METHODS

Patients

All patients were prospectively enrolled on a clinical trial specifically designed for transplantation of adults and children with DOCK8 deficiency. Recipients were enrolled to 1 of 2 arms depending on donor source: (1) matched related or unrelated donor or (2) haploidentical related donor (ClinicalTrials.gov NCT01176006). This report focuses on the outcomes of patients who underwent haploidentical related donor transplantation. 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 after transplantation and reverses the clinical phenotype of severe recurrent infections. The secondary objective of the study was to evaluate the safety of this regimen including, transplantation-related toxicity, the incidence of acute and chronic 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 were obtained for all patients and donors, with parental permission obtained for minors.

The inclusion criteria for recipients enrolled on the haploidentical arm included the following: (1) age of 6 to 40 years with confirmed homozygous or compound heterozygous mutations in the *DOCK8* gene performed by a Clinical Laboratory Improvements Amendments–certified laboratory, (2) 1 or more life-threatening infections, a viral-driven lymphoma, or squamous cell carcinoma, and (3) adequate organ function. Recipients who received a haploidentical donor source included only those patients for whom a 10/10-matched related or unrelated donor could not be identified. Exclusion criteria consisted of active CNS involvement by malignancy, chronic active hepatitis B, human immunodeficiency virus, or those who were pregnant or lactating. Donor selection was based on prioritizing identification of the best available adult donor over a minor-aged donor, and other selection criteria based on cytomegalovirus (CMV)/Epstein-Barr virus exposure and ABO blood type. Mutation on 1 allele of *DOCK8* did not represent a donor exclusion criterion. Additionally, all recipients were screened for the presence of donor-specific anti-HLA antibodies against potential donors and were excluded if positive, given the concern for primary graft failure [24].

Transplantation Conditioning Regimen

The pretransplantation conditioning regimen consisted of cyclophosphamide 14.5 mg/kg/day on days -6 and -5, fludarabine 30 mg/m^2/day on days -6 to -2, busulfan 3.2 mg/kg/day on days -4, -3, and -2 (pharmacokinetically targeted based on a test dose of .8 mg/kg of busulfan given approximately 1 week before the start of conditioning), and 200 cGy total body irradiation (TBI) on day -1 (Figure 1). The dose of fludarabine was based on actual body weight and adjusted for renal dysfunction. Busulfan was dose adjusted, based upon a test dose of .8 mg/kg of busulfan—according to the lower of the actual or the ideal body weight—given before the start of the preparative regimen [25-27] to determine the conditioning dose needed to target an area under the curve (AUC) of 3600 to 4800 μmol/minute. Busulfan was given as an i.v. infusion over 3 hours once daily for 3 days.

Stem Cell Collection

All donors underwent bone marrow harvest with a planned fresh infusion on day 0. The target minimum dose was 2×10^8 total nucleated cells per kilogram recipient body weight.

Post-Transplantation GVHD Prophylaxis

GVHD prophylaxis consisted of PT/Cy 50 mg/kg/day i.v. once daily × 2 doses on days +3 and +4 (based on the lesser of the actual or ideal body weight in obese patients), tacrolimus i.v./per oral from day +5 to day +180 (goal levels, 5 to 10 ng/mL), and mycophenolate mofetil 15 mg/kg i.v./per oral every 12 hours from day +5 to day +35. Immunosuppression was tapered or stopped at 6 months after transplantation if there was no evidence of GVHD.

Lymphoid and Myeloid Engraftment

$CD4^+$ and $CD8^+$ T lymphocytes, B cells, and natural killer cells were quantified by flow cytometry before transplantation and at designated intervals after transplantation, including at days +30, +60, and +100 and at 6, 12, 18, and 24 months after transplantation. *Neutrophil engraftment* was defined as a neutrophil count of $> .5 \times 10^9$ cells/L for 3 consecutive days. *Platelet engraftment* was defined as a nontransfused platelet count of $>20 \times 10^9$ cells/L for 7 consecutive days.

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 determined by studies using mixtures of known proportions of allogeneic DNA samples.

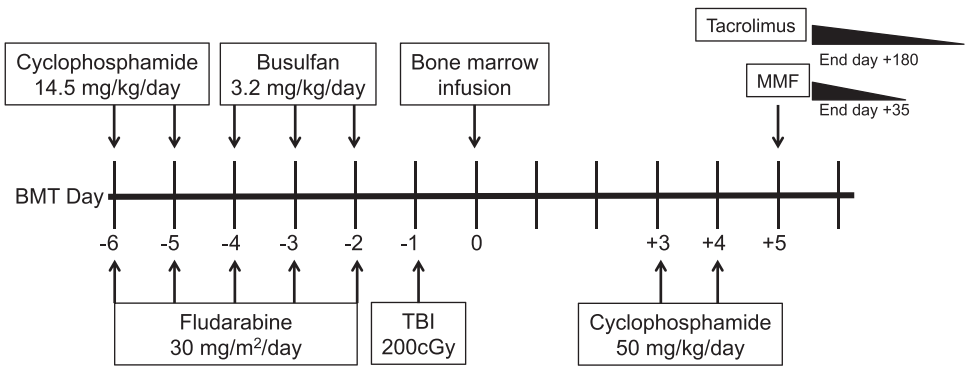

Figure 1. Schema of conditioning regimen for haploidentical related donor recipients.

Supportive Care

We followed standard guidelines for supportive care and infectious disease prophylaxis established at the National Institutes of Health Clinical Research Center for patients undergoing allogeneic HSCT. These guidelines specifically included the incorporation of pre-emptive CMV-directed therapy for low-level CMV reactivation to prevent CMV-related disease and weekly/biweekly surveillance of adenovirus, CMV, Epstein-Barr virus, and toxoplasmosis. Herpes simplex virus/varicella zoster virus prophylaxis was used in all adults, regardless of herpes simplex virus/varicella zoster virus serostatus and was used in pediatric patients who were seropositive or had a prior history. Antibacterial management for fever and neutropenia was guided based on patient's allergy history and pretransplantation colonization and risk factors. Pretransplantation sinopulmonary infections were conservatively managed with antimicrobial support to optimize and reduce the pretransplantation inflammation and bacterial burden, and invasive interventions were not used immediately before transplantation in this cohort of patients. Given the history of prior stroke and critical areas of CNS vasculopathy and stenosis, specific precautions taken for subjects 2 and 5 included institution of once-daily aspirin (81 mg), which was held during thrombocytopenia, maintaining a minimum platelet goal of 50,000 and avoidance of hypotension. Both patients were closely followed by neurology and cardiology consultations during their inpatient hospitalizations for optimization of supportive care. In addition, for those 2 patients, seizure prophylaxis was maintained through day 100. Filgrastim (5 μg/kg/day) was administered once daily from day +5 to neutrophil engraftment (absolute neutrophil count >1000 for 3 days).

RESULTS

Patient Characteristics

The clinical characteristics of the 7 haploidentical related donor recipients with DOCK8 deficiency are summarized in Table 1. The median age at the time of transplantation was 20 years (range, 7 to 25 years). All recipients shared features of DOCK8 deficiency, including the presence of recurrent DNA viral infections, recurrent bacterial infections (sinusitis, pneumonia, or otitis), and eczema. IgE levels were markedly elevated in all patients. Distinct comorbidities were present in individual subjects. Patient 1 had a history of diffuse large B cell lymphoma, treated 4 years earlier with etoposide, prednisone, vincristine, cyclophosphamide, hydroxydaunorubicin, and rituximab chemotherapy and was in remission at the time of transplantation, cardiomyopathy (ejection fraction of 30%), and chronic renal disease with renal artery stenosis. Patient 2 had a history of vulvar squamous cell carcinoma, previous right middle cerebral artery strokes (Figure 2A), and diffuse arterial calcifications (Figure 2C). Patient 3 had a history of Hodgkin lymphoma (nodular sclerosis type), whose treatment was complicated by bleomycin-induced radiographic evidence of pretransplantation pulmonary fibrosis (pretransplantation pulmonary function testing revealed normal flows and volumes with a moderate reduction in diffusion with a diffusing capacity of lung for carbon monoxide/alveolar volume adjusted of 57% and an forced expiratory volume in 1 second of 92% and patient had normal oxygen saturations on room air), recurrent pancreatitis, and chronic cholestatic liver disease. Patient 5 had critical basilar artery stenosis and CNS vasculopathy (Figure 2B). Patient 6 required treatment for *Pneumocystis jirovecii* pneumonia before HSCT.

Five patients were compound heterozygotes and 2 patients were homozygous for *DOCK8* mutations (Table 1).

Donor Characteristics

The median donor age was 43 years (range, 24 to 50 years) (Table 2). The donors consisted of 3 mothers, 3 fathers, and 1 brother. Two donors were 6/10-HLA matched and the

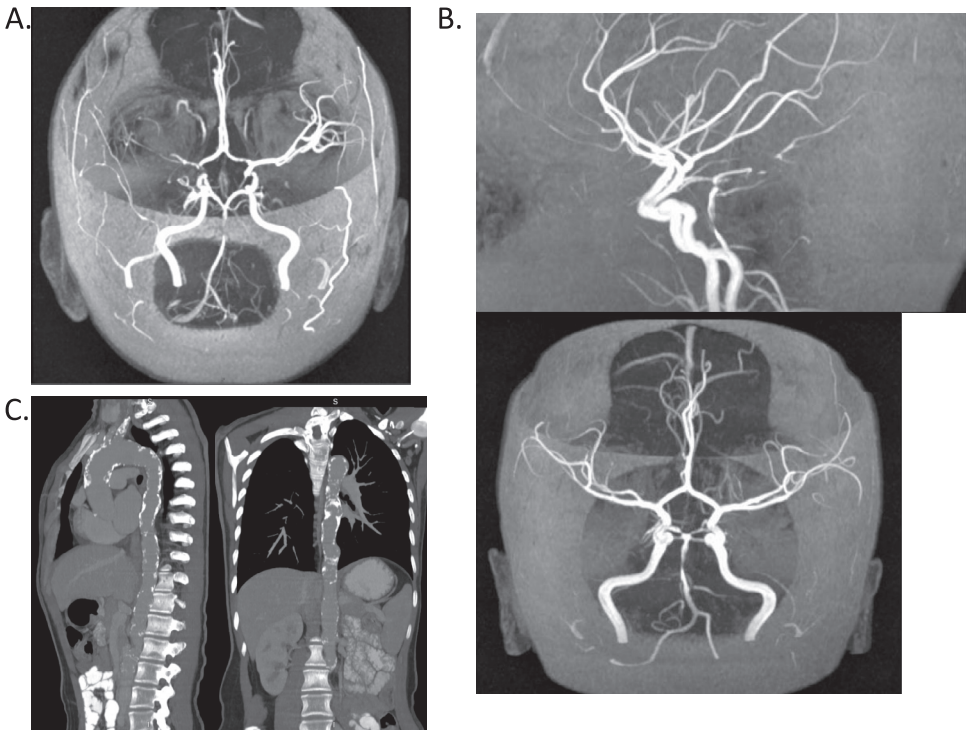

Figure 2. Vasculopathy seen in patients with DOCK8 deficiency. CNS vasculopathy (A) and aortic calcifications (C) in 19-year-old female. Critical basilar artery stenosis in 7-year-old male (B). (A) MRA brain: the right M1 segment demonstrates irregularity and long segment stenosis. Branches of the right middle cerebral artery are significantly diminished in caliber compared to branches on the left side. Branches of the left posterior cerebral artery are diminished in the caliber compared to branches on the right side. There may be narrowing of the left vertebral artery just before the confluents. There is probably narrowing of the basilar artery just after the confluence. The right vertebral artery is dominant. Most of the major intracranial arteries demonstrate irregularity. (B) MRA brain: multiple critical stenosis and vascular irregularities are noted within the posterior circulation, most notably within the basilar and right posterior cerebral arteries. (C) CT chest and abdomen: ectatic aorta and major branches with wall calcification advanced for patients of this age and gender.

Table 1
Baseline Characteristics of Patients with DOCK8 Deficiency Receiving Haploidentical HSCT*

Patient	Donor	Age at HSCT, y/Sex	Type of Infections		Pulmonary Complications	Other	IgE	*DOCK8* Mutation
			Viral	Other				
1	Brother	20/F	HPV-skin, *M. contagiosum*	Recurrent bacterial sinusitis, pneumonias/otitis, candida vaginitis (azole resistant), *Pseudomonas* colonization,	Bronchiectasis	DLBCL with CNS involvement at age 15, eosinophilic esophagitis, renal artery stenosis, nonischemic cardiomyopathy (EF 30% to 35%), chronic kidney disease, mild eczema	5970	Compound het; Del exons 1-13, R249X
2	Mother	19/F	HPV-skin, HSV reactivation	Recurrent bacterial sinusitis/ pneumonias/otitis	Bronchiectasis	Vulvar SCC, CNS vasculitis, right MCA stroke × 2 (ages 17 and 18), ectatic thoracic aorta and focal narrowing and wall thickening of the abdominal aorta, severe eczema	>6000	Homozygous, Del exon 37
3	Mother	25/F	HPV-skin, CMV viremia	Recurrent bacterial sinusitis/ pneumonias, MRSA infections (skin), candidiasis (vaginal), abnormal Pap smears	Asthma, pulmonary fibrosis secondary to bleomycin	Hodgkin lymphoma (nodular sclerosing), bleomycin lung toxicity, alopecia, recurrent pancreatitis, chronic cholestatic liver disease, severe eczema	639	Compound het, c.325-1A > G, c.325-2delAinsTG
4	Father	20/M	HPV-skin, *M. contagiosum,* EBV viremia, varicella	Recurrent bacterial sinusitis/ pneumonias		Eczema	1536	Compound het, c.1805G>A,p.W602X, c.4540delG,p.E1514Kfs8
5	Father	7/M	EBV viremia, varicella	Recurrent bacterial sinusitis/ pneumonias/otitis, oral candidiasis	Asthma	Cerebral artery stenosis of basilar and right posterior cerebral arteries, prior left PCA CVA	1128	Compound het, c.3194delC,P.T1065fs
6	Mother	10/M	EBV viremia, CMV viremia	Recurrent bacterial sinusitis/ pneumonias, *Pneumocystis jirovecii* pneumonia	Asthma, eosinophilic pneumonia	Eczema	794	Homozygous, Del exons 1-36
7	Father	18/M	Varicella, *M. contagiosum*	MRSA skin infection, recurrent bacterial sinusitis/pneumonia, osteomyelitis	Pneumonia	Severe eczema	4849	Compound het, Del exons 1-45

F indicates female; M. contagiosum, Molluscum contagiosum; DLBCL, diffuse large B cell lymphoma; EF, ejection fraction; het, heterozygote; HPV, human papilloma virus; HSV, human herpes simplex virus; SCC, squamous cell carcinoma; MCA, middle cerebral artery; MRSA, methicillin-resistant staphylococcus aureus; M, male; EBV, Epstein-Barr virus; CVA, cerebrovascular accident; PCA, posterior cerebral artery.

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

Patient	Haplo Donor	Cell Source	HLA Match	CMV Match (R/D)	Busulfan Dose AUC Targeted BU Dose, mg/kg/d x 3 Days	Composition of Donor Graft $CD34^+$, $\times 10^6$/kg	Composition of Donor Graft $CD3^+$, $\times 10^7$/kg	Day of Engraftment Neutrophil	Day of Engraftment Platelet	Infections after HSCT	Acute GVHD†	Outcome/ Follow-Up, mo
1	Brother	BM	5/10	+/-	AUC 4500 3.1 mg/kg/day	6.7	3.8	13	24	HHV6 viremia and CSF without clinical sequelae, BK viremia	Acute grade I skin	Alive/31.7
2	Mother	BM	6/10	+/+	AUC 4100 3.2 mg/kg/day	6.1	6.8	17	21	CMV reactivation	None	Alive/24.7
3	Mother	BM	5/10	+/+	AUC 3800 2.8 mg/kg/day	2.8	4.8	18	35	CMV reactivation, BK viruria with cystitis	Acute grade 2 GI, grade 1 skin, grade 2 lung	Death/5.5
4	Father	BM	5/10	+/+	AUC 3800 2.2 mg/kg/day	4.8	10.4	15	14	CMV reactivation, BK cystitis	Acute grade 3 GI	Alive/20.9
5	Father	BM	6/10	+*/-	AUC 4000 3 mg/kg/day	7.7	7.6	14	17	None	None	Alive/20.6
6	Mother	BM	5/10	+/+	AUC 3769 3.6 mg/kg/day	7.5	9.2	15	14	CMV reactivation	None	Alive/15.1
7	Father	BM	5/10	+/+	AUC 4000 2.3 mg/kg/day	6.2	3.9	14	17	CMV reactivation, adenoviremia, BK hemorrhagic cystitis	Acute grade 2 GI, grade 1 skin	Alive/9.5

R indicates recipient; D, donor; BM bone marrow; HHV-6, human herpes virus 6; CSF; GI, gastrointestinal.
* CMV IgG negative before initiation of IVIG replacement.
† No patients have developed chronic GVHD.

remaining 5 were 5/10-HLA matched. All donors underwent a bone marrow harvest with general anesthesia. All of the donors were heterozygous for a *DOCK8* mutation.

Busulfan Dosing and Stem Cell Infusion

The busulfan dose was based on a test dose administered at least 1 week before the start of conditioning. Confirmatory pharmacokinetics were not determined during the actual preparative regimen. The targeted AUC ranged from 3769 μmol/minute to 4500 μmol/minute with a trend towards a lower targeted AUC in patients who underwent transplantation more recently (Table 2). The mean total dose of busulfan administered was 8.6 mg/kg (range, 6.6 mg/kg to 10.8 mg/kg).

The median doses of total nucleated cells, $CD34^+$, and $CD3^+$ cells per infused product were 5.8×10^8, 6.2×10^6, and 6.8×10^7/kg recipient body weight, respectively (Table 2). No recipient had specific antibodies to the selected donor.

Engraftment

All recipients attained neutrophil and platelet engraftment, with a median times to engraftment of 15 days (range, 13 to 18 days) and 19 days (range, 14 to 35 days), respectively (Table 2). This is comparable to what observed in the matched setting, where the times to neutrophil and platelet engraftment occurred at a median of 10.5 and 18 days, respectively [13]. All recipients attained greater than 95% donor chimerism in the whole blood, myeloid, and CD3 subcomponents by day 30 (Table 3).

Clinical Outcomes

All recipients experienced noninfectious, self-limited high fever in the several days after transplantation, a well-characterized feature of T cell–replete haploidentical HSCT [28-33]. No patient experienced hemodynamic instability during this period and steroids were not required.

The outcome of transplantation on the clinical manifestations of DOCK8 deficiency was similar to that seen in the matched related and matched unrelated donor setting. Specifically, all recipients displayed transient worsening of their pretransplantation sinopulmonary disease during engraftment without any development of idiopathic pneumonia syndrome, followed by steady improvement in all manifestations with intermittent uses of both systemic and local antimicrobial therapies. Pre-existing eczema resolved after transplantation (Figure 3) and improvement in other viral-associated infections was seen as well (Figure 3). In 1 case with pre-existing eosinophilic esophagitis, there was full resolution after HSCT. One patient developed symptomatic hemorrhagic cystitis that improved with conservative management.

Despite the extensive and unique comorbidities in this cohort of patients, there was no worsening of pre-existing vasculopathy or CNS events after HSCT. Rather, in patient 1, the baseline cardiomyopathy improved after transplantation as previously described [34], and vascular imaging of the midaortic narrowing and bilateral renal artery stenosis was stable at 18 months after transplantation. For the 2 patients with a history of CNS vasculopathy and prior stroke, neither had any major CNS complications, and follow-up imaging studies showed no progression. In particular, patient 5's follow-up imaging at approximately post-transplantation day 100 demonstrated stable to slightly improved basilar artery stenosis, with stable findings at 1 year after transplantation. At 1 year after transplantation, vascular imaging for patient 2 was stable, and she had suffered no further neu-

Table 3
Donor Chimerism at 30 Days and > 100 Days after HSCT

Patient	Donor	Peripheral Blood Day +30, %				Peripheral Blood >100 Days, %			
		Myeloid	CD3+	CD 19 +*	NK	Myeloid	CD3+	CD 19+	NK
1	Brother	99	99	—	98	100†	100†	100†	100†
2	Mother	100‡	100‡	100‡	100‡	100†	100†	100†	100†
3	Mother	100	100	—	100	N/A	N/A	N/A	N/A
4	Father	100	100	—	100	100	100	100	100
5	Father	100	99	—	100	100	100	100	100
6	Mother	100	100	100	100	99	100	98	99
7	Father	100	100	—	100	100	100	100	100

NK indicates natural killer; N/A, not applicable, too few cells.
* Too few cells to sort chimerism. For patient number 3, 6-month studies not available because of early death.
† Results identical at 6 months and 1 year after HSCT.
‡ Chimerism at day +60.

rologic events. Additionally, oral human papilloma virus–associated lesions showed dramatic improvement in patient 2, starting as early as 2 months after HSCT (Figure 3), similar to the marked improvement in methicillin-resistant Staphylococcus aureus skin lesions that showed a similarly rapid resolution (Figure 3). Patient 3, however, had a more complicated course, which included the development of pulmonary symptoms with engraftment (new hypoxia and infiltrates), which was steroid responsive, but with subsequent worsening pulmonary function. Symptoms included the development of exertional dyspnea, hypoxia requiring intermittent oxygen, and recurrent pulmonary infections with concern for organizing pneumonia and focal interstitial fibrosis, which was exacerbated by continued tobacco use.

GVHD

Acute GVHD was seen in 4 recipients and was limited to maximum grade 3 gastrointestinal GVHD in 1 patient (patient 4). GVHD was responsive to steroids in all cases. Three patients with gastrointestinal GVHD received systemic steroids. Five patients are more than 1 year since transplantation and all are off immunosuppression. There were no cases of chronic GVHD.

Infection

We routinely monitored for viral reactivation with plans for pre-emptive treatment for CMV viremia, with all patients responding to pre-emptive therapy with foscarnet or ganciclovir. Two subjects had intermittent CMV viremia before transplantation. Other viremias included human herpesvirus 6 and BK, for which there were no long-term clinical consequences, although 3 subjects did have transient BK-related cystitis, 1 of whom developed hemorrhagic cystitis. One subject developed low-level adenoviremia while on systemic steroids for acute GVHD that responded to cidofovir and reduction of immunosuppression.

Survival

With a median follow-up time of 20.6 months (range, 9.5 to 31.7 months), 6 of 7 patients are alive and well. Patient 3 died at day +165 after transplantation from a multifactorial process, including worsening of pulmonary fibrosis from re-

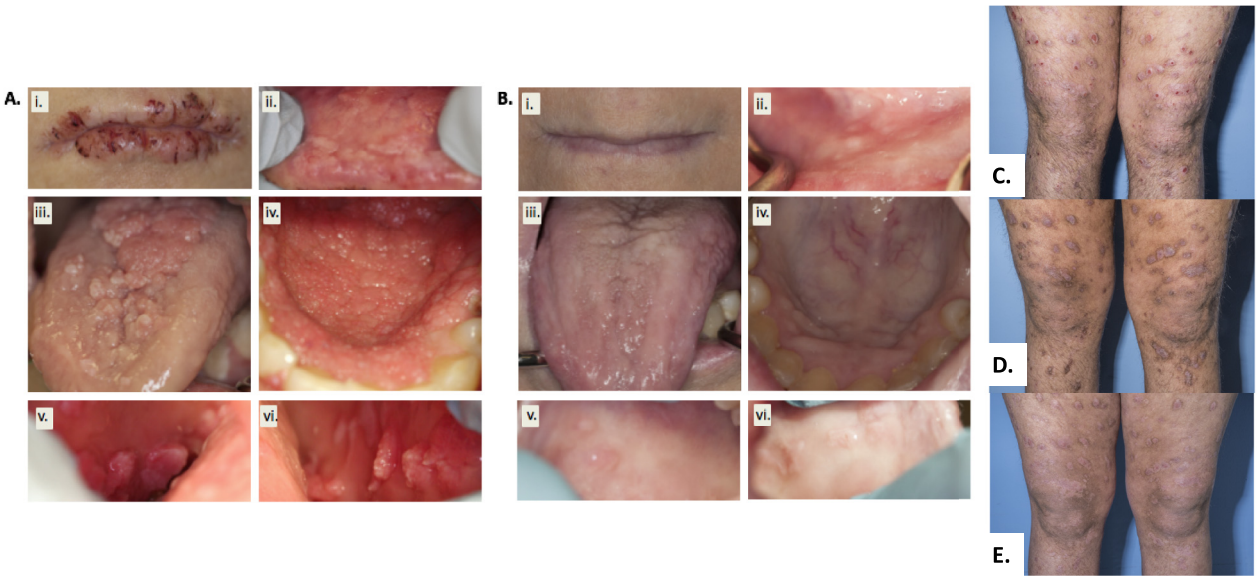

Figure 3. Resolution of oral HPV lesions and DOCK8 skin disease after transplantation. Images of oral mucosa HPV-associated papillomas shown in patient 2 (19-year-old female) before (A) and 1 year after (B) HSCT. Areas show in both panels (i) lips, (ii) labial mucosa, (iii) tongue dorsum, (iv) hard palate, (v) right, and (vi) left buccal mucosa. Images of recalcitrant, eroded lichenified, eczematous plaques on the legs of patient 7 (18-year-old male) with DOCK8 deficiency before transplantation (C), with significant resolution in cutaneous lesions and symptoms at day 30 (D) and at day 100 (E) after transplantation.

current infections, tobacco use, and potentially organizing pneumonia.

Immune Reconstitution

Five patients have data available to 12 months or greater after transplantation. Lymphocyte recovery was seen by 6 months after transplantation (Figure 4). Specifically, absolute CD4 counts exceeded pretransplantation values in 3 of 4 evaluable patients by 6 months after transplantation, and in the last patient the $CD4^+$ cell count exceeded pretransplantation values by 12 months after transplantation. Similarly, despite remaining low, all 4 patients had $CD8^+$ cell recovery that exceeded pretransplantation values by 6 months after transplantation. Natural killer cell recovery was seen in 2 patients by 6 months after transplantation, with 1 patient achieving levels matching those before transplantation by 6 months and 5 subjects exceeding pretransplantation levels by 12 months after transplantation. B cell recovery was delayed, with 2 subjects exceeding pretransplantation levels at 12 months after transplantation and beyond, and was similar to the experience seen in the matched setting [13]. The role that PT/Cy plays in B cell recovery in the haploidentical transplantation is an ongoing study [35]. In 3 subjects with available data, response to vaccination with diphtheria, tetanus, and/or pneumococcal has also been demonstrated.

Improvements in IgE levels and eosinophilia were marked. The IgE level improved almost immediately in all patients by day 30 after HSCT. With a median IgE of 1539 (range, 639 to > 6000 IU/mL) before transplantation, for those patients with 1-year follow-up, the median IgE had decreased to 77.6 (range, 9.1 to 151). Similarly, the median absolute eosinophil count immediately before initiation of the conditioning regimen was 920 (range, 400 to 7610 cell/μL), which decreased in all patients by day 30. Post-HSCT day 30 eosinophil values were a median of 10 (range, 0 to 980 cells/μL).

Changes in lymphocyte subsets and immunoglobulin levels are shown (Supplemental Table S1) for 4 patients with complete data available. By 1 year after transplantation, increases to normal levels were seen in the CD4 central memory and $CD8^+$ naïve T cell pools. Improvement in immunoglobulin levels are seen as well, including normalization of pretransplantation values in 2 subjects and not requiring replacement therapy by 1 year after transplantation.

DISCUSSION

In this study, we report the outcome in 7 patients who received a fludarabine/busulfan-based haploidentical related donor HSCT with T cell–replete bone marrow grafts followed by PT/Cy. All 7 patients attained full donor engraftment, had minimal GVHD, and with minimal infection-related complications, supporting the feasibility of this approach in DOCK8 deficiency when a matched related or unrelated donor is not available [13]. This regimen resulted in immune reconstitution, a critical feature in immunodeficiency patients, many of whom undergo HSCT with active infectious disease. The low incidence of GVHD was particularly beneficial as alloreactivity provides no therapeutic benefit in nonmalignant diseases.

The major questions related to HSCT in DOCK8 deficiency include the optimal donor type, conditioning regimen, GVHD prophylaxis, and level of donor chimerism in the individual compartments required to reverse the phenotype. These considerations are likely to be disease specific.

Donor selection for patients who lack an HLA-matched donor include a partially mismatched related or unrelated donor, umbilical cord blood (UCB) units, or a haploidentical related donor. The use of 9/10 HLA–matched donors is associated with a higher rate of rejection as well as GVHD. The latter is particularly problematic in diseases such as DOCK8 deficiency, where alloreactivity confers no advantage in terms of a graft-versus-leukemia effect. UCB has the advantage of a short time between initiation of the donor search and the acquisition of the donor product, and units can be found for nearly all patients; however, the low cell doses in UCB lead to delays in immune reconstitution and an increased risk of graft failure. In this regard, in 2010 we carried out HSCT on a 20-year-old patient with DOCK8 deficiency (the older sister of patient 1 in this study) using a reduced-intensity conditioning regimen and double UCB units (D. Hickstein, unpublished). However, before a second transplantation could be carried out, primary graft failure led to viral reactivation and death. Haploidentical related donors have the advantage of donors who are readily available for nearly all patients; there is the ability to select the optimal donor in terms of blood type, CMV status, etc.; additional cell products can be collected from the donor, if needed; and there is the ability to proceed to HSCT expeditiously if necessary. The drawback has been the strong alloreactivity due to T lymphocytes that recognize major class I and class II disparities between donor and host.

Recent studies utilizing PT/Cy for haploidentical HSCT have shown safety and efficacy [36,37], with outcomes comparable to transplantation with matched unrelated donor sources [38]. In a recent comparison of outcomes of T cell–replete allografts for hematologic malignancies from HLA-haploidentical donors using PT/Cy (n = 53) to outcomes of matched related (n = 117) and matched unrelated donors (n = 101), nonrelapse mortality, acute GVHD, chronic GVHD, and overall survival were comparable [39].

The use of PT/Cy provides a number of benefits. First, it obviates the need for graft manipulation. Second, it avoids the use of serotherapy in a cohort of patients with preexisting active viral infections [40-42], and it preserves antiviral immunity after transplantation. PT/Cy capitalizes on the ability of the drug's selective in vivo depletion of alloreactive proliferating T cells, while sparing resting regulatory T cells and stem cells, and, thus, facilitates engraftment [43-45]. In the setting of a T cell–replete graft, proliferating T cells that have been recently induced by exposure to antigen can be eliminated by the timely administration of post-transplantation administration of cyclophosphamide, thereby reducing the risk of GVHD.

Experience with PT/Cy after haploidentical transplantation for nonmalignant conditions is limited. Klein et al. recently reported on their experience with 11 pediatric patients carrying a host of life-threatening nonmalignant diseases, who all received reduced-intensity–conditioned transplantation with alternative donors (including 4 haploidentical donors) and PT/Cy alone or in combination with tacrolimus and mycophenolate mofetil [46]. In their series, there was only limited acute GVHD, no transplantation-related mortality, and successful engraftment, leading to reversal of the disease manifestations in all patients [46]. Parta et al. also described a child with chronic granulomatous disease who underwent haploidentical transplantation utilizing PT/Cy for GVHD prophylaxis who had successful engraftment and limited acute GVHD (grade 2) and CMV viremia, both of which were well controlled [23]. Our cohort

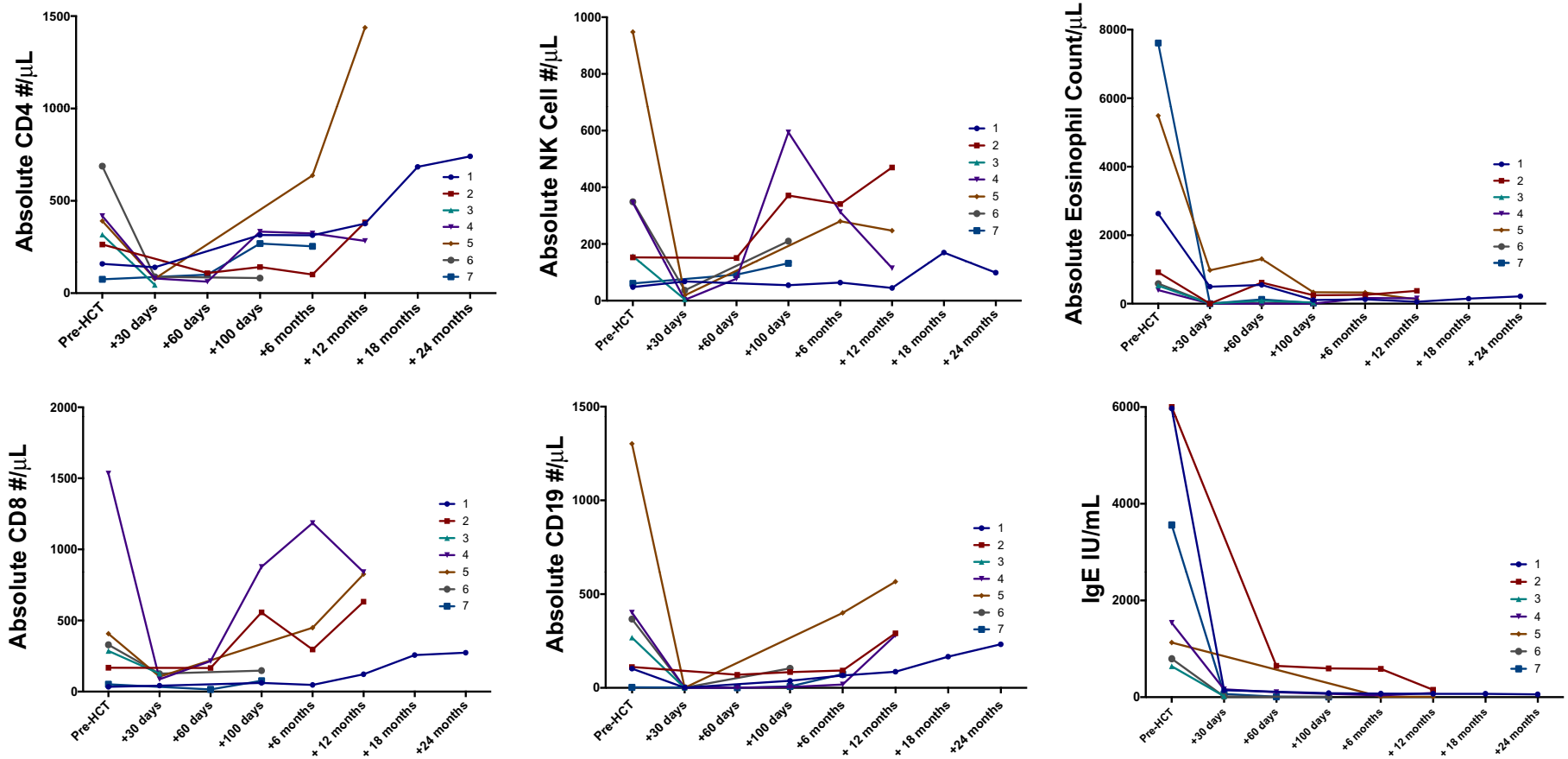

Figure 4. Changes in IgE, absolute eosinophil count, CD4, CD8, NK cell, and CD19 counts before and after transplantation.

of patients with DOCK8 deficiency had pre-existing viral and bacterial complications and other significant comorbidities, but they engrafted rapidly and had minimal GVHD, indicating the promise of this transplantation approach.

The second major question in HSCT for DOCK8 deficiency involves the type and amount of conditioning and the need for serotherapy. We have used a busulfan/fludarabine-based regimen without serotherapy for our 10/10 HLA–matched related and unrelated donor HSCT for patients with DOCK8 deficiency with excellent results [13]. Similarly, in the matched related and unrelated donor HSCT for patients with DOCK8 deficiency, we have used a test dose of busulfan to target an AUC of 3600 μmol/minute to 4800 μmol/minute, with a trend towards targeting a lower AUC in more recent patients, as we have had reliable engraftment at the higher AUC. In the current haploidentical related donor regimen, we incorporated busulfan rather than serotherapy because of concern for disseminated viral infections in this cohort of patients who already harbor DNA viruses due to DOCK8 deficiency. Adding 3 days of busulfan to the Johns Hopkins nonmyeloablative regimen resulted in a mean total busulfan of 8.6 mg/kg. This amount of busulfan was less than the amount used in a recent trial of reduced-intensity conditioning for chronic granulomatous disease, where the median administered total busulfan dose was 10.5 mg/kg [22]. The busulfan dose in our study resulted in a projected median cumulative AUC of 11,600, which corresponds to the 45 to 65 mg/L/hour in the Gungor study (55% to 75% of a full myeloablative dose) [22]. However, the addition of 200 cGy TBI to the busulfan dose in our regimen likely resulted in a myeloablative regimen. The uniform engraftment we observed confirmed results from murine models where a busulfan exposure of 45 to 65 mg/L/hour inhibited most granulocyte macrophage colony–forming units and $CD34^+$ hematopoietic stem cells [47]. In future haploidentical related donor HSCT for DOCK8 deficiency, we anticipate targeting an AUC of 3000 to 4000 μmol/minute to further reduce the risk of end-organ damage.

The type of GVHD prophylaxis represents an important component of any HSCT regimen. PT/Cy appears to convey a favorable risk to benefit ratio in DOCK8 deficiency as well as other diseases, particularly in the haploidentical related donor setting.

Regarding the extent of donor chimerism necessary to reverse the disease phenotype, a retrospective study of 11 patients with DOCK8 deficiency who underwent HSCT was recently reported [14]. Although the 7 patients receiving matched related donors all had high levels of donor chimerism, several of the mismatched patients, who received a nonmyeloablative regimen, had high levels of donor $CD3^+$ chimerism but low levels of myeloid and B cell chimerism. Nevertheless, switched memory B cells were preferentially donor derived and antibody production was achieved, suggesting a survival and/or differentiation advantage for the donor B cells. In this regard, it has been shown that DOCK8 functions as an adaptor molecule that links toll-like receptor signaling to B cell activation and promotes B cell proliferation and differentiation via a STAT3-dependent mechanism [48]. Overall, these data confirm previous observations in mice and patients where normal donor T cells appeared to have a selective advantage in DOCK8 deficiency [6,49]. Longer follow-up will be required to determine if this survival of low levels of donor B cells is durable.

Allogeneic HSCT clearly provides a curative therapy for DOCK8 deficiency and allows for long-term survival; however, 1 potential criticism of the current protocol is the possibility of late effects and long-term toxicity [50-53]. Given that the current regimen provides rapid and complete donor engraftment with minimal complications with GVHD and that there is no known risk of hematologic malignancies in DOCK8, moving toward chemotherapy dose-reduction and deintensification of therapy represents a desirable objective, particularly in the consideration of long-term fertility preservation and reduction of late effects. In this regard, our future goal is to target a lower busulfan exposure and removal of TBI to reduce toxicity.

Haploidentical transplantation is increasingly becoming the standard of care when a matched donor is not available [39,54,55]. It is more frequently being used in the primary immunodeficiency setting [18], either with [16,23] or without high-dose cyclophosphamide [17,20], for GVHD prophylaxis. In our experience, despite extensive comorbidities in this patient population, haploidentical transplantation with PT/Cy for GVHD prophylaxis is effective and results in minimal regimen-related toxicity, absence of steroid-refractory GVHD despite complete donor chimerism, and reconstitution of the deficient lymphocyte compartments, leading to complete reversal of the infection susceptibility phenotype. With the widespread use of genetic testing, DOCK8 deficiency will likely be diagnosed earlier in life—leading to improved outcomes, before the development of life-threatening infections, end-organ damage, and virally driven malignancies, which was contributory to the ultimate demise of the 1 patient who had pre-existing bleomycin-related pulmonary fibrosis. Thus, the outcomes with HSCT in DOCK8 deficiency should continue to improve. The use of PT/Cy approach will allow for nearly all identified children with DOCK8 deficiency to undergo HSCT despite the lack of a matched donor.

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SUPPLEMENTARY DATA

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