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Dose-adapted post-transplant cyclophosphamide for HLA-haploidentical transplantation in Fanconi anemia

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Abstract

We developed a haploidentical transplantation protocol with post-transplant cyclophosphamide (CY) for *in vivo* T-cell depletion using a novel adapted-dosing schedule (25 mg/kg on days +3 and +4) for Fanconi Anemia. With median follow-up of 3 years (range, 37 days to 6.2 years), all six patients engrafted. Two patients with multiple co-morbidities and late referrals to transplant died from sepsis (n=2) and chronic graft-versus-host disease (GVHD) (n=1). Four patients without pre-existing co-morbidities and early transplant referrals are alive with 100% donor chimerism and excellent performance status. We conclude that modulated-dosing post-transplant CY is effective *in vivo* T-cell depletion to promote full donor engraftment in patients with Fanconi anemia.

Keywords

Fanconi's anemia; transplantation; BMT pediatric; haploidentical

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CONFLICT OF INTEREST

The authors have no pertinent financial disclosures relating to this study.

AUTHOR CONTRIBUTIONS

M.T. designed the trial, wrote the protocol, enrolled patients, analyzed the data, and wrote and edited the manuscript.
C.B. enrolled patients, analyzed data, and edited the manuscript.
M.W. enrolled patients, analyzed data, and edited the manuscript.
R.P. enrolled patients, analyzed data, and edited the manuscript.
R.S. designed the trial and edited the manuscript.
B.S. designed the trial, analyzed the data, enrolled patients, and edited the manuscript.
L.B. analyzed the data and edited the manuscript.
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INTRODUCTION

Fanconi anemia (FA) is the most common of the rare, inherited marrow failure disorders, with a prevalence of 1 in 360,000 live births and a carrier frequency as high as 1:181.[1] The only cure for the fatal hematological manifestations of this disease is hematopoietic cell transplantation (HCT), and the best results occur after human leukocyte antigen (HLA)-matched sibling HCT[2] with fludarabine (FLU)-containing regimens.[3] When well-matched donors are unavailable, patients receive supportive care or alternative donor transplantation when there is progression to significant neutropenia, transfusion dependence, or leukemia. HLA-haploidentical HCT traditionally incorporates *ex vivo* T-cell depletion (TCD) to reduce the risk of GVHD in patients with FA. A recent publication using the conventional haploidentical transplant approach of using CD34+ selected cells demonstrated a 5-year overall survival of 83%; however, engraftment was observed in only 75% of patients, which is a limitation of extensive removal of donor T cells.[4] Based on the promising haploidentical HCT results with post-transplant CY for *in vivo* selective TCD in both malignant[5] and non-malignant[6] diseases, we tested this method in a small cohort of patients with FA, but used a modulated dose of CY to reduce the risk of toxicity seen in FA.[7] Here, we update results from our multi-center pilot trial[8] evaluating the safety and efficacy of HLA-haploidentical HCT in individuals lacking a well-matched donor. We modified our Seattle-based non-myeloablative conditioning regimen incorporating FLU[9, 10] and coupled this with an adjustment of the Hopkins-based post-transplant CY dose for *in vivo* TCD.[5, 11, 12]

PATIENTS AND METHODS

Patient and donor characteristics

Six patients with marrow failure caused by FA, as confirmed by chromosomal fragility testing, who lacked well-matched donors, were enrolled in this study. Subjects had consent documented by local Institutional Review Board-approved forms. Each related donor was HLA-matched at one haplotype, with any number of HLA mismatches in the second haplotype. Haploidentical donors were chosen per institutional guidelines of donor selection. The protocol was later modified to allow 10/10 HLA-matched unrelated donors, with single class I allele mismatch allowable (Patient #6). Bone marrow was stipulated as the stem cell source, and each patient had a negative donor lymphocytoxic crossmatch. Patient and donor characteristics are shown in Table 1.

Transplant characteristics

Patients were enrolled in the multi-institutional study Protocol 2064 at the Fred Hutchinson Cancer Research Center, Seattle, WA (n=1); Universidade Federal do Parana, Curitiba, Brazil (n=2); UCSF Benioff Children's Hospital Oakland, Oakland, CA (n=2); and Children's Hospital of Wisconsin, Milwaukee, WI (n=1). The conditioning regimen consisted of CY (5 mg/kg) on days −6 and −5, FLU (30 mg/m2) from days −6 to −2, and 2 Gy total body irradiation (TBI) on day −1. Marrow was infused on day 0, followed by post-transplant CY (25 mg/kg/day, on days +3, +4). To protect against hemorrhagic cystitis, MESNA was administered at 100% of the CY dose. In patients #3 through #6 (n=4), the conditioning regimen was modified by eliminating pre-transplant CY in order to reduce the

severity of mucositis. However, the same dose of post-transplant CY was administered in these subjects. Patients received granulocyte colony stimulating factor (G-CSF) at 5 μg/kg/day IV or SC from day +5 until the absolute neutrophil count (ANC) was >500/μL for 3 consecutive days. Postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine commenced on day +5 for and extended until days +35 and +84, respectively. Cyclosporine was tapered off by day +180 if there was no graft-versus-host disease (GVHD). Mucositis was graded per FA-specific guidelines as published by Zanis-Neto et al.[13] and GVHD was graded by established methods.[14] Demographics are displayed in Table 1.

RESULTS

Patients

Six patients were transplanted at a median of 11.1 (range, 6.9–13.9) years of age and at 1.9 (range, 0.6–7.3) years after the diagnosis of FA was established. All patients were transplanted for marrow failure, and one patient had concurrent cytogenetic abnormalities [6,XY,der(19)t(1;19)(q23;p13)[2]/46,XY[19]]. No patient had myelodysplasia. Two patients were referred to transplant late in their courses; both were heavily transfused (one having a ferritin > 14,000 μg/L and one had marked clinical virilization due to androgen use. The remaining four patients were referred to transplant early in their clinical courses with minimal transfusions or other supportive care before HCT.

Transplant outcomes

Outcomes are summarized in Table 2. Early toxicity in the form of mucositis was seen in all patients, with the degree of mucositis improving in the last three patients transplanted with CY in the conditioning. No patient developed VOD, and one patient developed mild late hemorrhagic cystitis which resolved with supportive care over one week's duration. With a median follow-up of 3 years (range, 37 days to 6.2 years), 4 of 6 patients survived after transplantation. One patient died 37 days after HCT from multi-system organ failure caused by disseminated toxoplasmosis and CMV. However, autopsy did not demonstrate any GVHD in target organs. A second patient developed severe acute grade III GVHD of the skin, gut, and liver, which progressed to severe chronic GVHD involving the lungs and oral mucosa. This chronic GVHD flared with tapering of immune suppression, and during this time she also developed insulin-dependent diabetes mellitus. At 6.2 years after transplant, she died secondary to bacterial sepsis. She maintained 100% donor chimerism at last follow-up. Both of these patients were referred to transplant late in the courses of their diseases. Furthermore, these two patients did not have access to the IV formulation of MMF, and there was concern for inadequate early immune suppression from poor oral absorption due to mucositis. Among four patients referred to transplant early, one developed grade II acute GVHD (anorexia only) and two developed grade I acute GVHD (skin only). Two of these four patients developed mild chronic GVHD with xerophthalmia and lichenoid changes in the buccal mucosa, respectively. Of the four patients who are alive, all have 100% donor chimerism, transfusion independence, and excellent performance status, with no active GVHD and on no active immunosuppressive treatment. None have developed secondary malignancies.

DISCUSSION

Here, we present results of our pilot study testing a novel approach of HCT in patients with FA who have no suitable HLA-matched donors. We reasoned that the DNA repair defect in individuals affected by FA would preclude the typical dose of CY (50 mg/kg) administered to accomplish *in vivo* T-cell depletion after HLA-haploidentical HCT. Thus, we required a dose that would be sufficient to target highly proliferative, alloreactive normal donor T cells but not cause untoward toxicity in patients with FA. Our compromise was to split the dose of 50 mg/kg on day +3 which was used historically[5] into two 25-mg/kg doses on days +3 and +4. This allowed us to stay within safe limits of CY administration for patients with FA, in whom a total dose up to 60 mg/kg is safe.[2] Our results show that the four patients with no pre-transplant comorbidities had only grade I (n=2) and grade II (n=1) acute GVHD and mild chronic GVHD (n=2), all of which have resolved. These four patients remain off immune suppression and are alive and in good health between 2.6 to 5 years after transplant. Conversely, in two patients who underwent transplantation late after diagnosis of FA and thus with significant pre-transplant iron overload and in one patient, virilization, we observed transplant-related mortality. Inadequate absorption of oral MMF may have contributed to the severe acute GVHD seen in one patient. Thus, in most cases, our strategy of modulating the CY dose post-transplant appears to have elicited an equivalent biological effect on donor T cells that was sufficient to control GVHD and promote engraftment. This is note-worthy, as the original preclinical studies supporting this strategy did not test sequentially lower limits of CY needed to delete alloreactive donor T cells. Another earlier preclinical study evaluated sequential doses of post-transplant CY as low as 7.5 mg/kg and concluded that doses this low were not an effective strategy for GVHD prophylaxis.[15] Thus, our results support the rationale that lower doses of post-transplant CY should be studied in a prospective manner. Our current dosing strategy also could be investigated for other rare diseases such as dyskeratosis congenita, ataxia-telangiectasia, DNA Ligase IV Deficiency, and Nijmegen Breakage Syndrome, which are susceptible to DNA damage from cross-linking agents leading to organ toxicity. A recent publication of alternative donor transplantation for FA evaluating *ex vivo* TCD marrow from related and unrelated 7–8/8 HLA-allele-matched donors or 4–6/6 HLA-matched unrelated cord blood demonstrated a 1-year survival of 63%. In this mixed group of patients from sequential trials from 1995–2012, improved survival was seen in those using FLU-based regimens having a younger age <10 years old, no prior opportunistic infections, and no prior red cell or platelet transfusions.[16] Our findings also suggest that proceeding to transplant at the first signs of marrow failure results in excellent outcomes. Our four patients with early referral to transplant had the best results, while the two patients with transfusion dependence, iron overload, and, in one, severe virilization from androgens, experienced the most significant complications of our study (transplant-related mortality and severe GVHD). This important observation highlights the vital need to transplant patients with FA as early as possible, and to not be dissuaded when only alternative donors are available. We further speculate that inadequate absorption of oral MMF due to mucositis may have contributed to the severe GVHD seen in one patient, and thus our recommendation is to use IV formulations of all immune suppression drugs during the early post-transplant period. While our transplant strategy appears promising, we recognize that with our low patient numbers, additional studies are warranted

to evaluate how this approach compares to other alternative donor sources, such as cord blood. In conclusion, our study is the first to apply the HLA-haploidentical, post-transplant CY approach to FA and can be a model for other genetic diseases requiring lower doses of alkylating agents.

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Table 1

Patient and donor characteristics

Patient #	Age at Diagnosis (years)	Age at Transplant (years)	Pre-HCT Co-morbidities	Recipient / Donor			HLA-Matching	CD34/kg × 10^6
				Sex	ABO	CMV status		
Patient 1	10.62	13.9	Iron overload (ferritin > 14,000)	F/F	B+/B+	+/+	6/10 Haplo (half-sib)	2.21
Patient 2	4.35	6.9	None	F/F	O−/O−	+/+	5/10 Haplo (mother)	3.69
Patient 3	9.88	11.1	Iron overload, androgen-induced virilization	F/M	AB+/O+	+/+	9/10 Haplo (cousin)	4.41
Patient 4	7.64	8.4	None	F/F	O+/O+	−/−	8/10 (mother)	14.1
Patient 5	4.80	11.9	None	F/F	A+/A+	−/+	10/10 (unrelated)	6.08
Patient 6	10.50	11.1	None	M/M	O+/O+	−/−	8/10 (sister)	3.90

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Table 2

Transplant outcomes of interest

Patient #	Mucositis Grade	Hemorrhagic cystitis / VOD	Infections through day +100	Day of Engraftment	Day platelet trans-fusion indepen-dence	1 month CD3 chimerism	CD3 Chimerism last follow-up	Max Grade of Acute GVHD	Grade of Chronic GVHD (Max/Last follow-up)	PFS at last follow-up	Last follow-up
Patient 1	3b	N / N	Acinetobacter baumanni(+9); disseminated CMV and toxoplasmosis (+27)	+15	N/E	100%	100%	None	N/E	N/E	Dead at Day +37 from multi-system organ failure due to disseminated toxoplasmosis and CMV
Patient 2	3b	N / N	CMV reactivation (+69)	+16	+25	100%	100%	Grade II (anorexia)	None	100%	Alive and well at 5 years after HCT
Patient 3	3b	Mild requiring 1 week of supportive care (day +30) / N	Fever of unknown origin (+18) resolved on broad-spectrum antimicrobials	+14	+29	100%	100%	Grade III (skin, gut, liver)	Severe (pulmonary, oral) / Moderate	80%	Dead at 6.2 years post-HCT from sepsis related to being immune compromised due to GVHD treatment
Patient 4	2	N /N	BK viremia (+62)	+14	+15	91%	100%	Grade I (skin)	Mild (xerophthal-mia) / None	100%	Alive and well at 2.6 years after HCT
Patient 5	2	N / N	CMV reactivation (+46)	+14	+15	81%	100%	Grade I (skin)	Mild (oral) / None	100%	Alive and well at 3.2 years after HCT
Patient 6	2	N / N	Parainfluenza upper and lower tract infection(+22)	+14	+27	100%	100%	None	None	100%	Alive and well at 3 years after HCT