

# Comparison of immune reconstitution between anti-T-lymphocyte globulin and posttransplant cyclophosphamide as acute graft-versus-host disease prophylaxis in allogeneic myeloablative peripheral blood stem cell transplantation

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## ABSTRACT

Anti-T-cell lymphocyte globulin (ATLG) and posttransplant cyclophosphamide (PTCy) are now widely used strategies to prevent graft-versus-host disease (GVHD) after allogeneic stem cell transplantation. Data comparing immune reconstitution (IR) between ATLG and PTCy is scarce. This retrospective study conducted at the University Medical Center Hamburg-Eppendorf (UKE) compares PTCy (n=123) and ATLG (n=476) after myeloablative allogeneic peripheral blood stem cell transplant. Detailed phenotypes of T, B natural killer (NK), natural killer T (NKT) cells were analyzed by multicolor flow at day 30, 100 and 180 posttransplant. Incidence of infections, viral reactivations, GVHD and relapse were collected. Neutrophil engraftment was significantly delayed in the PTCy group (median day 12 vs. day 10,  $P < 0.001$ ) with a high incidence of infection before day+100 in the PTCy arm but a higher Epstein-Barr virus reactivation in the ATLG arm and comparable cytomegalovirus reactivation. Overall incidence of acute GVHD was similar but moderate/severe chronic GVHD was seen more often after PTCy (44% vs. 38%,  $P = 0.005$ ). ATLG resulted in a faster reconstitution of CD8+ T, NK, NKT and  $\gamma\delta$ T cells while CD4 T cells and B cells reconstituted faster after PTCy. Similar reconstitution was observed for T-regulatory cells and B cells. Non-relapse mortality relapse incidence, disease-free survival, and overall survival did not differ significantly between both arms. Even though differences in IR were related to a decreased incidence of infection and moderate/severe cGVHD in the ATLG group they had no impact on any of the other long-term outcomes. However, it remains undetermined which regimen is better as GVHD prophylaxis.

## Introduction

Allogeneic stem cell transplantation (allo-SCT) is a potentially curative treatment strategy for hematological diseases.<sup>1</sup> This is attributed mainly to the graft-versus-tumor effect derived from transferring the donor's immune system to the recipient.<sup>2</sup> However, the benefit of allo-SCT may be offset by increased transplant-related mortality (TRM) especially due to graft-versus-host disease (GVHD).<sup>3</sup> In an attempt to decrease the incidence of GVHD physicians employ a multitude of strategies, including *in vivo* T-cell depletion (TCD) with pretransplant anti-T-lymphocyte globulin (ATLG)<sup>4,5</sup> and/or posttransplant cyclophosphamide (PTCy).<sup>6-8</sup>

GVHD prophylaxis with PTCy decreases the incidence of graft rejection and GVHD by impairing the function of alloreactive T cells.<sup>9</sup> However, data is scarce on the effect of PTCy effect on immune reconstitution (IR) post-allo-SCT, especially when compared to the standard use of ATLG as a TCD tool.<sup>4,5,10,11</sup> In this

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study we aim to compare the IR kinetics and transplant outcomes between ATLG and PTCy as TCD strategies in patients undergoing allo-SCT with myeloablative conditioning (MAC) and peripheral blood stem cells (PBSC).

## Methods

This retrospective study conducted at the University Medical Center Hamburg-Eppendorf (UKE) with a primary outcome to compare IR between PTCy *versus* ATLG *in vivo* TCD in adult patients who received MAC PBSC allo-SCT. Secondary outcomes included incidence of viral reactivations, engraftment, infections, acute GVHD (aGVHD), chronic GVHD (cGVHD), non-relapse mortality (NRM), progression-free survival (PFS), overall survival (OS). All patients signed written informed consents for treatment, and the study was approved by the Institutional Review Board of UKE.

MAC regimens were defined according to working group definition.<sup>12</sup> ATLG (Grafalon®, Neovii, Switzerland) was given at a dose of 30 mg/kg for related donor or 60 mg/kg for unrelated donor with a trend in recent years to give the lower dose for both groups. All ATLG doses were fractionated between days -4 to -1. PTCy was administered as 50 mg/kg/day. Posttransplant immunosuppression was given on days +3 and +4 combined with calcineurin inhibitor (tacrolimus for unrelated donor or haplo-identical donor, and cyclosporine for related donor) and mycophenolate mofetil for mismatched transplants. Similar supportive care was used for all patients per institutional guidelines including antimicrobial prophylaxis consisting of fluoroquinolone for bacterial infections, trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis jirovecii*, micafungin for fungal infections and acyclovir for viral infections. Patients were screened weekly for cytomegalovirus (CMV) and Epstein-Barr virus (EBV) by blood polymerase chain reaction (PCR).

Neutrophil engraftment was defined as the first 3 consecutive days with a measure of an absolute neutrophil count  $>0.5 \times 10^9/L$ . Platelet engraftment was defined as the first consecutive days with a platelet count  $>20 \times 10^9/L$  without transfusion support.

Acute GVHD was graded according to standard criteria.<sup>13</sup> Chronic GVHD was graded according to National Institute of Health (NIH) criteria routinely at every visit after transplantation.<sup>14</sup>

### Infections were defined as any microbial testing with a positive result and requiring therapy.

As per institution guidelines, blood samples were collected for each patient on days 30, 100 and 180 post-allo-SCT. Samples were used directly after red blood cell lysis following 10 minutes of incubation with erythrocyte lysing reagent without fixative. Immunophenotypes were assessed using four color cytometry using mouse anti-human antibodies for the following cells: T lymphocytes (CD3+), activated T lymphocytes (CD3+HLADR+), T-helper cells (CD3+/CD4+), cytotoxic T cells (CD3+/CD8+), B lymphocytes (CD19+), B-lymphocyte subpopulations (CD19+CD5+CD1d+)(CD19+CD27+), naïve B cells (CD19+CD27-CD10+), natural killer (NK) cells (CD56+CD3-), natural killer T (NKT) cells (CD56+CD3+), naïve T-helper cells (CD4+CD45RA+), memory T-helper cells (CD4+CD45R0+), naïve cytotoxic T cells (CD8+CD45RA+), memory cytotoxic T cells (CD8+CD45R0+),  $\gamma\delta$ T cells ( $\gamma\delta$ TCR+, CD3+), regulatory T cells (CD4+CD25+CD127low-neg).

### Statistical analyses

All data was retrospectively collected, and was summarized

by standard descriptive statistical methods.  $\chi^2$  test was used to compare categorical variables, whereas continuous variables were compared using Student's *t*-test. We defined disease-free survival (DFS) as survival without relapse or progression of hematological disease; we censored patients without disease or progression at the time of the last follow-up. We defined OS and NRM as death from any cause, and without evidence of relapse respectively. We used the Kaplan-Meier method to calculate the probabilities of moderate/severe cGVHD relapse-free survival, DFS and OS; and the cumulative incidence functions were used to estimate RI and NRM in a competing risk setting. All analysis was performed using SPSS version 26.0 and ACCorD.

## Results

### Patients and transplant characteristics

In order to have comparable groups we selected only patients receiving myeloablative conditioning for their first allo-SCT with only PBSC as a stem cell source. From 2005 to 2019, 599 patients were included in the study. Four hundred and seventy-six patients received ATLG, with 34% and 66% receiving 30 mg/kg and 60 mg/kg ATLG respectively. One hundred and twenty-three patients received PTCy. The median age at transplant was 53 years (range, 18-75 years) in both groups. Seventy nine percent and 72% were transplanted from a full match donor (HLA10/10) in the ATLG and PTCy group, respectively. All patients, donor and transplant characteristics are listed in Table 1.

### Transplant outcomes

All transplant outcomes are summarized in Table 2.

### Engraftment

Platelet and neutrophil engraftment were significantly delayed in the PTCy group when compared to the ATLG group; with a median of 12 days (range, 8-36 days) to neutrophil in the ATLG *versus* 16 days (range, 12-27 days) in PTCy group ( $P < 0.001$ ); and a median of 15 days (range, 8-99 days) to platelet engraftment in the ATLG *versus* 21 days (range, 9-99 days) in the PTCy group ( $P = 0.024$ ).

### Cytomegalovirus infections and Epstein-Barr virus reactivation

We observed no significant differences in incidence of CMV reactivation before day 100 (ATLG 46%, PTCy 50%). The overall incidence of infection before day 100 was significantly higher in the PTCy group (91%) when compared to the ATLG group (75%),  $P < 0.001$ . The incidence of EBV reactivation before days 100 in the ATLG group was higher than the PTCy group (33% *vs.* 16%,  $P < 0.001$ ).

### Graft-versus-host disease

The cumulative incidences of aGVHD grade 2-4 and 3-4 were similar between the two groups with 36% and 15% in the ATLG group and 40% and 12% in the PTCy group.

The cumulative incidence of all grade cGVHD were similar between the two groups, 15% and 27% in the ATLG and PTCy groups respectively, however we observed a higher cumulative incidence of moderate and severe cGVHD in the PTCy group, 38% ATLG *versus* 44% PTCy ( $P = 0.005$ ).

**Table 1. Patients and transplant characteristics.**

Patients	ATLG N (%)	PTCy N (%)	P
Total patients	476 (100)	123 (100)	
ATLG dose			
30 mg/kg	162 (34)		
60 mg/kg	314 (66)		
Mean age (Standard Deviation)	50 (14)	50 (13)	NS
Disease			<0.001
ALL	27 (10)	35 (29)	
AML	206 (43)	23 (19)	
CML	16 (3)	1 (1)	
MDS	43 (9)	2 (2)	
MDS-MPN	6 (1)	4 (3)	
HL	4 (1)	2 (2)	
NHL	75 (16)	13 (11)	
MM	64 (13)	38 (31)	
PMF	12 (3)	2 (2)	
Other AL	3 (1)	3 (2)	
ECOG			NS
0	115 (30)	23 (20)	
1	241 (63)	81 (72)	
2	23 (6)	9 (8)	
3	3 (1)	0 (0)	
Mean KI at SCT (Standard Deviation)	86 (12)	83 (11)	0.011
Mean donor age (Standard Deviation)	36 (12)	37 (14)	NS
Donor/ recipient CMV serology			NS
D-/R-	151 (32)	41 (34)	
D+/R+	194 (41)	58 (47)	
D-/R+	62 (13)	9 (7)	
D+/R-	68 (14)	15 (12)	
Donor-Recipient sex			NS
No mismatch			
Male-Male	241 (51)	59 (48)	
Female-Female	81 (17)	16 (13)	
Mismatch	154 (32)	48 (39)	
Male - Female	101 (21)	32 (26)	
Female - Male	53 (11)	16 (13)	
ABO incompatibility			NS
Isogroup	182 (39)	57 (47)	
Minor	127 (27)	23 (19)	
Major	111 (24)	33 (27)	
Bidirectional	48 (10)	8 (7)	
Median year of transplant (range)	2013 (2005-2019)	2015 (2005-2019)	<0.001
Type of transplant			
Related	77 (16)	45 (37)	<0.001
Unrelated	399 (84)	78 (63)	
Full match (HLA10/10)	377 (79)	88 (72)	NS
Mismatch (HLA <10/10)	99 (21)	35 (29)	NS
MRD	74 (16)	31 (25)	<0.001
MMRD	3 (1)	14 (11)	
MUD	303 (64)	57 (46)	
MMUD	96 (20)	21 (17)	
Mean CD34 x10 <sup>6</sup> /kg (SD) infused	11.55 (64)	7.18 (2)	NS
Conditioning details			<0.001
Busulfan based	256 (54)	29 (24)	
TBI based	130 (27)	55 (45)	
Other	90 (19)	39 (32)	
TBI	146 (31)	55 (45)	0.003
TBI dose			
≤10 Gy	44 (9)	21 (17)	0.001
>10 Gy	102 (21)	34 (28)	NS

continued from previous column

Immune suppression	ATLG N (%)	PTCy N (%)	P
CNI+MMF	413 (87)	77 (62)	<0.001
CNI+MTX	57 (12)	0 (0)	
CNI	1 (0.2)	23 (19)	
other	5 (1)	23 (19)	

ATLG: anti T-cell lymphocyte globulin; PTCy: and post-transplant cyclophosphamide; NS: statistically not significant; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; MDS-MPN: myelodysplastic syndrome - myeloproliferative neoplasm; HL: Hodgkin lymphoma; NHL: non Hodgkin lymphoma; MM: multiple myeloma; PMF: primary myelofibrosis; Other AL: other acute leukemia; ECOG: Eastern Cooperative Oncology Group performance status; KI: Karnofsky index; CMV: cytomegalovirus; D-: donor with negative CMV serology; D+: donor with positive CMV serology; R-: recipient with negative CMV serology; R+: recipient with positive CMV serology; MRD: matched related donor; MMRD: mismatched related donor; MUD: matched unrelated donor; MMUD: mismatched unrelated donor; SD: standard deviation; TBI: total body irradiation; CNI: calcineurin inhibitor; MMF: mycophenolate mofetil; age in years.

**Table 2. Transplant outcomes.**

Transplant outcomes	ATLG N (%)	PTCy N (%)	P
Patients	476 (100)	123 (100)	
Leukocytes engraftment median days (range)	12 (8-36)	16 (12-27)	<0.001
Platelet engraftment median days (range)	15 (8-99)	21 (9-99)	0.024
CMV reactivation [15 in ATLG, 54 in PTCy missing]	214 (46)	60 (50)	NS
EBV reactivation [34 in ATG missing, 4 in PTCy missing]	131 (33)	19 (16)	<0.001
Overall incidence of infection by day 100	344 (75)	109 (91)	<0.001
NRM at 3 years	16%	30%	0.006
Relapse incidence	34%	29%	NS
DFS at 3 years	50%	42%	NS
OS at 3 years	65%	58%	NS
Moderate/severe cGVHD relapse-free survival	40%	27%	NS

NS: statistically non-significant; NRM: non-relapse mortality; OS: overall survival; DFS: disease-free survival; cGVHD: chronic graft-versus-host disease; EBV: Epstein-Barr virus; CMV: cytomegalovirus; PTCy: posttransplant cyclophosphamide; ATLG: anti T-cell lymphocyte globulin; SD: standard deviation.

**Non-relapse mortality**

PTCy was associated with a higher NRM when compared to ATLG on univariate analysis, in addition a positive patient CMV serology, patient age >52 years, donor age >34 years and female donor were also associated with higher NRM. On multivariate analysis, NRM was not affected by ATLG and PTCy; only donor sex, patient age and CMV serology had a significant impact on NRM (Table3).

**Relapse, disease-free survival and overall survival**

After a median follow-up of 16 months (range, 1-169 months) we observed no significant differences in terms of DFS (at 3 years ATLG 51%, PTCy 42%, P=0.3), relapse incidence (ATLG 34% vs. PTCy 29%, P=0.261), OS (65% vs. 58%, P=0.663) or moderate/severe cGVHD relapse-free survival at 3 years was (ATLG 40% vs. PTCy 27%, P=0.063) between the two groups.

**Immune reconstitution**

We observed a faster reconstitution of CD3 T lympho-

cytes (CD3+) ( $P < 0.05$ ) and activated T cells (CD3+/HLADR+) after ATLG than PTCy ( $P < 0.05$ ) (Figure 1A and B). In addition, as shown in Figure 2 and the *Online Supplementary Table S1* the reconstitution of cytotoxic T cells (CD3+/CD8+) and also of naïve cytotoxic T cells (CD3+/CD8+/CD45RA+) ( $P = 0.017$ ) was significantly faster in the ATLG group (on day 90,  $P = 0.002$ ), while the reconstitution of memory cytotoxic T cells (CD3+/CD8+/CD45RO+) was comparable (Figure 2). In contrast to cytotoxic CD8+ positive cells, CD3 helper cells (CD3+/CD4+) had a trend for faster reconstitution in the PTCy group (Figure 3), which was sustained for naïve (CD4+/CD45RA+) ( $P = 0.002$ ) as well as for memory helper cells (CD4+/CD45RO+) ( $P < 0.001$ ). The reconstitution of B cells (CD19+) was similar in the ATLG and PTCy group (Figure 4A) and a trend for faster reconstitution of naïve B-cells (CD19+/CD27-/CD10+) was observed in the PTCy group (Figure 4B). NK-cell reconstitution was faster on day 30 in the ATLG group ( $P < 0.001$ ), however the values on day 100 and 180 were similar after ATLG and PTCy (Figure 5A). Furthermore, NKT cells and  $\gamma\delta$ T cells reconstitution was faster in the ATLG group ( $P < 0.05$ ) at all time points (Figure 5B and C), while there were no significant differences in regulatory T-cell immune reconstitution between the two groups.

All our data is summarized in the *Online Supplementary Table S1*. All our findings were confirmed in a donor subgroup analysis (*Online Supplementary Table S2*).

## Discussion

Although ATLG and PTCy are widely used for GVHD prevention in allo-SCT, data is scarce on their impact on immune reconstitution and only one small prospective study using RIC PBSC has compared immune reconstitution PTCy to ATLG so far.<sup>15</sup> In this retrospective study, we compared the influence of ATLG to PTCy on immune reconstitution and transplant outcomes after myeloablative allogeneic PBSC transplant. In our study, we observed some strong differences in terms of cell counts, immune reconstitution, infections moderate/severe cGVHD and EBV reactivation between the two groups.

Since NK and  $\gamma\delta$ T cells have a protective role against many bacterial and viral infections including CMV<sup>16-32</sup> the longer period of aplasia and the decreased numbers of NK and NKT cells in the PTCy group can explain the higher incidence of infections before day 100 in this group.

One of the most recent studies of  $\gamma\delta$ T-cell recovery and their association with transplant outcomes was conducted on 102 pediatric patients with acute leukemia.<sup>35</sup> They reported significantly improved PFS and OS in patients with elevated  $\gamma\delta$ T cells, these findings have also been reported in adults.<sup>34,35</sup> In addition they reported a significantly lower incidence of infections with a total absence of bacterial infections in the high  $\gamma\delta$ T-cell group.<sup>35</sup> Our findings fall in line with the literature. We observed an early recovery of the  $\gamma\delta$ T-cell population in both groups independent of the donor subtype. In addition,  $\gamma\delta$ T cell were consistently higher in the ATLG group in all evaluations when compared to the PTCy group, which may explain the decreased overall incidence of infections in this group. Even though our study was not designed for long term outcomes, we observed no significant difference in DFS or OS between the two groups.

**Table 3. Multivariate non-relapse mortality**

Multivariate	NRM HR (95% CI) P-value
ATLG vs. PTCy	1.6 (0.98-2.48) 0.061
Patient CMV serology negative vs. positive	1.73 (1.09-2.75) 0.02
Patient age <52 vs. $\geq$ 52	1.69 (1.05-2.73) 0.03
Donor Age <34 vs. $\geq$ 34	1.32 (0.81-2.13) 0.26
Donor sex Male vs. Female	1.61 (1.01-2.59) 0.048
CD34 $\times 10^6$ /kg <7.2 vs. $\geq$ 7.2	0.748 (0.46-1.2) 0.24
ECOG 3 vs. 0-2	1.4 (0.87-2.14) 0.17

NRM: non-relapse mortality; HR: hazard ratio; CI: confidence interval; ATLG: anti T-cell lymphocyte globulin; PTCy: posttransplant cyclophosphamide; ECOG: Eastern Cooperative Oncology group.

Retiere *et al.* observed a rapid NK recovery within day 30 after allo-HSCT in the ATLG and PTCy group, however while they reported an increase in NK-cell counts in the ATLG group, they did not observe any effect of the donor type on these values and concluded the recovery rate was a direct effect of the difference in GVHD prophylaxis between the two groups.<sup>15</sup> Our results fall in line with Retiere's, we observed rapid reconstitution of NK cells at day 30, and we observed a significantly higher percentage and absolute count of NK cells at day 30 in the ATLG group. This was validated by our donor subgroup analysis which allows us to conclude that this was a direct effect of the difference in the TCD strategy. This supports the hypothesis that ATLG spares NK cells while PTCy targets them.<sup>15</sup> Rubio *et al.* reported that early recovery of NKT cells post T-cell-repleted allo-SCT, and a high NKT-cell dose in the graft are associated with protection from aGVHD.<sup>36,37</sup> In addition Tae *et al.* report an increase in the incidence of aGVHD and of relapse in patients with lower NKT-cell counts post-allo-SCT.<sup>38</sup> Retiere *et al.* did not observe any significant differences in the NKT-cell population between ATLG and PTCy.<sup>15</sup> However, in our study, we observed a higher number and percentage of NKT cells in the ATLG group when compared to PTCy and this was confirmed by our subgroup donor analysis. Nonetheless, we did not observe any significant differences in the incidence of relapse or aGVHD, while we observed a significantly lower incidence of moderate and severe cGVHD in the ATLG group when compared to the PTCy group.

Servais *et al.* studied the impact of ATLG on IR post MAC PBSC allo-SCT.<sup>39</sup> They looked precisely at memory and naïve T cells and they observed that ATLG selectively depletes naïve CD4+ T and naïve CD8+ T cells whereas it does not significantly impact memory T cells.<sup>39</sup> Our results fall in line with Servais *et al.*, as we observed a progressive increase in the naïve to memory ratio both in the CD4+ T cells and CD8+ T cells, indicating that the effect of ATLG effect is more pronounced on naïve T cells than on memory T cells. However, in our study the effect of ATLG on naïve and memory CD4+ T cells was

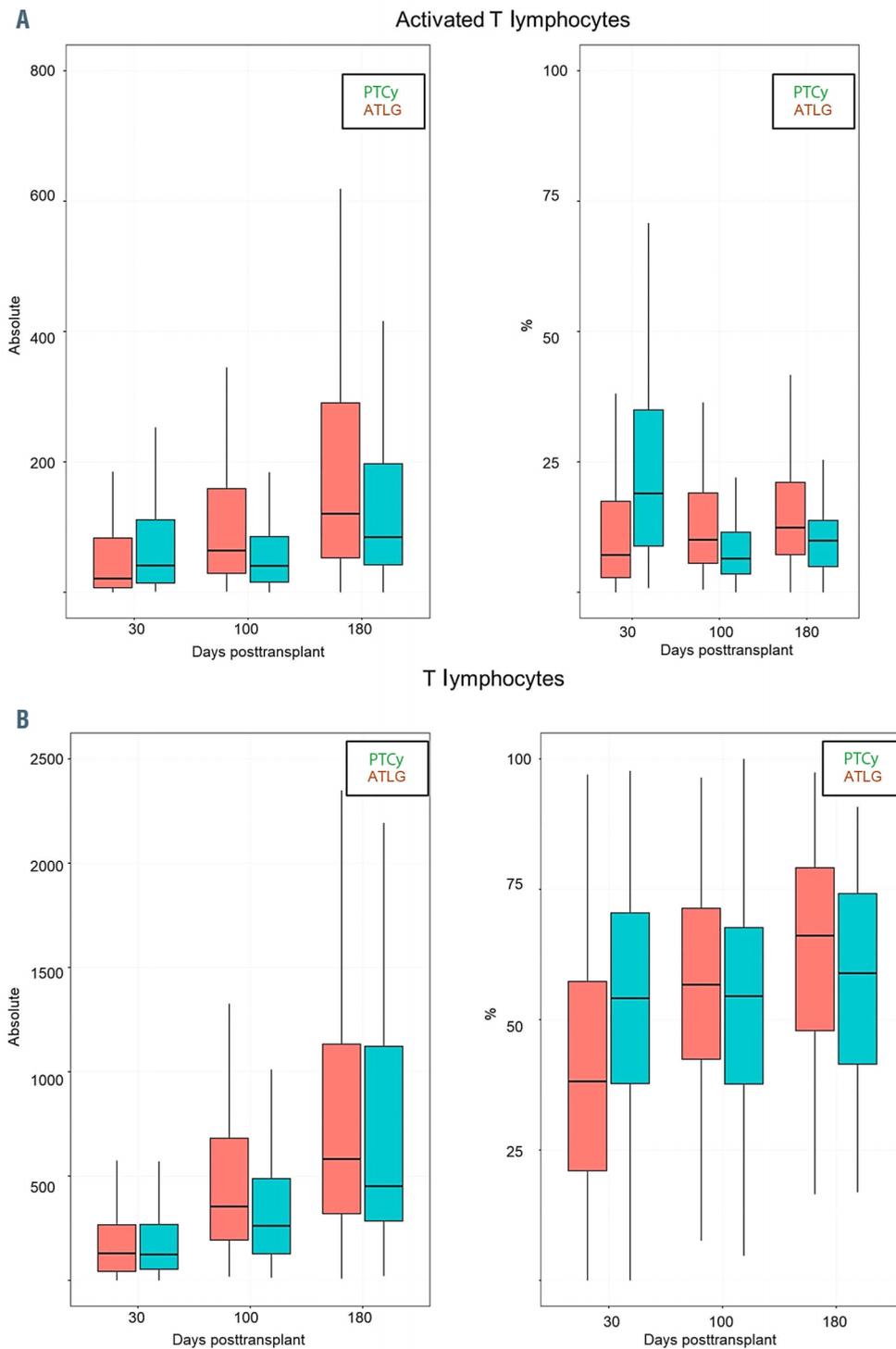


Figure 1. Comparison between ATLG and PTCy regarding immune reconstitution of (A) activated T cells CD3+/HLADR+ and (B) all T cells (CD3+).  $P^*$ =P-value at day 30;  $P^{**}$ =P-value at day 100;  $P^{***}$ =P-value at day 180; %: percentage of cells; Absolut: absolute number of cells. TLG: anti T-cell lymphocyte globulin; PTCy: and post-transplant cyclophosphamide.

more pronounced than PTCy. In our study the reconstitution of CD8+ T cells was faster than that of CD4+ T-cells with CD8+ T cells recovering by day 100, while the CD4+ T-cell reconstitution was not observed at the last evaluation of the immune profile on day 180. In addition, the CD4+/CD8+ ratio did not return to normal in either of the two groups, which indicates incomplete recovery of the CD4+ T-cell compartment. These findings were confirmed by subgroup analysis according to the donor.

It is well established that reconstitution of the T-cell compartment after allo-HSCT arises from both homeo-

static peripheral expansion (HPE) of donor T cells transferred with the graft and from the novel production of naïve T cells in the thymus.<sup>40,41</sup> In patients receiving MAC most of the T cells originate from HPE, and given that ATLG persist for several weeks in circulation,<sup>42,43</sup> it can be hypothesized that ATLG selectively depletes donor naïve T cells while it spares other T-cell populations. This differential cytotoxic activity of ATLG has been demonstrated *in vitro*.<sup>44</sup> In addition, HPE occurs more asymmetrically between T cells, with CD8+ T cells having higher proliferating potential by HPE when compared to CD4+ T cells.<sup>39</sup> This may explain the decreased

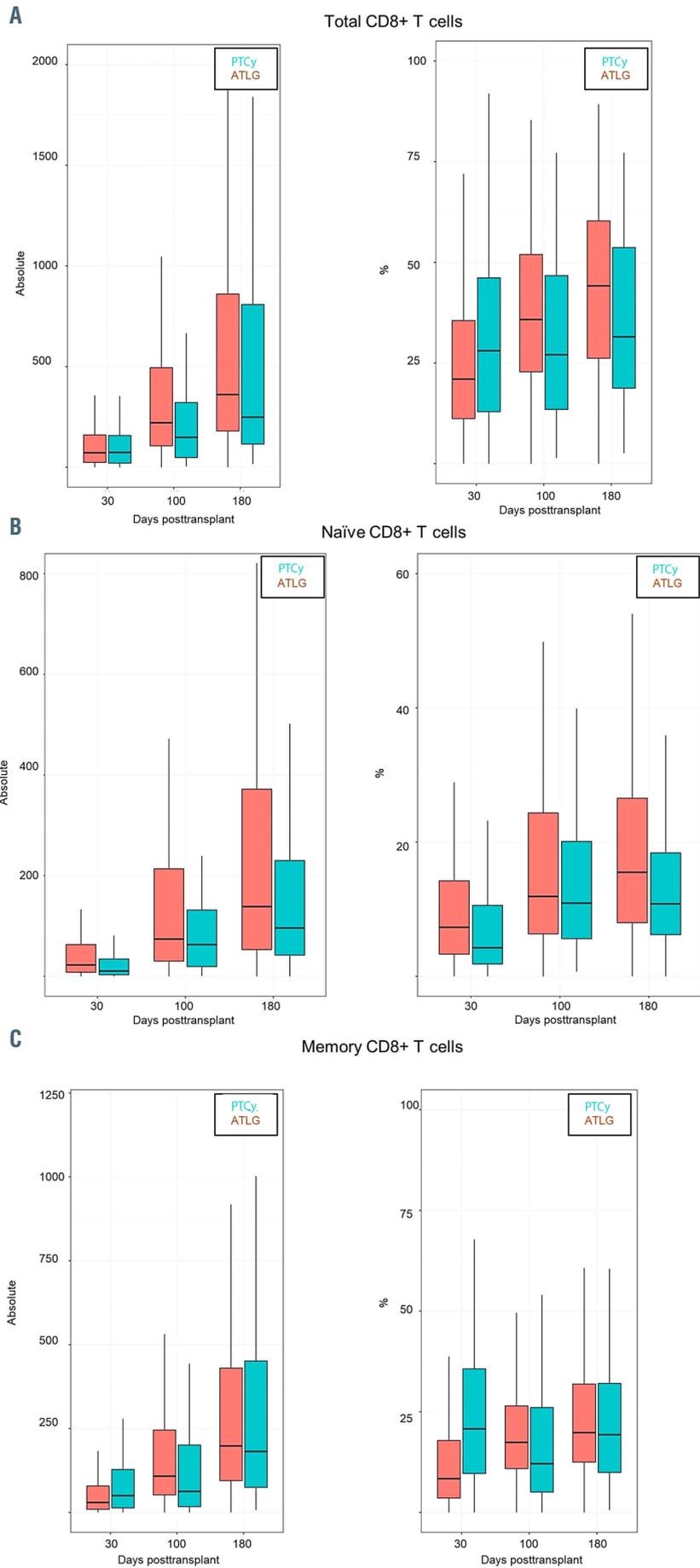


Figure 2. Comparison between ATLG and PTCy regarding immune reconstitution of CD8+ cells. (A) Total CD8+ T cells; (B) naïve CD8+ T cells; (C) memory CD8+ T cells.  $P^*=P$ -value at day 30;  $P^{**}=P$ -value at day 100;  $P^{***}=P$ -value at day 180; %: percentage of cells; Absolut: absolute number of cells. ATLG: anti T-cell lymphocyte globulin; PTCy: and post-transplant cyclophosphamide.

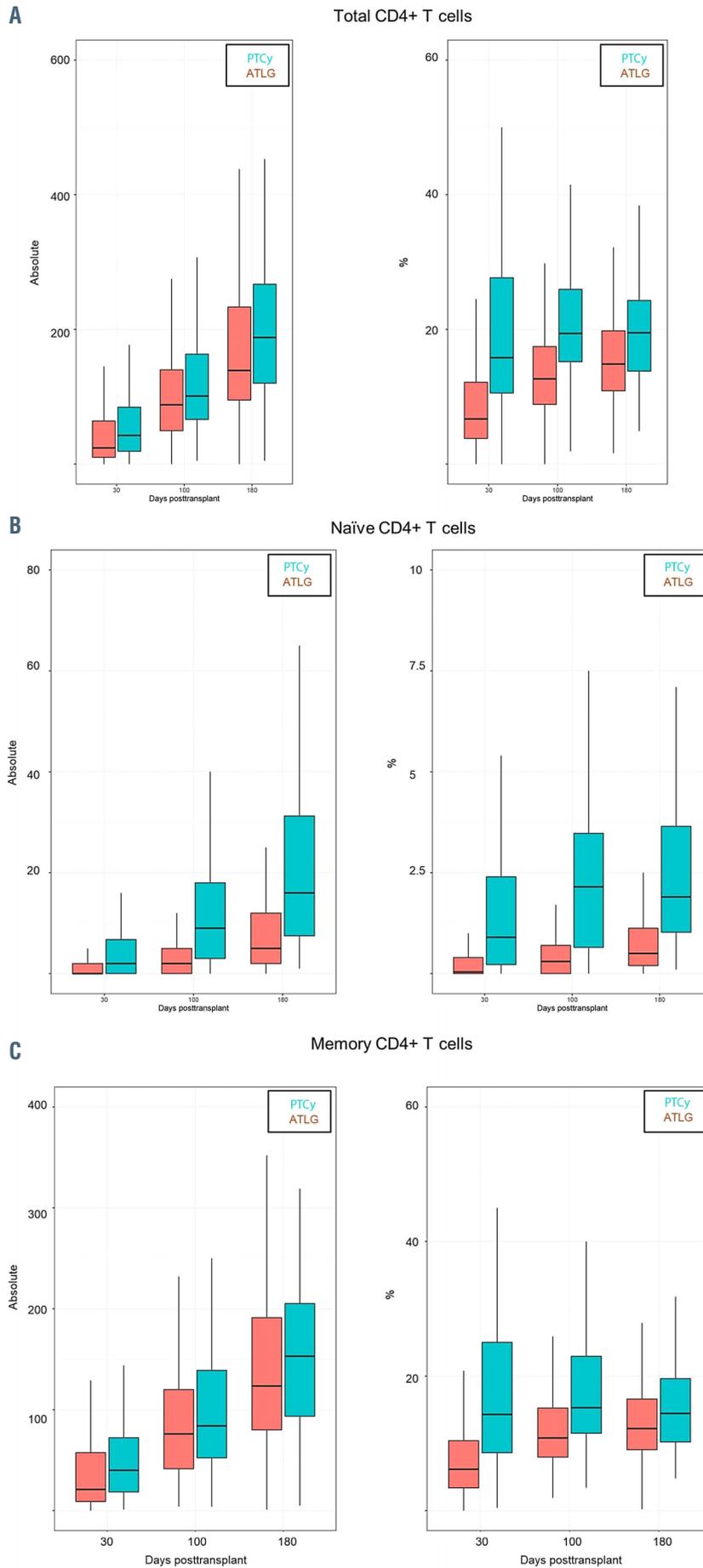


Figure 3. Immune reconstitution for CD4+ T-cell ATLG versus PTCy. (A) Total CD4+ T cells; (B) naïve CD4+ T cells; (C) memory CD4+ T cells.  $P^*$ = $P$ -value at day 30;  $P^{**}$ = $P$ -value at day 100;  $P^{***}$ = $P$ -value at day 180; %: percentage of cells; Absolut: absolute number of cells. ATLG: anti T-cell lymphocyte globulin; PTCy: and post-transplant cyclophosphamide.

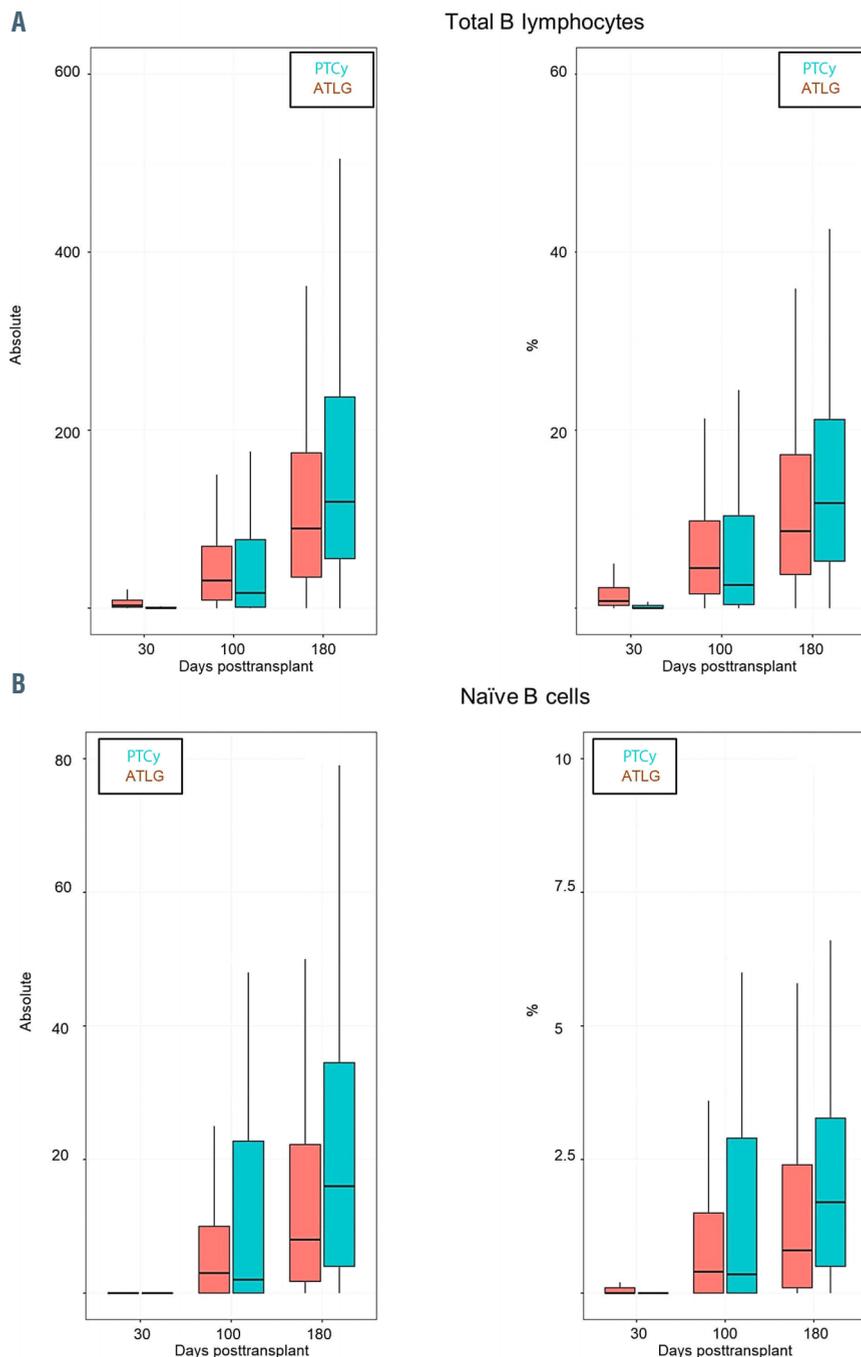


Figure 4. Comparison between ATLG and PTCy regarding immune reconstitution of B cells (A) Total B cells; (B) naïve B cells.  $P^*$ = $P$ -value at day 30;  $P^{**}$ = $P$ -value at day 100;  $P^{***}$ = $P$ -value at day 180; %: percentage of cells; Absolut: absolute number of cells. ATLG: anti T-cell lymphocyte globulin; PTCy: and post-transplant cyclophosphamide.

CD4+ T-cell population and increased CD8+ T-cell numbers in the ATLG group. Another explanation for the decreased number of CD8+ T cells in the PTCy group is the ability of PTCy to selectively target proliferating NK and CD8+ T cells more than CD4+ T cells.<sup>15</sup> These findings were all validated in the donor subgroup analyses which makes it safe to assume that the observed discrepancies between the ATLG and PTCy groups can be attributed to the difference in the TCD between the groups. From our findings, we can hypothesize that PTCy has less impact on all the CD4+ T cells, while it has increased activity against CD8+ T cells, which was expressed by higher proliferation of CD4+ T cells in the PTCy and CD8+ T cells in the ATLG group. In addition, we can hypothesize that ATLG has a more pronounced effect on memory CD8+ and CD4+ T cells than PTCy.

The higher percentage of T lymphocytes and activated lymphocytes in the ATLG group may be explained by the higher CD8+ T-cell and NTK-cell reconstitution observed in this group.

It has been proven in animal models that Tregs suppress GVHD without decreasing GVL,<sup>45</sup> and that they accelerate post-transplant T-cell immune reconstitution in murine models.<sup>46</sup> In our study Tregs persisted after transplant and we observed no significant differences in Treg reconstitution post-allo-HSCT between the two groups. This may be explained by the cyclophosphamide resistance of Tregs conferred by their increase in the expression of aldehyde dehydrogenase for cyclophosphamide detoxification which allow them to persist posttransplant in the PTCy setting,<sup>10,15</sup> and by the selective sparing of Tregs by ATLG.<sup>39</sup>

After allo-HSCT the numbers of total B cells normalize

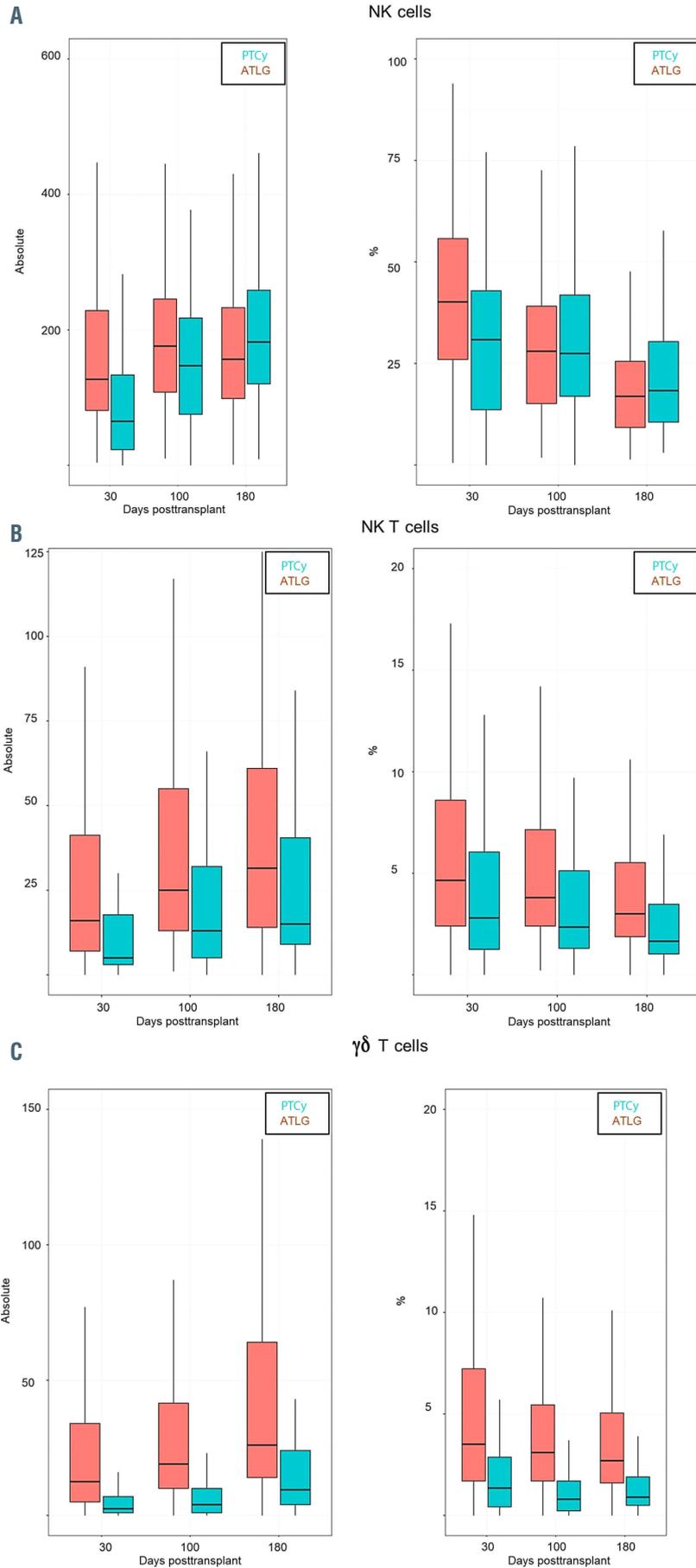


Figure 5. Comparison between ATLG and PTCy regarding immune reconstitution of innate immune system (A) B natural killer (NK) cells; (B) natural killer T (NKT) cells; (C)  $\gamma\delta$ T cells.  $P^*$ = $P$ -value at day 30;  $P^{**}$ =  $P$ -value at day 100;  $P^{***}$ = $P$ -value at day 180; %: percentage of cells; Absolut: absolute number of cells. ATLG: anti T-cell lymphocyte globulin; PTCy: and post-transplant cyclophosphamide.

within 3 months to 1 year.<sup>47-49</sup> However, the reconstitution in the first year compromise mainly transitional and naïve subsets and memory B cells occurs much later. Our results show normalization of the total B-lymphocyte count at day 60, but no complete recovery of naïve and memory B-cells at last follow-up. In addition, we observed a higher percentage and count of naïve B cells in the PTCy group at day 100.

Even though we observed a higher incidence of NRM in the PTCY group on univariate analysis, this did not persist on multivariate analysis (hazard ratio 1.6; 95% Confidence Interval: 0.98-2.48;  $P=0.061$ ). This difference can be explained by a higher proportion of high risk patients (higher proportion of mismatch transplants and ALL) in the PTCY group when compared to ATLG.

Given the retrospective nature of our study and the heterogeneity of our patient population especially the differences in donor types, ATLG dosing, immune suppression regimens and conditioning regimens, it should be noted that our study is prone to bias especially with univariate analysis. In an attempt to reduce bias and render the population more homogenous we selected consecutive patients undergoing allo-SCT only with MAC regimens and PBSC as a stem cell source. In addition, we conducted subgroup analysis in which we found anecdotal differences in clinical outcomes and IR between the 30 mg/kg and 60 mg/kg ATLG dose, and we confirmed our findings

in a subgroup analysis according to donor type. However, our findings should be confirmed in more homogenous prospective studies.

### Conclusion

Acknowledging the bias associated with our study especially its retrospective nature, while taking into consideration the large sample size, it is safe to conclude that a better CD8+ T-cell, NK-cell, NKT-cell and  $\gamma\delta$ T-cell reconstitution is observed in the ATLG group while improved CD4+ recovery is a hallmark of the PTCy group. Even though these findings have been translated into a decreased incidence of infection and moderate/severe cGVHD in the ATLG group they had no impact on any of the other long-term outcomes. So, it remains undetermined which TCD strategy is better to consider and results from well-designed randomized studies are needed.

### Disclosures

*No conflicts of interest to disclose.*

### Contributions

*RM and NK designed the study, analyzed data, interpreted results, and wrote the manuscript; NG, UFF, GZ, SH, CW, FA, and MC collected and analyzed data. All authors approved the final version of the manuscript.*

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