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A Modified Post-Transplant Cyclophosphamide Regimen, for Unmanipulated Haploidentical Marrow Transplantation, in Acute Myeloid Leukemia: A Multicenter Study

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A B S T R A C T

We report a modified post-transplant cyclophosphamide (PT-CY) regimen, for unmanipulated haploidentical marrow transplants, in 150 patients with acute myeloid leukemia (AML). All patients received a myeloablative regimen, cyclosporine A (CsA) on day 0, mycophenolate on day +1, and PT-CY 50 mg/kg on days +3 and +5. The median age was 51 (range, 17–74) years, 51 (34%) patients had active disease at transplant, and the median follow-up of surviving patients 903 (range, 150-1955) days. The cumulative incidence (CI) of engraftment, acute graft-versus-host disease (GVHD) grade II to IV, and moderate/severe chronic GVHD was 92%, 17%, and 15%, respectively. The 4-year CI of transplant-related mortality (TRM) and relapse was 20% and 24%, respectively. Four-year survival for remission patients was 72% (74% versus 67% for <60 or ≥60 years of age) and 26% for advanced patients (17% versus 41% for <60 or ≥60 years of age). In a multivariate analysis, active disease at transplant was the only negative predictor of survival, TRM and relapse. The original PT-CY regimen can be modified with CsA on day 0, still providing protection against GVHD, low toxicity, and encouraging low relapse incidence in AML patients, also over 60 years of age.

INTRODUCTION

The introduction of high-dose post-transplantation cyclophosphamide (PT-CY) for unmanipulated HLA haploidentical transplants [1] has been a major breakthrough of the past decade, perhaps the most significant one, and is largely responsible for the worldwide increase in unmanipulated haploidentical transplants [2]. It is based on pre-clinical studies [3] showing that donor alloreactive T cells will proliferate in the first 72 hours after transplantation, and will thus be killed when PT-CY is administered on days +3 and +4. For this reason the original Baltimore protocol calls for tacrolimus and mycophenolate to start on day +5, following the administration of PT-CY [4]: regulatory T cells and hematopoietic stem cells express aldehyde dehydrogenase that makes them resistant to PT-CY, and would be spared the effect of PT-CY [4-6]. One issue with the original Baltimore PT-CY protocol, based on a nonmyeloablative conditioning regimen, has been the risk of relapse in patients with acute leukemia [1], although further studies have shown a strong correlation of relapse with disease risk [7].

We reasoned that patients with AML required a fully myeloablative conditioning regimen, and were concerned of adding, on top of this, high-dose CY on 2 consecutive days. We therefore administered PT-CY on days +3 and +5 and started cyclosporine A (CsA) on day 0 and mycophenolate on day +1 [8]. We also reasoned that some alloreactive T cells

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exposed to CsA would not proliferate and would be spared the purging effect of PT-CY, possibly enhancing the graft-versus-leukemia effect in our initial advanced patients. The same reasoning leads to an increased risk of graft-versus-host disease (GVHD), which was however disproved by the observation of very little acute and chronic GVHD, very little toxicity and transplant-related mortality (TRM), and encouraging survival in our initial reports [8,9]. These results were achieved in a single center (Genova) [8], and the question was whether they would be reproduced also in a multicenter setting. We therefore retrospectively analyzed 150 AML patients, grafted in 7 different units, from a haploidentical donor, all receiving the same modified PT-CY protocol, with CsA on day 0 and PT-CY on days +3 and +5, following a myeloablative conditioning regimen.

PATIENTS AND METHODS

Patients' Characteristics

This retrospective study was approved by the institutional research board of the Istituto di Ematologia, Fondazione Policlinico Universitario Gemelli, Rome, Italy, and included 150 AML patients. Eligible for this study were AML patients, receiving unmanipulated haploidentical bone marrow, between 2010 and 2016, with a uniform prophylaxis for GVHD, as detailed subsequently. Patients were treated at different institutions as follow: 93 patients in Genova, 25 patients in Rome, 10 patients in Frankfurt, 7 patients both in Ancona and Pavia, and 5 patients in Cuneo. Clinical characteristics are shown in Table 1. High-resolution HLA typing was performed both on patients' and donors' DNA to confirm allele matching, and haplotypes were determined on family studies when possible. The donor had a major ABO mismatch in 17 pairs (12%), a minor ABO mismatch in 38 pairs (27%), and in 7 pairs (5%) there was a double ABO mismatch.

Conditioning Regimens and GVHD Prophylaxis

All patients received a myeloablative regimen: younger patients were eligible for full-dose total body irradiation (TBI), whereas older patients received a combination of thiotepa, busulfan, and fludarabine (TBF). The TBF regimen consisted of thiotepa (5 mg/kg) on days –6 and –5 (total dose 10 mg/kg); busulfan (BU) (3.2 mg/kg) on days –4, –3, and –2 (total dose 9.6 mg/kg); and fludarabine (FLU) (50 mg/m^2) on days –4, –3, and –2 (total dose 150 mg/kg). The TBI regimen consisted of FLU 120 mg/m^2, followed by 9 to 12 Gy TBI (FLU-TBI). The median age of patients receiving TBF (n = 114) was 55 (range, 17 to 74) years; the median age of patients receiving FLU-TBI (n = 28) was 38 (range, 20 to 58) years ($P < .0001$). Busulfan was capped at 2 days in patients over 60 years of age (Table 1). GVHD prophylaxis was uniform and consisted of intravenous CsA, from days 0 to +20, of 3 mg/kg, adjusting for blood levels (200 to 400 ng/mL), and then orally until day +180; mycophenolate (15 mg/kg every 12 hours) from days +1 to +28; and CY 50 mg/kg on days +3 and +5. Granulocyte colony-stimulating factor was started on day+6 until neutrophil recovery.

The stem cell source was unmanipulated bone marrow for all patients, and the median dose of cells collected and infused on day 0, was 3.1 (range, .8 to 6.7) $\times 10^8$ cells/kg.

Donor-specific antibodies (DSAs) were not routinely assessed pretransplant.

Table 1
Clinical characteristics of patients

n		150
Age, yr		51 (range 17-74)
Patients over 60 yr of age		42 (28%)
Male/female		76/74
Donors' age		36 (range 17-67)
ABO matched		80 (56%)
Disease phase		
CR1		68 (45%)
CR2		31 (21%)
Active disease at transplant		51 (34%)
ELN risk group	Low	4 (3%)
	Intermediate	51 (34%)
	High	95 (63%)
FLT3 positive		34 (22%)
Interval diagnosis transplant		201 (range 48-3004)
Conditioning regimen		
FLU-TBI		27 (19%)
TBF (BU3)		80 (53%)
TBF (BU2)		43 (28%)
Nucl. cells ($\times 10^8$ cells/kg)		3.1 (range 0.8-6.7)
Follow-up, d		903 (range 120-1955)

Data are presented as median (range) or n (%).
MMF indicates mycophenolate; BU3, busulfan 3.2 mg/kg/day × 3; BU2, busulfan 3.2 mg/kg/day × 2; ELN, European Leukemia Network; FLT3, FMS-like tyrosine kinase 3.

Supportive Care

Antimicrobial prophylaxis was given as per Institutional standard of care. Twice-weekly monitoring for cytomegalovirus, by PCR or antigenemia, was started on day –7 until day+100 and thereafter once a week, or at each outpatient examination; weekly Epstein-Barr virus monitoring by PCR was started on day +15 up to day+100.

Diagnosis and Treatment of GVHD

The clinical diagnosis of acute GVHD was made according to standard criteria, and confirmed histologically by skin and/or rectal/colon biopsies. First- and second-line therapy of GVHD was provided according to Institutional protocols.

Statistical Analysis

The NCSS 11 Data for Windows (NCSS LLC, Kaysville, UT), was used for contingency tables, rank sum test, cumulative incidence (CI) rates, and actuarial survival. When calculating the CI of TRM, the competing risk was relapse, and vice versa; when calculating the CI of engraftment or GVDH, the competing risk was death due to any cause. The log-rank test was used for differences between survival curves; Gray's test was used to assess differences between cumulative incidence curves. Multivariate Cox analyses on engraftment, GVHD, TRM, relapse, and survival were run with the following variables: nucleated cell dose, disease phase, donor, and patient age, TBI- or BU-based conditioning, number of nucleated cells infused, interval between diagnosis and transplant, and transplant center (Genova versus others). The number of patients who died of leukemia relapse in this study was compared with the number of leukemic deaths in a recent analysis of the Center for International Blood and Marrow Transplant Registry (CIBMTR) [10]: contingency table analyses were used to test for statistical significance.

RESULTS

Engraftment

The median number of nucleated bone marrow cells infused was 3.1 (range, .8 to 6.7) $\times 10^8$/kg; the 25th and 75th percentiles were 2.3 and 4.1 $\times 10^8$ cells/kg, respectively. Eight patients died before day +20 and could not be evaluated; 2 were in complete remission (CR) 1, 2 were in CR2, and 4 were in advanced disease at transplant. Six patients failed to achieve durable engraftment, one of whom could be rescued with a second transplant from the same donor: the overall CI of neutrophil engraftment at 80 days was 92% (Table 2): it was 90%,

Table 2
Main outcomes

			95% Confidence interval
Proportion engrafted	CI	92%*	88–98%
Acute GVHD grade II to IV	CI	17%*	12–24%
Acute GVHD grade III to IV	CI	5%*	2–10%
Chronic GVHD moderate/severe	CI	15%†	10–22%
TRM	CI	20%†	14–28%
Relapse	CI	24%†	18–33%
Actuarial survival	KM	57%†	49–66%
Actuarial survival for remission patients	KM	73%†	63–82%
Disease-free survival	KM	52%†	43–52%
Disease-free survival for remission patients	KM	67%†	55–79%
GRFS	KM	45%†	36–55%
GRFS for remission patients	KM	55%†	42–68%

GRFS indicates survival free of GVHD and relapse; KM, Kaplan-Meier.
* At 100 days.
† At 4 years.

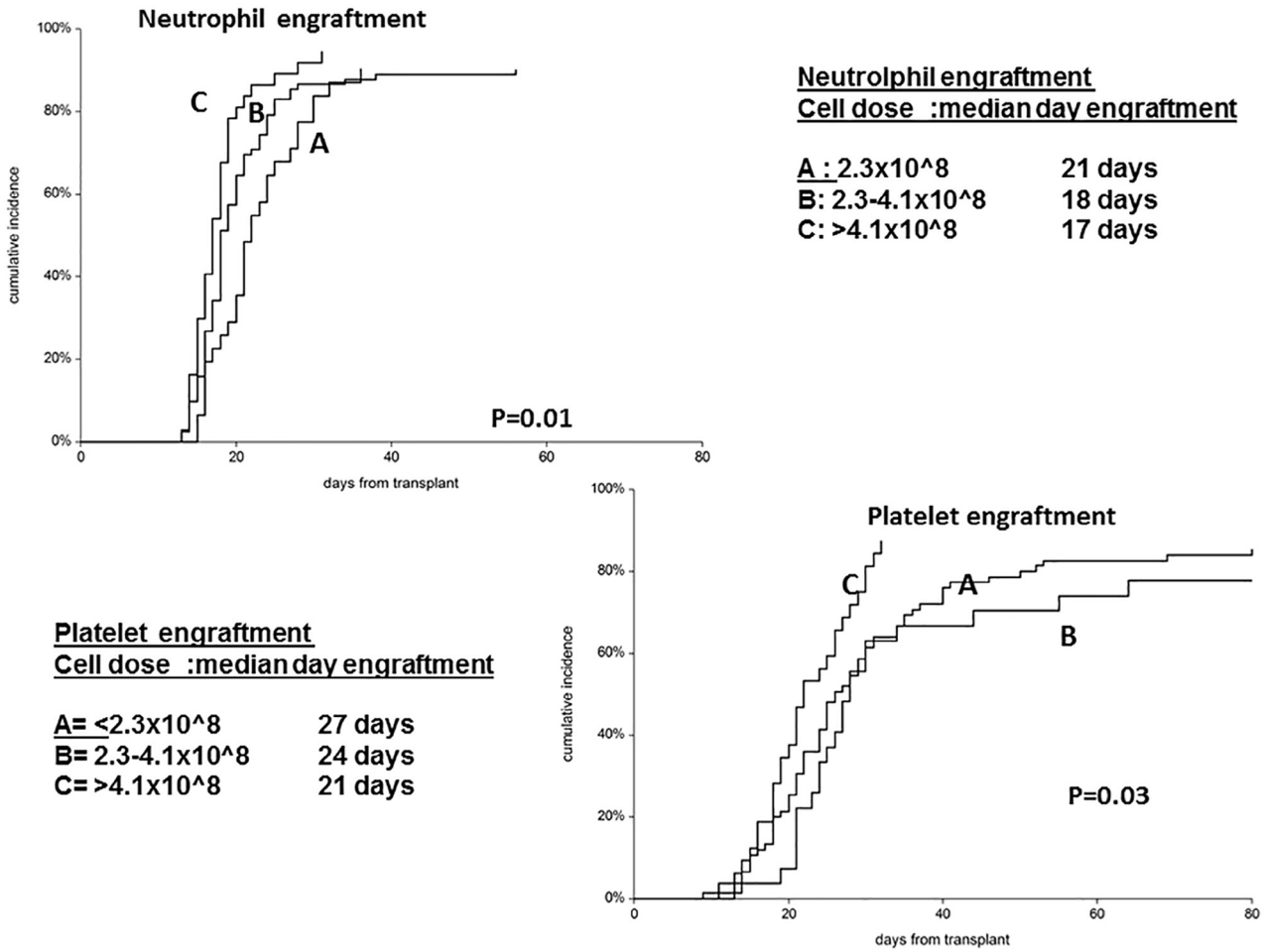

Figure 1. Cumulative incidence of neutrophil and platelet engraftment, stratified according to the nucleated bone marrow cells at transplant: (A) <2.3× 10^8 cells/kg, (B) 2.3 to 4.1 × 10^8 cells/kg, and (C) >4.1 × 10^8 cells/kg. A strong effect of the cell dose on time to engraftment is shown .

92%, and 95% for patients receiving <2.3 to 4.1 × 10^8 cells/kg and >4.1 × 10^8 cells/kg nucleated marrow cells ($P = .01$), respectively (Figure 1). The median day of neutrophil engraftment was day +21 (range, 15 to 36) versus day +18 (range, 13 to 56; $P = .004$) versus day +17 (range, 13 to 31; $P = .0007$), for the 3 groups, respectively. In a multivariate Cox analysis, factors predicting neutrophil engraftment were the nucleated cell dose infused, entered as a continuous variable, with a risk ratio (RR) of 1.37 ($P = .001$) and advanced disease, with a lower probability of neutrophil engraftment (RR, .6; $P = .03$) (Table 3). There was no effect of ABO matching. The CI of platelet engraftment was 87%: it was 85%, 88%, and 89% for patients receiving <2.3 to 4.1 × 10^8 cells/kg and >4.1 × 10^8 cells/kg nucleated marrow cells ($P = ·03$), respectively (Figure 1). The median day of platelet engraftment was day +27 (range, 13 to 180), for patients receiving ≤ 2.3 × 10^8 cells/kg versus day +24 (range, 9 to 90) for patients receiving 2.4 to 4.0 × 10^8/kg cells/kg ($P = .057$) versus day +21 (range, 13 to 31; $P = .01$) for patients receiving ≥4·1 × 10^8 cells/kg (Figure 1).

Table 3
Multivariate Cox analysis on outcome

Variable	Baseline Value	Compared Value	PMN		TRM		Relapse		OS	
			RR	*P*	RR	*P*	RR	*P*	RR	*P*
Phase	CR1	CR2	.8	.5	1.9	.3	1.5	.5	1.8	.2
		Advanced	.6	.03	3.2	.03	11.3	.000	9.0	.000
Donor age	< 35	≥35 yr of age	.8	.4	.6	.3	.6	.2	.6	.1
Recipient age	< 60	≥ 60 yr of age	.7	.5	2.0	.1	.4	.23	.6	.3
Conditioning	TBI	TBF-BU3	.7	.3	2.4	.2	.5	.3	.8	.8
TBF-BU2	.8	.5	1.2	.8	.8	.7	.7	.7		
Cell dose		Continuous	1.37	.001	.8	.2	.9	.6	.8	.1
In DxTx		Continuous	1.0	.3	.9	.4	.9	.08	.9	.07
Center Genova		Other	.8	.5	.9	.8	.5	.1	.7	.3

PMN indicates neutrophils; OS, overall survival; DxTx, interval diagnosis transplant.

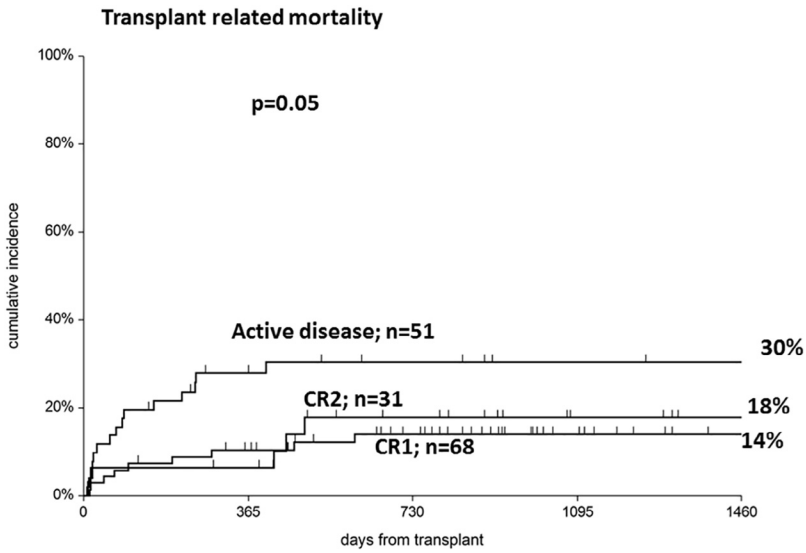

Figure 2. Cumulative incidence of TRM, in patients stratified according to disease phase: first remission (CR1), second remission (CR2), or active disease.

Graft-Versus-Host Disease

Acute GVHD grade I to IV was diagnosed in 56, 19, 5, and 1 patients, respectively: the CI of acute GVHD grade II to IV was 17%, and 5% for grades III to IV (Table 2). In a multivariate analysis, patients with advanced disease had a lower probability of grade II to IV acute GVHD ($P = .01$), with no other significant predictor, including donor and patient age. There was no effect of cell dose on acute GVHD: patients receiving the highest cell dose had 0% rate of grade III to IV GVHD. Chronic GVHD was classified as minimal, moderate, or severe in 47, 14 and 6 patients, respectively. The CI of moderate-severe chronic GVHD was 15% (Table 2), and there were no significant predictors in multivariate analysis, including cell dose; older donors (>36 years of age) were associated with a higher risk of chronic GVHD (RR, 1.79), but this did not reach statistical significance ($P = .19$). Sixty-five patients were available for examination at 1 year, and 41 (63%) were off immunosuppressive treatment.

Transplant Related Mortality and Relapse

At a median follow-up of 903 (range, 120 to 1955) days, 89 patients were alive, 29 had died of TRM, and 32 had died of recurrent disease. The overall cumulative incidence of TRM at 100 days was 9%, at 1 year was 15%, and at 4 years was 20% (Table 2); it was 14% and 18% in patients in CR1 and CR2, respectively, and 30% in patients with active disease ($P = .05$) (Figure 2). In multivariate analysis advanced disease (RR, 3.2; $P = .03$) was the only negative predictor of TRM.

Thirty-five patients relapsed, and 32 patients died of recurrent leukemia. The 4-year CI of relapse was 24% (Table 2); it was 13% for remission patients (14% and 10% for CR1 and CR2, respectively) and 47% for patients with active disease at transplant ($P = .00006$) (Figure 3). In multivariate analysis advanced disease was the strongest negative predictor of relapse (RR, 11.3; $P < .0001$) (Table 3); there was a trend for a lower relapse in patients with a longer interval diagnosis to transplant. Sixty-two patients had Wilms tumor 1 (WT1)

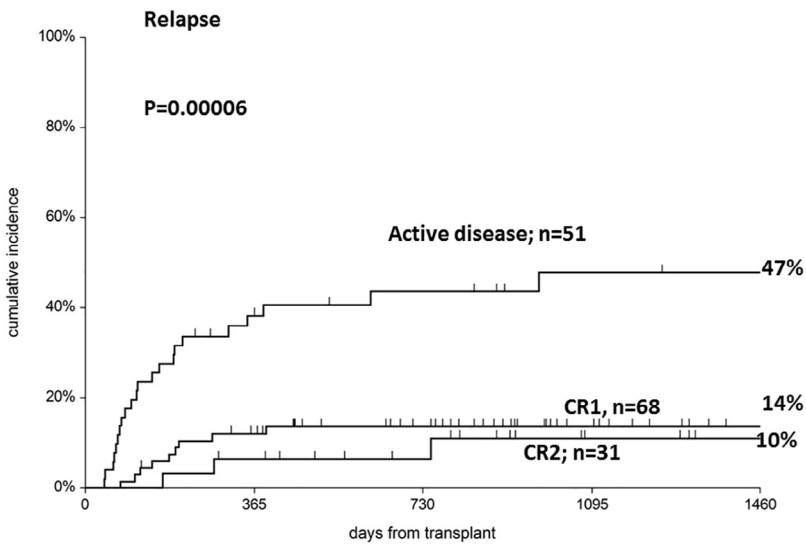

Figure 3. Cumulative incidence of leukemia relapse, in patients stratified according to disease phase.

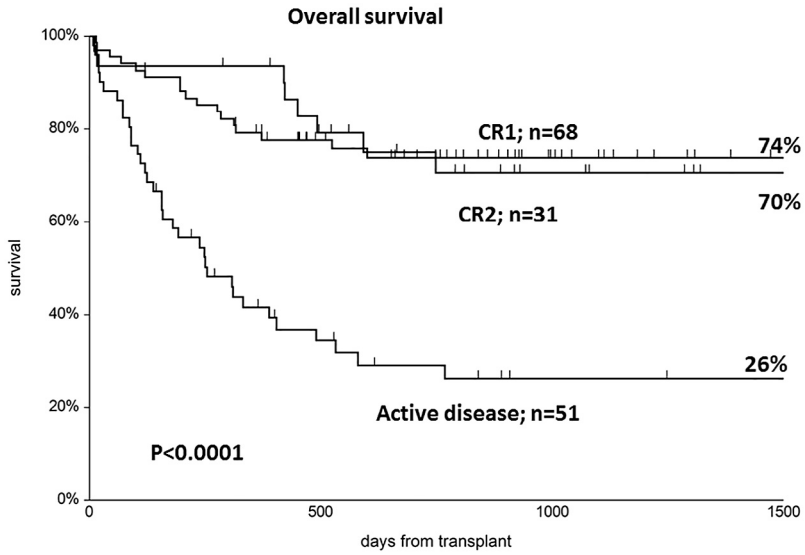

Figure 4. Overall survival in patients stratified according to disease phase.

levels before transplant: relapse was seen in 3 of 52 with WT1 copies <100 copies/10^4 ABL versus 2 relapses of 10 patients with WT1 levels >100 copies/10^4 ABL ($P = .1$). The number of events is very small and limits the potency of the test.

Survival

The overall actuarial 4-year survival is 57% (Table 2): it is 74%, 70% and 26% for patients grafted in CR1, CR2, or with active disease, respectively ($P < .0001$) (Figure 4). When comparing patients grafted in Genova (n = 93) or in other centers (n = 57), 4-year survival for remission patients was 73% versus 72%, and for patients with active leukemia it was 23% versus 30%, respectively. Figure 5 outlines actuarial survival of patients stratified by age (≤60 or >60 years of age) and remission status (74% and 67% for younger and older remission patients, 41% and 17% for older and younger advanced patients). The 4-year probability of survival free of GVHD and relapse for remission patients was 55% (Table 2). In a multivariate analysis, advanced disease was the only significant negative predictor of survival (Table 3). There was no effect of the conditioning regimen (TBI based versus BU based), despite a significant difference in age (37 years of age versus 55 years of age), with comparable survival, both for remission patients (69% versus 75%; $P = .7$) and relapsed patients (25% versus 27%; $P = .5$).

The causes of death were as follows: relapse (n = 32), infections (n = 14), graft failure (n = 5), multiorgan failure (n = 2), acute GVHD (n = 1), chronic GVHD (n = 2), second tumor (n = 1), and interstitial pneumonia (n = 4). The proportion of different causes of death was not different ($P = .8$) in patients receiving TBI or TBF, although graft failure was reported in 0 of 27 and 5 of 123, patients respectively (0% versus 4%).

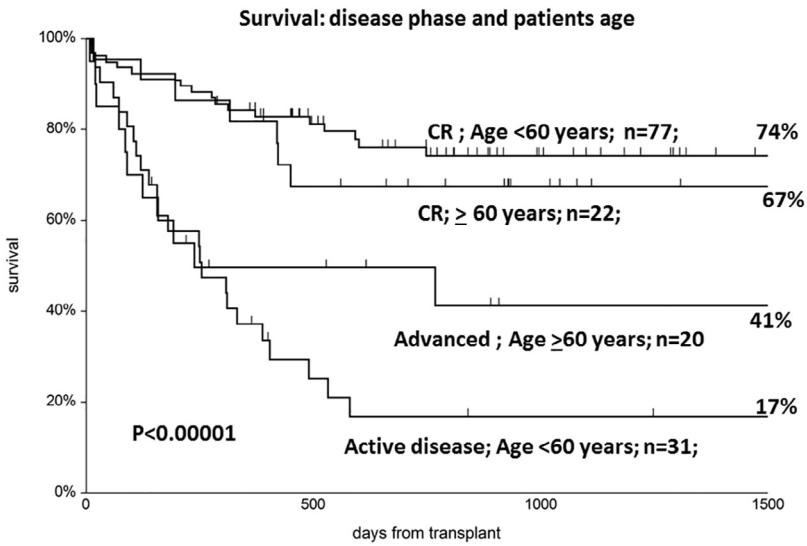

Figure 5. Overall survival in patients stratified according to remission status and age: a strong effect of disease phase can be seen, for both young and older patients (>60 years of age). Of note is the comparable survival of remission AML patients aged <60 years of age or ≥60 years of age.

DISCUSSION

We have shown in the present study that the Baltimore protocol of high dose PT-CY for haploidentical bone marrow transplants can be modified and still retain a favorable profile, in terms of GVHD protection and effective graft versus leukemia. The results can be summarized as follows: high rate of engraftment, low incidence of GVHD and transplant mortality, very low relapse in remission patients, encouraging survival, including patients over 60 years of age, no center effect.

As to the first point, all patients received unmanipulated marrow, and the cumulative incidence of engraftment was 92%: in 5 (3.3%) patients rejection was reported as the primary cause of death, and DSAs were detected in 3 of them. We found a strong effect of cell dose on time to engraftment, but not on the risk of rejection: the median cell dose, for 6 patients rejecting, was 3.4×10^8 cells/kg, compared with 3.0×10^8 cells/kg for patients engrafting. In keeping with this observation, a recent study on unmanipulated haploidentical transplants shows a comparable rate of engraftment for patients receiving marrow (92%) or peripheral blood grafts (95%) [11]. The issue of graft rejection in haploidentical stem cell transplantation has been recently reviewed [12], and does not seem to be linked to the stem cell source, but rather to the presence of DSA. To prevent graft rejection it is important to test for DSA, to select for the best possible donor.

As to the second point we have seen a low incidence of GVHD, despite the use of CsA before PT-CY. Perhaps the use of marrow for all patients might have played a role, as higher rates of GVHD grades III to IV have been reported with the use of peripheral blood [11]. The standard PT-CY regimen (days +3 and +4) following a nonmyeloablative regimen produces a risk of GVHD grade II to IV and III to IV of 34% and 6%, respectively [1], which is quite similar to what we have seen in our series of patients with PT-CY given on days +3 and +5, but following a myeloablative conditioning regimen. Our low GVHD rates were associated with relatively low rates of TRM, both in young patents, as well as in patients over 60 years of age: the overall TRM was 18% and 23% in patients less than or over 60 years of age ($P = .3$); for remission patients these figures are 13% and 18%, respectively, suggesting that also older AML patients can be scheduled for a , respectively graft with a relatively low toxicity profile. Overall TRM is reported to be low, also in other studies, both with myeloablative or nonmyeloablative regimens, with figures ranging from 3% [13] to 21% [14]. We not have data on the use of peripheral blood as a stem cell source with this platform: it may be that the early use of CsA before PT-CY will expose patients to a high risk of acute and chronic GVHD; therefore, these results cannot be extrapolated to unmanipulated haploidentical peripheral blood.

Relapse remains one of the key problems of allogeneic transplantation for leukemia, and has remained unchanged over the past decades [15]: in this study we have seen a very low rate of relapse in remission patients (13%), perhaps lower when compared with other series of patients, and the use of PT-CY following CsA administration might have played a role. In a study of the CIBMTR, on 104 myeloablative haploidentical grafts in AML with PT-CY on days+3 and +4 [10], the proportion of patients in first remission was 46%, 20% were in second remission, and the median follow-up was 900 days, and 41 patients (39%) died of leukemia relapse. In the present study on 150 AML patients, 45% were in first remission and 21% in second remission, and the median follow-up was identical (900 days): 32 patients died of leukemia relapse (21%), which is significantly lower when compared with the CIBMTR study (Fisher $P = .002$). There may be a role for the myeloablative conditioning regimen, given in our study, as the CIBMTR included also reduced intensity regimens. Nevertheless, relapse remains high for patients with advanced disease, 47% in our series, and new strategies are needed to prevent leukemia relapse. A pre-emptive approach with cellular therapy [16], or with azacytidine [17], is one option, but requires close monitoring of a marker for minimal residual disease (MRD). Post-transplant prophylaxis of relapse is another possibility, as shown in a German cooperative study with panobinostat [18], or again with azacytidine [19-22]. Salvage strategies may need to be personalized in haploidentical graft recipients, as relapse can occur with loss of the HLA mismatched haplotype [23].

Older age has long been a contraindication for allogeneic transplantation, especially from alternative donors. A recent report has shown encouraging outcome for patients older than 55 years of age, with both acute and chronic hematologic malignancies [24], receiving haploidentical grafts, mainly with peripheral blood as a stem cell source, and the original PT-CY protocol days+3 and +4. We were thus interested in looking at the outcome of our AML patients over 60 years of age: for remission AML, survival in older patients was comparable to younger patients (67% versus 74%). For advanced AML, there was a trend for a reduced risk of relapse in the older group, leading to improved survival (41% versus 17%), despite an equal distribution of high European Leukemia Network risk: among 20 advanced patients over 60 years of age, there were 5 relapses (25%) compared with 17 of 31 in younger patients (51%; $P = .03$).

Finally, all major outcomes were comparable for patients grafted in Genova or elsewhere, and there was no center effect in a multivariate Cox model.

In conclusion, the original Baltimore PT-CY regimen can be modified with CsA administered before PT-CY in AML patients undergoing an unmanipulated haploidentical marrow graft, following a myeloablative conditioning regimen. In a multicenter setting we have seen a low incidence of GVHD and TRM, and relapse has been lower than in other series. These results were obtained with marrow as a stem cell source, and may not translate automatically to unmanipulated peripheral blood. It could be speculated that CsA given before PT-CY has prevented some alloreactive T cells from PT-CY purging, thus increasing the graft-versus-leukemia effect. This hypothesis is being tested in an European Society for Blood and Marrow Transplantation registry-based study comparing CsA following PT-CY given on days +3 and +4 with CsA preceding PT-CY given on days +3 and +5.

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