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ORIGINAL ARTICLE

Grade 2 acute GVHD is a factor of good prognosis in patients receiving peripheral blood stem cells haplo-transplant with post-transplant cyclophosphamide

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ABSTRACT

Background: The impact of acute graft versus host disease (GVHD) on survivals for patients receiving a haploidentical allogeneic stem-cell transplant (Allo-SCT) with peripheral blood stem-cells (PBSC) complemented by post-transplant cyclophosphamide (PTCY) is ill-known.

Material and methods: This retrospective study included 131 patients who received a PBSC haplograft in order to precise the impact of acute GVHD on outcomes. There were 78 males and 53 females and the median age for the whole cohort was 59 years (range: 20–71). Thirty-five patients were allografted for a lymphoid disease and 96 for a myeloid malignancy, including 67 patients with acute myeloid leukemia (AML).

Results: The cumulative incidence (CI) of day 100 grade 2–4 and 3–4 acute GVHD was 43.4 + 4.6% and 16.7 + 3.4%, respectively. The 2-year CI of moderate/severe chronic GVHD was 10.1 + 2.8%. The only factor affecting the occurrence of GVHD was GVHD prophylaxis. Indeed, CI of day 100 grade 2–4 (but not grade 3–4) acute GVHD was significantly reduced when adding anti-thymoglobulin (ATG) to PTCY. However, in multivariate analysis, grade 2 acute GVHD was significantly associated with better disease-free (HR: 0.36; 95%CI: 0.19–0.69, $p = .002$) and overall (HR: 0.35; 95%CI: 0.1–0.70, $p = .003$) survivals. The same results were observed when considering only AML patients.

Conclusion: Acute grade 2 GVHD is a factor of good prognosis after PBSC haplotransplant with PTCY. Further and larger studies are needed to clarify the complex question of GVHD prophylaxis in the setting of haplo-transplant, especially that of combining ATG and PTCY.

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Introduction

The prophylaxis and management of graft versus host disease (GVHD) have dramatically improved in recent years [1]. The most spectacular breakthrough has been represented by the use of high-dose post-transplant cyclophosphamide (PTCY), that has shown a significant efficacy at reducing the incidence of both acute (aGVHD) and chronic GVHD, initially in the haplo-identical hematopoietic stem cell transplant (SCT) setting [2,3], and nowadays also for patients receiving an allogeneic SCT (Allo-SCT) from matched or 9/10 mismatched donors [4–7]. Historically, PTCY supplementation was first considered for haplo-identical Allo-SCT with bone marrow (BM), considering that BM grafts contained low levels of donor lymphocytes, the first effector cells responsible for GVHD. However, for practical reasons, a number of institutions have more recently considered peripheral blood stem cells (PBSC) as a better source of hematopoietic stem cells after mobilization. Comparison of the efficacy of BM vs PBSC Allo-SCT, for matched or haploidentical SCT with PTCY supplementation has shown PBMC to be responsible for a significantly higher incidence of grade 2–4 (40/50%) [8,9], and grade 3/4 (14% vs 4%) [9] aGVHD or chronic GVHD (41% vs 20%) [8]. Yet, these results can be counterbalanced by a significant lower risk of relapse in leukemic patients, suggesting that PBSC should be preferred rather than BM to favor a graft-versus-leukemia (GVL) effect.

Recently, the Baltimore group has shown that the development of grade 2 aGVHD was associated with better survival after matched or haplo-identical BM Allo-SCT using PTCY as GVHD prophylaxis [10,11]. The conclusion was that the ability of PTCY to limit grade 3–4 aGVHD while maintaining grade 2 aGVHD/Graft-versus-disease effect could contribute to the efficacy of this schedule, while further attempts to reduce aGVHD may be detrimental. By contrast, an Italian study did not find any impact of grade 2 aGVHD after BM

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haplo-identical SCT with PTCY [12]. These controversial results led us to further examine the impact of aGVHD on outcomes when patients are transplanted with PBSC instead of BM while receiving PTCY as GVHD prophylaxis.

Patients and methods

Study design, patient selection and data collection

The main objective of this retrospective study was to decipher the impact of aGVHD (especially grade 2) on survivals in a cohort of haplo-identical Allo-SCT patients receiving PBSC as source of graft and PTCY as GVHD prophylaxis. Cases from two French centers (Nantes and Besançon University Hospitals) were combined for the purpose of this study which included 131 patients transplanted between December 2013 and September 2019 (Besançon $n = 37$; Nantes $n = 94$). Follow-up for all patients was updated as of March 2020. All patients had provided informed consent for data entry into the European Bone Marrow Transplantation (EBMT) group registry database for observational/retrospective studies. The study was performed in accordance with the Declaration of Helsinki. The institutional review boards of Nantes and Besancon reviewed and approved the study.

Conditioning, graft source and GVHD prophylaxis

Three conditioning regimens were used: (i) a Baltimore-based RIC regimen with fludarabine [2] or clofarabine [13]; (ii) a CloB2 regimen [14] or (iii) a TBF regimen [15]. The three conditioning regimens were used in Nantes while the Besançon team used TBF only. All patients received PBSC as stem cell source and PTCY 50 mg/kg/d on $d + 3$ and $d + 4$ with cyclosporine and mycophenolate mofetil (MMF) as GVHD prophylaxis. Moreover, anti-thymoglobulin (ATG, Thymoglobuline®, Sanofi) 2.5 mg/kg on d −1 was used for all patients conditioned by the CloB2 regimen and some of the patients who received a TBF regimen.

Supportive care

In both transplant centers, patients received standard broad antimicrobial prophylaxis combining antifungal, antibiotic, antiviral and antiparasitic agents: a beta-lactam-based prophylaxis for *Pneumococci* and encapsulated bacteria initiated after aplasia, acyclovir or valacyclovir to prevent herpes and varicella infections, cotrimoxazole or atovaquone for *Pneumocystis* infections, fluconazole for fungal infections or posaconazole in case of GVHD.

Statistical analyses

The clinical and biological outcomes studied were overall survival (OS), disease-free survival (DFS), GVHD-free/relapse-free survival (GRFS), relapse incidence (RI), non-relapse mortality (NRM), day + 100 acute GVHD incidence (grade 2–4 and 3–4) in engrafted patients (meaning patients with neutrophil recovery and donor chimerism >5% and thus excluding early death in aplasia), moderate/severe chronic GVHD incidence in engrafted patients alive at day +100, primary/secondary graft failure, hematologic recoveries and infections.

OS was defined as the time from day 0 of Allo-SCT to death or last follow-up. DFS was defined as time from day 0 of Allo-SCT to the duration of survival without evidence of relapse, disease progression or death, censored at the date of relapse, death or last follow-up. Acute and chronic GVHD were diagnosed and graded according to standard criteria [16,17]. GRFS was defined as time from day 0 of Allo-SCT to time without evidence of grade III–IV acute GvHD (aGVHD), extensive chronic GvHD, relapse or death, censored at the date of grade III–IV aGvHD, extensive chronic GvHD, relapse, death or last follow-up [18]. Relapse was defined as any event related to re-occurrence of the disease. NRM was defined as death from any cause without previous relapse nor progression. Primary graft failures were defined by donor chimerism <5% at day 30 while secondary graft failure was defined by donor chimerism down to <5% after documentation of engraftment at day 30. Neutrophil recovery was defined as the first of three consecutive days with an absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L. Platelet recovery was defined as the first of three consecutive days achieving platelets $\geq 50 \times 10^9$/L unsupported by platelet transfusion for 7 days.

Probabilities of OS, DFS and GRFS were calculated using the log-rank test and Kaplan-Meier graphical representation. Cumulative incidences (CI) of relapse and NRM were analyzed together in a competing risks framework. For acute and chronic GVHD, death without aGVHD before day 100 and death without chronic GVHD were competing risks, respectively.

Univariate analyses were performed using the log rank test for OS, DFS and GRFS. Characteristics considered for univariate analysis were: gender (male vs female), age (< or ≥ median = 60 years old), disease (lymphoid vs myeloid), disease-risk index (DRI) [19] (low/intermediate vs high/very-high), conditioning regimen (Baltimore-based vs CloB2 vs TBF), GVHD prophylaxis (PTCY vs PTCY + ATG), donor age (< or ≥ the 40 years old median), D/R CMV status (–/– vs others), ABO compatibility (compatibility vs minor/major incompatibility) and acute GVHD (grade 2 vs grade 0/1 vs grade 3/4). Multivariate analyses were performed using the Cox proportional-hazard model. Factors with a p value <0.20 by univariate analysis or of interest for the study were included in multivariate analysis.

All tests were two-sided and p values <.05 were considered as indicating a statistically significant association. Analyses were performed in April 2020 using the Medcalc (Ostend, Belgium) and XLSTAT Life Sciences (Paris, France) statistical software.

Results

Patients

Among the 131 patients there were 78 males and the median age for the whole cohort was 59 years old (range:

20–71). Thirty-five patients received Allo-SCT for a lymphoid disease and 96 for a myeloid malignancy, including 67 patients with acute myeloid leukemia (AML). Patient characteristics are given in Table 1. Outcomes of the whole cohort and AML patients are given in Table 2.

Univariate analysis

Engraftment

The incidence of primary/secondary graft failure was significantly higher with the CloB2 regimen, with an incidence of 20.6% *versus* 1.7% after Baltimore-based and 2.1% after TBF regimens ($p = .0009$). No other factor was associated with graft failure.

Acute and chronic GVHD

The median time of occurrence of grade 2–4 aGVHD was 35 days (range: 15–133) from transplant. Grade 2 aGVHD was observed in 39 patients, involving the skin only ($n = 23$), the gastrointestinal (GI) tract ($n = 8$), both skin and GI ($n = 7$), GI and liver ($n = 1$). Grade 3–4 aGVHD occurred in 17 patients with involvement of skin only ($n = 4$), GI tract ($n = 9$), liver ($n = 1$) or both skin and GI ($n = 3$). CI of day 100 grade 2–4 and 3–4 aGVHD were 43.4 ± 4.6% and 16.7 ± 3.4%, respectively. The 2-year CI of moderate/severe chronic GVHD was 10.1 + 2.8%.

GVHD prophylaxis was the only factor associated with acute GVHD. Indeed, the CI of grade 2–4 acute GVHD at day 100 was significantly reduced in patients receiving PTCY + ATG (27 ± 6%) compared to patients who did not

Table 1. Characteristics of patients.

	Haplo patients N = 131
Median Follow-up: months (range)	37.4 (6.5-73.3)
Period of transplant	December 2013–September 2019
Median year of transplant	2017
Gender: male/female	78/53
Median age: years (range)	59 (20-71)
<60 years	68
Diseases	
AML	67
MDS	19
Myelofibrosis	4
MDS/MPS	2
ALL	7
NHL	18
HL	6
CLL	4
BPDCN	3
CML	1
Lymphoid/myeloid	35/96
DRI: low/int vs high/very high	2/78
	49/2
Previous allograft	17
Previous autograft	16
Conditioning	
Baltimore-based	56*
T(1d/2d)BF	46 (28/18)
CloB2	29
GVHD prophylaxis	
PTCY/CsA/MMF	75
PTCY/ATG/CsA/MMF	46
Donors	
Median age: years (range)	40 (20-72)
Brother/sister	35/22
Son/daughter	40/19
Father/mother	7/2
Nephew/niece	4/1
Cousin/cousin	1/0
Sibling	0
Matched unrelated	0
9/10 mis-matched	0
Female to male	26
ABO compatibility	
Compatibility	91
Minor inc	20
Major inc	20
D/R CMV status	
−/−	65
−/+	25
+/−	14
+/+	27
PBSC graft counts: median (range)	
CD34+: 10^6/kg	6.99 (2.88-19.94)
CD3+: 10^7/kg	24 (3.93-66.75)
CD45+: 10^8/kg	8.07 (3.2-25.36)

*fludarabine $n = 28$; clofarabine $n = 28$.

Table 2. Outcomes after PBSC transplant with PTCY.

	Whole cohort =131	AML patients $n = 67$
Primary/secondary graft failure	6/2	2/1
Neutrophils recovery: days (range)	18 (8-32)	18 (8-32)
Platelets recovery: days (range)	26 (10-134)	26.5 (10-131)
2-year OS	55.3+-4%	50.5+-6%
2-year DFS	51.9+-4%	48.7+-6%
2-year GRFS	37.7+-4%	33.5+-6%
Total relapses	32 (24.4%)	15 (22.3%)
Total deaths	60 (45.8%)	34 (50.7%)
Causes of death		
Relapse	23	15
Infection	18	11
Infection + GVHD	3	1
GVHD	7	4
Cardiac toxicity	2	0
Neurologic toxicity	3	2
Pulmonary toxicity	1	1
Polymyositis	1	0
Multiple organ failure	2	0

receive ATG (56 ± 6%, $p = .02$) (Figure 1(A)). However, it was the case neither for CI of grade 3–4 aGVHD (9 ± 9% vs 18 ± 4%, $p = .11$) (Figure 1(B)), nor the 2-year CI of moderate/severe chronic GVHD (27 ± 5% vs 29 ± 6%, $p = .65$) (Figure 1(C)). No other factor was associated with grade 2–4 acute GVHD. No factor either was associated to grade 3–4 acute GVHD.

When considering the AML haplo-SCT sub-group ($n = 67$), the incidences of grade 2–4 and grade 3–4 aGVHD were 41.7% and 13.4%, respectively. Again, the incidence of grade 2–4 aGVHD was significantly lower for patients receiving PTCY + ATG (24.3% vs 63.3%, $p = .002$). There was also a trend for lower grade 3–4 acute GVHD when using PTCY + ATG (5.4% vs 23.3%, $p = .07$).

Finally, DRI was the sole factor associated with moderate/severe chronic GVHD with a lower CI at 2-years for high/very-high DRI patients (12 ± 7% vs 33 ± 6%, $p = .05$).

Relapses

The TBF regimen was associated with a significantly lower CI of relapse at 2 years (5.3 ± 4%% vs Baltimore-based: 32.8 ± 10%% vs CloB2: 35.7 ± 7%27.5%, $p = .007$). This was also the case for patients with low/intermediate DRI (16 ± 4% vs 42 ± 8%, $p = .003$). The rates of relapse for patients receiving 1 or 2 days of thiotepa in the TBF sub-group were 7.1% (T1BF $n = 2/28$) and 5.5% (T2BF $n = 1/18$), respectively.

Nrm

A younger donor was associated with a lower 2-year CI of NRM (18.5 ± 5% vs 34.6 ± 6%, $p = .02$). The same was true for patients with lymphoid diseases (11 ± 5% vs 32 ± 5%, $p = .04$). The 2-year CI of NRM using the TBF regimen was 38.5 ± 7%. It was 21.4 ± 8% when using a Baltimore-based regimen and 19.7 ± 5% when using a CloB2 regimen. Surprisingly, the death rate was 50% when using one day of thiotepa vs 16.6% when using 2 days of thiotepa in the TBF sub-group ($p = .04$).

Survivals

A better 2-year OS was observed for patients with low/intermediate DRI (67.8 ± 5% vs 36.3 + 6%, $p = .0001$), those with

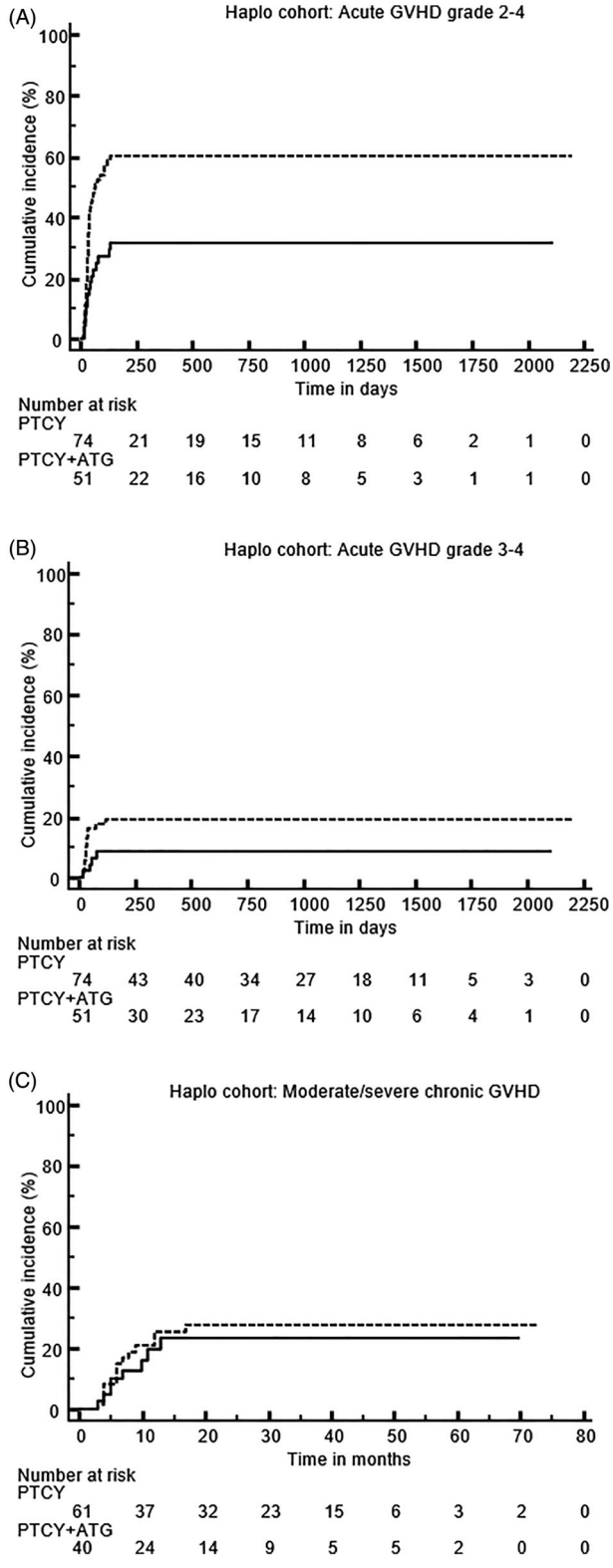

Figure 1. Cumulative incidence of grade 2–4 (A) and 3–4 (B) acute GVHD and moderate/severe chronic GVHD (C) between PTCY and PTCY + ATG sub-groups for haplo (left) and matched (right) transplants. Dotted line: PTCY; solid line: PTCY + ATG.

lymphoid disease (70.8 ± 7% vs 49.5 ± 5%, $p = .03$) and those with a younger donor (<40 years old) (64.5 ± 6% vs 46.6 ± 6%, $p = .02$). Low/intermediate DRI was still associated with a better 2-year DFS (61.8 ± 5% vs 35 ± 6% %, $p = .002$) but not 2-year GRFS ($p = .37$). The 2-year DFS was 58.6 ± 4%

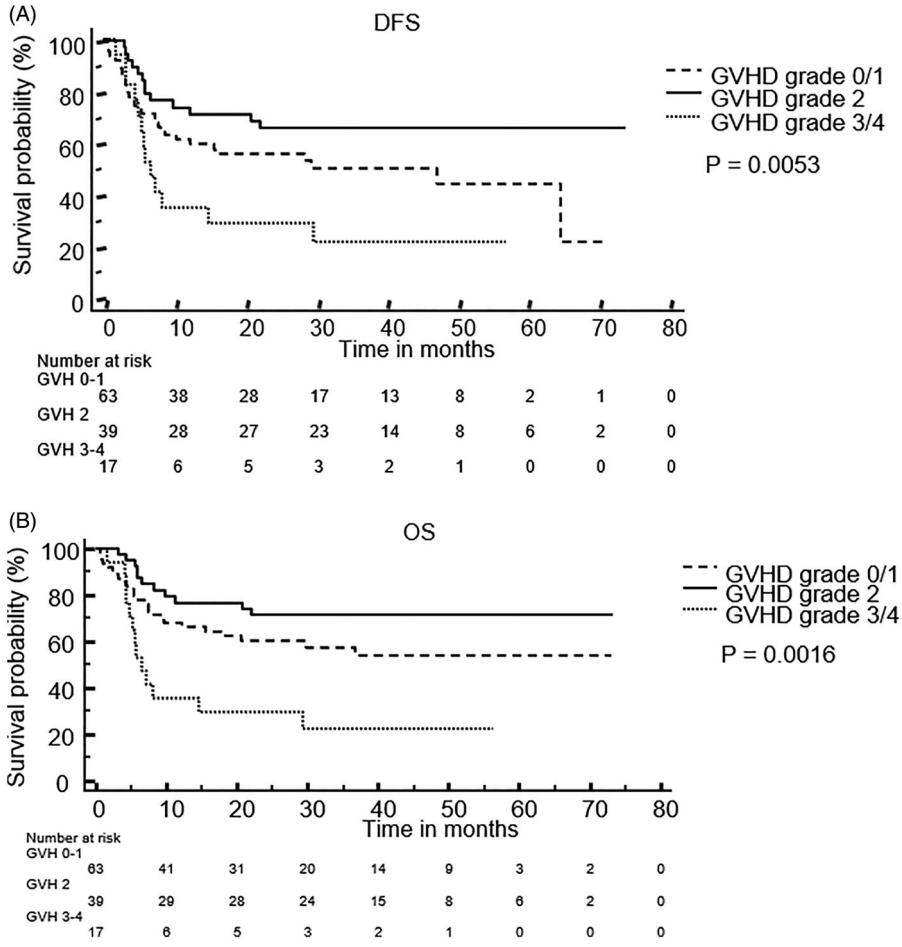

Figure 2. Comparison of DFS (A) and OS (B) according to patients developing grade 0/1, grade 2 or grade 3–4 acute GVHD after haplo-transplants.

when considering a younger donor (<40 years old) vs 45.5 ± 5% for older donors ($p = .08$). No factor was associated with better GRFS.

Very interestingly, 2-year OS was significantly higher for patients developing grade 2 acute GVHD ($n = 39$, 71 ± 7%) compared to patients developing grade 0/1 ($n = 63$, 60.4 ± 6%) or grade 3-4 acute GVHD ($n = 17$, 29.4 ± 11) ($p = .0007$, Figure 2(A)). Similarly, 2-year DFS was significantly higher for patients developing grade 2 acute GVHD ($n = 39$, 66.2 ± 7%) compared to patients developing grade 0/1 ($n = 63$, 56 ± 6%) or grade 3–4 acute GVHD ($n = 17$, 29.4 ± 11) ($p = .005$, Figure 2(B)).

In the AML haploSCT sub-group, patients with grade 2 acute GVHD also had the best survival rates. For 2-year OS, the percentages of survivors for patients who developed grade 2 aGVHD, versus grade 0/1 or grade 3/4, were respectively 67.3% (± 10%), 49.7% (±8%) and 22.2% (±13%, $p = .01$). Similarly, 2-year DFS were 67.3% (±10%), 45.2 (±8%) and 22.2% (±13%, $p = .02$).

Finally, the incidence of death due to GVHD was 25% in the PTCY without ATG sub-group., while it was only 4.1% in patients receiving PTCY + ATG.

Multivariate analysis

In multivariate analyses, taking into account the type of GVHD prophylaxis, myeloid or lymphoid disease, conditioning, donor age, DRI and acute GVHD grade, grade 2 acute GVHD remained significantly associated with better DFS (HR: 0.36; 95%CI: 0.19–0.69, $p = .002$) and OS (HR: 0.35; 95%CI: 0.1–0.70, $p = .003$). Conversely, an older donor (PFS HR: 1.93; 95%CI: 1.12–3.33, $p = .01$; and OS HR: 2.56; 95%CI: 1.42–4.60, $p = .001$) and high/very-high risk DRI (PFS HR: 2.72; 95%CI: 1.58–4.66, $p = .0003$; OS and HR: 3.55; 95%CI: 2.00–6.33, $p < .0001$) both remained associated to a significantly poorer prognosis. Neither the type of conditioning, disease nor GVHD prophylaxis were associated with survivals.

Infections

Although PTCY + ATG reduced significantly the incidence of grade 2 aGVHD, this could have been associated with a higher incidence of infections or deaths by infection compared to patients receiving PTCY without ATG. Supportive care was similar in the two institutions. In fact, more patients developed *Enterococcus* spp. bacterial infections in the PTCY group ($p = .03$) while more patients required preemptive treatment for CMV ($p = .02$) and EBV ($p = .05$) reactivations after receiving PTCY + ATG. The number of deaths due to infections was not significantly different between the PTCY and PTCY + ATG groups (30.5% of total deaths vs 41.6%, $p = .54$). Details are given in the Table 3.

Table 3. Infections and deaths from infections in the haplocohort depending on the GVHD prophylaxis.

	PTCY $n = 75$	PTCY + ATG $n = 56$	p value
Bacterial Infections			
Sepsis not documented	32 (42.6%)	34 (60.7%)	.06
Gram-positive bacilli: *Clostridium difficile*	11 (14.6%)	3 (5.3%)	.15
Gram-positive cocci: *Staphylococcus spp.*	19 (25.3%)	10 (17.8%)	.41
Enterococcus spp	13 (17.3%)	2 (3.5%)	.03
Gram-negative bacilli:			
Enterobacteriaceae spp.	11 (14.6%)	9 (16%)	1
Pseudomonadaceae spp.	4	0	
Stenotrophomonas maltophilia	1	1	
Nocardia	0	3	
Mycobacterium tuberculosis	0	1	
Viral infections			
CMV reactivation with preemptive treatment	At risk $n = 31$ 16 (51.6%)	At risk $n = 21$ 18 (85.7%)	.02
CMV disease	4	0	
EBV reactivation with preemptive treatment	7 (9.3%)	13 (23%)	.05
EBV-induced PTLD	0	0	
Adenovirus reactivation	4	4	
BK-virus cystitis (requiring cidofovir)	15 (1) (20%)	16 (2) (28.5%)	.35
HHV-6 reactivation	36/56 informative (64%)	13/27 informative (48%)	.24
VZV	0	1	
Fungal infections			
Aspergillosis	8 (10.6%)	9 (16%)	.51
Candidemia	1 (krusei)	1 (lusitaniae)	
Mucormycosis	2	2	
Cryptosporidium	1 (stool)		
Pneumocystis	1	1	
Parasitic infections			
Toxoplasma gondii	2	4	
Deaths from infections	11 (14.6%)	10 (17.8%)	.57
Septic shock not documented	4	5	
Septic shock by *E coli*+ adenovirus	1	0	
Septic shock by *E coli*	0	1	
Adenovirus	0	1	
Ileocolitis by *Pseudomonas aeruginosa*	1	0	
Cerebral toxoplasmosis	1	0	
Pneumocystis pneumoniae	1	0	
Respiratory syncytial virus pneumoniae	1	0	
Acute respiratory syndrome not documented	2	0	
Aspergillosis	0	1	
Tuberculosis	0	1	
Encephalitis	0	1	

Discussion

PTCY as GVHD prophylaxis has revolutionized the practice and care for patients benefiting from Allo-SCT. After showing great usefulness in the context of haplo-transplant with BM as source of graft, PTCY is now largely used for both matched and unmatched transplants using PBSC. The observation that PBSC grafts led to a higher incidence of aGVHD in haploidentical Allo-SCT with PTCY was rapidly recognized. However, the question remains whether this could not be useful in terms of survivals. Indeed, development of grade 2 aGVHD has been reported to be associated with better survivals in allotransplant using BM and PTCY [10,11]. Our question here was thus to demonstrate that, using PBSC as source of graft, the development of such aGVHD may represent also an advantage for the patients. Our results effectively show that developing grade 2 aGVHD, a generally manageable complication, results in better OS and DFS, not only by univariate but also multivariate analyses, after haplo-transplant with PTCY. The reason is probably that there is a relationship between this event and a GVL effect, as already reported with BM grafts [10,11]. The protective effect of developing grade 2 aGVHD also seems to be a particular feature of Allo-SCT followed by PTCY. Indeed, no such data have been reported in transplants using other GVHD prophylaxis, where it is rather chronic GVHD that is associated with better survivals [20]. Of course, because of the retrospective nature of this study and a contradictory report showing no impact of grade 2 aGVHD after haplotransplant with BM/PTCY [12], these results have to be confirmed by other larger prospective studies.

Therefore, and paradoxically, it seems that proposing alternative strategies for GVHD prophylaxis in order to reduce the incidence of aGVHD after PBSC haplo-transplant with PTCY may not be the right thing to do. Among these strategies, the major one that emerged was to add ATG to PTCY. Several groups have demonstrated that this combination seems to be associated with a lower incidence of acute or chronic GVHD [21–25]. Here, the schedules used present the advantage, similarly to a recent publication [25], of comparing these two prophylaxis regimen (PTCY vs PTCY + ATG 2.5 mg/kg, both with cyclosporine and mycophenolate mofetil) in a large cohort of patients. Haplo-transplants were first considered, which allowed to demonstrate that PTCY + ATG indeed significantly reduces the incidence of acute grade 2–4 GVHD (27% vs 56% at day 100). However, this turned out not to be true when considering only grade 3–4 aGVHD

or moderate/severe chronic GVHD. This result (reduced incidence of grade 2 aGVHD) was obtained while there was no increase in the incidence of relapse, infections (except CMV and EBV reactivations) or death by infection. This study thus confirms previous results and indicates that reducing grade 2 aGVHD can be reached using a lower dose of ATG (2.5 mg/Kg). Indeed, Salas *et al*. [21] have reported an incidence of 21.2% of grade 2–4 aGVHD at day 100 with PTCY + ATG 4.5 mg/Kg while for Peric at al [26], with 82% of transplants performed with PBSC, this incidence was 30% using ATG at 10 mg/kg.

Since reaching grade 2 aGVHD in the particular setting of PBSC haplotransplant with PTCY seems to be of interest, treatments potentially inducing GVHD in patients with grade 0/1 aGVHD might be proposed. This refers mainly to donor lymphocyte infusion, which has been already been proven to be safe and efficient to reduce relapse after haplotransplant [27]. Reducing grade 2 aGVHD may be only possible by applying strategies enhancing and preserving the GVL effect without aggravating GVHD, that should be considered systematically after transplant. This could involve pharmacological agents, such as tyrosine kinase inhibitors against BCR-ABL or FLT3-ITD for leukemia, monoclonal antibodies, hypomethylating agents, checkpoint inhibitors or adoptive infusion of NK cells, CAR T cells or leukemia-specific cytotoxic T lymphocytes [28]. However, as ATG was used here only after TBF and CloB2 conditioning regimens, it cannot be excluded that the combination of PTCY + ATG could be beneficial with a Baltimore-based conditioning. This strategy is currently tested by the Nantes team.

This cohort also allowed to analyze the impact of the conditioning regimen in haplo-Allo-SCT with PTCY. Surprisingly, CloB2 was associated with a higher rate of graft failure, suggesting that this regimen is not immunosuppressive enough, although ATG was systematically administered with PTCY/cyclosporine and MMF for these patients. Conversely, CloB2 was confirmed to be very efficient for matched grafts, especially in AML [14]. CloB2 conditioning should thus probably not be prescribed for patients receiving a haplo-identical graft. Regarding the TBF regimen, we confirmed a low incidence of relapse [26]. However, NRM was high in this group, resulting in similar OS and DFS compared to CloB2 or Baltimore-based regimens. This was not due to the use of a higher thiotepa dosage (2 days instead of 1) but perhaps to the use of PBSC. Indeed, NRM has been reported to be only of 13% after haplo-Allo-SCT with PTCY and BM as source of graft [15]. Other studies [26,29] have also observed a high incidence of TRM after haplo-Allo-SCT with TBF conditioning, including one showing, as for us, no influence of the intensity of the conditioning regimen [29]. Thus, other regimens with low TRM may be preferred when considering PBSC haplo-Allo-SCT with PTCY, such as a reinforced Baltimore-based regimen with clofarabine (11% of NRM) [13] or treosulfan-based regimen (17% of NRM) [30].

Besides grade 2 aGVHD, two other factors have been identified as predicting a better OS for haplo-Allo-SCT recipients in this series: choice of a younger donor and low/intermediate DRI at transplant. Recommendations regarding donor selection in haplo-Allo-SCT [31,32] already indicate that the use of a younger donor is preferable, probably because of a lower toxicity, as observed in our series, but also possibly because of the infusion of more immunocompetent cells, a larger repertoire and potentially lower clonal hematopoiesis, that might translate in a lower risk of relapse and infection [33]. As for age, DRI is a fixed parameter at the time of transplant. Here again, we confirm previous results showing that DRI is the most powerful factor predicting survivals after PBSC haplo-Allo-SCT [30,34,35]. As expected, high/very-high DRI were here associated with a higher risk of relapse, suggesting that it could be useful to consider post-transplant strategies in order to avoid relapse. This higher risk of relapse is probably due to a lower GVL effect that can be indirectly suspected by a lower incidence of moderate/severe chronic GVHD, as reported in this series and others [36].

Finally, our results with or without ATG are similar to what has already been reported in terms of infections in the haplo-identical setting [37,38]. However, more patients required preemptive therapy for CMV and EBV reactivations with the use of PTCY + ATG, suggesting a strengthened viral monitoring in such patients, even if no death was reported to be linked to these infections.

In conclusion, results of this study suggest that acute grade 2 GVHD is an independent prognostic factor for better survivals in haplo-Allo-SCT using PBSC and PTCY. Further and larger studies are needed to clarify the complex question of GVHD prophylaxis in the setting of haplo-transplant, especially the combination of ATG with PTCY. This combination should also be evaluated in the matched setting where the use of PTCY alone is already known to be associated with acceptable rates of GVHD [39,40].

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Author contributions

PC and AB designed, performed, coordinated the research, analyzed, interpreted the data, and wrote the manuscript.

MCB performed statistical analyses, generated the figures and helped writing the manuscript. PP, AG, ALB, BMI, ED, BM, VD, NB, CT, TG, AL, BT, SV, YD, CB, AD, ME, YLB, AD, CM, ED, PM, SLG, and TG contributed data and commented on the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical standards statement

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of informed consent

Informed consent was obtained from all patients for being included in the study.

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