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Disease-specific Hematopoietic Cell Transplantation: Nonmyeloablative Conditioning Regimen for Dyskeratosis Congenita

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Abstract

Dyskeratosis congenita (DC) is characterized by reticular skin pigmentation, oral leukoplakia, and abnormal nails. Patients with DC have very short telomeres and about one-half have mutations in telomere biology genes. A majority of patients with DC develop bone marrow failure (BMF). Hematopoietic cell transplantation (HCT) represents the only known cure for BMF in DC, but poses significant toxicities. We report six patients who underwent allogeneic HCT with a novel nonmyeloablative conditioning regimen specifically designed for DC patients. Graft sources included related peripheral blood stem cells (1), unrelated bone marrow (2), and unrelated double umbilical cord blood (3). Complete donor engraftment was achieved in 5 of 6 patients. One patient had initial autologous hematopoietic recovery, which was followed by a second transplant that resulted in 88% donor chimerism. With a median follow-up of 26.5 months, four patients are alive, three of whom were recipients of unrelated grafts. We conclude with this small study that encouraging short-term survival can be achieved with HCT in patients with DC utilizing a preparative regimen designed to promote donor engraftment and minimize life-threatening disease-specific complications such as pulmonary fibrosis. Long-term follow-up will be crucial with respect to individualized patient care with each of the transplanted individuals.

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Conflict of Interest

The authors declare no conflict of interest.

Introduction

Dyskeratosis congenita (DC) is characterized by the clinical triad of reticular skin pigmentation, oral leukoplakia and abnormal nails. Patients with DC have abnormally short telomeres and about one-half have mutations in genes important in telomere biology genes. The clinical complications of DC are broad and include bone marrow failure (BMF), cancer, pulmonary fibrosis, liver abnormalities, and esophageal stenosis[1–3]. Telomeres consist of nucleotide repeats, $(TTAGGG)_n$, and a protein complex at chromosome ends; they are critical for the maintenance of chromosome stability[4]. Mutations that result in DC occur in genes that directly interact with telomerase, the reverse transcriptase that adds nucleotide repeats to chromosome ends, (*DKC1, TERC, TERT, NOP10*, and *NHP2*), or with one component of the shelterin telomere protein protection complex (*TINF2*)[5]. The most severely affected patients often have the diagnostic triad, very short telomeres (<1st percentile for age), BMF, and other complications. Other, less severely affected patients may still have very short telomeres, but fewer clinical findings.

Hematopoietic cell transplantation (HCT) is a life-saving measure for patients with malignant and non-malignant diseases. However, the fully myeloablative and immunosuppressive regimens typically prerequisite for donor cell engraftment are commonly associated with significant tissue injury. This systemic toxicity is frequently extreme in patients with inherited defects in genome maintenance, which cause several BMF syndromes, including DC and Fanconi Anemia (FA), and for whom HCT is at present the only definitive BMF therapy[6–9]. This patient population is also at higher risk of developing MDS and AML[10, 11]. In addition, previous studies have estimated that BMF and its associated immunodeficiency are responsible for 60-70% of premature mortality in DC patients[9].

Past efforts to correct BMF in DC by allogeneic HCT have resulted in unacceptable transplant-related mortality, especially from pulmonary, vascular and hepatic complications[7, 9, 12–14]. Therefore, we hypothesized that a nonmyeloablativeconditioning regimen designed with regard to the clinical complications of DC may result in better outcomes. We designed a prospective DC-specific nonmyeloablative transplantation regimen to determine whether engraftment can be achieved with less toxicity. Our approach utilized the incorporation of fludarabine, reduction in cyclophosphamide dose, use of low-dose TBI, and use of alemtuzumab instead of anti-thymocyte globulin (ATG). Rationale behind these choices is expanded in the discussion. Here we report successful short-tem outcomes, including the ability to achieve favorable results with unrelated donor sources of hematopoietic stem cells, which has been an even greater challenge for these patients in the past[7, 9, 12].

Patients and Methods

Six patients underwent allogeneic HCT with nonmyeloablative conditioning specifically designed for DC patients. This transplant protocol was approved by the Institutional Review Board at the University of Minnesota and the University of Colorado at Denver, and informed consent was obtained from the subjects or their guardians prior to HCT.

The clinical trial has been registered on www.clinicaltrials.gov since 30 March 2007 (NCT00455312). Follow-up is reported through 30 September 2009.

The classification of a patient with DC has evolved since the advent of clinical telomere length testing and genetic testing. Initially, the diagnosis was based only on the presence of the classic clinical triad. The diagnosis of DC was suspected clinically in 5 of our 6 patients (ages 2, 5, 18, 24 and 29) with BMF by the presence of at least 2 features of the classic triad or a feature of the triad plus another condition also seen in patients with DC. The diagnosis was then supported by documentation of very short telomeres with automated multicolor flow cytometry fluorescent *in situ* hybridization (flow FISH) of leukocyte subsets (less than 1st percentile of normal for age)[15]. Research and clinical gene sequencing was also performed as described[5]. A 6th patient (age 25) was classified as "DC-like"[3] as a result of BMF with very short telomeres but no features of the DC diagnostic triad or other physical findings and no identifiable mutation in a known DC gene.

The nonmyeloablative regimen included a single dose of cyclophosphamide (50 mg/kg) intravenously (IV) on day −6, fludarabine (40 mg/m^2) IV once daily for five consecutive days from day −6 to day −2, alemtuzumab (0.2 mg/kg) IV once daily for five consecutive days from day −10 to day −6, and a single 200 cGy dose of total body irradiation (TBI) on day −1. The low-dose TBI was delivered side-to-side, instead of anterior-posterior, with the patient in a seated position and the arms resting at the side of the thoracic cage. This enabled the arm to provide a natural pulmonary compensation with respect to the delivered radiation. There was no other shielding provided. In the case of patient 1, following the inability to achieve cord blood donor derived hematopoiesis with the initial transplant, a second set of cord blood was infused after a preparative regimen consisting of a 5-day course of ATG, supplemented by a 28 day course of prednisone.

Graft-versus-host disease (GVHD) and graft failure prophylaxis consisted of cyclosporine and mycophenolate mofetil. Cyclosporine was started on day −3 and adjusted to maintain a level of greater than 200 mg/L (initial dose 2.5 mg/kg every 12 hours for patients weighing 40 kg or more, or 2.5 mg/kg every 8 hours for patients weighing under 40 kg) until day +180 with a subsequent taper over 10 weeks unless GVHD was present. Mycophenolate mofetil was started on day 0 using a dose of 15 mg/kg (maximum 1 gram) three times a day until day +30, at which time it was stopped unless GVHD was present.

Stem cell sources included one human leukocyte antigen (HLA) matched related peripheral blood stem cell graft (6/6 HLA match, patient 2), two from unrelated donor bone marrow grafts (one 7/8 HLA match, one 8/8 HLA match, patients 3 and 4, respectively), and three from double umbilical cord blood grafts (one set of 4/6 and 4/6 HLA match, two sets of 4/6 and 5/6 HLA match, patients, 1, 5, and 6, respectively). HLA typing of the patient and donor was performed at the allele level for HLA-A, B, C and DRB1 in the case of marrow or peripheral blood stem cells. In the case of umbilical cord blood, HLA typing was performed at antigen level for HLA-A, and B and allele level for DRB1. All grafts were unmanipulated. Hematopoietic chimerism was assessed on peripheral blood leukocyte DNA by competitive PCR analysis of variable tandem repeat regions[16].

The Kaplan-Meier product limit estimator was used to calculate actuarial survival probabilities and cumulative incidences in cases reported from the literature in the absence of competing risks. Subjects were censored at death. Subgroup survivals were compared using the log-rank rest for equality of survivor functions[17]. Stata10 was used for these analyses. A p-value of ≤0.05 was considered to be significant.

Results

The clinical characteristics and pre-transplant evaluations are shown in Table 1. Two of 6 patients had no evidence of the characteristic clinical mucocutaneous triad at the time of presentation with BMF, although one of those patients fit criteria for Hoyeraal-Hreiderasson Syndrome, a severe form of DC. Only 2 of 6 patients had an identifiable genetic mutation associated with DC (patient 1 had a mutation in *DKC1* and patient 2 had a mutation in *TINF2*), but all 6 had very short leukocyte subset telomere length measurements by flow FISH. All patients had progressive pancytopenia with evidence of marrow hypocellularity on biopsy, but normal cytogenetic analysis on bone marrow aspirates. No patients had evidence of myelodysplasia or leukemic transformation.

Graft characteristics and transplant outcomes are provided in Table 2. Complete donor engraftment after the initial stem cell infusion was achieved in 5 of 6 patients, with patient 1 showing autologous hematopoietic recovery. Following a second transplant 88% donor chimerism was achieved in this patient. This was documented on a bone marrow aspiration performed on day +20 after the second transplant.

Four patients are alive (67%) with a median follow-up of 26.5 months (range 12 months to 45 months). Three of the surviving patients received unrelated donor stem cell grafts. There were two lethal infectious complications in the immediate post-transplant period. Patient 4 died of adenoviral sepsis 3 months post-HCT. Patient 1 died of sepsis after leaving the hospital against medical advice on day +21, having received a second umbilical cord blood transplant following prior graft failure. His last documented total white blood cell count was 0.3 $x10^9$/L. To date, there has been no evidence of pulmonary, hepatic or vascular complications through this transplant process in our patients. Follow-up PFT data on patient 5 is available at 6 months post-transplant showing similar changes to pre-transplant evaluation. Patient 6 has follow-up PFT data available at 1 year post-transplant showing similar changes to pre-transplant evaluation.

Other significant transplant related morbidity included grade II acute skin GVHD (patient 4), grade IV acute gastrointestinal GVHD (patient 5), and limited chronic skin GVHD (patient 2), all of which were successfully treated with systemic and topical steroids. Patient 2 also had CMV reactivation at low levels that was treated successfully with IV ganciclovir followed by oral valganciclovir. Patient 6 had skin manifestations of Varicella Zoster Virus, which was successfully treated with IV and oral acyclovir.

In order to better understand the HCT outcomes in this study and the literature, we also reviewed the literature on HCT in DC. There have been some promising results using reduced intensity conditioning (RIC) or nonmyeloablative preparations in DC.

Various regimens have been examined, but there is not yet a commonly accepted standard[9, 12, 14, 18–30]. Table 3 summarizes the reported experience of HCT for patients with DC using RIC, including specifics of donors, regimens, and outcomes. Kaplan-Meier analysis revealed that overall cumulative survival was 65%, with related donor recipients having better survival compared with unrelated donor transplants (91% versus 30% respectively, p = 0.05). However, the follow-up intervals for the two donor types are short and not very different from other historical data[10].

Discussion

This study was designed to assess clinical complications and outcomes of a nonmyeloablative regimen that included agents known to promote donor engraftment in other BMF disorders (e.g., fludarabine) and excluded agents (e.g., busulfan and high-dose TBI) that can lead to life-threatening disease-specific extra-medullary side effects, such as pulmonary fibrosis. Preliminary data suggest that this strategy may result in improved short-term outcomes in patients with DC. Ongoing follow-up is required to assess the long-term success of this nonmyeloablative regimen in DC.

Historical results with myeloablative conditioning resulted in death in 14 of 21 patients with DC; there were no survivors who received unrelated sources of stem cells[2, 9, 22]. In a review of 65 cases of allogeneic HCT for DC with all intensity types, matched related donor (MRD) transplants fared better, but long-term outcome was poor with late pulmonary complications[10]. The overall survival was 24% at 11 years after HCT. The MRD group had 71% cumulative survival at over 5 years, while the alternative donor group had a cumulative survival of 31% at 2 years. The longest survivor in the MRD group died at 20 years after HCT from pulmonary fibrosis, and the longest survivor in the unrelated group died from pulmonary fibrosis at 10 years[10].

There are 4 important elements to the design of our nonmyeloablative strategy: 1) incorporation of fludarabine, 2) reduction in cyclophosphamide dose, 3) the use of low-dose TBI, and 4) the use of alemtuzumab instead of anti-thymocyte globulin (ATG). Patients with DC are at high risk of pulmonary fibrosis and liver dysfunction due to their disease. Thus, we specifically dose-reduced or excluded agents known to be associated with severe organ-specific toxicity.

The use of fludarabine in HCT preparative regimens has been important in the reduction of intensity in several settings. This strongly immunosuppressive, less myeloablative agent has been used successfully in RIC for a variety of settings including acquired severe aplastic anemia[30], other BMF syndromes such as FA[8], and previously in DC[19–22, 25, 26]. Fludarabine is generally well tolerated with limited extra-medullary toxicity.

Conventional HCT agents that may have severe organ-specific toxicity, especially pulmonary and hepatic complications, include busulfan[31, 32] and melphalan[33, 34]. The exclusion of these medications from the preparative regimen is highly desirable based on the prior experience with them for DC patients. While the use of cyclophosphamide at higher doses may result in significant morbidity and potentially mortality, reduction in the

total dose by inclusion of agents like fludarabine has resulted in reduction in toxicity and better survival[21, 22, 26].

Use of TBI in DC patients is also considered undesirable due to its toxicity profile, including potential pulmonary, hepatic and dermatologic complications[9, 35]. This is described primarily with full-dose TBI, as opposed to the low-dose TBI used in this protocol, which has previously been successful in DC patients[21, 25]. The additional consideration of patient positioning to achieve partial pulmonary compensational shielding is an important aspect to the continued inclusion of low-dose TBI in the RIC regimen. While the elimination of TBI may result in decreased toxicity and late complications, this may increase the risk for graft failure. This has been demonstrated in patients with FA in the MUD setting when low-dose TBI is removed[6].

The incorporation of alemtuzumab, a humanized monoclonal antibody directed at CD52, was chosen as a method of achieving *in vivo* T-cell depletion. It is more immunosuppressive than alternatives, including ATG, as it targets T-cells, B-cells, monocytes and macrophages[36]. T-cell depletion has been shown to be important in reducing the risk of graft versus host disease (GVHD) in other BMF settings such as FA[8]. As with fludarabine, immunosuppression with alemtuzumab has the potential to provide for de-escalation of regimen intensity while still achieving donor derived engraftment. In addition, the use of alemtuzumab in transplantation has previously been shown to reduce GVHD more effectively than ATG[37], and may reduce the risk of post-transplant lymphoproliferative disorder (PTLD) compared with ATG[38]. The major concern with the use of alemtuzumab is infectious complications[37], including viral pathogens such as adenovirus[39], which proved fatal in one of our patients. New approaches utilizing alemtuzumab in combination with a second monoclonal antibody directed at CD45 have also shown promise. Two DC patients were treated with this combination in a different study, one with a MUD who died and one with a MRD who is still alive[18].

Our data show encouraging overall survival of 4 of 6 patients (67%), including 3 of 5 patients (60%) from unrelated donor sources, a source that has been difficult in the past[9, 10, 12, 14, 18, 28]. In addition, of particular note with our nonmyeloablative regimen has been the absence of pulmonary or liver complications during and immediately after HCT. The mortality associated with this regimen was limited to infectious etiologies. One episode of adenovirus sepsis occurred within the first 3 months post-transplant occurred in the patient with Hoyeraal-Hreiderasson Syndrome, who came to HCT with multiple co-morbid conditions. The second infection-related death was due to uncharacterized sepsis in a neutropenic patient who left the hospital against medical advice. Survivor follow-up remains relatively short (between 12 and 45 months), and thus long-term complications cannot yet be discussed.

It also remains to be seen whether resolution of bone marrow failure substantially alters the natural history of the disease[9]. The risk of cancer has been reported to be 10-fold greater than in the general population with a cumulative incidence up to 50% by age 50 in the DC population[10]. These are primarily skin, head and neck, and anogenital cancers[10], resembling what is seen in patients with Fanconi Anemia[40, 41]. Cancer and pulmonary disease follow

BMF as the causes of premature mortality for DC patients[9, 10]. . It is important to identify these patients early in life in order to screen for BMF, MDS, AML and solid tumors. It is possible that early diagnosis could lead to improved outcomes. It is also important to screen patients that present with aplastic anemia for DC since some patients can present with BMF alone, as occurred with one of our patients.

Patients with DC require modification of their HCT conditioning due to the increased risks associated with full myeloablative preparative regimens. In such instances we would recommend a disease-specific protocol using nonmyeloablative conditioning to help avoid the potential hepatic and pulmonary complications. Carrier detection in family members is important for both genetic counseling and the donor selection process for HCT as treatment for bone marrow failure. If a pre-symptomatic individual with DC was used as the donor source, there would be a high risk for failure of engraftment[15, 42, 43].

Since the underlying genetic defect cannot yet be corrected in non-hematopoietic lineages, patients with DC should continue to be followed by a comprehensive, multidisciplinary team in the post-transplant period. Follow-up should include regular skin, oral, pulmonary, and genitourinary exams[3, 7]. Concurrent with a better understanding of treatment and surveillance there is an ongoing need for continued research into the underlying etiologies, molecular biology, and genetics of telomere shortening to address the basic nature of this disease.

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Table 1:

Patients' characteristics.

Patient	Age (years) Sex	Presence of Clinical Triad	Telomere Length	Co-morbid Conditions	PFT
1	24 Male	Skin, Nail, Oral	VL	Epiphora, early gray hair, bipolar	Moderately decreased DLCO
2	29 Female	Skin, Nail, Oral	VL	Epiphora, early gray hair	Normal
3	5 Female	Nail, Oral	VL	None	N/A
4	2 Male	None	VL	HH syndrome, delayed development, ataxia, microcephaly, cerebellar hypoplasia, IUGR, retinal hemorrhages	N/A
5	18 Female	Skin, Nail, Oral	VL	None	Mild reversible obstruction
6	25 Male	None	VL	Mild hypogonadism, short stature and learning disabilities	Mildly decreased DLCO

PFT, pulmonary function tests; DLCO, carbon monoxide diffusing capacity; N/A, not applicable due to young age; VL, very low (<1%) in granulocytes, lymphocytes, naïve T cells, memory T cells, and B cells (granulocytes too low to test in patient 4); HH, Hoyerdaal-Hreidarsson; IUGR, intrauterine growth restriction

Table 2:

Transplant characteristics and outcomes.

Patient	Graft and match	NC dose/kg (x10 ^{8}/kg)	CD34+ Cell dose/kg (x10^{6}/kg)	Follow Up (outcome)	Donor Chimerism	Myeloid Recovery Day[1]	Platelet Recovery Day[2]	Acute GVHD	Chronic GVHD
1	URD dUCB (4/6) (4/6)	0.55, 0.39	0.5, 0.43	1 m (died from unknown sepsis outside of hospital)	88% *	None	None	No	No
2	REL PBST (6/6)	13.93	4.43	3 y, 9 m (alive)	100%	+9	+33	No	Limited Skin
3	URD BM (7/8)	1.38	1.55	3 y, 5 m (alive)	100%	+13	+29	No	No
4	URD BM (8/8)	5.92	2.31	3 m (died from adenoviral sepsis)	100%	+12	+35	Grade II Skin	No
5	URD dUCB (4/6) (5/6)	0.32, 0.2	2.39, 1.62	12 m (alive)	100%	+23	+49	Grade IV GI	No
6	URD dUCB (4/6) (5/6)	0.2, 0.39	0.23, 1.14	12 m (alive)	100%	+25	+46	No	No

[1] Myeloid recovery: absolute neutrophil count of more than or equal to 0.5 x10^{9}/L, first of 3 days.

[2] Platelet recovery: untransfused platelet count of more than or equal to 50 x10^{9}/L, first of 7 days.

URD, unrelated donor; REL, related; dUCB, double umbilical cord blood; BM, bone marrow; PBST, peripheral blood stem cell transplant; NC, nucleated cell; GI, gastrointestinal; GVHD, graft versus host disease; m, month; y, year.

* Despite not meeting criteria for myeloid recovery, a bone marrow biopsy on day +20 was used to evaluate chimerism.

Table 3:

Reduced Intensity Conditioning Regimens for Dyskeratosis

Patient	Age	Sex	Source	Cell Dose	GVHD ppx	CY	FLU	BU	MEL	ATG	ALEM	TBI	Outcome	Complications	Reference
1	18	M	MRD	4.6	CSA/MTX	80		0.8					Alive, > 6 y	Skin cGVHD	[23]
2	21	F	MRD	4	CSA/MTX	80		0.8					Alive, > 5 y	Skin/GI GVHD	[23]
3	1	M	MRD	6.6	CSA/Pred.		300		40	5			Alive, 1 y		[20]
4	5	M	MRD	6.82	CSA/ATG	60				52			Alive, 6 m		[24]
5	10	F	MRD	10	CSA/MMF		90					2000	Alive, 2 y		[25]
6	2	M	MRD	3	CSA	40	120			15			Alive, 2 y		[26]
7	5	F	MRD	2.06	CSA/MMF	40	120			15			Alive, 17 m		[22]
8	6	M	MRD		Tacrolimus	X	X			X		200	Alive, 9 m	3rd Transplant PTLD, GVHD	[27]
9	33	M	MRD	3.3	CSA/MTX	140				90			Alive, > 463 d	CMV Retinitis	[29]
10	22	M	MRD	2.6	CSA/MTX	140				90			**Died, 44 d**	GI/Liver GVHD	[29]
11	29	F	MRD	13.93	CSA/MMF	50	200				1	200	Alive, 44 m	Skin cGVHD	This Report
12	2	F	MRD	2.5	CSA	40	150				0.3		Alive, 40 m	Skin GVHD	[18] *
13	3	M	**MUD**	5.7	CSA/Pred.	120	180			160			Alive, 15 m		[19]
14	8	F	**MUD**	6	CSA/Pred.	120	180			160			Alive, 16 m	EBV	[19]
15	5	F	**MUD**	1.38	CSA/MMF	50	200				1	200	Alive, 40 m		This Report
16	24	M	**MUD**	5.92	CSA/MMF	50	200				1	200	**Died, 3 m**	Skin GVHD	This Report
17	15	M	**MUD**	3.8	CSA/MTX	120	180			160			**Died, 45 d**	GVHD, TTP	[14]
18	24	M	**MUD**	3.4	CSA		150		140		100		**Died, 15 m**		[12]
19	1	M	**MUD**	12.5	CSA/MMF	40	150				0.6		**Died, 19 m**		[18] *
20	3	F	**UCB**	1.15	CSA/MMF	50	200			90		200	Alive, 2 y	cGVHD, CMV	[21]
21	18	F	**dUCB**	0.52	CSA/MMF	50	200				1	200	Alive, 11 m	GI GVHD	This Report
22	25	M	**dUCB**	0.59	CSA/MMF	50	200				1	200	Alive, 11 m		This Report
23	2	M	**dUCB**	0.94	CSA/MMF	50	200				1	200	**Died, 1 m**		This Report
24	26	NK	**dUCB**	0.18	CSA/Pred.	50	100						**Died, 2 m**	2nd Transplant	[28]

GVHD, graft versus host disease; ppx, prophylaxis; CY, cyclophosphamide; BU, busulfan; MEL, melphalan; ATG, anti-thymocyte globulin; ALEM, alemtuzumab; TBI, total body irradiation; MRD, matched related donor; MUD, matched unrelated donor; UCB, umbilical cord blood; dUCB, double UCB; CSA, cyclosporine; MTX, methotrexate; Pred, Prednisone; MMF, mycophenolate mofetil; PTLD,

post-transplant lymphoproliferative disorder; EBV, Epstein-Barr virus; TTP, thrombotic thrombocytopenic purpura; CMV, cytomegalovirus; GI, gastrointestinal; NK, not known; Cell Dose, CD34+ cells x 10e6/kg; Cyclophosphamide, Busulfan, ATG, and Alemtuzumab dosing in mg/kg; Fludarabine and Melphalan dosing in mg/m^2; TBI dosing in cGray

*Also used anti-CD45 monoclonal antibody in preparative regimen