Thiotepa Cyclophosphamide Followed by Granulocyte Colony-Stimulating Factor Mobilized Allogeneic Peripheral Blood Cells in Adults With Advanced Leukemia


Thirty-one patients (median age, 44 years) with advanced hematologic malignancies were given thiotepa 15 mg/kg, and cyclophosphamide 120 (n = 14) or 150 (n = 17) mg/kg followed by unfractionated peripheral blood stem cell transplants (PBSCT) from genotypically identical siblings (n = 28) or one antigen mismatched family donor (n = 3). Donors were mobilized with granulocyte colony-stimulating factor 5 to 10 μg/kg/d for 6 days and underwent two to three leukapheresis on days +5, +6, +7. The median cell yield per donor expressed/kg of recipients body weight was as follows: nucleated cells 13 × 10^6/kg; CD34+ cells 6 × 10^6/kg; colony-forming unit-granulocyte macrophage 38 × 10^6/kg, and CD34+ cells 449 × 10^6/kg. The diagnoses were chronic myeloid leukemia (n = 4), acute myeloid (n = 9) or lymphoid leukemia (n = 2), acute myelofibrosis (n = 2), multiple myeloma (n = 1), lymphoma (n = 6), chronic lymphocytic leukemia (n = 1) and myelodysplasia (n = 6). Twenty-eight patients had advanced disease, 29 patients were first grafts, and 2 were second transplants 3 and 9 years after the first. Neutrophil counts of 0.5 × 10^9/L and platelet counts of 30 × 10^9/L were both achieved on day +14 (median). Engraftment could be proven by sex markers or DNA polymorphism in 29 of 31 patients: one had early leukemia relapse and one patient was unengrafted because of early death. Acute graft-versus-host disease (GVHD) was scored as minimal or absent (grade 0 to I) in 14 patients, moderate (grade II) in 12, and severe (grade III to IV) in four. Causes of death were leukemia (n = 4), acute GVHD (n = 4, with associated cytomegalovirus infections in three), sepsis (n = 1), liver failure (n = 1), multiorgan failure (n = 1), and hemorrhage (n = 1). The actuarial transplant mortality is 29%, the actuarial relapse rate 22%. Nineteen patients survive with a median follow up of 288 days (100-690). The actuarial 2-year survival is 57%. Three patients received PBSCT from family donors mismatched for one class II antigen: all engrafted, one developed grade I aGVHD; one died of leukemia on day +155; two are alive disease free 267 to 290 days postgraft. This study suggests that thiotepa cyclophosphamide followed by unfractionated PBSC allograft may be an alternative form of transplant for adults with advanced leukemia, also in the setting of one antigen mismatched donor. The engraftment is rapid with acceptable GVHD and relatively low transplant-related mortality.

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PATIENTS UNDERGOING allogeneic marrow transplantation from an HLA identical sibling experience profound immunodeficiency in the first months post-bone marrow transplant (BMT), often associated with poor hematopoietic function. This leads to a high rate of viral and fungal infections with considerable morbidity and mortality. One way of improving graft function would be to transplant larger numbers of hematopoietic progenitors: it is uncertain whether this will then translate into lower transplant mortality. The data in the literature on this issue is scarce and conflicting. We have recently reported a series of 38 patients with leukemia receiving an HLA identical sibling T-cell replete marrow transplant after standard conditioning with cytoxan and total body irradiation: although efforts were made to optimize the marrow harvest, there was a wide variation of colony-forming unit-granulocyte-macrophage (CFU-GM) content from donor to donor, and the median number of infused CFU-GM was 2.4 × 10^9/kg. In that study, there was a significant survival advantage for patients receiving high CFU-GM numbers. Large numbers of hematopoietic progenitors can be given with granulocyte colony-stimulating factor (G-CSF) or granulocyte macrophage (GM)-CSF mobilized peripheral blood stem cell transplant (PBSCT).

We have thus conducted a pilot study, in adults with advanced malignancies, to look at the feasibility of unfractionated allogeneic PBSCT. The possible risk of inducing severe graft-versus-host disease (GVHD), because of greater numbers of infused T cells, especially in elderly patients, was of concern and we, therefore, elected to use a conditioning regimen not including total body irradiation (TBI) in an attempt to reduce tissue damage. We were also unwilling to use busulfan because of the high rate of liver complications seen with this agent in our unit, as well as in other centers, and we chose thiotepa based on its potent myeloablative effect. The combination of thiotepa and cyclophosphamide has already been described as preparative regimen for autologous marrow transplants. We are now reporting 31 consecutive patients allografted in this program.

MATERIALS AND METHODS

Patients. Eligible for this trial were patients with advanced leukemia, having failed two or three lines of therapy or relapsed after autotransplants and second allotransplants after leukemia relapse. Three acute myeloid leukemia (AML) patients had early disease but cytogenetic abnormalities (t(4;13), 8 +11 +19 and −3). Clinical details are listed in Table 1.

Mobilization procedures. Donors were HLA genotypically identical siblings in 28 and one antigen mismatched (class II) family members in three (a parent in two patients, a sibling in one). In 29 cases, it was a first stem cell donation, in two cases it was a second donation, BM having been obtained 3 and 9 years before. No donor had a central venous catheter placed, with the exception of one obese

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Recipients prophylaxis consisted of Cyclosporin A (CyA) 1 mg/kg/day as a collection was infused on day 0, the second day on days -3, -2 (total dose 120 mg/kg) (n = 31) of eloid leukemia; Interval Dx Tx, interval diagnosis-transplant. Levels were reported as colonies X10^4/kg of recipients' body weight. Cells were assayed for clonogenic precursors. Briefly 10^5 mononuclear cells (MNC) were plated as follows: 1.1 mL consisting of Iscove's modified Dulbecco's medium (IMDM) Clone) in direct immunofluorescence and the positivity determined by flow cytometry using a Coulter Profile scan (Coulter, Hialeah, FL).

In vitro colony assay. Light density cells from leukapheresis were assayed for clonogenic precursors. Briefly 10^4 mononuclear cells (MNC) were plated as follows: 1.1 mL consisting of Iscove’s modified Dulbecco’s medium (IMDM) + 0.9% methylcellulose 30% fetal calf serum (FCS) (HyClone) + 100 ng GM-CSF (Sandoz, Basle, Switzerland). After 14 days of incubation in humidified atmosphere at 37°C in 5% CO2, colonies were classified and counted using an inverted microscope (Zeiss, West Germany). Colonies were counted with an Olympus IM inverted microscope, and the number of CFU-GM was determined.

Conditioning regimen and GVHD prophylaxis. Patients received thiopeta 5 mg/kg every 12 hours on day -7 and 5 mg/kg on day -6 (total dose 15 mg/kg), and cyclophosphamide (CY) 60 mg/kg/d on days -3, -2 (total dose 120 mg/kg) (n = 14) or cyclophosphamide 50 mg/kg/d on days -4, -3, -2 (n = 17). The first PB collection was infused on day 0, the second on day +1. GVHD prophylaxis consisted of Cyclosporin A (CyA) 1 mg/kg/day as a continuous infusion from day -1 to day +20 + methotrexate (MTX)

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<th>No. of patients</th>
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<td>Cells × 10^9/kg</td>
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<td>CD34+ cells × 10^9/kg</td>
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<td>CD8+ cells × 10^5/kg</td>
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* Rank Sum Mann Whitney. † Median (range).

RESULTS

Side effects for the donor. Fifteen donors complained of bone pain, six complained of headache during the administration of G-CSF. Leukapheresis procedures were overall well tolerated. One patient had a profound hypocalcemia that required admission to the emergency department for 1 day. Average WBC counts were 7 × 10^9/L before G-CSF, 52 × 10^9/L (range, 34 to 76) on day +5, and 11 × 10^9/L (range, 3 to 26) on day +10. Average platelet counts on day 0, +5, and +10 were 209 × 10^9/L (121-280), 147 × 10^9/L (34-282), and 143 × 10^9/L (98-183).

PBSC collections. Twenty-six donors underwent leukapheresis twice, three donors three times, and two donors only once: the latter had significant problems during the first leukapheresis, such as difficult venous access and hypocalcemia. The yield of collections for each donor is expressed in billions/kg of recipients' body weight and is listed in Table 1.

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Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; Interval Dx Tx, interval diagnosis-transplant.

Engraftment. Engraftment could be proven by sex markers or DNA polymorphism in 29 patients: one did not clear leukemia, and one patient was unevable because of early death. Most patients had uneventful trilineage engraftment.
THIOTEPA CYCLOPHOSPHAMIDE FOLLOWED BY G-CSF

the median day to reach an absolute count of \(0.5 \times 10^9/L\) neutrophils (PMN) was 14 (range, 12 to 20), for \(30 \times 10^9/L\) platelets, it was 14 days (range, 10 to 76). The median platelet count on day +100 was 110 \(\times 10^9/L\) (range, 15 to 220). Three patients received a second infusion of mobilized PBSC on day +57, +102, and +105 because of persisting thrombocytopenia (15 to 20 \(\times 10^9/L\)): the infusions were given without conditioning regimen and were, respectively, 5.7, 8.1, 4.3 \(\times 10^9/kg\) cells. All three patients had increments of platelet counts together with grade I-II acute GVHD. All three are alive and well with functioning grafts 8 to 12 months post-PBSCT.

Immune reconstitution. Absolute CD3, CD4, and CD8 counts for each patient were scored weekly: the median value for all patients was calculated on day +20, day +50, day +100, and day +150. CD3* cells/mm² were 142, 539, 625, and 634, respectively, CD4* cells/mm² were 98, 235, 237, and 285, respectively, and CD8* cells were 48, 235, 440, and 420, respectively. When compared with concurrent marrow transplant recipients, the recovery of CD3* cells on day +20, +50, +100, and +150 was similar (127, 400, 547, and 772/mm²). There was a very significant difference in absolute counts of CD4* cells on day +20 (45/mm²; \(P = 0.02\)), on day +50 (758/mm²; \(P = 0.0001\)), on day +100 (83/mm²; \(P = 0.0001\)), and on day +150 (159/mm²; \(P = 0.0008\)). The difference in CD8 counts was not so pronounced: day +20 (40/mm²; \(P = 0.03\)), day +50 (208/mm²; \(P = 0.1\)), day +100 (384/mm²; \(P = 0.7\)), and day +150 (572/mm²; \(P = 0.7\)). The CD4/CD8 ratio for PB versus BM recipients was also significantly different: on day +20, it was 2 versus 1.1 (\(P = 0.7\)); on day +50, it was 1 versus 0.36 (\(P = 0.0001\)); on day +100, it was 0.5 versus 0.21 (\(P = 0.0001\)); and on day +150, it was 0.67 versus 0.27 (\(P = 0.004\)) (Fig 1).

Cytomegalovirus (CMV) infections. Sixteen patients developed CMV antigenemia at a median interval of 50 days from transplant (range, 34 to 107). Six patients had 1 to 4 CMVAg* cells and were treated with either ganciclovir (n = 3) or foscarnet (n = 3). Ten patients had five or more positive cells and were given combined therapy with foscarnet and ganciclovir. The actuarial risk of developing CMV antigenemia is 61% at 180 days. Four patients had prolonged diarrhea for over 8 weeks, positive for CMV-DNA. All four patients have died of complications caused by associated acute GVHD.

GVHD. Acute GVHD was scored as minimal or absent (grade 0 to I) in 14 patients, moderate (grade II) in 13, and severe (grade III to IV) in four. The latter four patients all had gut GVHD, in two patients in association with CMV infections. Cystitis was present in two patients, one of which recurred on day +100 and required hospitalization with catheterization. Twenty-eight patients evaluable for chronic GVHD were scored as absent in six, limited in 15, and extensive in seven patients. At last follow up, there were 20 patients evaluable: chronic GVHD was scored as absent in eight patients, limited in eight, and extensive in two patients.

Liver complications. The median bilirubin level within day +10 of transplant was 0.85 mg% (.1 to 3.1), the median of maximum level within day +30, 1.4 mg% (.04 to 1.1), and on day +100, it was 0.5 mg% (.2 to 20). Maximum transaminase (ALT) levels within day +30 were 48 (8 to 1,045) and on day +100 it was 83 (18 to 585). Six patients had a maximum bilirubin level that exceeded 5 mg% (7.5 to 20), and two patients died of liver failure: both of these patients had anti-hepatitis C virus (HCV) positive donors, and one was HBsAg* herself.

Leukemia relapse. One patient died too early and could not be evaluated. Of the remaining 30 patients, five have relapsed between day +29 and day +141, and four have died of leukemia: the actuarial risk of relapse at 2 years is 22% (Fig 2).

Causes of death. Overall there were 12 deaths, 4 caused by leukemia (1 with liver failure), and 8 due to transplant-related causes: acute GVHD was the primary cause of death in four patients, with associated CMV infections in three, sepsis in one patient, liver failure in one, multiorgan failure in one, and hemorrhage in one. The actuarial 1-year transplant related mortality is 29% (Fig 2). It is 46% for 17 patients receiving cyclophosphamide 150 mg/kg in the conditioning regimen and 16% for patients receiving cyclophos-
phamide 120 mg/kg (P = .1). There was no difference in transplant mortality for patients receiving more or less than 449 \times 10^6/kg CD3+ cells (median infused number of CD3+ cells).

Survival. Nineteen patients survive with a median follow-up of 288 days (100 to 690). The actuarial 2-year survival is 57% (Fig 2).

DISCUSSION

The recent introduction of allogeneic PBSCT raises two issues, one on donor compliance and possible risks, the other on cell yield and early/late posttransplant events. For the donor, the major concern is clearly the administration of hematopoietic growth factors. G-CSF has now been used for some years in a large number of patients with little side effects, at least in the short-term: these include bone pain, malaise, headache, and a propensity for thrombosis. However, we currently ignore the possibility of long-term side effects. Despite initial encouraging results, growth factors promoting maturation and proliferation of hematopoietic cells should be used with caution. The Italian Group for Bone Marrow Transplantation (GITMO) has addressed this issue and has recently proposed guidelines for the use of allogeneic PBSC grafts. The in vivo administration of G-CSF to normal donors is thought to be indicated in three different settings: (1) contraindication or refusal of the donor to undergo general anesthesia, (2) second donation after rejection of a marrow graft, and (3) to overcome the HLA barrier with high a number of T-cell depleted cells, as recently shown by the Perugia group. In other circumstances and outside clinical trials, the donor should be informed of the potential short-term and long-term effects of G-CSF, of the potential problems of leukapheresis, and of the risk of general anesthesia. The donor should be given the opportunity to choose one or the other form of donation.

Once the choice of performing an allogeneic PBSCT has been taken, one has to consider several variables: the use of a central venous catheter (CVC), the dose of G-CSF, the number of days of treatment, the maximum allowed level of circulating white blood cells, the days when leukapheresis should be started, and the total number of leukapheresis. In the present report, we have chosen not to place a CVC. With one exception, we have used G-CSF doses between 5 and 10 \mu g/kg. We have performed usually two leukaphereses, starting on day +6. We have also tried to keep the donor's WBC count below 60 \times 10^9/L, without other apparent clinical problems. Other investigators have used larger doses of G-CSF, between 10 and 16 \mu g/kg/d, and have usually performed leukaphereses on day +4 and +5, at which time there appears to be a peak in circulating hematopoietic progenitors.

The conditioning regimen used in this pilot study, thiotepa and cyclophosphamide, was chosen because we wished to avoid TBI and busulfan in an attempt to reduce organ toxicity. The association of thiotepa and cyclophosphamide has already been reported as tolerable in similar doses in autologous transplants. In this study, we were impressed with the lack of major side effects. Despite the fact that it was an allogeneic setting with large numbers of T cells and elderly patients, we had acceptable liver toxicity with a median bilirubin level of 0.85 within day +10 and a median maximum level of 1.4 mg% within day +30. There were two deaths caused by liver failure in patients receiving PBSC from anti-HCV+ donors. Other problems were transient cystitis in two patients and mucositis in 20 patients.

Trilineage engraftment was fast, as already shown in other studies, although three patients were given a second infusion of PBSC because of persisting thrombocytopenia between day +57 and +105. They all had complete recovery of platelet counts in the following 2 to 6 weeks. It is interesting to note that full engraftment could be obtained in a patient with advanced CML, grafted with PBSC from a sibling who had received chemoradiotherapy for Hodgkin's disease the year before. Chimerism studies have shown mixed chimerism in two patients for over 100 days, possibly because of the persistence of leukemic cells. This was the reason for increasing the dose of cyclophosphamide from 120 mg/kg to 150 mg/kg in the last patients. GVHD was observed and was the primary cause of death in four patients, but the majority of patients developed no or mild GVHD. The same was true for chronic GVHD, which was absent or limited in 16 of 19 patients surviving, as scored at last follow-up. This, despite the large number of CD3+ cells infused, sevenfold greater than normal marrow harvest, there is no good explanation for the lack of increased acute GVHD with very large numbers of infused CD3+ cells (up to 1,500 \times 10^6/kg), although a soft conditioning regimen may be protective. Indeed, other investigators have reported an increased risk of acute and especially chronic GVHD in recipients of allo-PSBCT following conventional preparation with radiation or busulfan.

Immune reconstitution is a crucial problem in allogeneic marrow transplantation, and we were hoping to see faster recovery of the immune system because of the large numbers of mature T cells infused. Recovery of CD3+ lymphocytes, in terms of absolute counts, was very similar to concurrent marrow transplant recipients. However, there were important differences in the kinetics of CD4+ and CD8+ cells recovery. The major difference was in the absolute number of CD4+ cells, which were significantly lower in BMT patients at all time intervals (day +20, +50, +100, and +150), whereas CD8 counts were similar. The CD4/CD8 ratio was again significantly different at all time intervals. This may or may not be relevant to the development of acute GVHD. It did not protect against CMV reactivation. The actuarial risk of CMV antigenemia was 61%, which is not different from our concurrent marrow transplant patients. This may be because of a greater viral load at the time of transplant, especially when donors are CMV+, because of the very large number of nucleated cells infused.

Leukemia relapse was seen in five patients and was the cause of death in four. The actuarial risk of relapse is 22% at 2 years, and this is very encouraging in a cohort of patients mostly with advanced or resistant disease, including patients with myelodysplastic syndrome (MDS), relapsed AML, and advanced chronic lymphocytic leukemia (CLL). The latter patient was a 56-year-old man grafted with over 80% B lymphocyte marrow infiltrate, thrombocytopenia and anemia.

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requiring weekly transfusions, and is now 1-year posttransplant with full donor chimerism. In 3 of 4 CML patients, the Philadelphia chromosome had a slow clearance with positive metaphases still present on day +100.

In conclusion, this study suggests that thiotepa cyclophosphamide followed by infusion of G-CSF mobilized is well tolerated also by elderly patients with advanced disease, short-term side effects of PBSC collection in donors were acceptable, and the cell yield was greater than with conventional marrow harvest. Hematopoietic engraftment and CD4+ cell recovery was fast. Whether allogeneic PBSC grafts will prove superior to BM transplants will have to be shown in prospective randomized trials.

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Thiotepa cyclophosphamide followed by granulocyte colony-stimulating factor mobilized allogeneic peripheral blood cells in adults with advanced leukemia

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