Allogeneic stem cell transplantation for patients with acute myeloid leukemia (AML) in second complete remission (CR2) transplanted from unrelated donors with post-transplant cyclophosphamide (PTCy). A study on behalf of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

Nagler, Arnon; Labopin, Myriam; Swoboda, Ryszard; Kulagin, Alexander; Labussière-Wallet, Hélène; Rovira, Montserrat; Blaise, Didier; Vydra, Jan; Yakoub-Agha, Ibrahim; Choi, Goda

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ARTICLE

Allogeneic stem cell transplantation for patients with acute myeloid leukemia (AML) in second complete remission (CR2) transplanted from unrelated donors with post-transplant cyclophosphamide (PTCy). A study on behalf of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

Arnon Nagler[1]✉, Myriam Labopin[2,3], Ryszard Swoboda[4], Alexander Kulagin[5], Hélène Labussière-Wallet[6], Montserrat Rovira[7], Didier Blaise[8], Jan Vydra[9], Ibrahim Yakoub-Agha[10], Goda Choi[11], Péter Reményi[12], Yener Koc[13], Jaime Sanz[14], Fabio Ciceri[15] and Mohamad Mohty[16]

Post-transplant cyclophosphamide (PTCy) is being increasingly used as graft-versus-host disease (GVHD) prophylaxis post allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with acute myeloid leukemia (AML) transplanted in first complete remission (CR1). However, results may differ in patients transplanted in CR2. We retrospectively evaluated transplant outcomes of adult AML patients transplanted between 2010–2019 from 9–10/10 human leukocyte antigen (HLA)-matched unrelated donor (UD) in CR2. In total, 127 patients were included (median age 45.5 years, 54% male). Median follow-up was 19.2 months. Conditioning was myeloablative (MAC) in 50.4% and the graft source was peripheral blood in 93.7% of the transplants. Incidence of acute (a)GVHD II-IV and III-IV was 26.2% and 9.2%. Two-year total and extensive chronic (c)GVHD were 34.3% and 13.8 %, respectively. Two-year non-relapse mortality (NRM), relapse incidence (RI), leukemia-free survival (LFS), overall survival (OS), and GVHD-free, relapse-free survival (GRFS) were 17.2%, 21.1%, 61.7, %, 65.2%, and 49.3%, respectively. Time from diagnosis to transplant (>18 months) was a favorable prognostic factor for RI, LFS, OS, and GRFS while favorable risk cytogenetics was a positive prognostic factor for OS. The patient's age was a poor prognostic factor for NRM and cGVHD. Finally, the female-to-male combination and reduced intensity conditioning (RIC) were poor and favorable prognostic factors for cGVHD, respectively. We conclude that PTCy is an effective method for GVHD prophylaxis in AML patients undergoing allo-HCT in CR2 from UD.

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INTRODUCTION

Acute and chronic graft-versus-host disease (cGVHD) continues to be a major cause of transplant-related mortality (TRM) in allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acute myeloid leukemia (AML) [1, 2]. Acute (a)GVHD is commonly observed in 40 to 60% of patients after allo-HSCT with an overall survival (OS) of 10 to 25% in patients with severe grades III–IV aGVHD. Therefore, a primary goal in allo-HSCT for AML is to prevent and reduce the incidence and severity of GVHD, which will most probably lead to a significant reduction in TRM and improvement in transplantation outcomes [3]. The standard regimen for the prevention of GVHD after allo-HSCT consists of

[1]Division of Hematology, Sheba Medical Center, Tel Hashomer, Israel. [2]ALWP of the EBMT Paris office, Paris, France. [3]Sorbonne University, Department of Haematology, Saint Antoine Hospital, INSERM UMR 938 Paris, France. [4]Department of Bone Marrow Transplantation and Onco-Hematology, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland. [5]First State Pavlov Medical University of St. Petersburg, Raisa Gorbacheva Memorial Research Institute for Paediatric Oncology, Hematology, and Transplantation, St, Petersburg, Russia. [6]Centre Hospitalier Lyon Sud, Pavillon Marcel Bérard, Service Hematologie, Lyon, France. [7]Hospital Clinic, Department of Hematology, Institute of Hematology and Oncology, Barcelona, Spain. [8]Programme de Transplantation & Therapie Cellulaire, Centre de Recherche en Cancérologie de Marseille; Institut Paoli Calmettes, Marseille, France. [9]Institute of Hematology and Blood Transfusion, Prague, Czech Republic. [10]CHU de Lille, Univ Lille, INSERM U1286, Infinite, 59000 Lille, France. [11]Deptartment of Hematology; University Medical Center Groningen, University of Groningen, Groningen, The Netherlands. [12]Dél-pesti Centrumkórház –Országos Hematológiai és Infektológiai Intézet; Dept. Haematology and Stem Cell Transplant, Albert, Budapest, Hungary. [13]Medicana International Hospital Istanbul; Bone Marrow Transplant Unit, Istanbul, Turkey. [14]Hospital Universitari i Politècnic La Fe, Valencia, Spain. [15]Ospedale San Raffaele s.r.l. Haematology and BMT, Milano, Italy. [16]Sorbonne University, Department of Clinical Hematology and Cellular Therapy, Hospital Saint Antoine, AP-HP, INSERM UMRs 938, Paris, France. Data presented in part at the ASH 2021 Hybrid Congress (publication number 3916). ✉email: arnon.nagler@sheba.health.gov.il

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a combination of a calcineurin inhibitor (CNI) and a short course of methotrexate [3–5]. The introduction of post-transplant cyclophosphamide (PTCy) as a GVHD prophylaxis has revolutionized the approach to allo-HSCT using a haploidentical donor (Haplo-HCT) [6, 7] has proven to be highly effective in preventing GVHD and reducing non-relapse mortality (NRM) rates in Haplo-HCT transplantation [6–8]. In addition to PTCy being associated with low rates of GVHD, it was shown to be accompanied by a low frequency of graft rejection and better immune tolerance and immune reconstitution [9, 10]. Although the mechanism of action of PTCy in preventing GVHD is not completely clear, PTCy given early after HLA-mismatched graft infusion is able to selectively inhibit rapidly proliferating alloreactive T cells in both the graft-versus-host and host-versus-graft directions, while preserving non-dividing hematopoietic stem cells and the slowly dividing memory and regulatory T cells in the graft, owing to their high aldehyde dehydrogenase content [9–13]. Moreover, PTCy upregulates regulatory T cells mediating long-term immune tolerance and GVHD control [12, 13]. As a consequence, PTCy is increasingly being used in other HSCT settings for patients with AML, including human leukocyte antigen (HLA)- matched unrelated donors (UD), and mismatched UD as well as HLA-matched sibling donors (MSD) [14–19]. However, most of these studies were in AML patients transplanted while in first complete remission (CR1). So far, no studies have evaluated PTCy in AML patients transplanted in second CR (CR2). Transplantation outcomes may differ in AML patients who relapse subsequent to a CR1 and thus undergo the allo-HSCT in CR2 achieved with additional anti-leukemic therapy in comparison to those transplanted in CR1 [20, 21]. From the theoretical point of view, the biology of the graft-versus-leukemia (GVL) effect and the interplay between the GVL effect and the usually accompanied GVHD, may also differ in AML patients transplanted in CR2 [20, 21]. We recently assessed transplantation outcomes in 1879 AML patients that underwent allo-HSCT from MSD and UD while in CR2, comparing myeloablative conditioning (MAC) versus reduced intensity conditioning (RIC) and demonstrated worse cGVHD after RIC-allo-HSCT (32% vs 39%) in patients ≥50 years old, while leukemia-free survival (LFS), and relapse incidence (RI) did not differ [21]. In a subsequent analysis of 1042 adult patients with AML undergoing MSD and UD transplants in CR2, the 2-year RI was higher in patients with positive measurable residual disease (MRD) pre-allo-HSCT versus those with negative MRD (40% vs 24%, $p < 0.001$), which translated into better LFS in the MRD negative group (57% vs 46%, $p = 0.001$) [21]. In both studies, GVHD prophylaxis was the conventional CNI-based prophylaxis. Given the different mechanism of action of PTCy compared to standard CNI-based GVHD prophylaxis, we aimed to study the outcomes of allo-HSCT with PTCy in AML patients transplanted in CR2.

SUBJECTS AND METHODS

Study design and data collection

This was a retrospective, multicenter analysis. Data were provided by the registry of the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). The EBMT is a non-profit, scientific society representing more than 600 transplant centers, mainly located in Europe, which are required to report all consecutive stem cell transplantations and follow-ups once a year. Data are entered, managed, and maintained in a central database. EBMT centres commit to obtain informed consent according to the local regulations applicable at the time of transplantation and report pseudonymized data to the EBMT. The validation and quality control program includes verification of the computer print-out of the entered data, cross-checking with the national registries, and on-site visits to selected teams. The study was approved by the ALWP of the EBMT institutional review board and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Criteria for selection

Eligibility criteria for this analysis included adult patients ≥18 years of age with de novo AML who underwent a first HSCT from a 9/10 or 10/10 HLA-matched UD in CR2 between 2010 and 2019. Anti -GVHD prophylaxis was PTCy with or without in vivo T-cell depletion (anti-thymocyte globulin [ATG]). Pre-transplantation preparative regimens were RIC or MAC. Donor cell sources were bone marrow (BM) or peripheral blood (PB). All other donor sources were excluded. Other exclusion criteria were previous history of HSCT, disease status other than CR2 before transplantation, Haplo or MSD as well as transplantations with ex vivo T-cell depletion.

Data collected comprised recipient and donor characteristics including age, gender, and cytomegalovirus (CMV) serostatus, disease characteristics, year of transplant, type of conditioning regimen, stem cell source, and GVHD prophylaxis regimen. The conditioning regimen was defined as MAC or RIC, based on the reports from individual transplant centers as per previously established criteria [22]. The conditioning regimen was defined as MAC when containing total body irradiation (TBI) with a dose >6 Gy or a total dose of busulfan >8 mg/kg or >6.4 mg/kg when administered orally or intravenously, respectively. All other regimens were defined as RIC [22]. Regimens for GVHD prophylaxis were per institutional protocols. Grading of aGVHD was performed using established criteria [23]. cGVHD was classified as limited or extensive according to published criteria. [24]. For this study, all necessary data were collected according to the EBMT guidelines, using the EBMT minimum essential data forms. The list of institutions contributing data to this study is provided in Appendix S1.

Statistical analysis

Median, minimum, and maximum values were used for quantitative variables, frequencies, and percentages for categorical variables [25]. The study endpoints were OS, LFS, RI, NRM, neutrophil recovery, aGVHD, cGVHD, and GVHD-free, relapse-free survival (GRFS). All endpoints were measured from the time of transplantation. Neutrophil recovery was defined as achieving an absolute neutrophil count (ANC) 0.5×10^9/L for 3 consecutive days. OS was defined as time to death from any cause. LFS was defined as survival with no evidence of relapse or progression. NRM was defined as death from any cause without previous relapse or progression. We used the modified GRFS criteria [26]. GRFS events were defined as the first event among grade III–IV aGVHD, extensive cGVHD, relapse, or death from any other cause [26]. The probabilities of OS, LFS, and GRFS were calculated using the Kaplan–Meier (KM) method. The aGVHD, cGVHD, RI, and NRM were estimated using cumulative incidence (CI) curves in a competing risk setting, death in remission being treated as a competing event for relapse. To estimate the CI of acute or cGVHD, relapse and death were considered as competing events. Univariate analyses were performed using the log-rank test for LFS, OS, and GRFS whereas Gray's test was used to compare CI estimates. Multivariate analyses were performed using the Cox proportional-hazards regression model [25]. All variables differing significantly between the groups, and factors known to influence outcomes were included in the Cox model. Continuous variables were included without categorization in the model. Then, we used a backward stepwise selection of variables with a non-restrictive *–p-value* threshold of 0.10 for removing variables. Results were expressed as the hazard ratio (HR) with a 95% confidence interval (95% CI) [25]. All p values were two-sided with a type I error rate fixed at 0.05. Statistical analyses were performed with SPSS 25 (IBM Corp) and R 4.0.2 (R Core Team 2020) [25, 27].

RESULTS

Patient, transplant, and disease characteristics

In total, 127 patients met the inclusion criteria. Median follow-up was 19.2 (95% CI, 14.8–27.5) months. Table 1 shows the baseline demographic and clinical characteristics. Median age was 45.5 (range 18.2–71.3) years and 54.3% of patients were male. The cytogenetic risk was favorable, intermediate, and adverse in 15.7%, 55.9%, and 5.5% of patients, respectively (cytogenetics were missing for 22.8% of the cases). Median time from diagnosis to transplantation was 20.4 (range 4.1–182.4) months. Donors were 10/10 and 9/10 HLA-matched unrelated in 60.6% and 39.4% of the allo-HSCT, respectively. Patients and donors were CMV seropositive in 77.8% and 47.2%, respectively. Karnofsky performance status (KPS) was ≥90 in 71.2% of the patients. Table 2 shows the transplant related characteristics. MAC was used in 50.4% while RIC was the regimen in 49.6%. The most frequent immunosuppression used in combination with PTCy as an anti

Table 1. Patient and donor characteristics.

Clinical parameter	$n = 127$
Median follow-up, months [95% CI]	19.16 [14.76–27.54]
Median age, years (min-max)	45.5 (18.2–71.3)
Patient sex:	
• male	54.3%)
• female	58 (45.7%)
Median time from diagnosis to allo-HSCT, months (min-max)	20.4 (4.1–182.4)
Cytogenetics:	
• favorable	20 (15.7%)
• intermediate	71 (55.9%)
• adverse	7 (5.5%)
• missing	29 (22.8%)
Donor:	
• UD 10/10	77 (60.6%)
• UD 9/10	50 (39.4%)
Donor sex	
• male	91 (72.2%)
• female	35 (27.8%)
• missing	1
Female to male transplantation	
• yes	107 (84.9%)
• no	19 (15.1%)
• missing	1
Karnofsky performance score	
• <90	36 (28.8%)
• >= 90	89 (71.2%)
• missing	2
Patient CMV serological status	
• negative	28 (22.2%)
• positive	98 (77.8%)
• missing	1
Donor CMV serological status	
• negative	67 (52.8%)
• positive	60 (47.2%)

Allo-HSCT allogeneic hematopoietic cell transplantation, *UD* unrelated donor, *CMV* cytomegalovirus, *CI* confidence interval, *min* minimum; *max* maximum

Table 2. Transplant characteristics.

Clinical parameter	$n = 127$
Median year of transplantation (min-max)	2017 (2011–2019)
Type of conditioning:	
• MAC	50.4%)
• RIC	63 (49.6%)
Conditioning regimen:	
• BuCy + /-other	8.7%)
• BuFlu + /-other	61.4%)
• FluMel + /-other	11.8%)
• TBI	11%)
• other	9 (7.1%)
Cell source:	
• BM	(6.3%)
• PB	119 (93.7%)
Associated immunosuppression	
• CsA	9 (7.1%)
• MTX	11 (8.7%)
• MMF	6 (4.7%)
• Tacro	12 (9.4%)
• CsA + MTX	8 (6.3%)
• CsA + MMF	27 (21.3%)
• MMF + Tacro	30 (23.6%)
• MMF + Siro	10 (7.9%)
• MTX + Tacro	1 (0.8%)
• other	13 (10.2%)
In vivo T cell depletion	
• no in vivo TCD	84 (66.1%)
• in vivo TCD	43 (33.9%)

MAC myeloablative conditioning, *RIC* reduced intensity conditioning, *Bu* busulfan, *Cy* cyclophosphamide, *Flu* fludarabine, *Mel* melphalan, *TBI* total body irradiation, *BM* bone marrow, *PB* peripheral blood, *CsA* cyclosporine A, *MTX* methotrexate, *Tacro* tacrolimus; *MMF* mycophenolate mofetil, *Siro* sirolimus, *TCD* T cell depletion

GVHD prophylaxis was tacrolimus with mycophenolate mofetil (MMF) (23.6%), followed by cyclosporine A (CsA) with MMF (21.3%). In vivo T-cell depletion was used in 33.9% of patients. The graft source was PB and BM in 93.7% and 6.3% of patients, respectively.

Transplantation outcome

Neutrophil recovery (ANC > 0.5×10^9/L) was achieved in 97.6% of the patients. On day +180, the incidence of aGVHD grades II-IV and III-IV was 26.2% and 9.2%, respectively (Table 3). The 2-year incidence of total and extensive cGVHD was 34.3% and 13.8%, respectively. Two-year NRM and RI were 17.2% and 21.1%, respectively (Table 3, Fig. 1). The 2-year LFS, OS, and GRFS was 61.7%, 65.2%, and 49.3%, respectively (Table 3, Fig. 1). In total, 40 (31%) of the 127 study patients died. The original disease was the main cause of death accounting for 41% of mortality, followed by infection (23.1%), GVHD (20.5%), and multiple organ failure (10.3%) (results not shown).

Table 3. Transplant outcomes.

Outcomes	Estimation [95% CI]	Number of events at estimation
180-day		
aGVHD grade II-IV	26.2 [18.6–34.5]	31
aGVHD grade III-IV	9.2 [4.8–15.2]	11
2-year		
cGVHD	34.3 [24.8–44]	32
Extensive cGVHD	13.8 [7.7–21.7]	13
RI	21.1 [13.4–30]	21
NRM	17.2 [10.7–25]	19
LFS	61.7 [51.1–70.6]	40
OS	65.2 [54.3–74.2]	34
GRFS	49.3 [38.8–59]	53

RI relapse incidence, *NRM* non-relapse mortality, *LFS* leukemia-free survival, *OS* overall survival, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *GRFS* GVHD-free and relapse-free survival, *CI* confidence interval

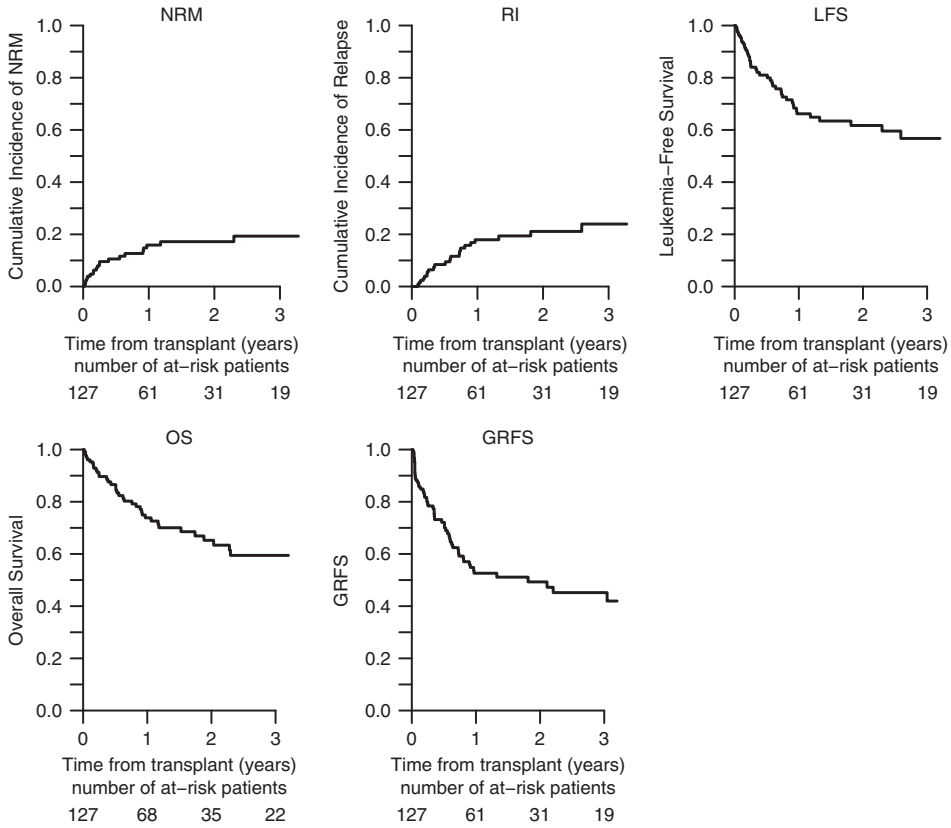

Fig. 1 Transplantation outcomes of allogeneic stem cell transplantation from an unrelated donor in patients with acute myeloid leukemia transplanted in second complete remission with post-transplant cyclophosphamide as anti-graft versus host disease prophylaxis. Non-relapse mortality (NRM), relapse incidence (RI), leukemia-free survival (LFS), overall survival (OS), and GVHD-free, relapse-free survival (GRFS).

In the multivariate analysis (Table 4), time from diagnosis to transplant was a significant prognostic factor for RI, LFS, OS and GRFS, with HR = 0.19 (95% CI 0.07–0.48, $p < 0.001$), HR = 0.30 (95% CI 0.16–0.56, $p < 0.001$), HR = 0.31 (95% CI 0.15–0.61, $p < 0.001$) and HR = 0.40 (95% CI 0.24–0.69, $p < 0.001$), respectively. Recent year of transplant was significantly associated with a better GRFS and a lower risk of aGVHD, grade II-IV: HR = 0.87 (95% CI 0.78–0.97, $p < 0.009$) and HR = 0.84 (95% CI 0.73–0.98, $p = 0.027$), respectively. Age (per 10 years) at time of transplant was a prognostic factor for NRM and cGVHD, HR = 1.83 (95% CI 1.24–2.69, $p = 0.002$) and HR = 0.68 (95% CI 0.51–0.90, $p = 0.008$), respectively, and favorable risk cytogenetics was a prognostic factor for OS, HR = 0.21 (95% CI 0.05–0.91, $p = 0.036$). Female donor to male recipient combination, and RIC were prognostic factors for cGVHD, HR = 0.15 (95% CI 0.03–0.64, $p = 0.011$), and HR = 3.74 (95% CI 1.52–9.18, $p = 0.004$), respectively. Duration of first CR, in vivo T cell depletion and HLA mismatch were not significantly associated with any transplantation outcome (data not shown).

DISCUSSION

In our current study we assessed outcomes of allo-HSCT from UD with PTCy as anti GVHD prophylaxis in a homogenous cohort of patients with AML transplanted in CR2. PTCy anti GVHD prophylaxis resulted in 180-day aGVHD grade II-IV and 2-year cGVHD incidences of 26.2% and 34.3%, respectively. These results are comparable with those that we have previously shown for AML patients transplanted in CR2 from both MSD and UD with mainly CNI based anti GVHD prophylaxis [20]. In that large EBMT registry study, we compared MAC with RIC before allo-HCT in AML patients transplanted in CR2. The GVHD prophylaxis was predominantly based on CsA with MTX or MMF (only 10.8% and 5.7% of patients receiving MAC or RIC, respectively, received PTCy). Overall, no significant differences in transplantation outcomes were observed between the study groups, except for reduced NRM and increased risk of cGVHD in patients aged ≥50 years old in the RIC group [20]. All other transplant outcomes in our study including RI, NRM, LFS and OS with PTCy as GVHD prophylaxis and 9–10/10 HLA matched UD transplants (21.1%, 17.2%, 61.7%, 65.2%, respectively) are roughly similar in comparison to the outcomes we previously observed in a similar cohort of AML patients transplanted in CR2 from MDS (40%) and 9–10/10 HLA matched UD with the conventional CNI based GVHD prophylaxis (28.9%, 19%, 52%, 58.7%, respectively) [20].

Allo-HSCT is a standard post consolidative therapy after achieving CR1 for intermediate and adverse risk AML and therefore most previous studies that have analyzed outcomes with PTCy-based anti GVHD prophylaxis, have focused on AML patients transplanted in CR1, while outcomes of transplantation with PTCy in AML patients undergoing transplantation while in CR2 are less known.

The favorable prognostic factors (time from diagnosis to allo-HSCT, and favorable cytogenetics) observed in the current study are in agreement with a subsequent EBMT study focusing on 1042 AML patients undergoing transplantation in CR2 from MSD or 10/10 HLA matched UD. Time from diagnosis to allo-HSCT was shown to be a prognostic factor for RI and OS, and favorable cytogenetics for RI, LFS and OS [21]. In another study assessing the outcomes of allo-HSCT with PTCy in AML patients transplanted in CR1 from different types of donor including MSD, UD or Haplo donor, we showed no significant differences in LFS and OS between studied groups, however patients treated with Haplo-HSCT had increased

Table 4. Multivariate analysis.

		HR	95% CI	*P* value
OS	age (per 10 years)	1.21	0.95–1.55	0.13
	favorable risk cytogenetics	0.21	0.05–0.91	0.036
	time from diag. to allo-HSCT > 18 mo	0.31	0.15–0.61	0.001
LFS	age (per 10 years)	1.11	0.87–1.40	0.40
	year of allo-HSCT	0.90	0.80–1.01	0.08
	favorable risk cytogenetics	0.34	0.10–1.14	0.08
	time from diag. to allo-HSCT > 18 mo	0.30	0.16–0.56	<0.001
RI	age (per 10 years)	0.83	0.61–1.12	0.22
	time from diag. to allo-HSCT > 18 mo	0.19	0.07–0.48	0.001
NRM	age (per 10 years)	1.83	1.24–2.69	0.002
aGVHD II-IV	age (per 10 years)	0.97	0.73–1.27	0.80
	year of allo-HSCT	0.84	0.73–0.98	0.027
	RIC vs MAC	2.16	0.96–4.83	0.062
cGVHD	age (per 10 years)	0.68	0.51–0.90	0.008
	female D to male R	0.15	0.03–0.64	0.011
	time from diag. to allo-HSCT > 18 mo	0.95	0.91–4.16	0.08
	RIC vs MAC	3.74	1.52–9.18	0.004
GRFS	age (per 10 years)	0.90	0.73–1.11	0.31
	year of allo-HSCT	0.87	0.78–0.97	0.009
	time from diag. to allo-HCT > 18 mo	0.40	0.24–0.69	0.001
	RIC vs MAC	1.81	0.99–3.28	0.052

Allo-HSCT allogeneic hematopoietic cell transplantation, *OS* overall survival, *LFS* leukemia-free survival, *RI* relapse incidence, *NRM* non-relapse mortality, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *GRFS* GVHD-free and relapse-free survival, *mo* months, *diag.* diagnosis, *RIC* reduced intensity conditioning, *MAC* myeloablative conditioning, *D* donor, *R* recipient, *HR* hazard ratio, *CI* confidence interval

risks of aGVHD and NRM, which was counterbalanced by a reduced risk of RI when compared to other donor types [19]. Results for the UD cohort were similar to the current study with incidence of aGVHD II-IV and III-IV being 28% and 8%, respectively, and 2-year total and extensive cGVHD of 32% and 18%, respectively. Other transplant outcome parameters including NRM, RI, LFS, OS and GRFS were 14%, 25%, 62%, 68% and 42%, respectively [19].

Of note, our current observed results in AML patients transplanted from UD in CR2 with PTCy as anti GVHD prophylaxis are similar to those observed in AML patients transplanted in CR1 from matched UD with PTCy with RI, NRM, LFS and OS incidences of 25.2%, 15.2%, 59.7% and 62.7%, respectively [16]. The similarity of transplantation outcomes regardless of being in CR2 or CR1 may speak to the unique biological properties of PTCy. Moreover, it may indicate that in allo-HSCT with PTCy, the GVL effect is not jeopardized despite the reduction in GVHD, and it may overcome the negative impact of the disease status [12, 13, 23, 28–30]. This may differ in allo-HSCT with conventional CNI- based anti GVHD prophylaxis as was recently demonstrated by Jentzsch *et al*. in a study of AML patients transplanted mostly from MSD and matched or mismatched UD. Outcomes in patients in CR2 were inferior to those of CR1, with higher relapse rates and inferior LFS [31].

The additional prognostic factors we observed for predicting transplantation outcome including adverse cytogenetics, increasing age, year of transplant and conditioning intensity were previously reported as poor prognostic factors for outcome of allo-HSCT in AML [15, 32–34]. This current retrospective study was transplantation registry-based, with several limitations including potential selection bias, the possibility of unavailable data that have not been considered, such as molecular, and measurable residual disease data, as well as missing cytogenetics for 23% of cases. We also lacked information on pre-transplant frontline treatments and the hematopoietic cell transplantation comorbidity index. In conclusion, the outcomes of allo-HSCT from UD with PTCy in AML patients transplanted in CR2 are similar to those previously reported for AML patients transplanted in CR1. PTCy is an effective method for GVHD prophylaxis in this setting with similar results to previously reported GVHD incidences in patients transplanted in CR2 using conventional CNI-based GVHD prophylaxis. Further studies, especially prospective in nature, are warranted to determine the role of PTCy in AML patients transplanted in CR2.

DATA AVAILABILITY

Professor Mohamad Mohty, and Dr Myriam Labopin from the ALWP will provide the data upon request.

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AUTHOR CONTRIBUTIONS

AN wrote the manuscript, designed the study, and interpreted the data. ML designed the study, performed the statistical analyses, interpreted the data, and edited the manuscript. MM designed the study, interpreted the data, and edited the manuscript. RS helped in writing the manuscript. AK, HLW, MR, DB, JV, IYA, GC, PR, YK, JS and FC reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

The scientific boards of the ALWP of the EBMT approved this study.

ADDITIONAL INFORMATION

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Correspondence and requests for materials should be addressed to Arnon Nagler.

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