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T Cell–Depleted Unrelated Donor Stem Cell Transplantation Provides Favorable Disease-Free Survival for Adults with Hematologic Malignancies

Ann A. Jakubowski,[1] Trudy N. Small,[2] Nancy A. Kernan,[2] Hugo Castro-Malaspina,[1] Nancy Collins,[3] Guenther Koehne,[1] Katharine C. Hsu,[1] Miguel A. Perales,[1] Genovefa Papanicolaou,[4] Marcel R. M. van den Brink,[1] Richard J. O'Reilly,[2] James W. Young,[1] Esperanza B. Papadopoulos[1]

We report a prospective phase II clinical trial in 35 adult patients (median age 40.5 years) with hematologic malignancies who received T cell–depleted, hematopoietic stem cell transplants from HLA-compatible, unrelated donors. The cytoreductive regimen consisted of hyperfractionated total-body irradiation, thiotepa, and fludarabine. The preferred graft source was granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC). PBSC were $CD34^+$ selected, followed by sheep erythrocyte rosetting to deplete residual T cells. Anti-thymocyte globulin provided graft rejection prophylaxis. No additional graft-versus-host disease (GVHD) prophylaxis was planned. Estimated disease-free survival at 4 years is 56.8% for the entire group and 75% in patients with standard-risk disease. The cumulative incidence of relapse is 6%. Acute GVHD grade II-III developed in 9% and chronic GVHD in 29% of patients. Fatal infections occurred in 5 of 35 (14%) patients. There was 1 late graft failure. This study demonstrates durable engraftment with a low overall incidence of GVHD. Its curative potential is reflected in the remarkably low relapse rate at 4 years.

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INTRODUCTION

Several studies have demonstrated the efficacy of T cell–depleted (TCD) allogeneic bone marrow (BM) [1-3] and TCD peripheral blood stem cell transplants (PBSCT) [4,5] from matched related donors (MRD) in patients with hematologic malignancies. In these reports, reduction in the incidence and severity of acute and chronic graft-versus-host disease (aGVHD, cGVHD) compared with T cell–replete transplantation, has not compromised the antitumor effect of the allograft. Furthermore, a 5-year follow-up of patients receiving TCD sibling transplants reported excellent performance status and quality of life [6]. In contrast, TCD transplants from unrelated donors have been less well studied [3,7,8].

Although the addition of anti-thymocyte globulin (ATG) addressed an early unacceptable rate of immune-mediated graft rejection and provided additional GVHD prophylaxis beyond that of TCD alone [9,10], it has resulted in delayed immune recovery [11]. We reported recently that conditioning with hyperfractionated total-body irradiation (HFTBI), thiotepa, and fludarabine in TCD PBSCT using MRD eliminated the need for ATG without increasing graft rejections. Furthermore, immune reconstitution improved with a reduction in opportunistic infections (OI) [5].

To extend curative transplantation options to patients without an MRD and to an older population at increased risk of GVHD, we utilized this regimen for matched or mismatched unrelated donor transplants. Only 2 doses of ATG were administered, and TCD was performed by automated $CD34^+$ stem cell selection followed by rosetting with sheep red blood cells (sRBC). We report the results of a trial in 35 patients, which evaluated the impact of this approach on transplant-related morbidity and mortality in patients with hematologic

From the [1]Adult Allogeneic Bone Marrow Transplant Service, Division of Hematologic-Oncology, Department of Medicine; [2]Department of Pediatrics; [3]Department of Clinical Laboratories; and [4]Infectious Disease Service of the Department of Medicine, Memorial Sloan-Kettering Cancer Center, Weill Medical College of Cornell University, New York, New York.

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Correspondence and reprint requests: Ann A. Jakubowski, PhD, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065 (e-mail: jakubowa@mskcc.org).

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malignancies. Secondary endpoints were to estimate disease-free survival (DFS) and overall survival (OS). We sought also to determine whether patients could achieve consistent engraftment with durable relapse-free survival (RFS) and whether T cell reconstitution could be improved with a low incidence of OIs.

MATERIALS AND METHODS

Patient Characteristics

Thirty-five adult patients with a variety of malignant hematologic diseases and treatment backgrounds were enrolled on this Memorial Sloan-Kettering Cancer Center (MSKCC) Institutional Review and Privacy Board–approved, phase II trial from July 1, 2001, to December 31, 2005, after obtaining informed consent. Analysis was performed as of December 31, 2008, after which there were no censored events. Eligibility included low level of disease or remission, availability of ≥7/10 HLA-matched unrelated donors, Karnofsky performance status (KPS) ≥70, no active infection or extramedullary disease, and satisfactory organ function as previously described [5].

Only those patients with intermediate- or high-risk acute myelogenous leukemia (AML) based on cytogenetics [12], and with acute lymphoblastic leukemia (ALL) high-risk cytogenetics (generally t(9;22) with p190 *bcr-abl* or t(4;11]) underwent transplantation in first complete remission (CR1). Disease status at transplantation determined standard- or poor-risk classification: AML-CR1, -CR2, ALL-CR1, or CML-first chronic phase were standard risk; all others were poor risk [5]. HLA matching for -A, -B, -C, -DRB1, and -DQB1 loci was established using DNA sequence–specific oligonucleotide probes. Donors were identified and recruited via the National Marrow Donor Program (NMDP) registry.

Preparative Regimen and Graft

The cytoreduction consisted of HFTBI followed by thiotepa, ATG, and fludarabine [5]. HFTBI was administered in 11 fractions of 125 cGy over 4 days, to a total dose of 1375 cGy. All patients had protective lung shielding after an initial 800 cGy, and overlying ribs received an additional 600 cGy boost. Male patients with acute leukemia or lymphoma received an additional 400 cGy testicular boost in a single fraction. After completion of HFTBI, thiotepa 5 mg/kg/day was administered over 4 hours on each of 2 consecutive days, with no adjustment for weight. Fludarabine 25 mg/m^2/day was administered over 30 minutes for 5 days, beginning on the first day of thiotepa. Patients received 2 doses of equine (60 mg/kg total) or rabbit (5 mg/kg total) ATG divided over the same 2 days as the thiotepa. The source of rabbit ATG was Sangstat (Fremont, California) until 2003 and thereafter Genzyme (Cambridge, Massachusetts).

Twenty-nine donors underwent G-CSF mobilization of PBSC according to NMDP guidelines. Targeted cell dose was 10^9 MNC/kg (3×10^6 CD34$^+$/kg) of recipient weight. CD34$^+$ cells were positively selected using the ISOLEX 300i Magnetic Cell Selection System (Baxter Healthcare Corp., New Providence, New Jersey), followed by sRBC-rosette depletion of T cells [5]. This achieved an approximately 5 $\log_{10}$ depletion of CD3$^+$ cells [13]. Six donors elected BM harvesting with TCD accomplished by sequential soybean lectin agglutination and sRBC-rosette depletion (SBA-E-) [14]. Fresh grafts were infused through a central venous catheter 24 to 48 hours after completing fludarabine.

GVHD Evaluation and Management

GVHD was diagnosed clinically and confirmed by biopsy whenever possible. Patients, who engrafted and survived >30 days, were evaluable for aGVHD, unless it had already been diagnosed before a terminal event. Scoring was based on Center for International Blood and Marrow Transplant Research (CIBMTR) criteria [15]. Patients surviving >100 days were evaluable for chronic GVHD using the Sullivan scoring criteria [16].

Supportive Care

Patients were managed clinically according to MSKCC standard guidelines [5], including infection prophylaxis for *Pneumocystis carinii*, Herpes viruses, and fungus. Patients who were seropositive for *Toxoplasma gondii* or whose donors were seropositive, also received atovaquone prophylaxis after transplantation. Patients who were cytomegalovirus (CMV) negative received seronegative blood products regardless of the donor's serologic status. If the patient or donor was seropositive, CMV-specific prophylaxis was administered beginning when the absolute neutrophil count (ANC) was self-sustaining >2000 cells/μL and continuing through day +100. This consisted of maintenance dosing of valganciclovir, as peripheral blood counts tolerated, and maintenance foscarnet dosing if they did not. Monitoring of CMV reactivation by CMV pp65 antigenemia assay of peripheral blood was performed regularly when either the patient or donor was CMV seropositive, generally once per week during the first 100 days. Epstein-Barr virus (EBV) was monitored similarly by qualitative polymerase chain reaction (PCR) until 2003 and thereafter by real-time PCR of the BNRF1-p143 locus (Roche Inc., Indianapolis, IN) [17].

Prophylactic antibacterial agents were not used until 2005. At that time, the practice of administering vancomycin prophylaxis against *Streptococcus viridans* at the development of neutropenia or no later than day −2 was initiated. This practice affected 4 patients on this study. Finally patients received granulocyte