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Post-Transplant Cyclophosphamide and Tacrolimus–Mycophenolate Mofetil Combination Prevents Graft-versus-Host Disease in Allogeneic Peripheral Blood Hematopoietic Cell Transplantation from HLA-Matched Donors

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A B S T R A C T

Allogeneic hematopoietic cell transplant (HCT) remains the only curative therapy for many hematologic malignancies but it is limited by high nonrelapse mortality (NRM), primarily from unpredictable control of graft-versus-host disease (GVHD). Recently, post-transplant cyclophosphamide demonstrated improved GVHD control in allogeneic bone marrow HCT. Here we explore cyclophosphamide in allogeneic peripheral blood stem cell transplantation (alloPBSCT). Patients with high-risk hematologic malignancies received alloPBSCT from HLA-matched unrelated/related donors. GVHD prophylaxis included combination post-HCT cyclophosphamide 50 mg/kg (days +3 and +4) and tacrolimus/mofetil mycophenolate (T/MMF) (day +5 forward). The primary objective was the cumulative incidence of acute and chronic GVHD. Between March 2011 and May 2015, 35 consecutive patients received the proposed regimen. MMF was stopped in all patients at day +28; the median discontinuation of tacrolimus was day +113. Acute and chronic GVHD cumulative incidences were 17% and 7%, respectively, with no grade IV GVHD events, only 2 patients requiring chronic GVHD immunosuppression control, and no deaths from GVHD. Two-year NRM, overall survival, event-free survival, and chronic GVHD event-free survival rates were 3%, 77%, 54%, and 49%, respectively. The graft-versus-tumor effect was maintained as 5 of 15 patients (33%) who received HCT with evidence of disease experienced further disease response. A post-transplant cyclophosphamide + T/MMF combination strategy effectively prevented acute and chronic GVHD after alloPBSCT from HLA-matched donors and achieved an unprecedented low NRM without losing efficacy in disease control or impaired development of the graft-versus-tumor effect. This trial is registered at clinicaltrials.gov as NCT02300571.

INTRODUCTION

Allogeneic hematopoietic cell transplant (HCT) remains the only curative therapy for many hematologic malignancies [1-3]. However, broad application of the procedure has been limited by the difficult control of graft-versus-host disease (GVHD), the principal complication and cause of mortality in allogeneic HCT [1,2]. The GVHD prophylaxis used most

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commonly in HCT is a calcineurin inhibitor combined with a short course of methotrexate, which, in an unrelated donor setting, is often supplemented by antithymocyte globulin (ATG). Even so, 30% to 80% of allogeneic HCT patients will develop GVHD [4-8], suggesting that development of strategies to control this potentially fatal complication is key to broadening its clinical applicability.

Cyclophosphamide given post-HCT is a novel and promising approach [9-11] that can be safely administered in high doses, even after allogeneic HCT, without hematopoietic stem cell toxicity. Therefore, it may be possible to exploit it to target early-proliferating alloreactive T cells involved in GVHD onset [10,11]. Post-transplant cyclophosphamide (PTCy) has already been proven safe and active in both haploidentical and unrelated bone marrow allografts [12-16]. On the contrary, PTCy has seldom been administered in the HLA-matched allogeneic peripheral blood stem cell transplant (alloPBSCT) setting, despite the use of this stem cell source in more than 75% of HCTs from unrelated adult donors [17-23]. This study explored the performance of PTCy infusion, measured by transplant morbidity and outcome, when added to tacrolimus/mofetil mycophenolate (T/MMF) as GVHD prophylaxis regimen in alloPBSCT.

METHODS

All patients underwent HCT from PBSCs and were matched for HLA-A, -B, -C, -DRB1, and -DQB1 alleles to either related or unrelated donors. The following were deemed acceptable levels of recipient–donor mismatch: an *allele match* for HLA-A, -B, -C, -DRB1, and -DQB1; a *single allele disparity* for HLA-A, -B, -C, -DRB1 or -DQB1; *two allele disparities* for HLA-A, -B, or -C; a *single allele disparity* for HLA-DRB1; and a *single antigen plus single allele disparity* for HLA-A, -B, or -C. The criteria for clinical eligibility included age ≤ 70 years, first remission at high risk of relapse, or second remission obtained after relapse or refractory hematologic malignancy. The principal exclusion criteria were refractory central nervous system disease, active infection, pregnancy, HIV-positive serology, or serious organ dysfunction (left ventricular ejection fraction < 45% or pulmonary forced vital capacity < 50% of predicted). All patients signed informed consent before study entry.

The study was approved by local Institutional Review Board and Ethics Committee. The trial is registered at clinicatrials.gov (NCT02300571). Our primary objective was to determine the capability of the drug combination to control GVHD both in acute (aGVHD) and chronic (cGVHD) manifestations based on their cumulative incidences, assuming an expected rate of aGVHD around 80% and cGVHD around 35% [8]. Secondary objectives were measures of nonrelapse mortality (NRM), infections, overall survival (OS), event-free survival (EFS), cGVHD EFS, and relapse rate. aGVHD was diagnosed based on standard criteria, whereas for cGVHD we applied both traditional and National Institutes of Health (NIH) criteria (defined as requiring systemic immunosuppressive treatment) [24-26]. Given the heterogeneity of patients, we also assessed disease risk index by the refined criteria, which takes into account disease status, stage and cytogenetics [27].

Conditioning Regimen, Post-Graft Immunosuppression, and Supportive Care

Conditioning regimens are reported in Table 1. Considering that the study objective was GVHD prophylaxis, the regimens adopted were disease-oriented. In 7 regimens cyclophosphamide was administered also before PBSC reinfusion on 2 consecutive days at a dose of 14.5 mg/kg (5 regimens) or 10 mg/kg (2 regimens). Immunosuppression began on days 3 and 4 after transplant with administration of intravenous cyclophosphamide (50 mg/kg/day). On day 5 onward, tacrolimus (.03 mg/kg in 2 daily doses; target trough levels 5 to 10 ng/mL) and MMF (15 mg/kg in 3 daily doses) were given. Both agents were continued until day +28 when MMF was discontinued and day +84 when a tacrolimus taper was started. Granulocyte colony-stimulating factor (5 μg/kg/day) was started on day 5 and continued until the absolute neutrophil count exceeded 1.0×10^9/L for 3 consecutive days.

Patients received prophylaxis for bacterial, fungal, and viral infections as well as for *Pneumocystis jiroveci* [28]. Standard cytomegalic virus (CMV) monitoring by PCR was started on day +10 and continued until day +365. Treatment with ganciclovir or valganciclovir began when the number of CMV-DNA copies rose above 100/mL (unrelated donors) or 500/mL (related donors) for 2 consecutive measurements or after a viral load change of >.5 log IU/

Table 1
Patient and Donor Characteristics (N = 35)

Characteristics	Value
Age at transplant , yr	
Median	49
Range	23-69
Sex	
Male	24 (69%)
Female	11 (31%)
Disease	
AML	16 (46%)
De novo AML	13 (37%)
Relapsed AML	3 (9%)
ALL	5 (14%)
De novo ALL	3 (8%)
Relapsed ALL	2 (6%)
Multiple myeloma	8 (23%)
Non-Hodgkin lymphoma	3 (8%)
MDS	2 (6%)
Hodgkin lymphoma	1 (3%)
Disease status at BMT	
1† CR	15 (43%)
>1† CR	5 (14%)
Active disease	15 (43%)
CIBMTR risk group	
High	20 (57%)
Intermediate	13 (37%)
Low	2 (6%)
Source of stem cell	
PBSC	35 (100%)
Sex mismatch	
No	22 (63%)
Yes	13 (37%)
Female into male	9 (26%)
Donor age , yr	
Median	33
Range	(20-68)
Source of graft	
Sibling	10 (29%)
Unrelated	25 (71%)
HLA match	
10/10	20 (57%)
9/10	8 (23%)
8‡/10	7 (20%)
CMV serology	
CMV D–R–	1 (3%)
CMV D+R–	0 (0%)
CMV D–R+	13 (37%)
CMV D+R+	21 (60%)
Conditioning regimen*	
Busulfan + cyclophosphamide	13 (37%)
Treosulfan + cyclophosphamide	5 (14%)
Melphalan + cyclophosphamide	4 (11.5%)
Treosulfan + cyclophosphamide + TBI 2 Gy†	4 (11.5%)
Melphalan + cyclophosphamide + TBI 2 Gy†	3 (8.5%)
Fludarabine + thiotepa + cyclophosphamide	3 (8.5%)
Thiotepa + treosulfan	2 (6%)
Treosulfan + fludarabine + cyclophosphamide	1 (3%)
Infused cell dose‡	
Median $CD34^+$ cell $\times 10^6$/kg	7.4 (range, 2-15)
Median $CD3^+$ cell $\times 10^8$/kg	3.01 (range, 1.240-9.788)
Median total nucleated cells $\times 10^8$/kg	12.1 (range, 6.9-16.9)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CR, complete response; CIBMTR, Center for International Blood and Marrow Transplant Research; TBI, total body irradiation; CMV, cytomegalovirus; D, donor; R, recipient.

* Cyclophosphamide was given also before PBSCT at 14.5 mg/kg on 2 consecutive days.

† Cyclophosphamide was also given before PBSCT at 10 mg/kg on 2 consecutive days.

‡ $CD34^+$ cell doses were available for all patients but $CD3^+$ doses only for 71% of patients.

mL in peripheral blood plasma. Epstein-Barr virus was monitored by PCR via biweekly plasma samples [29].

Surveillance weekly blood cultures were drawn until patient discharge; in cases of fever (>38.5°C), blood and urine cultures were collected and wide-spectrum antibiotic coverage (ie, piperacillin/tazobactam 4.5 g i.v. every 8 hours, vancomycin 500 mg i.v. every 6 hours) was undertaken until pathogen identification or clinical control was achieved. Diagnostic and invasive procedures were performed when clinically indicated. All specimens submitted for bacterial and fungal cultures were performed according to standard methods. Blood and platelet transfusions followed institutional protocols [28].

Monitoring after Transplant

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count > $.5 \times 10^9$/L after transplant, whereas platelet engraftment was defined as a platelet count of 20×10^9/L with no transfusion during the preceding 7 days. The degree of donor chimerism was assessed on days +30, +56, +90, +180, and +360 post-transplant on circulating myeloid and $CD3^+$ lymphocytes. Chimerism was determined using PCR on a panel of informative variable number tandem repeat regions, with full chimerism defined as more than 95% donor $CD3^+$ cells. aGVHD and cGVHD were graded as described elsewhere [24-26].

Statistical Analysis

OS, EFS, and cGVHD-EFS were estimated using the Kaplan-Meier method with their respective 95% confidence intervals (CIs) [30-32]. Patient death from any cause constituted an OS event, whereas relapse or death from any cause was characterized as an EFS event. Most broadly defined were cGVHD-EFS events, which included any form of cGVHD (defined per NIH criteria) [33], relapse, or death. OS, EFS, and cGVHD-EFS values were calculated from transplant date to date of event occurrence or upon censor at final follow-up for patients without an observed event. Discontinued immunosuppression time was determined from the date patients ended their taper from immunosuppressive drugs without subsequent resumption. NRM encompassed all deaths that occurred without evidence of relapse.

Standard methods were used to estimate rates of aGVHD and c GVHD, relapse or progression, and NRM. Death was treated as a competing risk for all other endpoints. Relapse was treated as a competing risk for NRM. The study was conceived as observational aiming to understand the timing and role of cyclophosphamide as a tool to prevent GVHD, assuming an expected rate of aGVHD around 80% and of cGVHD around 35% [8]. Categorical variables were expressed as proportions, and continuous variables were expressed as medians within their respective ranges. All statistics were computed using IBM SPSS Statistics (v.20; SPSS Inc., Chicago, IL) and GraphPad Prism (v.5; GraphPad Software, Inc., La Jolla, CA).

RESULTS

Engraftment and Immune Reconstitution

Between March 2011 and April 2015 we enrolled 35 consecutive patients (characteristic summary in Table 1) with high-risk hematologic malignancies treated at our center. All the 10 related donors and 10 (40%) of the unrelated ones were 10/10 matched. Among the other 15 unrelated donors, 8 (32%) and 7 (28%) were 9/10 and 8/10 matched, respectively. Sustained engraftment was documented in 34 of 35 patients (97%) with median times to neutrophil and platelet recovery of 15 (range, 12 to 32) and 18 days (range, 16 to 32), respectively. Only 1 patient (3%) who developed multiresistant *Pseudomonas aeruginosa* septicemia experienced primary graft failure.

Donor chimerism was >97% from day +28 and sustained in all nonrelapsing patients. Absolute lymphocyte counts measured 400/μL (range, 40 to 1980) on day +28 after HCT, 1020/μL (range, 50 to 4900) on day +56 after HCT, and 1300/μL (range, 400 to 5200) on day +84 after HCT (Table 2), with $CD3^+$ cells being 310/μL (range, 26 to 1670), 680/μL (range, 28 to 3200), and 890/μL (range, 70 to 4000), respectively.

After transplant the median time of discharge was 22 days (range, 11-36). Three patients (9%) required readmission at days +29, +38, and +46, respectively, because of fever (n = 2, 6%) or pneumonia (n = 1, 3%). In all cases complications were controlled and transfer to the outpatient clinic afterwards.

Infections and Toxicity

Six of 35 patients (17%) experienced septicemia during the engraftment phase (days 0 to 26). *Staphylococcus* spp. was isolated in 3 of 35 patients (9%) and gram-negative bacilli (*Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, and *Klebsiella pneumoniae*) in 3 of 35 patients (9%). Treatment with the appropriate antibiotic therapy resulted in complete control of all but 1 infection. Two of 35 patients (6%) colonized with *K. pneumoniae* carbapenemase–producing bacteria before HCT suffered transient aplasia after transplant. They experienced fevers of unknown origin that were successfully treated

Table 2
Post-Transplant Data

Data	Value
Median engraftment time	
Neutrophil engraftment > $.5 \times 10^9$/L	15 days (range, 12-26)
Platelet engraftment > 20×10^9/L	18 days (range, 16-60)
Peripheral blood lymphocyte count*	
Median day +28, U/μL	400 (range, 40-1.980)
Median day +56, U/μL	1.020 (range, 50-4.900)
Median day +84, U/μL	1.300 (range, 400-5.200)
Median day +180, U/μL	1.900 (range, 580-4.200)
Chimerism†	
Day +28	>97% of patients alive and not relapsed
Day +56	>97% of patients alive and not relapsed
Day +84	>97% of patients alive and not relapsed
CMV reactivation	
Incidence	21/35 (60%)
Median day of reactivation	38 (range, 22-54)
Bloodstream infection during engraftment (days 0-26)	
Incidence	6/35 (17%)
Toxicity (grades 3-4)‡	
Liver enzymes elevation	5/35 (14%)
Hyperbilirubinemia	1/35 (3%)
Mucositis	7/35 (20%)
Hemorrhage§	3/35 (9%)
Sinusoidal obstruction disease	1/35 (3%)

* Peripheral blood lymphocyte count was available on days +28, +56, and +84 for all patients.
† Chimerism on peripheral blood was available for all patients alive without disease relapse.
‡ Toxicities were graded according to standard National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.
§ Hemorrhagic cystitis.

with antibiotics (meropenem 2 g every 8 hours, gentamycin 80 mg every 8 hours, and tigecyline 50 mg every 12 hours), which allowed both patients to be discharged after engraftment (days +18 and +23, respectively). No patient developed a pulmonary fungal infection during transplant or follow-up.

CMV reactivation occurred in 21 of 35 patients (60%) at a median +38 days (range, 22 to 54). No case of primary CMV infection was reported, and only 1 patient (3%) had late CMV reactivation (day +232). In all CMV cases preemptive therapy was successful. No Epstein-Barr virus–related disease was observed. Hemorrhagic cystitis with BK viriuria was witnessed in 3 of 35 patients (9%) on day +24, +41, and +46, respectively. However, complete resolution of the infection was achieved in all patients within 4 to 6 weeks.

Three of 35 patients (9%) were hepatitis B virus–positive before HCT. Of these, 2 (67%) suffered viremia reactivation after transplant. The first patient, who was on entecavir treatment for a mutated hepatitis B virus form (YMDD) at the time of transplant, experienced a viremia flare-up on day +84. Association therapy (entecavir/tenofovir) was initiated and prompt control achieved. The second patient, for whom lamivudine had been discontinued 12 months post-HCT, was diagnosed on day +540 with hepatitis B virus–mutated hepatitis (codon M250LM) that was successfully treated with tenofovir.

Grades 3 and 4 toxicities that occurred during the first 100 days after transplant are listed in Table 2. Grade 3 mucositis (20%, 7/35) and liver enzyme elevation (14%, 5/35) emerged most often. Mild sinusoidal occlusion syndrome occurred in 1 patient (3%) [34].

Immunosuppression, GVHD, and Graft-versus-Tumor Effect

After discontinuation of MMF (day +28 for all patients) and tacrolimus (median, +113 days; range, 49 to 276), only 2 of 21 patients (9%) alive without disease progression required immunosuppression restart. The overall cumulative incidence across all aGVHD grades was 17% (95% CI, 2% to 45%), of which 12% were grades II to III (95% CI, 1% to 48%), and none was grade IV. The median aGVHD onset was +75 days (range, 22 to 98) (Figure 1A). No cases of late-onset aGVHD were reported. Three of 35 patients (9%) who required steroid therapy responded well, such that it was discontinued after a median 75 days (range, 36 to 200). At 2 years the cumulative incidence of NIH-defined cGHVD requiring systemic immunosuppression was 7% (95% CI, 1% to 51%) (Figure 1B). We also analyzed the cumulative incidence of overall (limited + extensive) cGVHD defined by traditional criteria. At 2 years the incidence was 11% equally due to limited (1 patient) and extensive cGVHD (2 patients). No patient died from GVHD. Because of the low event rate an analysis to assess the possible role of donor source on GVHD incidence was not conducted. After we enrolled the first 35 patients, the observed activity (aGVHD, 17%; cGVHD, 7%) was much greater than expected both in magnitude and overall duration ($P < .0001$ and $P = .0033$, respectively).

Among the 21 of 35 patients (60%) alive without disease progression, 5 (24%) who were transplanted with evidence of disease (1 acute myeloid leukemia, 1 acute lymphoblastic leukemia, 1 myelodysplastic syndrome, 1 multiple myeloma, and 1 refractory follicular non-Hodgkin lymphoma) achieved and maintained complete response after alloPBSCT.

Outcomes

The median follow-up period for the entire population was 20 months (range for patients without an event, 9 to 67 months). The only patient who died of NRM accounted for the 2-year NRM cumulative incidence of 3% (95% CI, 0% to 61%) (Figure 1C). Estimated 1-year OS and EFS for all patients were 86% (95% CI, 69% to 94%) and 60% (95% CI, 42% to 74%), respectively; at 2 years they were 77% (95% CI, 59% to 88%) and 54% (95% CI, 37% to 69%), respectively (Figure 2A,B). The 2-year cumulative incidence of relapse was 46% (95% CI, 28% to 62%) across all patients and 25% (95% CI, 3% to 56%) for patients undergoing HCT in complete response (Figure 2C). For the 20 of 35 patients (57%) transplanted in complete response, only 4 (20%) relapsed: 3

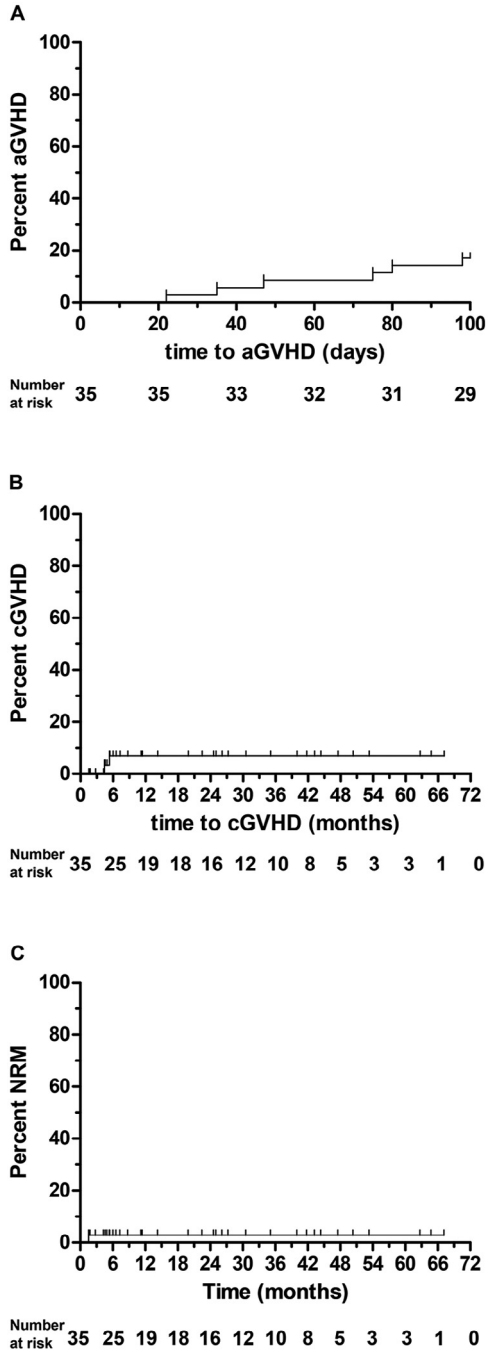

Figure 1. Transplant-related complications. (A) Cumulative incidence of aGVHD. (B) Cumulative incidence of cGVHD. (C) Cumulative incidence of NRM.

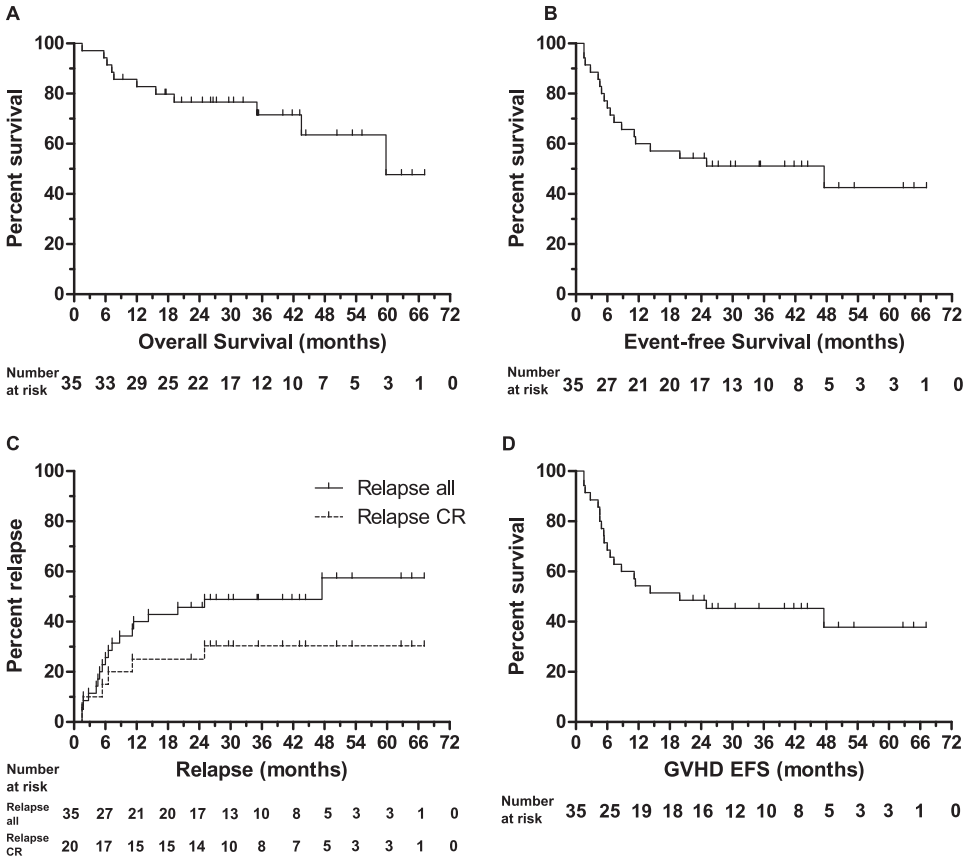

Figure 2. Kaplan-Meier survival curves. (A) OS. (B) EFS. (C) Relapse incidence in all patients (*solid line*) and in patients who underwent BMT with complete remission (CR) of the underlying disease (*dashed line*). (D) GVHD-EFS.

with acute myeloid leukemia (1 with FLT3-positive disease on day +67, 1 with NPM1-positive form on day +162, and 1 with central nervous system extramedullary relapse on day +706). At relapse all patients were treated with mitoxantrone, etoposide, and cytarabine regimen followed in the first patient by sorafenib and a second allogeneic HCT (intra–bone cord blood transplant), in the second patient by fludarabine, cytarabine, liposome-encapsulated-doxorubicin and granulocyte colony-stimulating factor and a second allogeneic HCT (intra–bone cord blood transplant), and in the third patient by FLAG-Myocet (2 cycles) followed by local conformational radiotherapy. Only 1 patient is still alive; the other 2 died from further relapse at days +245 and +1067, respectively. The fourth patient transplanted for myelodysplastic syndrome relapsed on day +128 and was treated with 5-azacitidine. Overall, 1- and 2-year rates of cGVHD-EFS were 54% (95% CI, 37% to 69%) and 49% (95% CI, 31% to 64%), respectively (Figure 2D). All employed patients returned to work after a median of 9 months (range, 168 to 462 days).

DISCUSSION

This study described the impact of a modified strategy to prevent GVHD by using cyclophosphamide in the early post-transplant days. We observed sharp reductions in aGVHD and cGVHD to 17% and 7%, respectively. Consistently, NRM was reduced to a mere 3%.

When allogeneic HCT was introduced into clinical practice during the early 1990s, it appeared to be a very effective therapy for many hematologic malignancies that were otherwise incurable [3]. However, the procedure was characterized by extremely high toxicity that resulted in a 30% to 40% mortality risk [35,36]. Over the years, deeper knowledge of the HLA system and transplant immunology, better selection and matching of donors and patients, and the advent of new immunosuppressive and antimicrobial drugs have led to a mortality risk reduction that is now approximately 15% to 20% [1]. Nonetheless, this rate still represents a burden that limits extensive application of HCT. Only by controlling GVHD will it be possible to reduce such toxicity to below 5%.

As a result of the introduction of PTCy in allogeneic HLA-matched bone marrow transplant as well as in the haploidentical setting, considerable progress in the prevention of GVHD has been made [9,12-16]. Only recently, initial data on the impact of this regimen after alloPBSCT in HLA-matched donors have been reported [17-19,37,38]. The first clinical experience described the role of PTCy as sole GVHD prophylaxis in 11 patients; the grades II to IV aGVHD incidence of 45% and the NRM in up 36% of cases discouraged further evaluation of this approach [18]. Subsequently, the Seattle group published on 42 patients treated with PTCy to which cyclosporine was added as GVHD prophylaxis. This prophylaxis did not consider HLA-mismatched unrelated donors—43% of our patients—and translated to a 70% incidence of grade II aGVHD but without any grades III to IV; the approach was revealed to be very active in protection toward cGVHD and NRM, with NIH-defined cGVHD and NRM rates of 16% and 14%, respectively [17]. Along with these studies,

Moiseev et al [37] reported on 86 patients affected by acute leukemias treated with PTCy and T/MMF. The main focus of the study was a retrospective comparison with an historical control group of patients treated with ATG, calcineurin inhibitors, and methotrexate. The incidence of grades II to IV aGVHD was 19% and of cGVHD 16%, and the NRM was as low as 16%. This study, however, did not described in detail the post-transplant clinical course in terms of immunosuppression taper, discontinuation, and number of patients requiring to restart it.

In addition to these 3 studies aiming to better define the ideal combination of PTCy and standard GVHD preventive regimens after alloPBSCT, 2 more studies were reported where PTCy was followed by an experimental GVHD prophylaxys with sirolimus. In the first study, among the 26 patients treated aGVHD grades II to IV ranged around 45% and cGVHD was quoted at 31% with 37% and 11% of patients still on immunosuppression at 1 and 2 years, respectively, whereas NRM at 2 years was 13% [19]. In the second study, where in unrelated patients MMF was added to cyclophosphamide and sirolimus, aGVHD grades II to IV was in the order of 30% to 35% and cGVHD 16% at 1 year. NRM was 14% at 1 year, but the median follow-up reported was 225 days, and this does not allow the drawing of any further conclusion [38].

Given these premises, the results reported in our study add more information to the general picture. First, PTCy after PBSCT resulted in rapid engraftment as observed with conventional GVHD prophylaxis [20,21,39]. Moreover, hematologic recovery made the procedure very tolerable, shortened the inpatient stay, and reduced the rate of hospital readmission. Second, the addition of T/MMF to PTCy was not detrimental to a rapid and sustained lymphocyte recovery that helped to contain severe infection incidence rates to that seen in similar previous PTCy studies, as opposed to historical allogeneic alloPBSCT data using standard GVHD regimens [9,12,15,18,40].

A third important finding led to another set of conclusions. PTCy after PBSCT resulted in high activity in aGVHD and cGVHD prevention. In fact, PTCy synergy with T/MMF determined a lower incidence of aGVHD compared with both conventional alloPBSCT (grades II to IV, 45% to 80%) [1,2,8,41] and reduced-intensity regimens (42% to 64%) [42] as well as to PTCy after allogeneic bone marrow transplantation (alloBMT; 43% to 51%) [9,12,13]. Furthermore, despite PBSC use, this strategy maintained a robust protection against cGVHD that, with conventional prophylaxis, may be as high as 48% [20]. This high control rate allowed lower steroid use compared with conventional calcineurin inhibitor–based strategies and earlier discontinuation of immunosuppressive therapy, staving off the need for its later reintroduction in most patients [7,43,44]. These data distinguish our study regimen from the previous ones of PTCy in either alloBMT or alloPBSCT [9,12,13,17-19]. In the BMT setting, at least 43% to 51% of patients were required to restart some form of immunosuppression after transplant; in the PBSCT setting, the Seattle group described that, regardless of high-grade matching requested in unrelated transplants, at 1 year 30% of the 33 patients still alive were still on immunosuppression and that, among those diagnosed with cGVHD, 6 of 7 (86%) were still on immunosuppression at the time of the report [9,17]. Our finding, if confirmed in a larger patient cohort, is extremely appealing because a post-allogeneic HCT state that requires no further immunosuppression may be a platform to develop future post-transplant cellular therapies that safely and specifically act on minimal residual disease.

Two outcome indicators demonstrated the direct consequence of GVHD control. One is the NRM of 3% (after conventional alloPBSCT and alloBMT with PTCy, NRM ranged between 21% to 30% and 15%, respectively) [9,12,20]. Notably, a 3% treatment-related mortality compares closely with what observed in the autologous setting [45]. The second indicator is the cGVHD-EFS that nearly overlapped EFS, thus confirming the long-term tolerability of this regimen.

Finally, “double” post-transplant immunosuppression might raise concerns about relapse incidence and the capability of generating an effective graft-versus-tumor (GVT) effect. In regard to the first point, although in our series relapse in patients transplanted not in complete response also remains a relevant issue, we reported an EFS and OS comparable with those described after conventional alloPBSC or alloBMT with PTCy, suggesting no impact of our strategy on the post-transplant outcomes [9,17,19,20]. The fast immunosuppression taper and the reduced need afterward of a new immunosuppressive treatment that we described, however, may lay the ground for future studies aiming to explore, in patients transplanted not in complete response, an early introduction of post-transplant cell therapies. In regard to the second point, GVT, although we did not give formal immune biologic evidence, we reported that 5 of 15 patients (33%) in partial response achieved and maintained complete response after transplant as a consequence of the allogeneic-based therapy. These data might support the intriguing concept that GVT is not sustained by early-proliferating donor T cells (targeted by early-phase immunosuppressive drugs) but rather by a different T cell population that needs time to develop and expand [46].

We acknowledge our data mandate further confirmation because are limited by the observational nature, the relatively small sample size (that is, however, very similar to other PTCy studies), heterogeneity of hematologic malignancies treated, and, consequently, conditioning regimens used [17,19]. Notwithstanding, these weaknesses are partly mitigated by the fact that the primary objective of the trial was GVHD control, and a valid GVHD prophylaxis should be widely reproducible in most transplant centers and should adapt to any disease and to specific conditioning regimens. For these reasons, despite the above-mentioned limitations, our results set the basis for the design of future clinical trials. This statement becomes more relevant in light of recent results achieved with ATG in related alloPBSCT [47]. In this setting, a large phase III trial showed that ATG inclusion produced both a clear reduction of cGVHD incidence and an improvement in cGVHD-EFS. However, at 2 years 25% of patients were suffering from cGVHD. Acknowledging the nonrandomized nature of our results, we did not observe any cGVHD late relapse both in related and unrelated donors, suggesting these 2 strategies should be compared in the near future; as also suggested by another recent large retrospective study [37].

In conclusion, the present study provides evidence that PTCy in association with T/MMF after alloPBSCT can substantially decrease both aGVHD and cGVHD, reducing NRM to less than 5%. If these results are confirmed in a larger clinical trial, then the application of allogeneic HCT might be broadened, and this strategy could be transformed into a safe immunologic platform for development of future cellular therapies aimed at generating a more effective and long-lasting GVT effect [48-50].

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Authorship statement: F.C.-S. conceived of the idea and planned the clinical trial project, wrote the protocol, cared for patients, analyzed clinical data, and wrote the manuscript. D.C. contributed to study design, coordinated the trial and day-to-day patient clinical management, and participated in data analysis and manuscript writing. S.G. assisted with day-to-day clinical management and participated in data analysis and manuscript writing. V.C., L.D.A., M.F., P.B., G.G., and D.R.-S. each contributed to patient accrual, patient care, and results analysis. E.V., F.N., and M.B. all took part in donor selection, donor registry management, and results analysis. L.G. worked with the Ethics Committee and Institutional Review Board submission, study approval, and results analysis. A.P., M.G., and A.S. were each involved in donor selection, PBSC collection and processing, and results analysis. L.G. and D.S. obtained peripheral blood lymphocytes, contributed to immune reconstitution study, and results analysis. F.F. and M.A. contributed equally to this study: they had the idea for the study, wrote the protocol, obtained funding, analyzed results, and revised the paper. All authors had access to the data, vouch for the completeness and accuracy of the data and analyses, and approved the final version of the manuscript. The corresponding author (F.C.-S.) had final responsibility for the decision to submit for publication.

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