Biol Blood Marrow Transplant 23 (2017) 310–317

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Clinical Research: Alternative Donors

Haploidentical Bone Marrow Transplantation with Post-Transplant Cyclophosphamide for Children and Adolescents with Fanconi Anemia

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Article history:
Received 5 July 2016
Accepted 3 November 2016

Keywords:
Haploidentical
Inherited bone marrow failure syndromes
Fanconi anemia

A B S T R A C T

We describe haploidentical bone marrow transplantation with post-transplant cyclophosphamide (PT-CY) for 30 patients with Fanconi anemia (FA). Twenty-six patients were transplanted upfront, and the preparatory regimens included fludarabine 150 mg/m^2 + total body irradiation 200 to 300 cGy ± CY 10 mg/kg without (n = 12) or with rabbit antithymocyte globulin (r-ATG) 4 to 5 mg/kg (n = 14). Four patients were rescued after primary or secondary graft failure after related or unrelated donor transplantation with the above regimen with (n = 2) or without r-ATG (n = 2). PT-CY at 25 mg/kg/day (total dose, 50 mg/kg) followed by cyclosporine and mycophenolate mofetil was given to all patients. All patients engrafted in the subgroup of patients who did not receive r-ATG (n = 14), but their transplant course was complicated by high rates of acute and chronic graft-versus-host disease (GVHD), and only 8 patients are alive. In the subgroup that received r-ATG (n = 16), 14 patients had sustained engraftment, severe GVHD rates were lower, and 13 patients are alive. Hemorrhagic cystitis occurred in 50% of patients, whereas cytomegalovirus reactivation occurred in 75%. One-year overall survival for the entire cohort was 73% (95% CI, 64% to 81%), and all surviving patients achieved full donor chimerism. In conclusion, haploidentical donor transplantation with PT-CY is a suitable option for FA patients without a matched related or unrelated donor.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment able to cure the hematologic complications related to Fanconi anemia (FA), and results are better when transplantation is performed early and with an HLA-matched unaffected sibling [1,2] If a matched family donor is not available, patients may be treated with androgens, but response is usually short-lived [3]. Although the results of HLA-matched unrelated donor transplantation for FA have improved during the past decade, long-term survival is suboptimal and donor availability remains a challenge for non-Caucasians [1,2,4,5]. In addition, in countries with modest to low Gross Domestic Product, costs associated with procurement of grafts from unrelated donor is prohibitive [5-8]. In a 2012 survey of Latin American HSCT practices, 4 countries had a national unrelated donor registry, 14 countries had an established HSCT program, and 7 countries were performing unrelated donor transplants. The Latin American Group of Bone Marrow Transplantation estimates only 10% of expected transplants are being performed in this region now [7].

Until recently, haploidentical donor transplantation required T cell–depletion techniques not widely available in Latin America. The recent success of haploidentical donor bone marrow transplantation with post-transplant cyclophosphamide (PT-CY) for graft-versus-host disease (GVHD) prophylaxis for hematologic malignancy has allowed this strategy to be extended for nonmalignant hematologic diseases [9-14]. Haploidentical graft acquisition costs are modest compared

Financial disclosure: See Acknowledgments on page 316.
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http://dx.doi.org/10.1016/j.bbmt.2016.11.006
1083-8791/

with unrelated donor sources (volunteer adults and banked cord blood) [15-18]. Herein we report the results of haploidentical transplantation for FA performed at a single institution in Brazil between 2008 and 2015.

METHODS

Patients

Between April 2008 and December 2015, 26 children and adolescents without a matched related or unrelated donor received a first haploidentical transplant at the Federal University of Parana, Curitiba, Brazil. Four additional patients were also treated for graft failure after failing a prior related (n = 1) or unrelated donor transplant (n = 3). All patients had failed at least 1 course of androgen therapy with oxymetholone or danazol. One patient (unique patient number [UPN] 1) was previously reported [9].

All donor–recipient pairs were mismatched at ≥2 HLA loci. Donors were not primed with granulocyte colony-stimulating factor before harvest, and unmanipulated bone marrow was the only graft used for transplantation. Informed consent was obtained from the patient or legal guardian before transplantation. The Ethical Committee of the Hospital de Clinicas, Federal University of Parana, Curitiba, Brazil approved the study.

The diagnosis of FA was confirmed by the diepoxybutane test and screened with a panel that included 11 previously described mutations. These mutations were c.2853-19del19, c.1115_1118delTTGG, c.2535_2536delCT, c.987_990delTCAC, and c.3788_3790delTCT in the *FANCA* gene; c.456 + 4 A > T, c. 67delG, c.1393C>T, and c.65G>A in the *FANCC* gene; and c.1077-2A>G and c.1480 + 1G>C in the *FANCG* gene.

Preparatory Regimen and GVHD Prophylaxis

Conditioning regimens varied by transplant period. Four regimens were used, and modifications were based on clinical observation. The regimens were (1) CY 10 mg/kg, fludarabine (FLU) 150 mg/m^2, and total body irradiation (TBI) 200 to 300 cGy (n = 5); (2) FLU 150 mg/m^2 and TBI 200 cGy (n = 9); (3) FLU 150 mg/m^2, TBI 200 cGy, rabbit antithymocyte globulin (r-ATG) 4 to 5 mg/kg (n = 13); and (4) FLU 150 mg/m^2, TBI 100 cGy, and r-ATG 4 to 5 mg/kg (n = 3). Mesna was co-administered with the CY (160% dose of CY in 5 divided doses at 0, 3, 6, 9, and 12 hours after CY infusion). All patients received GVHD prophylaxis that included PT-CY 25 mg/kg/day on days +3 and +4. On day +5, cyclosporine was initiated at a dose of 3 mg/kg/day i.v. adjusted to achieve therapeutic levels and mycophenolate mofetil (MMF) at a dose of 15 mg/kg/dose p.o. or i.v. 3 times daily with a maximum dose of 3 g/day.

Supportive Care

Antimicrobial prophylaxis with levofloxacin was started on day –8 and continued until broad-spectrum antibiotics were initiated and sulfamethoxazole-trimethoprim from day –8 and continued for at least 6 months post-transplant. Acyclovir and fluconazole or micafungin were given beginning on day –1. Cytomegalovirus (CMV) antigenemia was monitored twice a week, whereas adenovirus and Epstein-Barr virus (EBV) were monitored every 2 weeks and treatment instituted when appropriate. Ursodeoxycholic acid was started on day –8 and continued for 30 days post-transplantation. Filgrastim 5 μg/kg/day was started on day +5 and continued until neutrophil recovery. All patients were treated in protective isolation rooms with high-efficiency particle air filters.

HLA Typing and Donor Selection

Patients and potential donors (parents and siblings) were typed for HLA-A, -B, -C, -DRB1, and -DQB1 loci at medium resolution level by reverse sequence specific oligonucleotides using multiplex flow analysis LABType SSO (One Lambda Inc., Canoga Park, CA). An extended family search for a suitable haploidentical donor was performed whenever patients had specific HLA antibodies against parents or siblings. Donor-specific antibodies (DSA) identification and monitoring was performed by bead-based multiplexing technology using LABScreen Single Antigen (One Lambda Inc.). HLA Fusion software (One Lambda Inc.) was used for both HLA typing and antibody identification. The assignment of anti-HLA antibodies took in consideration median fluorescence intensity values (reactions > 1000 were positive) under the light of epitope analysis. The mother was the preferred donor until 2014 and thereafter the father or a male relative when available [15].

Endpoints

Neutrophil recovery was defined as the first of 3 consecutive days with an absolute neutrophil count ≥ $.5 \times 10^9$/L and platelet recovery ≥ 20×10^9/L without platelet transfusion during the previous 7 days. Primary graft failure was defined as ≤5% donor chimerism in peripheral blood by day +30. Secondary graft failure was defined as loss of donor chimerism to ≤5% after achieving neutrophil recovery with >5% donor chimerism. Chimerism assays were PCR-based amplification of short tandem repeat regions on peripheral blood at days 30, 100, 180, and 365 and thereafter annually [19]. Mixed chimerism was defined as >5% and <95% donor and full chimerism as ≥95% donor. Acute GVHD was graded according to establish criteria [20], and chronic GVHD was graded according to the 2005 National Institutes of Health Consensus criteria [21]. The global severity score, mild, moderate, and severe, was calculated using the maximum severity score of chronic GVHD.

Statistical Methods

Descriptive data are presented in Tables 1 to 4. The primary outcome was overall survival, and death from any cause was considered an event. Overall survival was analyzed using the Kaplan-Meier method [22].

RESULTS

The characteristics of the 26 children and adolescents who received a first haploidentical bone marrow transplantation, their transplant conditioning regimens, donor characteristics, and outcomes are presented in Tables 1 and 2. Table 1 describes the population who did not receive r-ATG and Table 2, those who received r-ATG. Table 3 describes recipients of a second transplantation for graft failure.

Among the 26 recipients of a first haploidentical transplant, 22 were transplanted for marrow failure. Other indications include refractory cytopenia with PAPA syndrome (pyogenic arthritis, pyoderma gangrenosum, and acne; n = 1) and acute myeloid leukemia (AML; n = 3). Two patients with AML received pretransplant chemotherapy with 1 cycle of mini-Fludarabine, Aracytin and G-CSF (n = 1) [23] and 1 cycle of low-dose aracytin plus tioguanine (n = 1). None was in remission at transplantation. The patient who did not receive chemotherapy had 70% of myelomonocytic blasts in the bone marrow and leukemia cutis. Only 5 of 25 patients studied had clonal cytogenetic abnormalities, and 3 had refractory cytopenia (n = 1) or AML (n = 2) at transplantation. Genetic subtypes were identified in 11 patients (*FANCA* in 8, *FANCC* in 1, and *FANCG* in 2). All others had a negative screening panel.

The median age at transplantation was 10 years (range, 4 to 16), and 81% were male. The median number of transfusions was 9 (range, 1 to >100). Two patients had no prior RBC transfusion. The median disease duration was 4.2 years (range, 1 to 10). Pretransplant exposure to CMV, EBV, and toxoplasma were common as demonstrated by seropositivity in 92% (n = 24), 81% (n = 21), and 77% (n = 20) of 26 patients, respectively. Haploidentical donors were siblings (n = 5) or parents (n = 21); the mother was the donor in 65% of transplants. Three patients had DSAs (median fluorescence intensity > 9000) and were treated with a single dose of rituximab (375 mg/m^2) and 3 to 6 sessions of plasmapheresis in the weeks preceding transplantation. Two patients responded and 1 did not (median fluorescence intensity > 15,000). The median number of total nucleated cells infused was 7.0×10^8/kg (range, 2.8 to 14.9).

Twenty-five of 26 patients demonstrated initial engraftment, including 3 patients with DSAs. The median time to neutrophil recovery was 15 days (range, 11 to 21) and platelet recovery, 18 days (range, 10 to 129). One patient experienced primary graft failure and 1 experienced secondary graft failure; both were in aplastic phase, had fewer than 10 previous blood transfusions, had no DSAs, and belonged to the r-ATG group (Table 2).

Protocol Evolution

The conditioning regimen for UPNs 1 and 2 comprised CY 10 mg/kg, FLU 150 mg/m^2, and TBI 200 cGy; UPNs 3 to 5 had AML and received the same regimen with the TBI dose increased to 300 cGy. All 5 patients had sustained engraftment,