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Improved Outcome of Refractory/Relapsed Acute Myeloid Leukemia after Post-Transplantation Cyclophosphamide-Based Haploidentical Transplantation with Myeloablative Conditioning and Early Prophylactic Granulocyte Colony-Stimulating Factor–Mobilized Donor Lymphocyte Infusions

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A B S T R A C T

We carried out post-transplantation cyclophosphamide (PTCy)-based haploidentical peripheral blood stem cell transplantation in 51 patients with refractory/relapsed acute myeloid leukemia not in remission. The first 10 patients received nonmyeloablative conditioning followed by planned granulocyte colony-stimulating factor (G-CSF)–mobilized donor lymphocyte infusions (DLIs) on days 35, 60, and 90. No patient developed graft-versus-host disease (GVHD), but 90% had disease progression between 3 and 6 months. A subsequent 41 patients received myeloablative conditioning (MAC); the first 20 patients did not receive DLIs (MAC group) and the next 21 patients received G-CSF–mobilized DLIs (G-DLI) on days 21, 35, and 60 (MAC-DLI group). The incidence of disease progression and progression-free survival at 18 months were 66% and 25% in the MAC group compared with 21.4% and 61.9% in the MAC-DLI group ($P = .01$). Chronic GVHD but not acute GVHD was increased in the MAC-DLI group (41.2% versus 11%, $P = .05$). Natural killer cell alloreactive donor was associated with lower incidence of disease progression in the MAC but not in MAC-DLI group. The only factor favorably influencing disease progression and progression-free survival was administration of G-DLI after myeloablative conditioning. Our study shows that early administration of G-DLI is feasible after PTCy-based haploidentical hematopoietic stem cell transplantation for refractory/relapsed acute myeloid leukemia and might be associated with improved survival after MAC.

INTRODUCTION

The outcome of allogeneic hematopoietic stem cell transplantation (HSCT) for acute myeloid leukemia (AML) in complete remission (CR) has improved over the years, and recent studies have highlighted the feasibility of haploidentical HSCT in this group of patients [1-3]. However, the long-term outcome of HSCT for AML not in CR (AML-nCR) remains abysmal, irrespective of the donor source [4].

Several studies have suggested that some of these patients might be salvaged with HSCT from matched related or unrelated donors [5-7]. Yet, lack of a matched related or unrelated donor and the time for procurement of the latter often render these patients ineligible for an allograft. Two large registry-based studies have attempted to identify subset of patients who might benefit from an allograft after induction failure with

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matched related or unrelated grafts [7,8]. However, very few studies have reported on the feasibility of haploidentical HSCT for AML-nCR and the effect of early intervention with donor lymphocyte infusions (DLIs) after an allograft. Most centers reserve this intervention for disease progression because of concern regarding graft-versus-host-disease (GVHD), and not many patients achieve a lasting response unless CR is achieved with further treatment before DLIs [9].

We developed a haploidentical HSCT program in 2011 for refractory and relapsed AML, based on the post-transplantation cyclophosphamide (PTCy) approach with peripheral blood stem cell grafts. We report on the outcome of haploidentical HSCT in 51 consecutive patients with AML-nCR and report on the feasibility and efficacy of planned prophylactic DLIs after both nonmyeloablative (NMA) and myeloablative conditioning (MAC).

METHODS

Primary refractory (PRef) AML was defined as persistence of >5% blasts despite at least 2 cycles of standard induction therapy. Relapsed refractory (RRef) AML was defined as failure to achieve < 5% blasts after 1 cycle of high-dose Cytarabine (Ara-C)–based salvage therapy. In a pilot study, from March 2011 to February 2015, 51 patients with PRef or RRef AML between the ages of 2 and 65 years without a matched family donor were offered haploidentical HSCT if they possessed a haploidentical family donor and did not have major organ dysfunction with an Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2. Written informed consent was obtained for all patients, and approval was obtained from Institute Ethics Committee in accordance with the Declaration of Helsinki.

Conditioning Regimen and GVHD Prophylaxis

The initial protocol (fludarabine, Cy, melphalan) consisted of cytoreductive chemotherapy with Campath-1H, followed by NMA conditioning based in principle on the Fred Hutchinson Cancer Research Center (FHCRC) protocol [10] replacing total body irradiation with melphalan 70 mg/m^2. This was piloted in the first 10 patients. These patients received planned DLIs as 1×10^6/kg of CD3$^+$ cells on days 35, 60, and 90, in the absence of GVHD. PTCy was administered 64 hours after infusion of the graft at 50 mg/kg twice at 24-hour intervals along with Mesna as described previously [11]. Another 24 hours after completion of Cy, i.v. cyclosporine (CSA) at 3 mg/kg in 2 divided doses and oral mycophenolate mofetil (MMF) at 15 mg/kg/dose 8 hourly were initiated. CSA doses were adjusted to maintain a trough level of 100 to 200 ng/mL and tapered over 4 weeks after day +90. MMF was tapered over 2 weeks after day +35.

The subsequent patients received MAC, consisting of fludarabine 30 mg/m$^2 \times 5$ days, i.v. busulfan 9.6 mg/kg over 3 days, and a single dose of melphalan 140 mg/m^2 on day −1 as previously published [12]. Unlike the NMA cohort, MMF was tapered between days 14 and 21 after transplant if there was no GVHD, and CSA was tapered from day +60 over 4 weeks. The first 20 patients treated on the MAC protocol did not receive DLIs (MAC group). The protocol was modified for the next 21 patients to receive DLIs as 1×10^6/kg of CD3$^+$ cells on day +21 and 5×10^6/kg on days +35 and +60 (MAC-DLI). DLI was contraindicated if the patient had disease progression, active GVHD, or received treatment for GVHD, even if the GVHD were quiescent without immunosuppression on the planned day of DLI. Filgrastim was not routinely used post-transplant in the NMA group from day + 5 until engraftment, but in the subsequent patients receiving MAC its use was restricted to patients with life-threatening sepsis and those not showing any 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 an average, 3 times the blood volume was processed with an average yield of 200 mL of final peripheral blood stem cell products. The target dose of CD34$^+$ cells was 5 to 10×10^6/kg with the minimum cell dose required being 3×10^6/kg.

Donor Lymphocyte Infusions

The cells were stored at multiple aliquots of CD3$^+$ T cells at 1×10^6/kg recipient body weight from the fraction of mobilized harvest and were cryopreserved at −196°C at vapor phase of liquid nitrogen. DLI was administered as per CD3$^+$ T cells/kg body weight of the patient as per the protocol. These cells were thawed at 40°C and infused immediately with only pheniramine maleate as premedication. All patients were on CSA when they received DLIs. In patients who received all 3 doses of DLIs and did not develop GVHD, CSA was tapered from day + 60 over next 4 weeks.

Supportive Care

All patients were treated in protective isolation rooms provided with high efficiency particle air filters. Antimicrobial prophylaxis was instituted as per the departmental guidelines. Cytomegalovirus (CMV) prophylaxis was guided by preemptive monitoring of viral CMV load by quantitative PCR twice a week until day 100.

HLA Typing, Natural Killer Cell Immunoglobulin-Like Receptor Haplotype Assignment, and B Scores

Patients and donors were typed for alleles at HLA-A, -B, -C,- DRB1, -DRB3/4/5, and -DQB1 by PCR amplification and oligonucleotide hybridization by molecular methods using commercial kits from Olerup (Stockholm, Sweden), which achieved intermediate resolution. Both parents, if available, were typed for HLA haplotypes.

Patients and donors were genotyped for 17 natural killer (NK) cell immunoglobulin-like receptor (KIR) genes and KIR-HLA ligands at HLA-B and -C loci by PCR amplification method using commercial kits from Olerup. B haplotype was defined as presence of at least 1 defining loci, that is, KIR2DL5, 2DS1, 2DS2, 2DS3, 2DS5, *or* 3DS1. Donors possessing the above were designated as haplotype Bx, whereas those lacking the same were assigned as haplotype AA. The KIR genotype was analyzed for the "B content" of KIR genes as proposed by Cooley et al. [13], based on centromeric or telomeric position of the inhibitory and activating genes. Accordingly, B scores of 0 to 4 were assigned depending on the alignment of KIR genes.

NK KIR Ligand Mismatch

NK alloreactivity was defined as mismatch of NK-KIR ligands C1/C2 or BW4 determined in the graft-versus-host direction based on "missing self" hypothesis as described previously [14]. KIR-HLA ligands C1, C2, and Bw4 were identified by PCR amplification method using commercial kits from Olerup.

Donor Selection

Mother or Non-Inherited Maternal Antigens (NIMA) mismatched sibling was the preferred donor. NK KIR ligand mismatch (NKLMM), NK KIR haplotype, and B scores were not taken in consideration during donor selection. Tissue cross-matching was done to rule out clinically significant antidonor HLA antibodies.

Chimerism Analysis

We assessed donor–recipient chimerism by the PCR-based amplification of a 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) on days 30 and 90 post-transplant.

Statistics

The cohort undergoing NMA conditioning were not considered for comparative analysis and were used for descriptive purposes only to demonstrate the basis for using early DLI in the MAC group. Analysis was carried out on an intention-to-treat basis in the MAC and MAC-DLI groups. Binary variables were compared between the 2 groups using the chi-square test, and the continuous variables were analyzed using independent sample *t*-tests, taking into account Levenes test for equality of variances. Probabilities of survival were estimated using the Kaplan-Meier product-limit method. The cumulative incidence rates of nonrelapse mortality (NRM), disease progression, acute GVHD (aGVHD), and chronic GVHD (cGVHD) were computed to take into account the presence of competing risks. An outcome was determined to be significantly different if the observed $P < .05$. Cox regression was carried out for multivariate analysis. All analyses were performed using the statistical software IBM SPSS Statistics Version 20 (Armonk, NY).

RESULTS

Patients Undergoing NMA Conditioning and DLI

Ten patients were treated in this cohort. The patient characteristics are detailed in Table 1. All engrafted at day +14 with donor chimerism > 95%. There were no extramedullary toxicities. All 10 patients achieved morphologic CR and received 3 doses of planned DLIs. There were no acute toxicities, and none developed aGVHD or cGVHD, despite 3 doses of DLIs. Only 4 of 10 patients developed CMV reactivation with no NRM. However, 9 of 10 patients relapsed between 95 and 156 days. The rates of overall survival (OS) and progression-free survival (PFS) were 10% only. This group was used as a reference and not included in further analysis.