

University of Groningen

**Should anti-thymocyte globulin be added in post-transplant cyclophosphamide based matched unrelated donor peripheral blood stem cell transplantation for acute myeloid leukemia? A study on behalf of the Acute Leukemia Working Party of the EBMT**

Spyridonidis, Alexandros; Labopin, Myriam; Brissot, Eolia; Moiseev, Ivan; Cornelissen, Jan; Choi, Goda; Ciceri, Fabio; Vydra, Jan; Remenyi, Peter; Rovira, Montserrat

*Published in:*  
Bone marrow transplantation

*DOI:*  
[10.1038/s41409-022-01816-1](https://doi.org/10.1038/s41409-022-01816-1)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2022

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Spyridonidis, A., Labopin, M., Brissot, E., Moiseev, I., Cornelissen, J., Choi, G., Ciceri, F., Vydra, J., Remenyi, P., Rovira, M., Meijer, E., Labussiere-Wallet, H., Blaise, D., Gorkom, G., Kroger, N., Koc, Y., Giebel, S., Bazarbachi, A., Savani, B., ... Mohty, M. (2022). Should anti-thymocyte globulin be added in post-transplant cyclophosphamide based matched unrelated donor peripheral blood stem cell transplantation for acute myeloid leukemia? A study on behalf of the Acute Leukemia Working Party of the EBMT. *Bone marrow transplantation*, 57, 1774–1780. <https://doi.org/10.1038/s41409-022-01816-1>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

## ARTICLE



# Should anti-thymocyte globulin be added in post-transplant cyclophosphamide based matched unrelated donor peripheral blood stem cell transplantation for acute myeloid leukemia? A study on behalf of the Acute Leukemia Working Party of the EBMT

Alexandros Spyridonidis<sup>1✉</sup>, Myriam Labopin<sup>2</sup>, Eolia Brissot<sup>3</sup>, Ivan Moiseev<sup>4</sup>, Jan Cornelissen<sup>5</sup>, Goda Choi<sup>6</sup>, Fabio Ciceri<sup>7</sup>, Jan Vydra<sup>8</sup>, Péter Reményi<sup>9</sup>, Montserrat Rovira<sup>10</sup>, Ellen Meijer<sup>11</sup>, Hélène Labussière-Wallet<sup>12</sup>, Didier Blaise<sup>13</sup>, Gwendolyn van Gorkom<sup>14</sup>, Nicolaus Kröger<sup>15</sup>, Yener Koc<sup>16</sup>, Sebastian Giebel<sup>17</sup>, Ali Bazarbachi<sup>18</sup>, Bipin Savani<sup>19</sup>, Arnon Nagler<sup>20</sup> and Mohamad Mohty<sup>3</sup>

© The Author(s), under exclusive licence to Springer Nature Limited 2022

In this registry-based study which includes acute myeloid leukemia patients who underwent a matched unrelated donor allogeneic peripheral-blood stem cell transplantation in complete remission and received post-transplant cyclophosphamide (PTCY) as graft-versus-host disease (GvHD) prophylaxis, we compared 421 recipients without anti-thymocyte globulin (ATG) with 151 patients with ATG. The only significant differences between PTCY and PTCY + ATG cohorts were the median year of transplant and the follow-up period (2017 vs 2015 and 19.6 vs 31.1 months, respectively,  $p < 0.0001$ ). Overall, 2-year survival was 69.9% vs 67.1% in PTCY and PTCY + ATG, respectively, with deaths related to relapse (39% vs 43.5%), infection (21.9% vs 23.9%) or GvHD (17.1% vs 17.4%) not differing between groups. On univariate comparison, a significantly lower rate of extensive chronic GvHD was found when ATG was added (9.9% vs 21%,  $p = 0.029$ ), a finding which was not confirmed in the multivariate analysis. The Cox-model showed no difference between PTCY + ATG and PTCY alone with respect to acute and chronic GvHD of all grades, non-relapse mortality, relapse, leukemia-free survival, overall survival, and GvHD-free-relapse-free survival between study cohorts. Our results highlight that the addition of ATG in PTCY does not provide any extra benefit in terms of further GvHD reduction, better GRFS or better survival.

*Bone Marrow Transplantation*; <https://doi.org/10.1038/s41409-022-01816-1>

## INTRODUCTION

The curative potential of allogeneic hematopoietic cell transplantation (allo-HCT) is hampered by graft-versus-host disease (GvHD) [1, 2]. Although allo-HCT has been routinely performed for more than six decades, the search for the ideal partner or alternative to conventional immunosuppressive (CIS) agents (such as calcineurin inhibitors, methotrexate, mycophenolate mofetil, sirolimus) to further reduce GvHD while sparing the graft-versus-leukemia (GVL) effect and immune-reconstitution is ongoing [3]. Anti-thymocyte globulin

(ATG) has proven beneficial in reducing GvHD and improving outcomes in the human leukocyte antigen (HLA)-matched unrelated donor (MUD) peripheral blood stem cell transplantation (PBSCT), thus being highly recommended and broadly used in Europe for this setting [4–7]. Post-transplantation cyclophosphamide (PTCY) has been developed over the past decade as a breakthrough technique for in vivo T-cell depletion demonstrating remarkable anti-GvHD efficacy in haploidentical HCT, whereas its value in the MUD-setting is still under intensive clinical investigation [8–12].

<sup>1</sup>Bone Marrow Transplantation Unit and Institute of Cellular Therapy, University of Patras, Patras, Greece. <sup>2</sup>EBMT Statistical Unit, Sorbonne Université, Hospital Saint-Antoine, Paris, France. <sup>3</sup>Department of Hematology, Hospital Saint Antoine, Paris, France. <sup>4</sup>Gorbacheva Research Institute, Pavlov Univ., St Petersburg, Russian Federation. <sup>5</sup>Erasmus MC Cancer Institute, University Medical Center, Rotterdam, Netherlands. <sup>6</sup>Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands. <sup>7</sup>Ospedale San Raffaele s.r.l., Haematology and BMT, Milano, Italy. <sup>8</sup>Institute of Hematology and Blood Transfusion, Prague, Czech Republic. <sup>9</sup>Dél-pesti Centrumkórház, Budapest, Hungary. <sup>10</sup>Hospital Clinic, Institute of Hematology & Oncology, Barcelona, Spain. <sup>11</sup>VU University Medical Center, Amsterdam, Netherlands. <sup>12</sup>Hôpital Lyon Sud, Hospices Civils de Lyon, Pierre Bénite, France. <sup>13</sup>Programme de Transplantation & Therapie Cellulaire, Centre de Recherche en Cancérologie de Marseille, Marseille, France. <sup>14</sup>University Hospital Maastricht, Maastricht, Netherlands. <sup>15</sup>University Hospital Eppendorf, Bone Marrow Transplant Centre, Hamburg, Germany. <sup>16</sup>Bone Marrow Transplant Unit, Medicana International Hospital, Istanbul, Turkey. <sup>17</sup>Maria Skłodowska-Curie National Research Institute of Oncology, Gliwice, Poland. <sup>18</sup>Bone Marrow Transplantation Program, Department of Internal Medicine, American University of Beirut-Medical Center, Beirut, Lebanon. <sup>19</sup>Vanderbilt University Medical Center, Nashville, TN, USA. <sup>20</sup>Hematology Division, Chaim Sheba Medical Center, Tel-Hashomer, Israel. ✉email: [spyridonidis@upatras.gr](mailto:spyridonidis@upatras.gr)

Received: 7 May 2022 Revised: 8 August 2022 Accepted: 19 August 2022

Published online: 07 September 2022

Following the demonstration that the use of PBSC haploidentical grafts result in more GvHD than bone marrow grafts [13, 14], groups have managed to reduce GvHD in haploidentical PBSCT by adding ATG to PTCY, however a concern of increased graft failure when reduced intensity conditioning (RIC) is used has been raised [15–17]. It is unknown whether such a dual PTCY + ATG T-cell depletion approach can also be safely applied and bring any added value in the MUD-PBSCT setting. In this retrospective registry-based study, we compared the outcomes of acute myeloid leukemia (AML) patients who underwent a MUD-PBSCT and received PTCY with or without ATG (PTCY vs PTCY + ATG)

## MATERIALS AND METHODS

### Study design and data collection

This is a retrospective, multicenter, registry-based analysis. Data were provided by the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT) registry in which >600 transplant centers report annually according to EBMT specific quality measures, all their consecutive HCTs. 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 study was conducted in accordance with the Declaration of Helsinki guidelines. Transplant data from allo-HCTs performed between 2010 and 2019 were initially screened for availability of detailed GvHD prophylaxis information. Included in the analysis were adult patients diagnosed with AML who underwent a first allogeneic PBSCT (allo-PBSCT) in first or second complete remission (CR1/CR2) from a 10/10 or 9/10 HLA-MUD (HLA A, B, C, and DRB1 and DQB1 allelic typing) using PTCY as GvHD prophylaxis. The outcomes of patients who received PTCY plus ATG were compared with outcomes of those receiving PTCY without any further *in vivo* T-cell depletion. *Ex vivo* T-cell depletion was an exclusion criterion. The list of institutions reporting data included in this study is given in the Appendix in the Supplementary data. Raw data used in this study can be requested from M.L.

### Definitions and statistical analysis

The primary objective of the study was the impact of the addition of ATG to PTCY on GvHD rates. Acute GvHD (aGvHD) and chronic GvHD (cGvHD) were defined and graded according to standard criteria. Death without evidence of relapse (REL) defined non-relapse mortality (NRM). GvHD, REL, and NRM were calculated using cumulative incidence curves in a competing risk setting. The probabilities of overall survival (OS) defined as time to death from any cause, leukemia-free survival (LFS) defined as time being alive without evidence of REL, and the refined GvHD-free, relapse-free survival (GRFS) defined as time being alive with neither grade III–IV aGvHD nor severe cGvHD nor disease REL at any time point were calculated from time of transplant using the Kaplan–Meier estimate [18]. The follow-up time was calculated using the reverse Kaplan–Meier method. Endpoints were censored at 2 years for all comparisons to take into account the difference of follow-up between the two groups. Univariate comparisons between groups were performed using the Chi-square and Fischer's exact test for categorical variables and the Mann–Whitney test for continuous variables, the Gray's statistic for cumulative incidence functions (GvHD, NRM, REL) and the log-rank test for survival outcomes (OS, LFS and GRFS). Multivariate analysis was performed using a Cox proportional-hazards model which included variables differing significantly between the groups, factors known to be associated with outcomes, plus a center frailty effect to take into account the heterogeneity across centers, as previously reported [18]. All tests were two-sided with the type 1 error rate fixed at 0.05. Statistical analyses were performed with SPSS 27.0 (SPSS Inc., Chicago, IL, USA) and R 4.1.1 (R Development Core Team, Vienna, Austria, URL:<https://www.R-project.org/>).

## RESULTS

### Characteristics of study population

Baseline patient, disease, and transplant characteristics are shown by study cohort (PTCY vs PCTY + ATG) in Table 1. In total, 572 patients with a median age of 50.9 years (range 18–75.6) were included in the analysis. With a median follow-up of 22.9 months (95% confidence interval [CI] 19.2–25.1) for the whole population, the cumulative incidences of grade II–IV and grade III–IV aGvHD

were 27.4% (95% CI 23.8–31.2) and 9.1% (95% CI 6.9–11.7), respectively, and of cGvHD (all grades) and of extensive cGvHD were 34.3% (30–40) and 17.6% (95% CI 14–21.8), respectively. At 2-years, the outcomes for the entire cohort were as follows: NRM 14.1% (95% CI 11–17.6), REL 28% 95% CI (23.5–32.5), LFS 63.9% (95% CI 59.2–68.3), and OS 69.3% (95% CI 64.7–73.5).

Four hundred and twenty-one patients received PTCY as GvHD prophylaxis and 151 received PTCY + ATG. The most frequently ATG formulation used was thymoglobulin (72.7%) given at a median dose of 5 mg/kg (range: 2.5–10; interquartile range: 5–7.5), whereas anti-Jurkat ATG (Grafalon, Neovii Pharmaceuticals) was given at a median dose of 40 mg/kg (range: 20–60; interquartile range: 30–60). PTCY and PCTY + ATG groups were well balanced regarding transplant and disease characteristics with the only significant difference between the two cohorts being the median year of transplant (2017 vs 2015,  $p < 0.0001$ ) and the corresponding median follow-up period (19.6 vs 31.1 months,  $p < 0.0001$ ), respectively. Very few patients did not receive any further CIS treatment (1.7% in PTCY vs 4.6% in PTCY + ATG). Similar proportions of PTCY and PTCY + ATG patients received either one (37.5% vs 36.4%) or two (60.8% vs 58.9%) CIS drugs, respectively ( $p = ns$ ). The combinations of these drugs varied between the PTCY and PTCY + ATG groups, being mainly cyclosporine or tacrolimus with mycophenolate mofetil (51.5% and 42.4%, respectively) (data shown in Supplementary Table 1).

### Univariate analysis of transplant outcomes with PTCY vs PTCY + ATG

The proportion of patients who experienced graft failure was low (2%) in both groups. In the univariate comparison, no significant differences in the cumulative incidence of aGvHD of all grades (II–IV, III–IV) and of overall cGvHD was found between PTCY and PTCY + ATG treated patients (Table 2 and Fig. 1). There was a significant reduction of extensive cGvHD from 21% (95% CI, 16–26.4) to 9.9% (95% CI, 5.2–16.4) in patients receiving ATG ( $p = 0.029$ ). The cumulative incidences of REL and NRM and the probabilities of 2-year OS, LFS and GRFS did not differ significantly between study cohorts with the corresponding survival curves being superimposable (Fig. 2). Two-year OS was 69.9% vs 67.1% in PTCY and PTCY + ATG, respectively, with the causes of death being disease recurrence (39% vs 43.5%), infection (21.9% vs 23.9%) and GvHD (17.1% vs 17.4%), respectively. The estimated cumulative incidence of death due to either infection or GvHD was not significantly different in the ATG group as compared to the non-ATG group. Cardiac toxicity accounted for only 2 deaths (both in the ATG-naive cohort). Other causes of death did not differ between groups and are given in Supplementary Table 2.

### Multivariate analysis of transplant outcomes with PTCY vs PTCY + ATG

The multivariate analysis did not confirm that adding ATG to PTCY reduced extensive cGvHD (Table 3). In the Cox model, the risks of aGvHD and cGvHD of all grades were not influenced by ATG (Supplementary Table 3). After adjustment for factors known to be associated with outcomes, the addition of ATG to PTCY was not found to significantly influence REL or NRM rates, nor to affect probabilities of OS, LFS and GRFS (Table 3). As expected, poor karyotype negatively affected the risk of REL (hazard ratio [HR] 2.59, 95% CI 1.74–3.86,  $p < 0.0001$ ), OS (HR 1.9, 95% CI 1.3–2.77,  $p = 0.0009$ ), LFS (HR 2.03, 95% CI 1.43–2.87,  $p < 0.0001$ ) and GRFS (HR 1.75, 95% CI 1.31–2.35,  $p = 0.0002$ ). An incremental age of 10 years was associated with increased NRM risk (HR 1.61, 95% CI 1.27–2.04,  $p < 0.0001$ ) and lower OS (HR 1.29, 1.11–1.48,  $p = 0.0006$ ). A good Karnofsky performance status score of  $\geq 90\%$  at the time of transplantation was associated with improved LFS (HR 0.66, 95% CI 0.46–0.94,  $p = 0.02$ ), OS (HR 0.66, 95% CI 0.46–0.96,  $p = 0.03$ ) and GRFS (HR 0.71, 95% CI 0.53–0.94,  $p = 0.019$ ) (Supplementary Table 4).

**Table 1.** Patient, disease and transplantation characteristics.

Variable		PTCy (N = 421)	PTCy + ATG (N = 151)	P
Age (years)	median (min–max) (IQR)	51 (18–75.6) (38.6–63.2)	49.8 (20.1–71.1) (37.2–57.5)	0.081
Year allo-HCT	Median (min–max)	2017 (2010–2019)	2015 (2010–2019)	<b>&lt;0.0001</b>
Follow-up	median (95% CI)	19.6 (16.4–23.2)	31.1 (27.4–48.0)	<b>&lt;0.0001</b>
Sex	M	247 (59%)	86 (57%)	0.71
	F	174 (41%)	65 (43%)	
Diagnosis	de novo	367 (87%)	129 (85%)	0.59
	sec AML	54 (12.8%)	22 (14.6%)	
Cytogenetics	not adverse	334 (79.3%)	123 (81.5%)	0.58
	adverse	87 (20.7%)	28 (18.5%)	
Status at HCT	CR1	350 (83.1%)	118 (78.1%)	0.17
	CR2	71 (16.9%)	33 (21.9%)	
Type of donor	UD 10/10	272 (64.6%)	99 (65.6%)	0.83
	UD 9/10	149 (35.4%)	52 (34.4%)	
ATG	Thymoglobulin	0	80 (72.7%)	
	Grafalon (Neovii)	0	30 (27.3%)	
	missing		41	
CIS	No drugs	7 (1.7%)	7 (4.6%)	0.13
	1 drug	158 (37.5%)	55 (36.4%)	
	2 drugs	256 (60.8%)	89 (58.9%)	
Conditioning	MAC	214 (51.1%)	87 (59.2%)	0.09
	RIC	205 (48.9%)	60 (40.8%)	
	missing	2	4	
KPS	<90	103 (25%)	38 (25.7%)	0.87
	≥90	309 (75%)	110 (74.3%)	
	missing	9	3	
HCT-CI	0	203 (59.2%)	52 (58.4%)	0.35
	1 or 2	50 (14.6%)	18 (20.2%)	
	≥3	90 (26.2%)	19 (21.3%)	
	missing	78	62	
F to M	no	358 (85.2%)	130 (86.7%)	0.67
	yes	62 (14.8%)	20 (13.3%)	
	missing	1	1	
Patient CMV	negative	107 (25.8%)	34 (22.8%)	0.47
	positive	308 (74.2%)	115 (77.2%)	
	missing	6	2	
Donor CMV	negative	233 (55.7%)	76 (50.7%)	0.28
	positive	185 (44.3%)	74 (49.3%)	
	missing	3	1	
Engraftment	graft failure	9 (2.2%)	3 (2%)	0.91
	engrafted	406 (97.8%)	146 (98%)	
	missing	6	2	

Cytogenetic risk according to MRC classification (Blood 2010;116: 354–65). Significant *p* values are given in bold.

AML Acute Myeloid Leukemia, sec AML secondary AML, ATG anti-T-cell globulin, CI 95% confidence interval, CIS conventional immunosuppression (cyclosporine, tacrolimus, mycophenolate mofetil, methotrexate, sirolimus), CMV cytomegalovirus, CR complete remission, F female, GvHD Graft versus Host Disease, GRFS GvHD-free, relapse-free survival, HCT allogeneic hematopoietic cell transplantation, HCT-CI HCT-Comorbidity Index, HR hazard ratio, IQR interquartile range, KPS Karnofsky Performance Status, LFS leukemia-free survival, M Male, MAC myeloablative conditioning, NRM non-relapse mortality, OS overall survival, REL relapse, PTCY post-transplantation cyclophosphamide, RIC reduced intensity conditioning, UD unrelated donor.

## DISCUSSION

To date, the question of the best combination of GvHD-preventing drugs in the MUD-PBSCT remains unanswered. ATG is recommended by an international expert panel for this setting, while PTCY is increasingly used potentially replacing

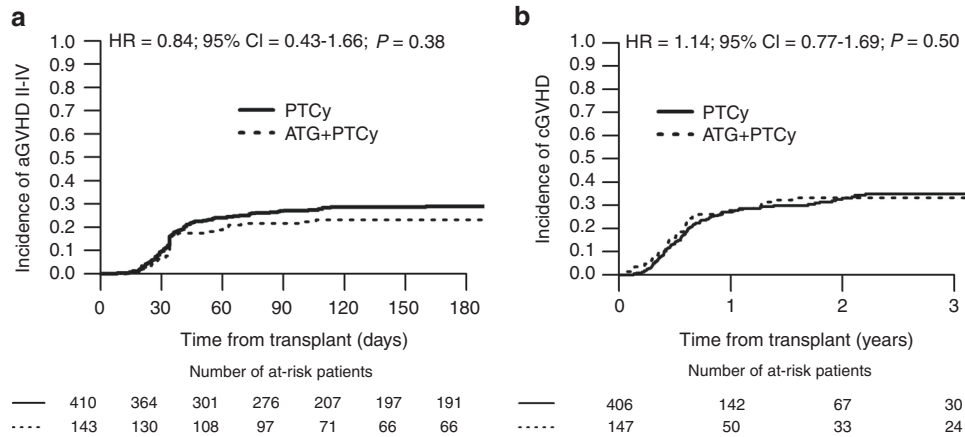
ATG [7–11]. The conceptual difference between ATG and PTCY is that the former depletes T-cells in a dose dependent manner, whereas the latter should eliminate only the proliferating alloreactive donor lymphocytes while preserving the resting memory T-cells. The rationale of combining ATG and PTCY is to

**Table 2.** Cumulative incidence (95% CI) of GvHD and 2-year survival outcomes.

	PTCY	PTCY + ATG	P
Acute GvHD, grade II–IV	28.9% (24.6–33.4)	23.1% (16.6–30.4)	0.2
Acute GvHD, grade III–IV	9.6% (7–12.7)	7.7% (4.1–12.8)	0.52
chronic GvHD, any grade	34.8% (29.3–40.4)	33.3% (25–41.7)	0.9
chronic GvHD, extensive	21% (16–26.4)	9.9% (5.2–16.4)	<b>0.029</b>
REL	26.7% (21.5–32.1)	31.1% (22.8–39.7)	0.2
NRM	14.8% (10.9–19.3)	13.1% (8.1–19.5)	0.95
LFS	64.7% (59–69.9)	61.2% (52–69.1)	0.17
OS	69.9% (64.3–74.9)	67.1% (58–74.6)	0.22
GRFS	49.5% (43.7–55)	53.1% (43.9–61.4)	0.94

Significant *p* values are given in bold.

AML Acute Myeloid Leukemia, sec AML secondary AML, ATG anti-T-cell globulin, CI 95% confidence interval, CIS conventional immunosuppression (cyclosporine, tacrolimus, mycophenolate mofetil, methotrexate, sirolimus), CMV cytomegalovirus, CR complete remission, F female, GvHD Graft versus Host Disease, GRFS GvHD-free, relapse-free survival, HCT allogeneic hematopoietic cell transplantation, HCT-CI HCT-Comorbidity Index, HR hazard ratio, IQR interquartile range, KPS Karnofsky Performance Status, LFS leukemia-free survival, M Male, MAC myeloablative conditioning, NRM non-relapse mortality, OS overall survival, REL relapse, PTCY post-transplantation cyclophosphamide, RIC reduced intensity conditioning, UD unrelated donor.

**Fig. 1** Cumulative incidence of GvHD in patients receiving PTCY or PTCY + ATG. **a** acute GvHD grade II–IV and **b** chronic GvHD.

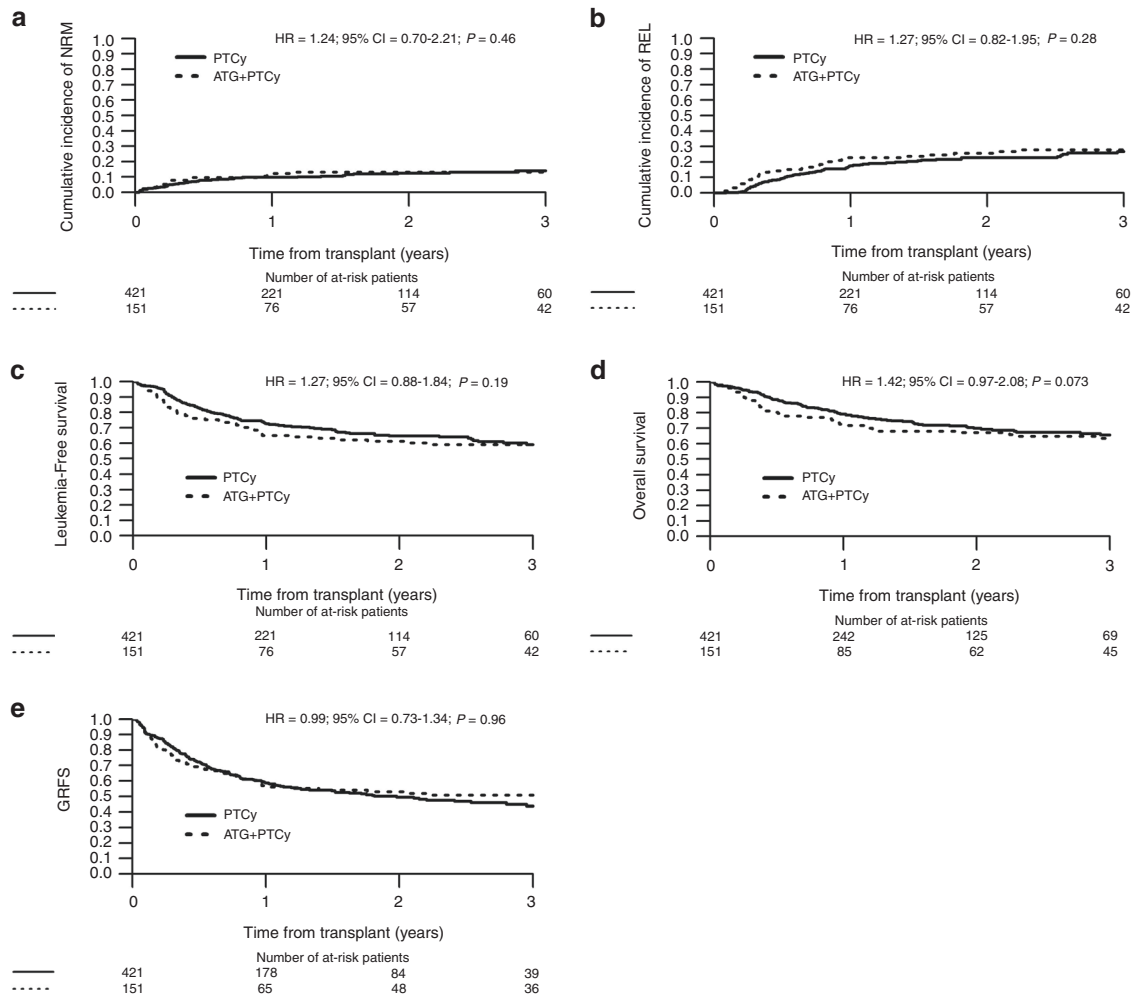
further reduce GvHD. Theoretically, such a combination is not synergistic as ATG depletes the T-cells which should allo-react and proliferate in order to become prone to cyclophosphamide elimination [19]. To our knowledge, this is the first large series study demonstrating the feasibility of the PTCY + ATG combination in MUD-PBSCT and analyzing the impact of adding ATG to PTCY in this setting.

We found that PTCY + ATG can be safely given in MUD-PBSCT without compromising engraftment and without modifying the risk of NRM when compared to PTCY-treated patients. Our findings concur with single center studies of HLA-matched PBSCT reporting reliable donor cell engraftment and low NRM rates with PTCY + ATG similar to standard CIS-treated historical controls [20–24]. We cannot exclude the possibility that addition of ATG to PTCY induced more viral reactivations and specific morbidity, as such information could not be captured in our retrospective analysis. Nevertheless, both the incidence of NRM (any cause) and NRM due to infections did not differ between PTCY and PTCY + ATG treated patients. Furthermore, there was no significant difference between NRM for CMV negative ( $n = 34$ ) vs positive ( $n = 115$ ) patients in the group receiving ATG + PTCY. As PTCY is associated with an increased risk of cytomegalovirus (CMV) infection, it remains to be seen whether the low NRM rates of the PTCY + ATG combination reported here will be replicated in centers where access to pre-emptive or prophylactic antiviral therapy is not ideal [25].

Though the univariate analysis showed an improvement of extensive cGvHD when ATG was added to PTCY, this effect was not apparent on multivariate analysis. Indeed, the Cox proportional-hazards model did not detect any difference between PTCY + ATG vs PTCY patients with respect to both aGvHD and cGvHD (of all grades). It must be noticed that we could not test whether PTCY + ATG may have remained significant in improving cGVHD in the Cox model if the National Institute of Health (mild, moderate, severe) instead of the 2-tier cGVHD grading (limited, extensive) was used. In contrast, ATG has been shown in randomized studies to reduce GvHD, especially cGvHD, when added to standard CIS in MUD-PBSCT [4–6]. On the other hand, one can assume that PTCY or ATG alone could control GvHD efficiently, making a combined PTCY + ATG in vivo T-cell depletion redundant. In support of this, our PTCY-treated patients who did not receive ATG ( $n = 421$ ) had a remarkably low cumulative incidence of grade III–IV aGvHD (9.6%) and extensive cGvHD (21%). Similar low severe aGvHD grade III–IV (2–6%) and extensive cGvHD (19–22%) rates have been recently reported in prospective HLA-matched (MRD/MUD) PTCY-based RIC-PBSCT studies [26, 27]. It is of note that in our series only 2.4% of patients did not receive any CIS; PTCY without any CIS has resulted in a high incidence of aGvHD in HLA-matched RIC-PBSCT [28, 29].

The addition of ATG could potentially be detrimental to the GVL effect. However, no differences were observed in REL rates between groups, with adverse cytogenetics remaining the most





**Fig. 2** Outcomes of patients according to the use of PTCy or PTCy + ATG. **a** Non-relapse mortality (NRM), **b** relapse (REL) incidence, **c** leukemia-free survival (LFS), **d** overall survival (OS) and **e** GvHD-free, relapse-free survival (GRFS).

important factor affecting REL in the multivariate analysis. This result should be taken with caution, as we focused only on AML patients transplanted in CR with less risk of REL. In agreement with our results, previous studies focusing on such populations (AML, CR) found that ATG given at the EBMT-recommended dose and schedule [30] (as expected to have been used in our transplanted cohort) does not impact REL [31, 32]. Another explanation for the comparable REL rates in the PTCy and PTCy + ATG groups could be related to their differential reconstitution of natural killer (NK) cells. PTCy + ATG vs PTCy-treated haploidentical recipients have a faster reappearance of NK cells which mature more rapidly and thus, the addition of ATG may have restored the possible delayed NK-mediated GVL effect seen with PTCy alone [16]. Future studies should aim to define more precisely the diverse and multifaceted effects of the PTCy + ATG combination on immune-reconstitution.

Considering the absence of any impact of the addition of ATG to PTCy on GvHD, NRM, and REL, it is not surprising that there was no significant difference in survival (OS, LFS, GRFS) between PTCy and PTCy + ATG treated patients. Clinically, these results call into question the rationale of intensifying GvHD prophylaxis with ATG in MUD-PBSCT when PTCy is used, a finding which should be validated in prospective trials. Though our registry data suggest a more frequent use of ATG in the earlier years (median year of PTCy + ATG 2015 vs 2017 for PTCy alone), this difference is most probably not because clinicians decreased their use of ATG, but rather because some centers started to use PTCy-alone GvHD

prophylaxis more recently. Indeed, the median year of transplant in centers ( $n = 25$ ) used both PTCy (280 pts) and PTCy + ATG (72 pts) was 2017 and 2017.5, respectively, whereas in centers using exclusively PTCy-alone was 2018 (38 centers, 213 pts). Of note, our study does not evaluate whether ATG or PTCy is better in MUD-PBSCT. While a prospective study answering this question is ongoing [10], retrospective EBMT studies have suggested a superiority of PTCy over ATG in terms of less aGvHD and better GRFS in 1 HLA-mismatched (9/10) unrelated HCT and comparable outcomes in well-matched (10/10) MUD-HCT [9, 11].

This study has all the inherent limitations of a retrospective registry-based analysis. Although we focused on a relatively uniform patient population (AML, CR, PBSCT, MUD) and tried to overcome further heterogeneity through multivariate modelling, there are still unmeasured or only partially measured factors (e.g., ATG pharmacokinetics [33], time of CIS withdrawal, MRD status) that could not be captured and adjusted for. Notwithstanding, this is the largest patient series showing that ATG can be safely combined with PTCy in the MUD-PBSCT setting enabling reliable donor cell engraftment, resulting in low rates of NRM and without detrimental effect on disease control in AML patients transplanted in CR. However, the addition of ATG to PTCy in this setting does not seem to provide any benefit in terms of GvHD reduction, meaningful improvement of quality of life (measured as better GRFS) or better survival. As the combination of ATG and PTCy is a feasible strategy for GvHD prevention in MUD-PBSCT, future

**Table 3.** Multivariate analysis for transplant outcomes.

PTCY + ATG vs PTCY	HR (95% CI)	P
Acute GvHD, grade II–IV	0.84 (0.56–1.25)	0.38
Acute GvHD, grade III–IV	0.84 (0.43–1.66)	0.62
chronic GvHD, any grade	1.14 (0.77–1.69)	0.5
chronic GvHD, extensive	0.58 (0.29–1.18)	0.13
REL	1.27 (0.82–1.95)	0.28
NRM	1.24 (0.7–2.21)	0.46
LFS	1.27 (0.88–1.84)	0.19
OS	1.42 (0.97–2.08)	0.073
GRFS	0.99 (0.73–1.34)	0.96

Variables included in the Cox proportional-hazards model were: PTCy + ATG vs PTCy, number of HLA mismatches (10/10 or 9/10), status at transplant (CR2 vs CR1), cytogenetic risk group (adverse vs other), sex matching (female donor to male recipient vs other), Karnofsky Performance Status ( $\geq 90$  vs  $< 90$ ), conditioning intensity (RIC vs MAC).

AML Acute Myeloid Leukemia, sec AML secondary AML, ATG anti-T-cell globulin, CI 95% confidence interval, CIS conventional immunosuppression (cyclosporine, tacrolimus, mycophenolate mofetil, methotrexate, sirolimus), CMV cytomegalovirus, CR complete remission, F female, GvHD Graft versus Host Disease, GRFS GvHD-free, relapse-free survival, HCT allogeneic hematopoietic cell transplantation, HCT-CI HCT-Comorbidity Index, HR hazard ratio, IQR interquartile range, KPS Karnofsky Performance Status, LFS leukemia-free survival, M Male, MAC myeloablative conditioning, NRM non-relapse mortality, OS overall survival, REL relapse, PTCY post-transplantation cyclophosphamide, RIC reduced intensity conditioning, UD unrelated donor.

prospective studies might prove that such a dual PTCY + ATG in vivo T-cell depletion in the early post-transplant period could allow the sparing of long-term immunosuppression with CIS agents.

#### DATA AVAILABILITY

AS, ML, BS, AN, and MM had full access to all the data in the study (available upon data-specific request).

#### REFERENCES

- Zeiser R, Blazar BR. Acute Graft-versus-Host Disease — Biologic Process, Prevention, and Therapy. *N Engl J Med*. 2017;377:2167–79.
- Zeiser R, Blazar BR. Pathophysiology of Chronic Graft-versus-Host Disease and Therapeutic Targets. *N Engl J Med*. 2017;377:2565–79.
- Spyridonidis A. How much immunosuppression do we need? *Blood*. 2017;129:1241–3.
- Chang Y-J, Wu D-P, Lai Y-R, Liu Q-F, Sun Y-Q, Hu J, et al. Antithymocyte Globulin for Matched Sibling Donor Transplantation in Patients With Hematologic Malignancies: A Multicenter, Open-Label, Randomized Controlled Study. *J Clin Oncol J Am Soc Clin Oncol*. 2020;38:3367–76.
- Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009;10:855–64.
- Walker I, Panzarella T, Couban S, Couture F, Devins G, Elemetry M, et al. Pre-treatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. *Lancet Oncol*. 2016;17:164–73.
- Bonifazi F, Rubio M-T, Bacigalupo A, Boelens JJ, Finke J, Greinix H, et al. Rabbit ATG/ATLG in preventing graft-versus-host disease after allogeneic stem cell transplantation: consensus-based recommendations by an international expert panel. *Bone Marrow Transpl*. 2020;55:1093–102.
- Bailén R, Kwon M, Pascual-Cascón MJ, Ferrà C, Sanz J, Gallardo-Morillo A, et al. Post-transplant cyclophosphamide for GVHD prophylaxis compared to ATG-based prophylaxis in unrelated donor transplantation. *Ann Hematol*. 2021;100:541–53.
- Battipaglia G, Labopin M, Kröger N, Vitek A, Afanasyev B, Hilgendorf I, et al. Posttransplant cyclophosphamide vs antithymocyte globulin in HLA-mismatched unrelated donor transplantation. *Blood*. 2019;134:892–9.
- Brissot E. Post-transplantation cyclophosphamide versus antithymocyte globulin after ric regimen allo-hct: first analysis of a prospective randomized multicenter trial in recipients of 10/10 matched donors. <https://ebmt2021.abstractserver.com/program/#/details/presentations/1316> (accessed Apr 2021).
- Brissot E, Labopin M, Moiseev I, Cornelissen JJ, Meijer E, Van Gorkom G, et al. Post-transplant cyclophosphamide versus antithymocyte globulin in patients with acute myeloid leukemia in first complete remission undergoing allogeneic stem cell transplantation from 10/10 HLA-matched unrelated donors. *J Hematol Oncol J Hematol Oncol*. 2020;13:87.
- Cytryn S, Abdul-Hay M. Haploidentical Hematopoietic Stem Cell Transplantation Followed by 'Post-Cyclophosphamide': The Future of Allogeneic Stem. *Cell Transplant Clin Hematol Int*. 2020;2:49–58.
- Bashey A, Zhang M-J, McCurdy SR, St Martin A, Argall T, Anasetti C, et al. Mobilized Peripheral Blood Stem Cells Versus Unstimulated Bone Marrow As a Graft Source for T-Cell-Replete Haploidentical Donor Transplantation Using Post-Transplant Cyclophosphamide. *J Clin Oncol J Am Soc Clin Oncol*. 2017;35:3002–9.
- Ruggeri A, Labopin M, Bacigalupo A, Gülbaz Z, Koc Y, Blaise D, et al. Bone marrow versus mobilized peripheral blood stem cells in haploidentical transplants using posttransplantation cyclophosphamide. *Cancer*. 2018;124:1428–37.
- El-Cheikh J, Devillier R, Dulery R, Massoud R, Al Chami F, Ghaoui N, et al. Impact of Adding Antithymocyte Globulin to Posttransplantation Cyclophosphamide in Haploidentical Stem-Cell Transplantation. *Clin Lymphoma Myeloma Leuk*. 2020;20:617–23.
- Makanga DR, Guillaume T, Willem C, Legrand N, Gagne K, Cesbron A, et al. Posttransplant Cyclophosphamide and Antithymocyte Globulin versus Post-transplant Cyclophosphamide as Graft-versus-Host Disease Prophylaxis for Peripheral Blood Stem Cell Haploidentical Transplants: Comparison of T Cell and NK Effector Reconstitution. *J Immunol Balt Md* 1950. 2020;205:1441–8.
- Wang Y, Wu D-P, Liu Q-F, Xu L-P, Liu K-Y, Zhang X-H, et al. Low-dose post-transplant cyclophosphamide and anti-thymocyte globulin as an effective strategy for GVHD prevention in haploidentical patients. *J Hematol Oncol J Hematol Oncol*. 2019;12:88.
- Kanate AS, Nagler A, Savani B. Summary of Scientific and Statistical Methods, Study Endpoints and Definitions for Observational and Registry-Based Studies in Hematopoietic Cell Transplantation. *Clin Hematol Int*. 2019;2:2–4.
- Nunes NS, Kanakry CG. Mechanisms of Graft-versus-Host Disease Prevention by Post-transplantation Cyclophosphamide: An Evolving Understanding. *Front Immunol*. 2019;10. <https://doi.org/10.3389/fimmu.2019.02668>.
- Alanazi W, Chen S, Lipton JH, Kim DD, Viswabandya A, Kumar R, et al. Post-Transplant Cyclophosphamide Combined with Anti-Thymocyte Globulin as Graft-versus-Host Disease Prophylaxis for Allogeneic Hematopoietic Cell Transplantation in High-Risk Acute Myeloid Leukemia and Myelodysplastic Syndrome. *Acta Haematol*. 2021;144:66–73.
- Kunacheewa C, Owattanapanish W, Jirabanditsakul C, Issaragrisil S. Post-Transplant Cyclophosphamide and Thymoglobulin, a Graft-Versus-Host Disease Prophylaxis in Matched Sibling Donor Peripheral Blood Stem. *Cell Transpl Cell Transpl*. 2020;29:963689720965900.
- Prem S, Atenafu EG, Al-Shaibani Z, Loach D, Law A, Lam W, et al. Low rates of acute and chronic GVHD with ATG and PTCy in matched and mismatched unrelated donor peripheral blood stem cell transplants. *Eur J Haematol*. 2019;102:486–93.
- Ruggeri A, Labopin M, Bacigalupo A, Afanasyev B, Cornelissen JJ, Elmaagacli A, et al. Post-transplant cyclophosphamide for graft-versus-host disease prophylaxis in HLA matched sibling or matched unrelated donor transplant for patients with acute leukemia, on behalf of ALWP-EBMT. *J Hematol Oncol J Hematol Oncol*. 2018;11:40.
- Salas MQ, Chen S, Lam W, Pasic I, Gerbitz A, Michelis FV, et al. Less Is More: Superior Graft-versus-Host Disease-Free/Relapse-Free Survival with Reduced-Intensity Conditioning and Dual T Cell Depletion in Acute Myelogenous Leukemia. *Biol Blood Marrow Transpl J Am Soc Blood Marrow Transpl*. 2020;26:1511–9.
- Goldsmith SR, Abid MB, Auletta JJ, Bashey A, Beitinjaneh A, Castillo P, et al. Posttransplant cyclophosphamide is associated with increased cytomegalovirus infection: a CIBMTR analysis. *Blood*. 2021;137:3291–305.
- Bolaños-Meade J, Reshef R, Fraser R, Fei M, Abhyankar S, Al-Kadhimi Z, et al. Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and cyclophosphamide; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced-intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203). *Lancet Haematol*. 2019;6:e132–e143.

27. De Jong CN, Meijer E, Bakunina K, Nur E, van Marwijk Kooij M, de Groot MR, et al. Post-Transplantation Cyclophosphamide after Allogeneic Hematopoietic Stem Cell Transplantation: Results of the Prospective Randomized HOVON-96 Trial in Recipients of Matched Related and Unrelated Donors. *Blood*. 2019;134:1–1.
28. Alousi AM, Brammer JE, Saliba RM, Andersson B, Popat U, Hosing C, et al. Phase II Trial of Graft-versus-Host Disease Prophylaxis with Post-Transplantation Cyclophosphamide after Reduced-Intensity Busulfan/Fludarabine Conditioning for Hematological Malignancies. *Biol Blood Marrow Transpl J Am Soc Blood Marrow Transpl*. 2015;21:906–12.
29. Bradstock KF, Bilmon I, Kwan J, Micklethwaite K, Blyth E, Deren S, et al. Single-Agent High-Dose Cyclophosphamide for Graft-versus-Host Disease Prophylaxis in Human Leukocyte Antigen-Matched Reduced-Intensity Peripheral Blood Stem Cell Transplantation Results in an Unacceptably High Rate of Severe Acute Graft-versus-Host Disease. *Biol Blood Marrow Transpl J Am Soc Blood Marrow Transpl*. 2015;21:941–4.
30. Penack O, Marchetti M, Ruutu T, Aljurf M, Bacigalupo A, Bonifazi F, et al. Prophylaxis and management of graft versus host disease after stem-cell transplantation for haematological malignancies: updated consensus recommendations of the European Society for Blood and Marrow Transplantation. *Lancet Haematol*. 2020;7:e157–e167.
31. Baron F, Labopin M, Blaise D, Lopez-Corral L, Vigouroux S, Craddock C, et al. Impact of in vivo T-cell depletion on outcome of AML patients in first CR given peripheral blood stem cells and reduced-intensity conditioning allo-SCT from a HLA-identical sibling donor: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transpl*. 2014;49:389–96.
32. Nagler A, Dholaria B, Labopin M, Socie G, Huynh A, Itälä-Remes M, et al. The impact of anti-thymocyte globulin on the outcomes of Patients with AML with or without measurable residual disease at the time of allogeneic hematopoietic cell transplantation. *Leukemia*. 2020;34:1144–53.
33. Admiraal R, van Kesteren C, Jol-van der Zijde CM, Lankester AC, Bierings MB, Egberts TCG, et al. Association between anti-thymocyte globulin exposure and CD4+ immune reconstitution in paediatric haemopoietic cell transplantation: a multicentre, retrospective pharmacodynamic cohort analysis. *Lancet Haematol*. 2015;2:e194–203.

## ACKNOWLEDGEMENTS

We thank the ALWP-EBMT staff for help with data management. The study was accomplished thanks to the contributing centers of the EBMT registry which provided patient data; a complete list appears in the Supplementary Appendix.

## AUTHOR CONTRIBUTIONS

AS, ML, and MM designed the study; ML performed the statistical analyses; AS wrote the paper; BS, AN, and MM revised the paper; EB, IM, JC, GC, FC, JV, PR, MR, EM, HLW, DB, GG, NK, YK, SG, AB, BS, AN, MM were the principal investigators at the centers recruiting the largest numbers of patients for the study. All authors reviewed the final version of the paper.

## COMPETING INTERESTS

The authors declare no competing interests.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the institutional review board at each site and complied with country-specific regulatory requirements. The study was conducted in accordance with the declaration of the Helsinki and Good Clinical Practice guidelines. Patients provide informed consent authorizing the use of their personal information for research purposes.

## ADDITIONAL INFORMATION

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41409-022-01816-1>.

**Correspondence** and requests for materials should be addressed to Alexandros Spyridonidis.

**Reprints and permission information** is available at <http://www.nature.com/reprints>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.