Biol Blood Marrow Transplant 22 (2016) 499–504

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Haploidentical Peripheral Blood Stem Cell Transplantation with Post-Transplantation Cyclophosphamide in Children with Advanced Acute Leukemia with Fludarabine-, Busulfan-, and Melphalan-Based Conditioning

Sarita Rani Jaiswal [1,2,*], Aditi Chakrabarti [2], Sumita Chatterjee [2,3], Sneh Bhargava [1], Kunal Ray [4,5], Paul O'Donnell [6], Suparno Chakrabarti [1,2]

[1] Department of Blood and Marrow Transplantation, Dharamshila Hospital and Research Centre, New Delhi, India
[2] Manashi Chakrabarti Foundation, Kolkata, India
[3] Apollo Gleneagles Hospital, Kolkata, India
[4] CSIR-Indian Institute of Chemical Biology, Kolkata, India
[5] Academy of Scientific and Innovative Research, CSIR-CRRI, New Delhi, India
[6] Fred Hutchinson Cancer Research Center, Seattle, Washington

Article history:
Received 14 September 2015
Accepted 14 November 2015

Key Words:
Haploidentical
Post-transplantation cyclophosphamide
Leukemia
Peripheral blood stem cells
Pediatric

A B S T R A C T

Post-transplantation cyclophosphamide (PTCY) therapy has made haploidentical transplantation a global reality in adults, but the literature is largely silent on the feasibility of this approach in children. We conducted a prospective study of 20 patients (median age, 12 years; range, 2-20 years) with advanced acute leukemia to evaluate the feasibility of PTCY-based haploidentical peripheral blood stem cell (PBSC) transplantation in children. The conditioning regimen comprised fludarabine, i.v. busulfan, and melphalan (Flu-Bu-Mel). PTCY on days +3 and +4 was followed by mycophenolate mofetil for 14-21 days and cyclosporine for 60 days. Thirteen patients (65%) had refractory or relapsed myelogenous leukemia, and the remainder had high-risk lymphoblastic leukemia. Prompt engraftment was noted at a median of 14 days, with full donor chimerism by day +28. The cumulative incidence of acute and chronic graft-versus-host disease was 35% and 5%, respectively. Nonrelapse mortality at 1 year was 20%. The incidence of disease progression was 25.7%. The actuarial overall survival at 2 years was 64.3% (95% confidence interval, 53.4%-75.2%). Our data suggest that Flu-Bu-Mel–based conditioning followed by PTCY-based haploidentical PBSC transplantation with reduced duration of immunosuppression is feasible in pediatric patients with advanced leukemia.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is often the sole curative option for children suffering from advanced hematologic malignancies refractory to conventional chemotherapy or relapsing thereafter [1]. However, the lack of a matched donor either in the family or in a volunteer unrelated donor registry remains a major hindrance to this option, particularly in patients from a non-Caucasian background [2]. Unrelated umbilical cord blood transplantation is likely the most viable option in such patients, given that stringent HLA matching is not essential [2]. The paucity of robust volunteer unrelated donor registries or public cord blood banks in the majority of developing nations renders allogeneic HSCT nonviable for 70%-80% of such children who otherwise could have benefited from this procedure.

The interest in HSCT from haploidentical family donors was revived by the Perugia group in the late 1990s, incorporating the concepts of megadose CD34-selected graft and natural killer (NK) cell alloreactivity to achieve universal engraftment without graft-versus-host disease (GVHD) and cure in a significant proportion of patients with high-risk leukemia [3]. Subsequently, several centers in Europe initiated T cell–depleted haploidentical transplantation in children with both malignant and nonmalignant disorders.

Financial disclosure: See Acknowledgments on page 504.

* Correspondence and reprint requests: Sarita Rani Jaiswal, Department of Blood and Marrow Transplantation and Hematology, Dharamshila Hospital and Research Centre, Vasundhara Enclave, New Delhi 110096, India.
E-mail address: drsaritaranij@gmail.com (S.R. Jaiswal).

http://dx.doi.org/10.1016/j.bbmt.2015.11.010
1083-8791/

Nonetheless, the procedure remained challenging and was considered a niche area, to be explored by only a few centers globally. A decade later, in a series of preclinical and clinical studies, a Johns Hopkins group pioneered the use of high-dose post-transplantation cyclophosphamide (PTCY) as GVHD prophylaxis in haploidentical HSCT [4,5]. The high incidence of engraftment with low incidence of both GVHD and nonrelapse mortality (NRM) reported by the innovator group made haploidentical family donor transplantation a global reality [6]. Despite a recent spate of reports on the use of PTCY in haploidentical HSCT in adults [7,8], the literature remains largely silent on the feasibility of this approach in children.

In the present study, we prospectively evaluated the feasibility and outcome of PTCY-based haploidentical peripheral blood stem cell (PBSC) transplantation in children with advanced acute leukemia using a chemotherapy-based conditioning regimen.

PATIENTS AND METHODS

Patients aged 2 to 20 years suffering from advanced acute leukemia without a matched family donor were eligible to undergo haploidentical HSCT if they had no major organ dysfunction and a Lansky score of ≥50 or an Eastern Cooperative Oncology Group performance status ≤2. Refractory disease was defined as evidence of >5% marrow blasts after 2 cycles of induction. Between October 2011 and February 2015, 20 such patients underwent haploidentical PBSC transplantation. Written informed consent and institutional ethical approval were obtained in accordance with the Declaration of Helsinki.

Conditioning Regimen and GVHD Prophylaxis

The concept of haploidentical PBSC grafting was adopted from the study by Raj et al [8]; however, the conditioning regimen was intensified, because the original protocol was associated with a high relapse rate and our study group comprised patients with advanced disease. In our pilot study, we replaced low-dose total body irradiation (TBI) with melphalan (Mel) 70 mg/m^2 and achieved engraftment in all 5 patients, with minimal toxicity and no GVHD [9]. The conditioning protocol for the current study comprised fludarabine (Flu) 30 mg/m^2 for 5 days from day −6, along with busulfan (Bu) 0.8 mg/kg every 6 hours for 12 doses and Mel 140 mg/m^2 on day −1. PTCY was administered 64 hours after graft infusion at 50 mg/kg twice at a 24-hour interval, along with Mesna. Another 24 hours after completion of PTCY, i.v. cyclosporine (CSA) 3 mg/kg was administered in 2 divided doses, with the patient later switched to oral form when appropriate. CSA doses were adjusted to maintain a trough level of 100 to 200 ng/mL. Because all patients had advanced disease, mycophenolate mofetil (MMF) 15 mg/kg 3 times daily was started along with CSA, but was discontinued between days +14 and +21 post-transplantation in the absence of GVHD. CSA was tapered from day +60 over 2 to 4 weeks. Filgrastim was not used routinely post-transplantation. Its use was restricted to patients with life-threatening sepsis and those showing no sign of engraftment by day +14.

Stem Cell Source and Harvest

Donors were treated with filgrastim 12 μg/kg/day in divided doses for 4 days before initiation of harvest on the fifth day. On average, 3 times the blood volume was processed, with an average yield of final PBSC products of 200 mL. The target dose of CD34$^+$ cells was 5×10^6/kg, with the minimum required cell dose of 3×10^6/kg.

Supportive Care

All patients were treated in protective isolation rooms provided with high-efficiency particle air filters. Levetiracetam 10 mg/kg twice daily oral or i.v. was started at 48 hours before the first dose of Bu and continued until 15 days post-transplantation. Ursodeoxycholic acid 15 mg/kg/day in 3 divided doses was started on day −7 and continued for 30 days post-transplantation. Antimicrobial prophylaxis was instituted in accordance with departmental guidelines with acyclovir, mold-active azoles or liposomal amphotericin, and fluoroquinolones. Cytomegalovirus (CMV) prophylaxis was guided by preemptive monitoring of viral CMV load by quantitative PCR twice weekly until day +100. Adenovirus and EBV load were monitored in peripheral blood once weekly.

HLA Typing, NK Cell Genotyping, and Ligand Mismatch

Patients and donors were typed for alleles at HLA-A, -B, -C, -DRB1, -DRB3/4/5, and -DQB1 by PCR amplification and for oligonucleotide hybridization by molecular methods using commercial kits from Olerup (Franzengatan, Sweden), which achieved intermediate resolution. Both parents, if available, were typed for HLA haplotypes. Patients and donors were genotyped for 17 NK cell KIR genes and KIR-HLA ligands at class 1 loci by PCR amplification using commercial kits from Olerup (Haeselstigen, Sweden). NK alloreactivity was defined as mismatch of NK-KIR ligands C1/C2 or BW4 determined in GVH direction based on the "missing self" hypothesis as described previously [10].

Donor Selection

Based on the initial experience in adults [11], an NK ligand-mismatched (NKLMM) donor was preferred, when available. Otherwise, the mother or a noninherited maternal antigen–mismatched sibling was chosen over the father or a noninherited paternal antigen–mismatched donor. Tissue cross-matching was done to rule out clinically significant anti-donor HLA antibodies.

Chimerism Analysis

We assessed donor–recipient chimerism through PCR-based amplification of polymorphic short tandem repeat regions FES, ACTBP, THO and VWA, followed by fragment separation by high-resolution capillary electrophoresis (ABI 3100 Genetic Analyzer; Life Technologies, Grand Island, NY) and quantitation using GeneScan Software (Life Technologies). This was carried out on whole marrow samples at days +30, +60, and +90 post-transplantation and every 3 months thereafter for 2 years.

Statistical Analysis

Probabilities of survival were estimated using the Kaplan-Meier product-limit method. The cumulative incidence rates of NRM, acute GVHD (aGVHD), and chronic GVHD (cGVHD) were computed to take the presence of competing risks into account. All analyses were performed using SPSS version 20 (IBM, Armonk, NY).

RESULTS

Patient Characteristics

Twenty patients aged 2 to 20 years (median, 13 years) underwent HSCT from a haploidentical family donor. Patient characteristics are detailed in Table 1. Thirteen patients were diagnosed with acute myelogenous leukemia (AML), including 8 with primary refractory disease and 5 with relapsed AML with 1% to 15% marrow blasts at the time of transplantation. Eight of the 13 patients with AML had adverse cytogenetics in the form of a complex karyotype (n = 5), monosomy 7 (n = 2), or t(6:9) (n = 1). Seven patients had high-risk acute lymphoblastic leukemia (ALL) {[t(9:22), n = 2]; [t(4:11), n = 2]; [absence of complete remission (CR) postinduction, n = 3)]} in morphological remission with high minimal residual disease (MRD). MRD was monitored in all patients before and after transplantation using 6-color flow cytometry. MRD was considered positive at a value >0.0001%. High MRD was defined as MRD >0.01% on 2 occasions after morphological CR.

Donor Characteristics

The median donor age was 37 years. The most common donor was the mother (n = 14), followed by a sibling (brother, n = 5; sister, n = 1). Thirteen donors (65%) were full haplotype-mismatched. Four donors and 3 patients were homozygous for more than 1 HLA allele. A noninherited maternal antigen–mismatched donor was available for 3 patients. One-third of the donors were ABO-mismatched.

Donor NK KIR Characteristics

Thirteen donors were KIR ligand–mismatched with the recipient. Bw4, C1, and C2 epitope mismatches were detected in 4, 5, and 3 donor–recipient pairs, respectively. All 13