Hematopoietic Cell Transplantation Using Reduced-Intensity Conditioning Is Successful in Children with Hematologic Cytopenias of Genetic Origin

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Article history:
Received 7 February 2015
Accepted 21 March 2015

Key Words:
Congenital hematologic cytopenias
Reduced-intensity conditioning
Hematopoietic cell transplantation

A B S T R A C T

Genetically derived hematologic cytopenias are a rare heterogeneous group of disorders. Allogeneic hematopoietic cell transplantation (HCT) is curative but offset by organ toxicities from the preparative regimen, graft rejection, graft-versus-host disease (GVHD), or mortality. Because of these possibilities, consideration of HCT can be delayed, especially in the unrelated donor setting. We report a prospective multicenter trial of reduced-intensity conditioning (RIC) with alemtuzumab, fludarabine, and melphalan and HCT in 11 children with marrow failure of genetic origin (excluding Fanconi anemia) using the best available donor source (82% from unrelated donors). The median age at transplantation was 23 months (range, 2 months to 14 years). The median times to neutrophil ($>500 \times 10^6$/L) and platelet ($>50 \times 10^9$/L) engraftment were 13 (range, 12 to 24) and 30 (range, 7 to 55) days, respectively. The day +100 probability of grade II to IV acute GVHD and the 1-year probability of limited and extensive GVHD were 9% and 27%, respectively. The probability of 5-year overall and event-free survival was 82%; 9 patients were alive with normal blood counts at last follow-up and all were successfully off systemic immunosuppression. In patients with genetically derived severe hematologic cytopenias, allogeneic HCT with this RIC regimen was successful in achieving a cure. This experience supports consideration of HCT early in such patients even in the absence of suitable related donors.

INTRODUCTION

Hematologic cytopenias due to bone marrow dysfunction (BMD) of genetic etiology include a heterogeneous group of disorders where genetic mutations result in abnormal or arrested hematopoiesis affecting 1 or more cell lines [1-3]. Conservative management of a lifelong defect in hematopoiesis is challenging and includes chronic transfusion therapy with inherent risks of infection, iron overload, anemia, bleeding, alloimmunization, susceptibility to life-threatening infections, and in many BMD, a predisposition to malignant transformation, usually acute myeloid leukemia [4-8].

Allogeneic hematopoietic cell transplantation (HCT) can cure the hematopoietic defects associated with BMD. The heterogeneity of BMD and associated organ involvement in some disorders present unique obstacles to successful HCT. In addition, the challenges of allogeneic transplantation, including identification of a suitable donor and optimization of preparative therapy to reduce regimen-related toxicity while ensuring successful engraftment, are important to overcome. Low rates of mortality, graft-versus-host disease (GVHD), and untoward late effects from HCT will promote consideration of transplantation earlier for BMD [9]. Safe and effective transplantation methods are a key to promoting long-term survival and maintaining quality of life after HCT at a young age before the increasing risks associated with conservative management of the marrow failure. Many BMD are associated with defective DNA repair or maintenance pathways and possess increased sensitivity to DNA-damaging agents, including chemotherapy and radiation. These sensitivities have resulted in increased HCT-related morbidity and mortality in BMD patients [2,9]. Further, the presence of genetic predisposition to malignant transformation requires development of transplantation approaches that minimize additional risks of late malignancies, which makes reduced-intensity conditioning (RIC) regimen an appealing choice.

Trials of HCT after RIC have recently targeted several groups of recipients for reasons such as age and pre-existing organ toxicity. Although RIC regimens are perhaps better tolerated, barriers to using RIC regimens and proceeding with HCT include risk of GVHD from unrelated donor grafts and potentially increased rates of graft rejection, especially in the immune competent and those exposed to multiple blood products [10].

This study describes the outcomes of a prospective, pediatric, multicenter, phase II HCT trial for children with BMD who had undergone RIC to achieve significant host immunosuppression. The primary objective was to determine donor engraftment, overall survival (OS), and event-free survival (EFS) after conditioning with alemtuzumab, fludarabine, and melphalan and HCT from the best available donor in patients with BMD and severe hematologic cytopenia. Fanconi anemia patients were excluded because of the use of the alkylating agent melphalan in the regimen and the unknown toxicity imparted by this drug on affected patients. Secondary objectives were to evaluate for

Financial disclosure: See Acknowledgments on page 1325.

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1083-8791/© 2015 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2015.03.019

immune reconstitution, infection, and transplantation-related toxicities.

METHODS

Patient and Donor Selection

BMD patients were enrolled at 4 transplantation centers. The protocol was approved by the institutional review board at each of the participating institutions (NCT00920972). Consent was obtained from legal guardians of all patients before enrollment and assents were obtained when appropriate. Eligibility included patients younger than 21 years of age with a diagnosis of a BMD other than Fanconi anemia. Patients with human immunodeficiency virus seropositivity, Lansky performance score ≤ 50, or uncontrolled active bacterial, viral, or fungal infections were ineligible.

Donors were selected based upon the best allele match for HLA-A, HLA-B, HLA-C, and DRB1 by high-resolution typing. In the absence of matched related donor (MRD) marrow, 7 to 8/8 allele/antigen matched unrelated donor (URD) marrow was considered eligible. Umbilical cord blood (UCB) products were required to be matched at a minimum of 4 of 6 HLA loci (low resolution at A and B; high resolution at DRB1) and have a pre-cryopreservation total nucleated cell number of $\geq 4.0 \times 10^7$ per kilogram recipient weight. Marrow products were infused fresh and unmanipulated except as required for ABO incompatibility between donor and recipient.

Conditioning Regimen and GVHD Prophylaxis

All patients received alemtuzumab intravenously (i.v.) daily for 3 days (10 mg, 15 mg, and 20 mg if >10 kg and 10 mg on each day if $\leq$ 10 kg) between day −21 and −19 after a 3 mg test dose per manufacturer recommendations on day −22. The total dose of alemtuzumab was 48 mg in children >10 kg and 33 mg in children $\leq$ 10 kg. Patients were discharged after alemtuzumab administration and readmitted on day −8. Fludarabine (30 mg/m^2 or 1 mg/kg if $\leq$ 10 kg) was administered i.v. daily between days −8 and −4. Melphalan (140 mg/m^2 or 4.7 mg/kg if $\leq$ 10 kg) was administered i.v. on day −3 [11]. GVHD prophylaxis consisted of cyclosporine or tacrolimus and short-course methotrexate (7.5 mg/m^2 on days 1, 3, and 6). Cyclosporine or tacrolimus was started on day −3 with regular monitoring to maintain therapeutic levels, continued to day +100, and tapered gradually to stop by day +180 in the absence of GVHD. In addition, prednisone was administered to all unrelated bone marrow (URD BM) recipients at 1 mg/kg/day from day +7 to +28 and subsequently tapered in the absence of GVHD. UCB recipients received a calcineurin inhibitor and mycophenolate mofetil (MMF) instead. MMF (1 g every 8 hours for children $\geq$ 50 kg or 15 mg/kg every 8 hours for children < 50 kg) was commenced on day -3 and continued through day +45 or for 7 days after engraftment, whichever came later. MMF levels were not monitored.

Supportive Care

All patients received antibiotic prophylaxis with oral ciprofloxacin, itraconazole (until day 100 for BM and day 180 for UCB), acyclovir (until 1 year if herpes simplex virus or varicella zoster virus positive) and trimethoprim-sulfamethoxazole (until 1 year) after engraftment. Patients were monitored weekly for cytomegalovirus (CMV) and Epstein-Barr virus (EBV) DNA until day +100. If CMV was detected, pre-emptive ganciclovir or foscarnet (if before engraftment) therapy was administered until CMV testing was negative × 2. Patients received transfusion of red blood cells if their hemoglobin levels fell below 70 g/L. Platelet transfusions were given for levels below 20×10^9/L. Granulocyte colony–stimulating factor at a dose of 5 μg/kg/day was commenced on day +7 and was continued until the absolute neutrophil count was greater than $.5 \times 10^9$/L for 3 consecutive days.

Endpoints/Statistical Evaluation

Primary endpoints were engraftment, OS, and EFS. *Neutrophil engraftment* was defined as the first of 3 consecutive days with an absolute neutrophil count greater than 500/μL, and *platelet recovery* was defined as the first of 7 consecutive days of a platelet count greater than or equal to 50,000/μL without a transfusion. Donor engraftment was determined by demonstrating chimerism by short tandem repeat analysis in BM and/or peripheral blood. The percentage of donor chimerism was assessed at 1, 3, 6, 9, and 12 months after HCT in the first year, every 6 months in the second year, and yearly thereafter. Formal assessments of immunologic recovery were made on days +100 and +180 and 12 months after HCT. Lymphocyte subpopulations were measured using flow cytometry to calculate absolute lymphocyte count, CD3, CD4, CD8, CD19, and CD16 + 56 cell numbers. Immunoglobulin levels (IgG, IgA, and IgM) were measured at the same intervals.

Statistical analyses were performed in Prism 5.03 (GraphPad, La Jolla, CA). The cutoff date for analysis was April 30, 2014. Continuous variables were summarized as medians and range and categorical variables as percentages. Cumulative incidence of neutrophil and platelet engraftment at 21 days, acute GVHD (aGVHD) at day +100 and chronic GVHD at 1 year were all estimated. OS, EFS, and treatment-related mortality were estimated using Kaplan-Meier estimators and comparisons between groups (MRD and URD) were carried out. *P* values less than .05 were considered significant.

RESULTS

Patient Characteristics

Eleven patients between 2 months and 14 years (median, 23 months) with BMD were enrolled in this study of RIC HCT. Patients, diagnoses, indication for transplantation, transplantation characteristics, and outcomes are summarized in Table 1. Nine were recipients of URD HCT. BM was the source of hematopoietic stem cells in all but 1 patient who received 4 of 6 matched UCB. The median total nucleated and CD34$^+$ cells per kg were 4.1×10^8 (range, $.98 \times 10^8$ to 7.92×10^8) and 4.36×10^6 (range, $.6 \times 10^6$ to 8.82×10^6), respectively.

Table 1
Patient Characteristics and Outcomes

Patient No.	Diagnosis (Indication for Transplantation)	Age at HCT, mo/gender	Follow- up, yr	Donor Source	HLA Match	TNC, per kg ($\times 10^8$)	CD34, per kg ($\times 10^6$)	aGVHD/ cGVHD	Outcome/On or Off IS at Last FU
1	CDA Type 1 (Red cell transfusion dependent)	23/M	10	URD UCB	4/6	.98	.6	0/0	A & W/off
2	DBA (transfusion dependent since 7 weeks of life - unresponsive to steroids)	12/F	8	URD BM	8/8	4.92	2.41	0/0	A & W/off
3	SDS (pancytopenia)	168/F	45 days	URD BM	8/8	3.8	4.2	Gr 4/NE	Died/on
4	Congenital BMD (red cell and platelet transfusion dependent since birth) [31]	2/M	6	MSD BM	8/8	5.8	7.8	0/0	A & W/off
5	CAMT (platelet transfusion dependent)	12/M	5	URD BM	8/8	3.95	4.82	0/0	A & W/off
6	X-linked thrombocytopenia (platelet transfusion dependent from 1 mo. of life)	12/M	7	URD BM	8/8	5.14	8.82	Gr 3/0	A & W/off
7	DBA (transfusion dependent since infancy -unresponsive to steroids)	72/M	6	MSD BM	8/8	3.64	5.2	0/0	A & W/off
8	CAMT (platelet transfusion dependent)	36/M	3.5	URD BM	8/8	3.5	3.31	0/0	A & W/off
9	SCN (severe neutropenia)	156/M	1.5	URD BM	8/8	4.1	3.68	0/0	A & W/off
10	X-linked thrombocytopenia (platelet transfusion dependent)	14/M	1.2	URD BM	8/8	7.92	5.92	0/0	A & W/off
11	DBA (transient response to steroids; recurrence with myelofibrosis at 2.5 years)	34/M	1	URD BM	8/8	7.41	4.36	Gr 4/NE	Dead/NE

TNC indicates total nucleated cells; cGVHD, chronic graft versus host disease; FU, follow-up; IS, immunosuppression; CDA, congenital dyserythropoeitic anemia; M, male; A & W, alive and well; SDS, Shwachman Diamond syndrome; F, female; Gr, grade; NE, not evaluable; CAMT, congenital amegakaryocytic thrombocytopenia.