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Immune reconstitution dynamics after unrelated allogeneic transplantation with post-transplant cyclophosphamide compared to classical immunosuppression with anti-thymocyte globulin: a prospective cohort study

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Running heads: Immune reconstitution after PTCy or ATG in unrelated HSCT

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Conflicts of interest

PB, LNK and The University of Texas MD Anderson Cancer Center have institutional financial conflict of interest with Takeda Pharmaceutical for the licensing of the technology related to CAR-NK cell research. MD Anderson has implemented an Institutional Conflict of Interest Management and Monitoring Plan to manage and monitor the conflict of interest with respect to MDACC's conduct of any other ongoing or future research related to this relationship.
PB participates on the Scientific Advisory Board for Dialectica.

Author contributions
MNK contributed with study design and methodology, data collection and analysis, results interpretation, writing of the manuscript draft and subsequent revisions.
FAR and PSC contributed with data collection and contribution to the execution of laboratory tests in flow cytometry
LJA contributed with statistical analyses of data and results interpretation
LNK, CMM and AAFR contributed with study conceptions, results interpretation and critical review of the manuscript drafts.
PB contributed with Cytobank analysis
LCM and NH contributed with study conception and methodology, general supervision, results interpretation and critical review of the manuscript drafts.
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Abstract

Post-transplant cyclophosphamide has contributed to the success of haploidentical hematopoietic stem cell transplantation (HSCT) and is also been used in transplantation from matched donors. However, limited data on the immune reconstitution after this type of immunosuppression is available. We aimed to evaluate immune reconstitution after HSCT from unrelated donors, comparing anti-thymocyte globulin (ATG) and post-transplant cyclophosphamide (PTCy). Consecutive patients undergoing HSCT from unrelated donors and receiving either ATG or PTCy were prospectively included. Immune reconstitution analyses were performed by flow cytometry pre transplant and on days 30, 60, 90, and 180 post-transplant. We included 36 patients, 20 in the ATG group and 16 in the PTCy group. In the early post-transplant period (D+30), the ATG group showed a higher number of total lymphocytes, T, B, and NK cells compared to the PTCy group. However, at D+180, the PTCy group exhibited a higher number of B cells. On D+60 and D+90, the ATG group displayed higher number of NK cells CD56dim compared to the PTCy group, while on D+180, the PTCy group showed higher number of CD56neg, CD16pos, and, NKG2Dpos NK cells. Naive CD4+, transition CD4+, and naive CD8+ T cells on D+60 were identified as risk factors for acute graft-versus-host disease (GVHD) grades II-IV, and a higher count of CD4+ memory cells on D+180 was identified as a risk factor for chronic GVHD. In the context of unrelated allogeneic transplantation, immunosuppression with PTCy was associated with later B, T and NK cells reconstitution compared to ATG.

Keywords: bone marrow transplantation, viral diseases, transplant, immune recovery, unrelated transplantation, thymoglobulin, Epstein Barr, Cy, immunosuppressor.

Introduction

Different immunosuppression protocols are employed in allogeneic hematopoietic stem cell transplantation (HSCT) to mitigate the acute and chronic graft-versus-host disease (GVHD) risk. These protocols are especially used in the scenario of transplants from unrelated donors, in which classical immunosuppression with calcineurin inhibitor and methotrexate are associated with high GVHD risk [1]. Some studies have shown that the association of anti-thymocyte globulin (ATG) in transplants protocols results in reduction in grades 2-4 acute GVHD and moderate to severe chronic GVHD [2-5].

The use of post-transplant cyclophosphamide (PTCy) has revolutionized haploidentical HSCT, with impressive outcomes related do GVHD, while it is relatively simple, cost-effective, easily reproducible, and eliminates the need for expensive T-cell depletion techniques. The PTCy strategy, when compared with haploidentical transplantation using depleted *in vitro* T-cells, yields better outcomes related to infectious complications [6-8]. Given the encouraging outcomes observed in haploidentical HSCT, especially concerning to GVHD, and the inherent assumption that PTCy could mitigate GVHD following a matched transplant, some trials are being conducted to investigate the value of PTCy in HLA-matched transplants [9-14], with encouraging results in the context of myeloablative conditioning regimens [15,16] or reduced intensity conditioning [17].

Poor immune reconstitution post-allogeneic HSCT is directly related to significant post-transplant morbidity and mortality, including viral, bacterial, and fungal infections, as well as relapse of the underlying disease. T-specific immunity is affected by a combination of factors, including the conditioning regimen used, thymic involution, patient age, graft type, type of T cell depletion (*in vivo* or *in vitro*), HLA mismatches between donor and patient, immunosuppression used, and GVHD [18]. In general, innate immunity recovers early after transplant, initially with the recovery of monocytes, followed by neutrophils. Natural killer (NK) are the first lymphoid cells to reconstitute and remain the predominant lymphocytes in circulation in the first three months post-transplant [19] and its early counts may be associated to transplant outcomes [20]. The recovery of adaptive immunity, especially T lymphocytes and B lymphocytes, is slower, potentially longer than one year [21]. The reconstitution of T lymphocytes occurs through two main mechanisms: 1) survival and peripheral expansion of donor-infused memory T lymphocytes, or 2) generation of donor T lymphocytes via the thymus from donor hematopoietic precursors.

However, immune reconstitution in the context of unrelated transplant, using PTCy, has still been little studied, with limited data comparing immune reconstitution with this immunosuppression strategy to the classical approach with methotrexate, calcineurin inhibitor, and ATG. The assessment of immunological profiles may allow preventive measures after transplant. Thus, our objectives were to evaluate the kinetics of immune reconstitution post-allogeneic HSCT from unrelated donors with two types of immunosuppression, ATG or PTCy. In addition, we aimed to correlate the impact of the immune reconstitution with complications in HSCT.

Methods

Study design, setting, and ethics

In this prospective cohort study, we included patients undergoing allogeneic HSCT from unrelated donors using immunosuppression with ATG or PTCy in the years 2019 to 2021 in a single private tertiary hospital (Hospital Israelita Albert Einstein). The study protocol was approved by the local ethics committee (number: CAAE 00527018.6.0000.0071). Informed consent for inclusion in this study was obtained from all research participants.

Participants eligibility and recruitment for this study

We included patients of any age undergoing their first allogeneic HSCT for hematological diseases with an unrelated donor, using an immunosuppression protocol with ATG or PTCy. The choice for one or another approach was based on the allocation in a concurrent study at the same institution, a clinical trial in which patients were randomized between ATG and PTCy (NCT03818334) or the clinical choice of the bone marrow transplant team. We excluded cases with both patient and donor with negative cytomegalovirus (CMV) serology pre-transplant. Clinical evaluations and treatments may be found in the supplemental material.

Immune reconstitution monitoring

Patients' peripheral blood samples were collected pre-transplant and on D+30, D+60, D+100 and D+180 post-transplant. Flow cytometric immunophenotyping for immune recovery was performed prospectively. For the analysis of mononuclear cells, an initial gate on CD45 was used. For T cells, CD3 and subsets CD3CD4 and CD3CD8 were used. For NK cells, CD3-, CD14-, CD11b-, CD19-, CD56+ or CD56-, CD16+ and NKG2D+ were used, and for B cells, CD3-, CD14-, CD11b-, CD19+. Monocytes included CD3-, CD14+, CD11b+. A subset analysis of CD4+ and CD8+ naive (CD45RA+), memory (CD45RO+) and transitional T cells (CD45RA+/-CD45RO+/-) was also performed. We also analyzed some patients data using the Cytobank platform to support and illustrate the findings of flow cytometry [22].

Outcomes

The primary endpoint in this study was B, T, and NK cells immune recovery in patients undergoing unrelated allogeneic transplantation using PTCy or ATG. The secondary endpoints studied included overall survival, cumulative incidence of TRM, 100-day mortality, aGVHD, CMV reactivation, and disease.

Statistical analysis

Categorical variables were summarized using frequencies and percentages, and continuous variables using means and standard deviations, or medians and ranges where appropriate, as well as the number of observations.

To compare categorical variables, the chi-squared or the Fisher's Exact tests were used, as appropriate. To compare continuous variables, the parametric t-Student test was used, if applicable, or the non-parametric Mann-Whitney test.

Overall survival was defined from the date of HSCT to the patient's death, and was estimated using the Kaplan-Meier method, with patients alive at the time of data

collection being censored. The log rank test was used for comparison of survival curves. Cumulative incidence curves (non-relapse mortality and relapse) were built with the Gray method. For relapse, non-relapse mortality was the competing event, and vice-versa. For uni and multivariable analyses for survival outcomes, we used the Cox model.

Statistically significant values were defined as P≤ 0.05. Statistical tests were performed using the R (Vienna, Austria) statistical software, version 4.3.2.

Results

Clinical Outcomes

The demographic characteristics and clinical outcomes of the included population and their donors are reported in Table 1. At 180 days post-transplant, acute GVHD grades II-IV occurred in 40% of the ATG group and 25% of the PTCy group (p=0.26). Chronic GVHD at 3 years was 31% for ATG and 19% for PTCy (p=0.19) (Supplementary figures 1 and 2). In a follow-up of 3.25 years, overall survival was 59% for ATG and 74% for PTCy (p=0.4) (Supplementary figure 3). CMV reactivation rates were 70% in the ATG group and 75% in the PTCy group within 180 days (p=0.98) (Supplementary figure 4), with similar median viral loads (median 959 Ui/ml in ATG and 932 Ui/ml in PTCy group) p=0.81. Second CMV reactivation occurred in 20% of ATG patients and 6% of PTCy patients, with no CMV-related disease in the ATG group compared to three cases in the PTCy group. Other viral infections are described in the Supplemental Material.

Immune reconstitution

There was no difference between the ATG and PTCy groups in terms of pre-transplant lymphomononuclear cell profile (represented in **Table 2** and **Figures 1, 2** and **Supplementary figure 5**). On D+30, in the ATG group, there was a significantly higher number of total lymphocytes (924 cells/uL versus 473 cells/uL, p=0.0004), T lymphocytes (238 cells/uL versus 82 cells/uL, p=0.022), B lymphocytes (17 cells/uL versus 2 cells/uL, p=0.004), and NK cells (385 cells/uL versus 96 cells/uL, p<0.001) compared with the PTCy group (two illustrative cases of patients from the ATG and PTCy groups, using the Cytobank platform, are represented in Figure 3 and supplemental material). On D+180, there was a higher count of B cells in the PTCy group (54 cells/uL versus 282 cells/uL, p=0.003).

The subtypes of T cells are represented in **Table 3**. A predominance of CD8 T cells was observed compared to CD4 T cells from the early stages of transplantation, persisting until D+180 in both analyzed groups. We observed a significantly higher population of double-positive CD4+ and CD8+ T cells in the PTCy group compared to the ATG group on D+180 (21 cells/µL vs. 98 cells/µL, p=0.003) **(Supplementary figure 6).**

Among the subsets of T cells, we found no difference between the groups regarding the profile of CD4+ and CD8+ T cells. Pre-transplant, there was a predominance of CD4+ memory T lymphocytes and naive CD8+ T lymphocytes in both groups. On D+30, in both groups, there was a predominance of CD4+ and CD8+

memory T lymphocytes. On D+60 and D+90 in both groups, we observed a persistence of predominance of CD4+ memory T cells, but with an increase in naive and transitional CD8+ T cells, while on D+180, naive CD8+ T cells were predominant in both groups (**Supplementary figure 7 and 8 and Supplementary table 1).**

An analysis of NK cell subsets was conducted based on the expression of CD56hi or CD56dim and the expression of CD16 **(Table 4)**. On D+30, there was a significantly higher number of all NK cell subtypes in the ATG group. On D+60 and D+90, there was a higher number of CD56dim cells in the ATG group. On D+180, there was a significantly higher number of CD56neg NK cells (CD3-, CD11b-, CD14-, CD19-, CD56 negative, CD16 positive, NKG2D positive) in the PTCy group (**Figure 4**).

Impact of immune reconstitution on clinical outcomes

Performance status of 90 or 100 in the Karnofsky scale was a protective factor for overall survival (HR 0.3 95%CI 0.1-0.96, p=0.042), and a higher absolute number of transitional CD4+ cells was associated with worse survival (HR 1.01 95%CI 1.01-1.04; p=0.0083).

Higher naive CD4+ cells (HR 1.04 95%CI 1-1.09 p= 0.047), CD4+ cells transitional stage (HR 1.04 95%CI 1.01-1.07 p= 0.012), and naive CD8+ cells (HR 1 95% CI 1-1.01 p= 0.026) on D+60 were risk factors associated with acute graft-versus-host disease (aGVHD) grades II-IV. On D+180 post-transplant, a higher quantity of memory CD4+ cells (HR 1.01 95%CI 1-1.02 p= 0.014) was a risk factor for chronic graft-versus-host disease (cGVHD). We also analyzed the impact of acute GVHD grades II-IV in immune reconstitution and found that patients who had GVHD had lower lymphocytes count on day+60 (939 cells/uL versus 476 cells/uL, p=0.009), lower B cells on day +90 (78.8 cells/uL versus 19.8 cells/uL, p=0.042) and lower CD4+ memory T cells on day +90 (76.5 cells/uL versus 36.8 cells/uL, p= 0.025) (Supplementary table 2).

Regarding graft source, peripheral blood, compared to bone marrow, was associated with higher counts of naïve CD4+ T cells on D+30 (2.24 cells/uL vs 0.44 cells/uL; p=0.03), higher counts of T cells on day +60 (523 cells/uL vs 188 cells/uL; p=0.03), higher CD4+ T cells on day +60 (39 cells/uL vs 22 cells/uL p=0.04), higher CD8+ T cells on day +60 (89 cells/uL vs 30 cells/uL p=0.01) and higher counts of NK cells CD56dim on day +60 (105 cells/uL vs 47 cells/uL p=0.04). We found no strong or median correlation with CD34 product counts and immune reconstitution on Spearman correlation test.

We also analyzed the impact of conditioning regimen on immune reconstitution and found that reduced intensity conditioning, compared with myeloablative, was associated with higher counts of CD4+ naïve T cells on day +30 (3.86 cells/uL vs 0.53 cells/uL, p=0.011), higher NK CD56high on day +30 (287.8 cells/uL vs 79.4 cells/uL, p= 0.01) and NK CD56dim on day +60 (137 cells/uL vs 52.9 cells/uL p= 0.03) while myeloablative conditioning was associated with higher NK cells CD56 neg, CD16 pos on day +90 (20.7 cells/uL vs 9.21 cells/uL p=0,03) and higher double positive CD4 and CD8 T cells on day +90 (34 cells/uL vs 7.4, cells/uL p=0.005).

Discussion

In the present study, we prospectively analyzed patients with onco-hematological diagnoses who underwent allogeneic transplantation with unrelated

donors and compared two different post-transplant immunosuppression approaches (classical immunosuppression with ATG or PTCy) according to post-transplant immune reconstitution. Early after the transplant, at day 30, the ATG group exhibited a higher number of total lymphocytes including T, B, and NK lymphocytes, as well as CD56 high, CD56dim and CD56neg, CD16pos, NKG2Dpos NK cells, compared with the PTCy group. However, we observed a greater increase in the total lymphocyte count in the PTCy group compared to the ATG group after D+90, mainly represented by CD8+ T lymphocytes. Additionally, there was a statistically significant higher number of B lymphocytes and CD56neg, CD16pos, NKG2Dpos NK lymphocytes in the PTCy group at D+180.

Rambaldi et al. [23] also assessed immune reconstitution in patients undergoing haploidentical transplantation with PTCy or matched transplantation with classic prophylaxis. Their study described a delayed reconstitution of T cells, with a significant reduction in naive T lymphocytes, and an early decrease in NK cells following haploidentical HSCT with PTCy. Furthermore, the authors observed a preferential expansion of immature CD56brightCD16 NK cells in the haploidentical group, compared to classic immunosuppression prophylaxis. The later reconstitution of total lymphocytes, B, T, and NK cells in the PTCy group may explain the higher grade 2 infection rate observed in the PTCy group in the Phase 3 study comparing PTCy and classical immunosuppression in matched and mismatched transplants with a reduced-intensity conditioning regimen [17] or the higher incidence of CMV reactivation in haploidentical and matched sibling transplants with PTCy compared to classical immunosuppression in sibling transplants in the retrospective CIBMTR analysis [24].

In the present study, at days D+60 and D+90, the ATG group showed a higher number of mature NK cells (CD56dim) cells compared to the PTCy group. Early-reconstituted NK cells exhibit an immature CD56bright phenotype, representing 40-50% of NK cells in the initial months, acquiring a predominantly CD56dim phenotype after several months, whereas, in healthy adults, CD56bright phenotype constitute approximately 5-10% [25]. It is believed that NK cell reconstitution primarily derives from the differentiation and maturation of progenitor cells rather than the expansion of mature NK cells within the graft. This idea is supported by the finding that NK cell reconstitution is mainly independent of graft type and its NK cell content [26]. The difference found in the NK maturation profile between the groups may be explained by the selective purging of dividing cells by PTCy, which has already been demonstrated by Russo et al and may be supported by the fact that proliferating NK cells after HSCT infusion do not express aldehyde dehydrogenase and may be killed by cyclophosphamide. [27] Previous studies have already shown an immature NK phenotype in haploidentical transplantation in more intensive immunossupression with positive CD34 selection [25,28].

In the present study, we identified a population of NK cells characterized as CD45pos, CD3neg, CD11bneg, CD14neg, CD19neg, CD56neg, CD16pos, NKG2Dpos that was higher in PTCy group on day +180. Human NK cells are generally defined as CD3-CD56+ lymphocytes. However, a subset of lymphocytes, CD56-CD16+ (CD56neg), displaying associations with NK markers, expands during chronic viral infections such as HIV-1 and HCV, and herpesvirus infections [29]. Many questions persist regarding the origin, development, phenotype, and function of the CD56neg NK cell population. Gaddy J et al have shown a subset of NK cells CD56 neg CD16 pos in cord blood

transplants that has low lytic activity and are possible precursors of mature NK cells [30]. A meta-analysis evaluating 757 individuals from a total of 28 studies found that CD56neg cells constitute 5.67% of NK cells in healthy peripheral blood, while HIV-1 infection increases this population to 10.69% with no evidence of increased exhaustion or decreased proliferation within the CD56neg subset [31]. In another study with health elderly donors, co-infection with CMV and EBV was associated with expansion of CD56 neg NK cells with reduced cytotoxic capacity, what correlated with end-stage-differentiated T cells and reduced CD4/CD8 ratio, reflecting immune risk profile [32]. The immune dysfunction after transplant and high prevalence of CMV and EBV serology in the population of the study may explain the identification of this NK cell in the present study.

The current study observed an increase in CD8+ T lymphocytes, leading to inversion of the CD4/CD8 ratio. CD4+ T lymphocytes at all evaluated time points and CD8+ T lymphocytes at D+30 were phenotypically represented mostly by memory lymphocytes, as previously described in patients who received PTCy immunosuppression [33]. We observed an elevation in CD8+ naive and transition T cells in both groups from D+90, with a predominance of CD8+ naive T cells at D+180 in both groups. This increase was accompanied by low counts of naive and transition CD4+ T lymphocytes compared to CD4+ memory T cells, indicating slow reconstitution of CD4+ T cells [19,34]. Kanakry et al. also described that the reconstitution of CD4+ and CD8+ T lymphocytes primarily involved effector memory cells (CD45RA-CCR7-) and terminally differentiated cells (CD45RA+CCR7-) in patients undergoing transplantation with PTCy [33]. It is known that the first "wave" of T cells post-transplant comprises donor T cells undergoing proliferation induced by lymphopenia and alloactivation, resulting in polyclonal T cells with a restricted TCR repertoire, limited antigenic specificity or alloreactivity activity, causing graft-versus-host disease (GVHD). Complete T cells reconstitution, with TCR diversity, requires thymic regeneration and endogenous T cells development, turning this process very slow [35].

Besides that, in the PTCy group, we found a higher number of double-positive T lymphocytes (CD4+CD8+) at D+180. The recovery of de novo T lymphocytes is dependent on a functional thymus. The infused hematopoietic stem cells (CD34), are able to recover the bone marrow function and generate de novo lymphoid progenitors that migrate to the thymus. These progenitors, initially double-negative (without CD4 and CD8 expression), undergo a maturation process, becoming double-positive. At this stage, these cells undergo negative selection, where T cells that recognize self-antigens undergo apoptosis, an essential step for GVHD control [36]. If double-positive T cells do not pass through negative selection, escaping thymic selection, it may be related to the development of GVHD. Hussen et al. [37] observed the development of double-positive T cells during xenogeneic GVHD and Hess et al. [38] identified, in an observational study with 35 patients submitted to PTCy, that CD4/CD8 double-positive cells is a predictive biomarker of GVHD. In a xenogeneic model, the authors identified this population as transcriptionally, metabolically, and functionally distinct from single-positive CD4 and CD8 T cells, demonstrating that these are chronically activated CD8 T cells, highly inflammatory, secreting IFN-γ, TNF-α, GM-CSF, IL-17A, IL-22, granzyme, and perforin. These cells acquire effector functions of the CD4 lineage and are directly implicated in the pathogenesis of GVHD. As all patients in this study received PTCy, it is uncertain whether the predictive value of GVHD would also apply to the classic

immunosuppression protocol. However, in our study, these lymphocytes were more frequent in the PTCy population.

In our study of the correlations of clinical outcomes with immune reconstitution, at D+30, a higher absolute number of CD4+ transition cells were associated with worse survival. CD4 transitional cells represent an intermediate stage between naive and memory T cells. The transition from CD45RA+ naive T cells to CD45RO+ memory T cells, initiated by TCR-mediated signaling in the G1 phase of the cell cycle, suggests that these transitional cells are in an active state of differentiation and proliferation [39]. This hyperactivation can lead to an increased risk GVHD, what may be related to increased mortality risk. Additionally, the immune reconstitution involving these highly reactive transitional T cells may not provide effective protection against infections due to their immature functional state, further contributing to increased mortality. Therefore, the presence of a higher number of transitional CD4+ T cells may indicate an imbalance in immune homeostasis, resulting in adverse clinical outcomes post-transplantation. Besides that, higher quantity of CD4+ naive, CD4+ transition, and CD8+ naive cells at D+60 were risk factors for acute GVHD. Additionally, a higher quantity of CD4+ memory cells at D+180 was a risk factor for chronic GVHD. In acute GVHD, after activation, alloreactive memory T cells cause damage to various tissues, such as the gastrointestinal tract, liver, skin, and thymus, through direct cytotoxicity or cytokine-mediated mechanisms. However, uncertainties persist regarding whether these effector memory cells present in the patient's blood with acute GVHD are derived from the graft, proliferating upon encountering the antigen, or if they differentiate from naive cells in the graft [40]. Some studies suggest that naive T cells are even more potent inducers of acute GVHD and may be associated with more severe forms, possibly due to being the most potent inducers of alloreactive responses *in vitro*. A possible explanation could be that naive T cells have a broader T cell receptor repertoire, and a higher proliferative capacity, resulting in a greater potential for clonal expansion and for recognizing antigens presented by antigen-presenting cells (APCs). A study evaluating the association of donor-infused CD4+ T cells with acute GVHD found that a higher quantity of infused CD4+ naive T cells was associated with a higher risk of grades II-IV acute GVHD and chronic GVHD [41]. The increased risk of GVHD associated with naive CD4+ T cells may explain the worse overall survival found in the present study with more CD4+ transition T cells at D+60.

We found higher counts of naïve CD4+ T cells on D+30 and higher counts of T cells, CD4+ T cells, and CD8+ T cells on D+60 in peripheral blood compared to the bone marrow graft source. Other authors have found higher CD4+ T cell counts with peripheral blood, which may be explained by the higher number of lymphocytes infused, approximately one log higher with peripheral blood compared to bone marrow [42,43]. Reduced-intensity conditioning was associated with higher counts of CD4+ naive T cells on day +30 and higher NK CD56high cells on day +30 and NK CD56dim on day +60. Petersen et al [43] also compared nonmyeloablative and myeloablative conditioning regimens in HLA-identical sibling donor transplants and found earlier normalization of NK cells, CD8+ T cells, and CD4+ T cells but delayed B cell recovery with less intensive conditioning.

The present study has some limitations, primarily related to the number of patients included in the analyses. However, this prospective study comprehensively analyzed the immune reconstitution of patients using ATG or PTCy, providing valuable

information for clinical practice regarding this innovative form of post-transplant immunosuppression.

In conclusion, in the context of unrelated allogeneic transplantation, the use of PTCy compared to classical immunosuppression with ATG is associated with a lower number of total lymphocytes, T cells, NK cells, and B cells on D+30, and a lower number of CD56dim NK cells on D+60 and D+90, but with a higher proportion of double-positive T cells, B cells, and a higher proportion of CD56negCD16posNKG2Dpos NK cells on D+180. These results may contribute to a deeper understanding of the differences in immune reconstitution and their impact on clinical practice, which is crucial for refining transplant strategies, such as vaccinations, minimizing complications, and improving patient survival. Collaborative research efforts hold the potential to further advance our comprehension of reconstitution biology and translate these findings into customized therapeutic interventions for HSCT recipients.

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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(3):e132-e143.
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Table1. Patient's characteristics and clinical outcomes

Variable		ATG	PTCY	p
		N= 20	N=16	
Age, years	Median [range]	45 [2-76]	37.8 [3-66]	0.319
Performance status	Karnofsky <90 [%]	6 [30]	2[12.5]	0.257
	Karnofsky 90-100 [%]	14 [70]	14 [87.5]	
Diagnosis	AML [%]	7 [35]	5 [31]	0.479
	MDS [%]	4 [20]	4 [25]	
	ALL [%]	2 [10]	4 [25]	
	CML [%]	2 [10]	3 [18.7]	
	Non-Hodgkin Lymphoma [%]	1 [5]	2 [12.5]	
	Others [%]	1 [5]	2 [12.5]	
Status of response	1st CR [%]	13 [65]	12 [75]	0.872
	2nd CR [%]	2 [10]	1 [6.2]	
	Active disease [%]	5 [25]	3 [18.8]	
CMV sorology	Patient IgG+ [%]	19 [95]	14 [87.5]	1.000
	Donor IgG+ [%]	18 [90]	14 [87.5]	
Conditioning regimen	Myeloablative [%]	12 [60]	14 [87.5]	0.133
	RIC [%]	8 [40]	2 [12.5]	
Graft source	BM [%]	9 [45]	10 [62.5]	0.296
	PBSC [%]	11 [55]	6 [37.5]	
Stem cell infused	CD34 x10^6/Kg [%]	5.4 [1.54-9]	4.6 [1.81-9.15]	0.286
Donor age	Median [range]	33.1 [19-55]	33.7 [22-49]	0.833
Donor sex	Male [%]	17 [85]	10 [62. 5]	0.146
Neutrophil Engraftment, days	Median [range]	12 [11-19]	16 days [12-21]	0.0001
Length of Hospitalization,. days	Median [range]	33 [17-103]	33 days [28-67]	0.26
Calcineurin inhibitor use, days	Median [range]	355 [10-1053]	279 [59-550]	0.3

ATG: anti-thymocyte globulin; PTCy: post-transplant cyclophosphamide; CR: complete remission; CMV: cytomegalovirus; RIC: reduced intensity conditioning; BM: bone marrow; PBSC: peripheral blood stem cells

Table 2. Lymphomononuclear profile

Variable		ATG	PTCy	P
		Cells/uL - mean [range]	Cells/uL - mean [range]	
Pre	Monocytes	156[45-294]	202[136-303]	0.466
	Lymphocytes	1177[565-4396]	1372[68-6486]	0.459
	T Lymphocytes	648[417-971]	717[314-1141]	0.890
	B Lymphocytes	42[16-183]	166[3-372]	0.835
	NK cells	126[90-242]	146[60-294]	0.972
D+30	Monocytes	379[212-477]	892[469-1362]	**0.037**
	Lymphocytes	924[282-3278]	473[176- 864]	**0.0004**
	T Lymphocytes	238[111-801]	82[47-160]	**0.022**
	B Lymphocytes	17[4-27]	2[1-7]	**0.004**
	NK cells	385[241-718]	96[59-196]	**<0.001**
D+60	Monocytes	204[74-386]	299[132-422]	0.488
	Lymphocytes	946[504- 3268]	706[261-1407]	**0.020**
	T Lymphocytes	406[174-717]	190[110-420]	0.100
	B Lymphocytes	50[13-60]	16[4-98]	0.338
	NK cells	319[184-597]	203[128-278]	0.074
D+90	Monocytes	192[127-387]	289[117-588]	0.428
	Lymphocytes	1193[342-2464]	874[211-3141]	0.415
	T Lymphocytes	368[263-839]	359[140-886]	0.597
	B Lymphocytes	37[14-81]	50[22-130]	0.571
	NK cells	282[185-440]	221[146-311]	0.082
D+180	Monocytes	195[83-361]	144[77-557]	0.790
	Lymphocytes	976[161-3821]	1688[485-3909]	0.085
	T Lymphocytes	513[304-602]	925[431-1416]	0.344
	B Lymphocytes	54[13-87]	282[138-553]	**0.003**
	NK cells	294[198-517]	257[155-245]	0.790

ATG: anti-thymocyte globulin; PTCy: post-transplant cyclophosphamide; NK: natural killer cells

Table 3. T cells subtypes

Variable		ATG	PTCy	p
		Cells/uL - mean [range]	Cells/uL - mean [range]	
Pre	CD4+CD8-	285[113-312]	107[50-257]	0.127
	CD8+CD4-	219[144-242]	325[130-559]	0.466
	CD4+CD8+	34[10-47]	91[29-167]	**0.048**
D+30	CD4+CD8-	28[12-125]	13[6-24]	0.083
	CD8+CD4-	82[21-435]	47[22-83]	0.270
	CD4+CD8+	14[3-39]	8[5-16]	0.551
D+60	CD4+CD8-	44[22-92]	23[20-32]	0.116
	CD8+CD4-	316[103-550]	95[48-324]	0.174
	CD4+CD8+	17[7-36]	20[13-28]	0.970
D+90	CD4+CD8-	56[47-132]	49[20-125]	0.521
	CD8+CD4-	265[159-550]	255[68-505]	0.514
	CD4+CD8+	17[6-35]	33[10-47]	0.260
D+180	CD4+CD8-	125 [43-173]	91[54-135]	0.827
	CD8+CD4-	240[202-353]	678[170-1035]	0.320
	CD4+CD8+	21[14-30]	98[57-134]	**0.003**

ATG: anti-thymocyte globulin; PTCy: post-transplant cyclophosphamide.

Table 4. Subsets of natural killer (NK) cells

Variable		ATG	PTCy	p
		Cells/uL - mean [range]	Cells/uL - mean [range]	
Pre	CD56dim	58[29-105]	60 [14-139]	0.890
	CD56hi	65 [33-126]	44 [24-70]	0.367
	CD56-CD16+NKG2D+	7[2-14]	27[8-36]	0.061
D+30	CD56dim	108[72-176]	25[9-48]	**<0.001**
	CD56hi	177[123-464]	62[10-121]	**0.003**
	CD56-CD16+NKG2D+	24[4-47]	3[2-23]	**0.041**
D+60	CD56dim	158[55-232]	50[39-59]	**0.009**
	CD56hi	116[86-155]	136[67-183]	0.986
	CD56-CD16+NKG2D+	21[8-51]	10[4-24]	0.164
D+90	CD56dim	148[81-234]	68[41-88]	**0.009**
	CD56hi	119[76-188]	90[68-164]	0.450
	CD56-CD16+NKG2D+	19[9-37]	21[15-50]	0.326
D+180	CD56dim	120[55-228]	87[52-127]	0.320
	CD56hi	133[63-157]	89[64-131]	0.790
	CD56-CD16+NKG2D+	10[4-19]	24[19-37]	**0.027**

ATG: anti-thymocyte globulin; PTCy: post-transplant cyclophosphamide.

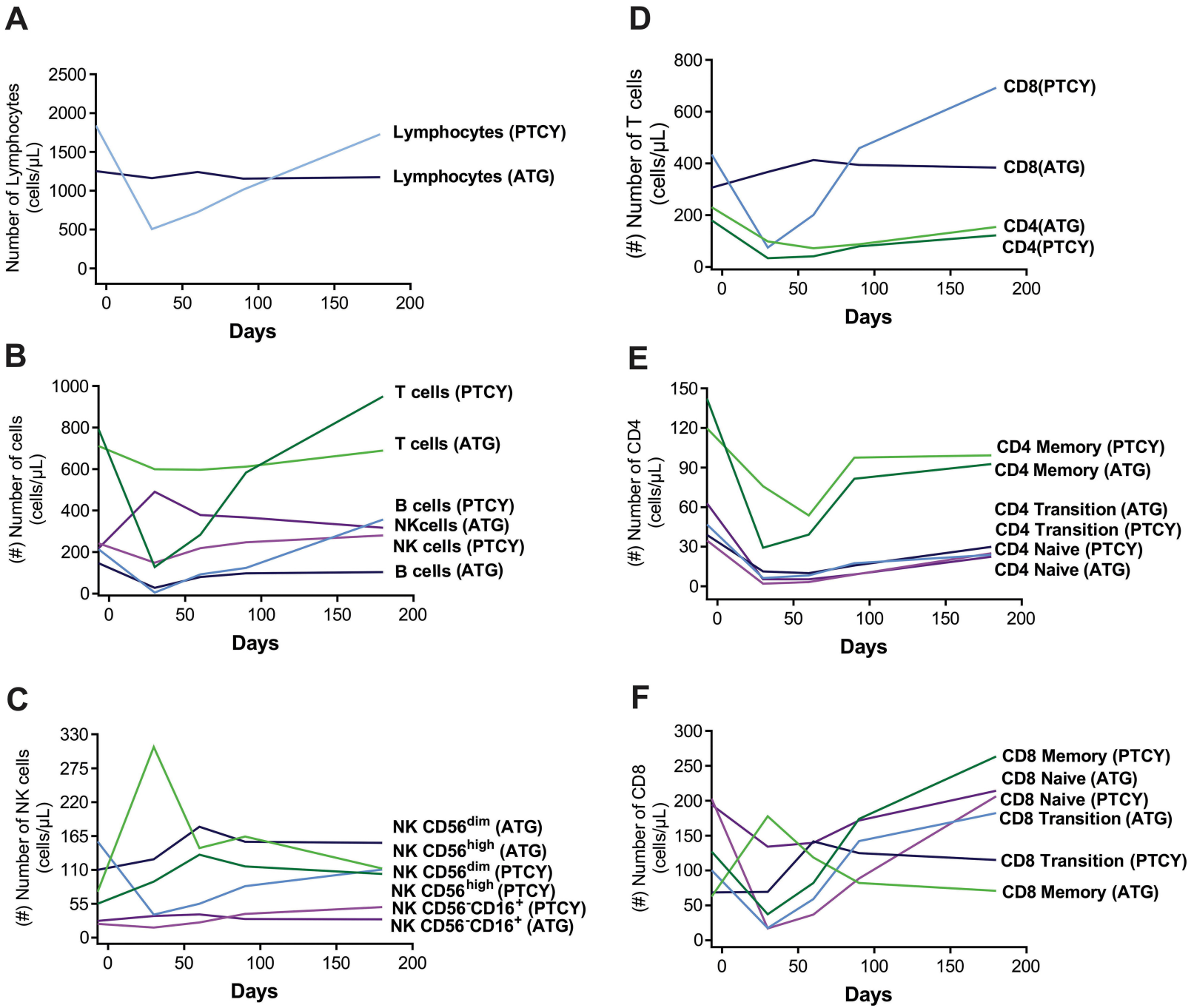
Figure legends

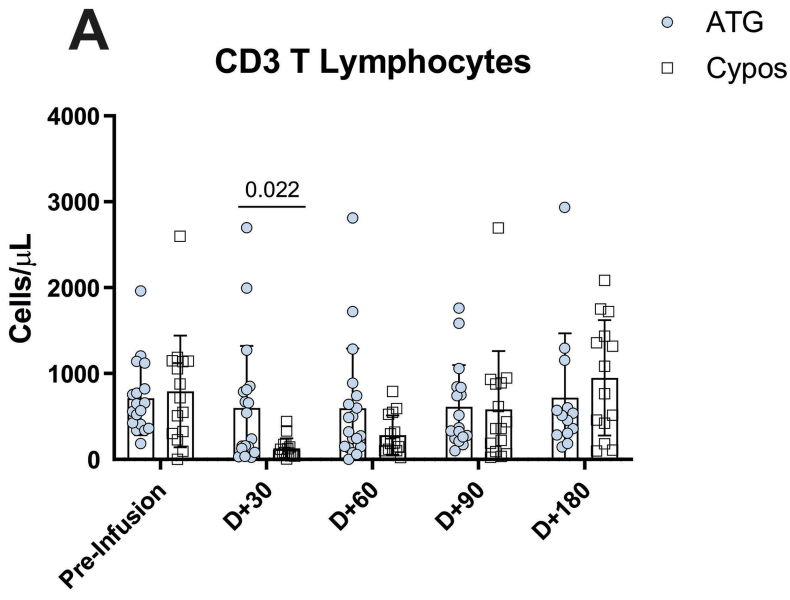
Figure 1. Total lymphocytes, T, B, and NK lymphocytes reconstitution
A. Earlier lymphocyte reconstitution was observed in the ATG group; however, there was a greater increase in lymphocytes in the PTCy group after D+100. B. There was a significant early reduction in T, B, and NK lymphocytes in the PTCy group on D+30, while lymphocyte recovery was observed on D+180. C. In NK cell subsets, there was an early increase in CD56High cells in the ATG group, which did not occur in the PTCy group, and higher counts of CD56dim on days +60 and +90 in ATG group compared with PTCy. D. There was an inversion of the CD4/CD8 ratio in both groups. E. CD4+ T cells were predominantly represented by memory CD4+ cells in both groups D. There was earlier recovery of transition and naive CD8+ cells in both groups

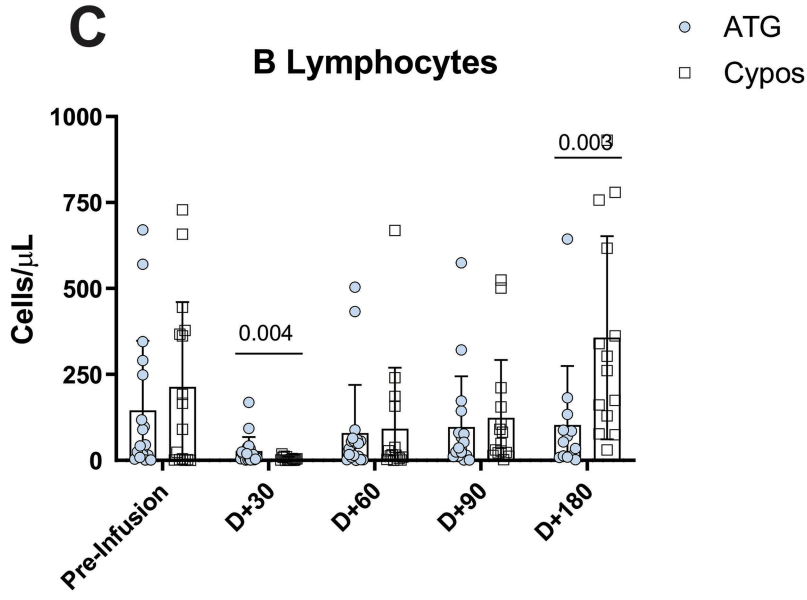
Figure 2. Distribution of T, B and natural killer (NK) lymphocytes by type of prophylatic immunosupression used in patients undergoing hematopoietic stem cell transplantation (HTSC): anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTCy) for pre transplant and 30, 60, 90 and 180 days
There was a higher number of T (label A), NK lymphocytes (label B) and B cells (label C) on D+30 in the ATG group compared to the PTCY group. There were no statistically significant differences between the groups on D+60 and D+90; however, on D+180, there was a higher number of B lymphocytes in the PTCY group compared to the ATG group (label C).

Figure 3. Cell clusters from the Cytobank platform showing differences between 2 patients, one who received anti-thymocyte globulin (ATG) and another who received post-transplant cyclophosphamide (PTCy) regarding markers of B, T, NK and monocytes cells on the day + 30
In the patient receiving ATG, there is a greater aggregate of CD56+ NK cells, a higher aggregate of CD3+ T cells, mainly represented by CD8+ cells, while in the patient who received PTCy, a higher population of CD14+ and CD11b+ monocytes can be visualized

Figure 4. Distribution of natural killer (NK) cells (panel A) and subtypes of $CD56^{high}$ (panel B), $CD56^{dim}$ (panel C), and CD56- CD16+ NK cells (panel D) by type of prophylatic immunosupression used in patients undergoing hematopoietic stem cell transplantation (HTSC): anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTCy) for pre transplant and 30, 60, 90 or 180 days
There is a higher number of total NK cells and CD56dim, CD56High, and CD56-CD16+ NK cells in the ATG group on D+30, a higher number of CD56dim cells in the ATG group on D+60 and D+90, and a higher number of CD56-CD16+ cells in the PTCy group on D+180.


A
Number of Lymphocytes (cells/µL)
2500
2000
1500
1000
500
0
0
50
100
150
200
Days
Lymphocytes (PTCY)
Lymphocytes (ATG)
B
(#) Number of cells (cells/µL)
1000
800
600
400
200
0
0
50
100
150
200
Days
T cells (PTCY)
T cells (ATG)
B cells (PTCY)
NKcells (ATG)
NK cells (PTCY)
B cells (ATG)
C
(#) Number of NK cells (cells/µL)
330
275
220
165
110
55
0
0
50
100
150
200
Days
NK CD56dim (ATG)
NK CD56high (ATG)
NK CD56dim (PTCY)
NK CD56high (PTCY)
NK CD56-CD16+ (PTCY)
NK CD56-CD16+ (ATG)
D
(#) Number of T cells (cells/µL)
800
600
400
200
0
0
50
100
150
200
Days
CD8(PTCY)
CD8(ATG)
CD4(ATG)
CD4(PTCY)
E
(#) Number of CD4 (cells/µL)
150
120
90
60
30
0
0
50
100
150
200
Days
CD4 Memory (PTCY)
CD4 Memory (ATG)
CD4 Transition (ATG)
CD4 Transition (PTCY)
CD4 Naive (PTCY)
CD4 Naive (ATG)
F
(#) Number of CD8 (cells/µL)
300
250
200
150
100
50
0
0
50
100
150
200
Days
CD8 Memory (PTCY)
CD8 Naive (ATG)
CD8 Naive (PTCY)
CD8 Transition (ATG)
CD8 Transition (PTCY)
CD8 Memory (ATG)


A
CD3 T Lymphocytes
ATG
Cypos
Cells/µL
4000
3000
2000
1000
0
0.022
Pre-Infusion
D+30
D+60
D+90
D+180


C
B Lymphocytes
ATG
Cypos
Cells/µL
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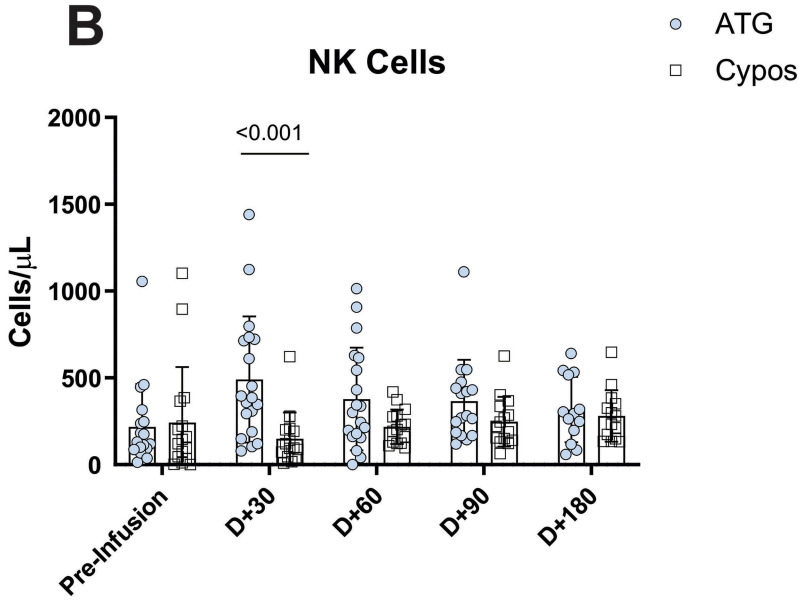
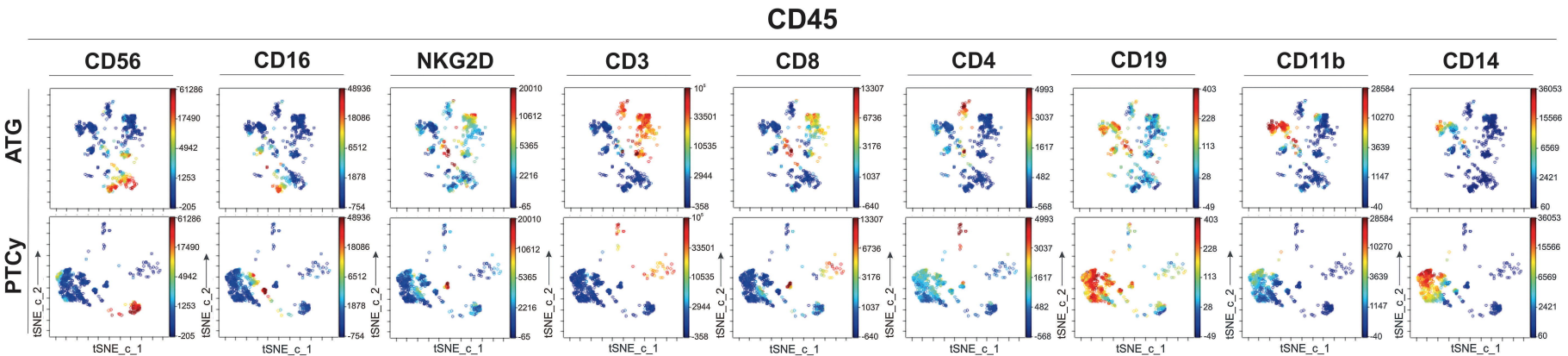
750
500
250
0
0.004
0.003
Pre-Infusion
D+30
D+60
D+90
D+180


B
NK Cells
ATG
Cypos
Cells/µL
2000
1500
1000
500
0
<0.001
Pre-Infusion
D+30
D+60
D+90
D+180


CD45
CD56
CD16
NKG2D
CD3
CD8
CD4
CD19
CD11b
CD14
ATG
PTCy
tSNE_c_1
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61286
17490
4942
1253
-205
48936
18086
6512
1878
-754
20010
10612
5365
2216
-65
10^4
33501
10535
2944
-358
13307
6736
3176
1037
-640
4993
3037
1617
482
-568
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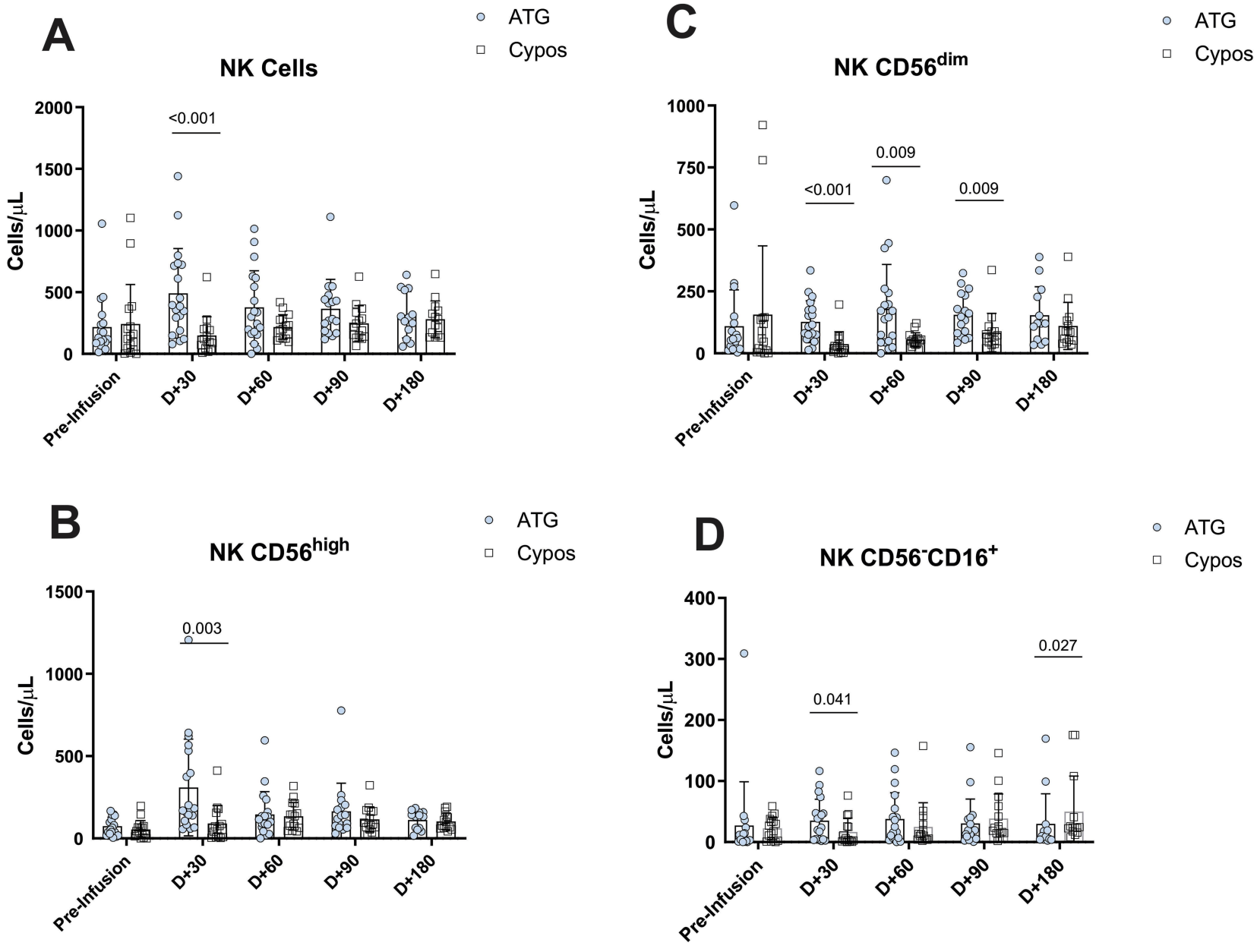
6569
2421
60


A
NK Cells
ATG
Cypos
Cells/µL
2000
1500
1000
500
0
<0.001
Pre-Infusion
D+30
D+60
D+90
D+180
C
NK CD56dim
ATG
Cypos
Cells/µL
1000
750
500
250
0
0.009
<0.001
0.009
Pre-Infusion
D+30
D+60
D+90
D+180
B
NK CD56high
ATG
Cypos
Cells/µL
1500
1000
500
0
0.003
Pre-Infusion
D+30
D+60
D+90
D+180
D
NK CD56-CD16+
ATG
Cypos
Cells/µL
400
300
200
100
0
0.041
0.027
Pre-Infusion
D+30
D+60
D+90
D+180

Supplemental material

Evaluations and treatments

As per protocol in our service, during pre-transplant evaluation, all patients underwent serological assessment for HIV, hepatitis A, B, and C, syphilis, Chagas disease, toxoplasmosis, HTLV, and herpesviruses, including CMV, EBV, herpes simplex, and varicella-zoster. Post-transplant viral monitoring was conducted as follows: quantitative polymerase chain reaction (PCR) for CMV twice a week, quantitative PCR for adenovirus weekly, and quantitative PCR for EBV every two weeks, while the patient was under immunosuppression. Quantitative PCR for polyomavirus in urine (BKV) and human herpesvirus 6 (HHV-6) were performed only in cases of clinical suspicion.

All patients received antimicrobial prophylaxis with trimethoprim-sulfamethoxazole until day -2. This antibiotic was resumed after hematological reconstitution. Intravenous pentamidine, fluconazole 400mg/day or micafungin 100mg/day was used as antifungal prophylaxis and, in cases of high risk for filamentous fungi infection, voriconazole 200mg every 12 hours was used at least until day +75 post-transplant. High-risk cases were considered when patients had high-risk acute myeloid leukemia (AML) or refractory disease, prolonged neutropenia, elderly patients, or previous filamentous fungal infection. Levofloxacin 500mg/day was used as bacterial prophylaxis until neutrophil recovery, and acyclovir 500mg/m^2 two to three times a day (intravenously) or 400mg orally every 12 hours or valacyclovir 500mg/day was used as viral prophylaxis for at least 6 months post-transplant. No patient used CMV prophylaxis with letermovir.

CMV infection was defined as any detection of CMV DNA in plasma. Recurrent CMV infection was defined as a new CMV infection in a patient with previous evidence of CMV infection without virus detection for 4 weeks during active surveillance. CMV disease was defined as the presence of symptoms and/or clinical signs related to the involved organ, with documented CMV in tissue (22). In cases of CMV infection, ganciclovir 5mg/Kg 12/12h until 2 negative CMV PCR tests or at least 3 weeks in case of CMV diasese was the first-line therapy. In cases of cytopenias or ganciclovir toxicity, foscarnet 90mg/Kg 12/12h was used. If EBV viremia exceeded 10,000 copies/mL, treatment with rituximab 375mg/m^2, four injections weekly, was initiated.

Hemorrhagic cystitis caused by BK virus was defined according to the European Conference on Infections in Leukemia (ECIL)(23) as: signs or clinical symptoms of cystitis, hematuria grade 2 or higher, and BKV viremia loads > 7 log 10 copies/mL. Cidofovir 3-5mg/Kg weekly with probenecid was the treatment of choice in cases of refractory and severe disease.

Neutrophil engraftment was defined as the first of three consecutive days with neutrophil count >0.5x10^9/L. Platelet engraftment was defined as seven consecutive days with platelet count above 20,000/uL, in the absence of platelet transfusion.

Regarding the intensity of the conditioning regimens used, myeloablative conditioning consisted of busulfan with an area under the curve (AUC) of 4000 to 5000 for 4 days (on days D-6 to D-3) and fludarabine 40mg/m^2 (D-6 to D-3) or cyclophosphamide 60mg/kg (D-5 and D-4) and total body irradiation (TBI) with 1200cGy (divided into D-3 to D-1) or 990 to 1200cGy divided on the days D-8, D-7, and D-6. Fludarabine 30mg/m² was given on days D-5, D-4, D-3, and D-2. Reduced intensity conditioning regimen consisted of busulfan AUC 5000 for 2 days (D-4 to D-3) and

fludarabine 40mg/m^2 (D-6 to D-3) or total marrow irradiation (TMI) 6Gy (D-8 and D-7), fludarabine 30mg/m^2 (D-6 to D-2) with busulfan AUC 4800 (D-5 and D-4).

GVHD prophylaxis consisted of cyclophosphamide 50mg/kg/day on D+3 and D+4; tacrolimus on D+5 (target concentration 5-15 ng/mL) and mycophenolic acid 15mg/kg/day orally from D+5 to D+35. In cases receiving ATG, prophylaxis consisted of rabbit ATG 3-7.5mg/kg (Sanofi Genzyme), methotrexate 5mg/m2 on D+1, D+3, D+6, and D+11, and initiation of calcineurin inhibitor on D-2. Calcineurin inhibitor was maintained until D+120 in both groups, when we started tapering immunosuppression if there were no GVHD.

Antibodies used in the Immune reconstitution

The antibodies used were CD19-FITC (clone:4G7), CD56-PE (clone:REA196), CD3-PE-CF594 (clone:UCHT1), CD314/NKG2D-PE-Cy7 (clone 1D11), CD45-PerCP-Cy5.5 (clone: 2D1), CD16-APC (clone:B73.1), CD4-APC-Cy7 (clone: SK3), CD8-Alexa1700 (clone:RPA-T8), CD11b-BV421 (clone:CBRM1/5), CD14-BV510 (clone: MfP9), CD62L-FITC (clone: SK11), CD45RO-PE (clone:UCHL1), CD127-PE-Cy7 (clone: HIL-7R-M21), CD25-APC (clone: M-A251), CD45RA-BV421 (clone: HI100), CD197/CCR7-BV510 (clone: 3D12), CD27-BV605 (clone: L128), CD28-BV650 (clone: L-293), CD45 and a violet viable dye - BUV393 (BD Horizon Fixable Viability Stain 440UV). All antibodies were provided by BD Biosciences, BD Pharmingen, BD Horizon, and Miltenyi. For the control of unspecific staining, fluorescence minus one (FMO) was used.

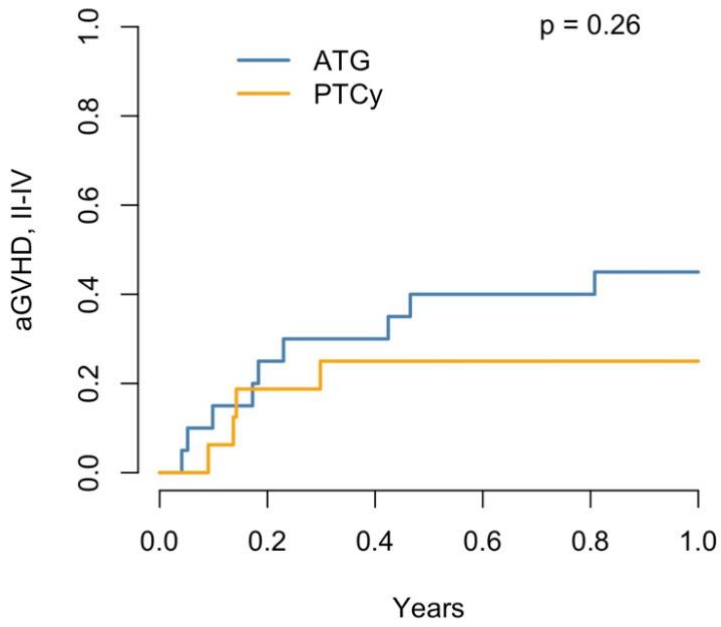
The samples acquisitions were performed using BD LSRFortessa Flow Cytometer (BD Biosciences). All data analysis was performed using the software FlowJo (BD Biosciences).

Clinical outcomes

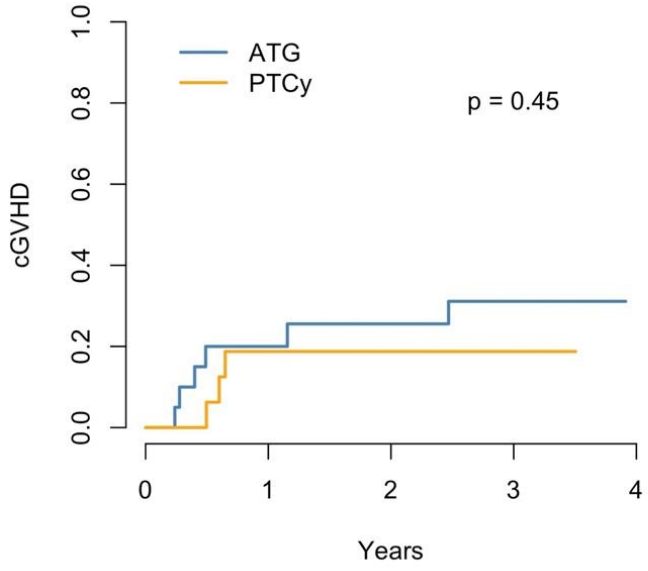
In the ATG group, 4 patients experienced non-relapse mortality. One patient died due to respiratory failure secondary to an infectious complication, another to septic shock before achieving neutrophil engraftment, and two died due to COVID-19 infection (31% at 3 years). In the PTCy group, there were 2 cases of non-relapse mortality (1 due to septic shock secondary to secondary graft failure and 1 due to acute gastrointestinal GVHD) (13% at 3 years) (p=0.30). In the ATG group, there were 5 deaths related to relapse of the underlying disease (25%), while in the PTCy group, there were 2 deaths related to relapse (12.5%).

Regarding other viral reactivations, in the ATG group, the second most frequent reactivation was EBV, which occurred in 7/20 (35%) patients, in a median of 60 days with Rituximab. Hemorrhagic cystitis by BK virus occurred in only 1/20 patient (5%) at 31 days post-transplant. Two patients (10%) had adenovirus infections, with one of them presenting with cystitis and viremia at 48 days post-transplant, and the other presenting with viremia, cystitis, and pulmonary involvement at 21 days post-transplant. In the cyclophosphamide group, 3/16 (18.7%) of the patients had hemorrhagic cystitis caused by BK virus, in a median of 36 days post-transplant. One patient had adenovirus viremia at 47 days post-transplant, and no patient had EBV-related viremia.

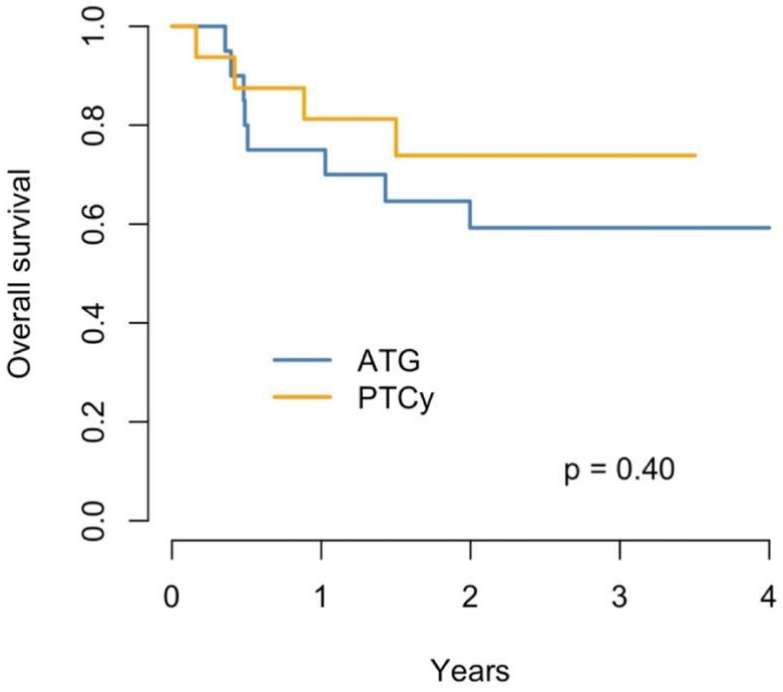
Supplementary Figure 1. Acute GVHD grades II to IV in patients who used ATG (blue line) or cyclophosphamide (orange line).



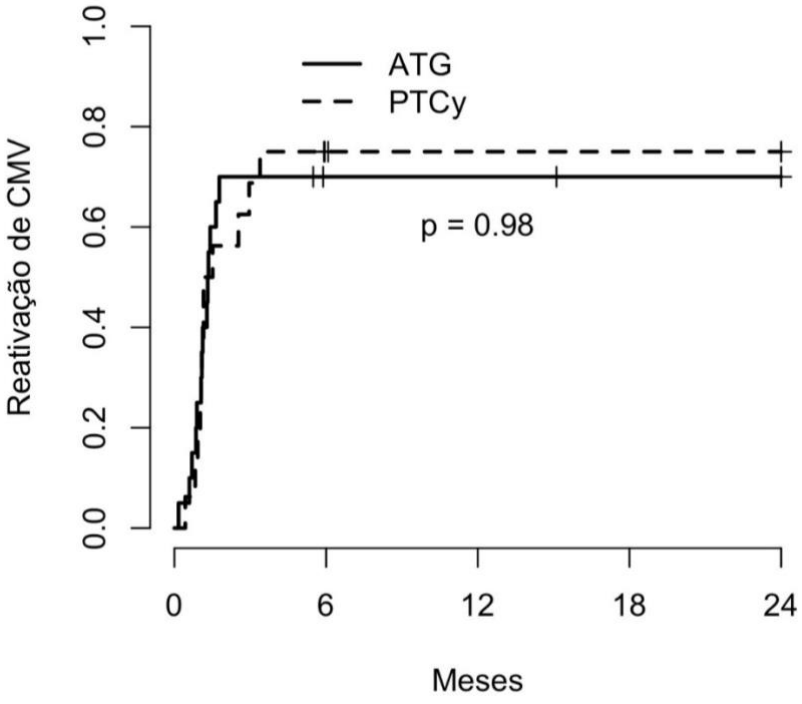
Supplementary Figure 2. Chronic GVHD in patients who used ATG (blue line) or cyclophosphamide (orange line)



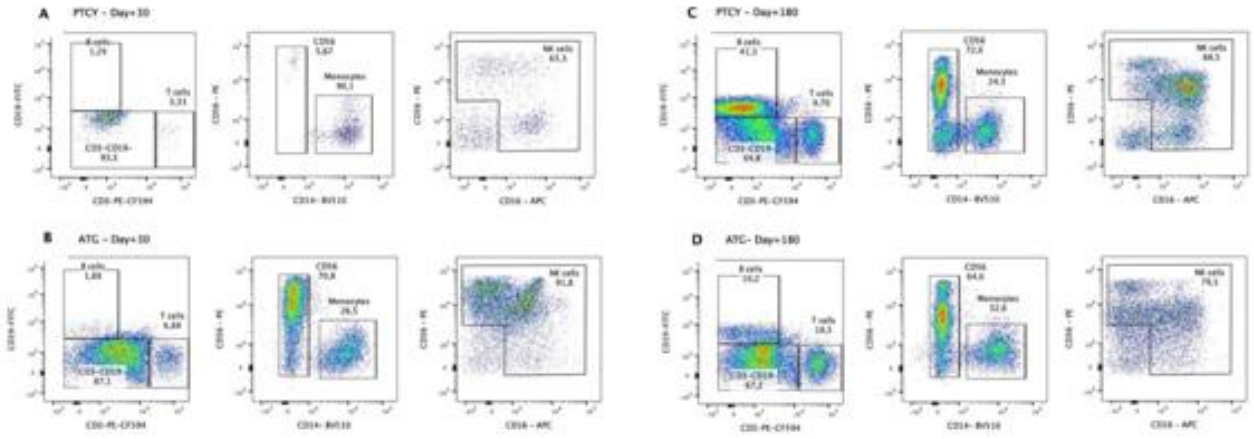
Supplementary Figure 3. Overall survival in patients who used ATG (blue line) or cyclophosphamide (orange line).



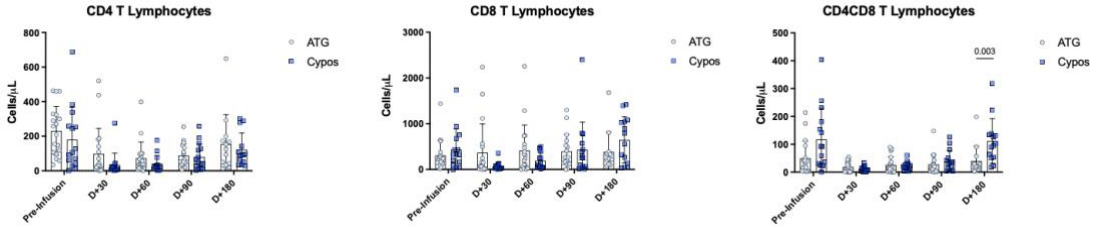
Supplementary Figure 4. CMV Reactivation in patients who used ATG (solid line) or cyclophosphamide (dashed line).



Supplementary Figure 5. Flow cytometry results of patients undergoing hematopoietic stem cell transplantation (HTSC) according to the type of immunosupression used: anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTCy) on 30 (D+30), panel A, or 180 (D+180) days after transplant, panel B

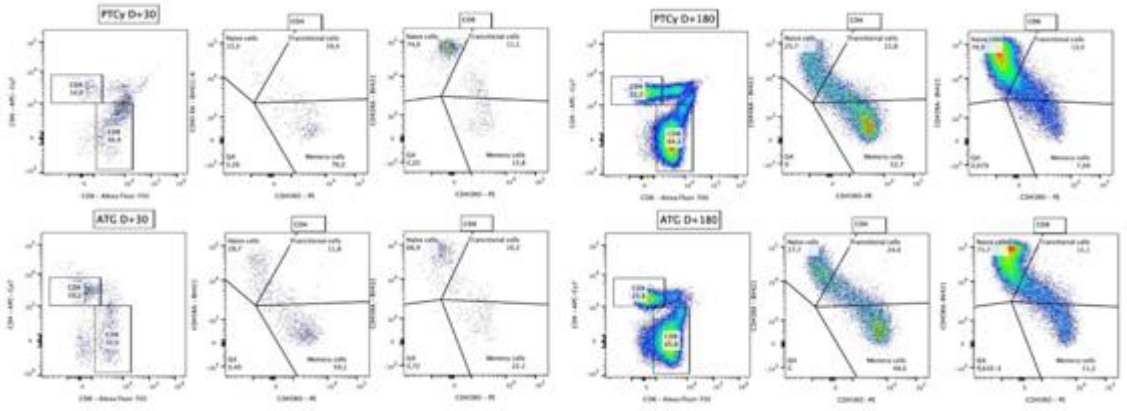


Supplementary Figure 6 . Distribution of T CD4+, CD8+ and CD4+CD8+ lymphocytes by type of prophylatic immunosupression used in patients undergoing hematopoietic stem cell transplantation (HTSC): anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTCy) for pre transplant and 30, 60, 90 and 180 days
There is an inversion of the CD4/CD8 ratio in both groups and a higher number of DP cells in the cyclophosphamide group on D+180



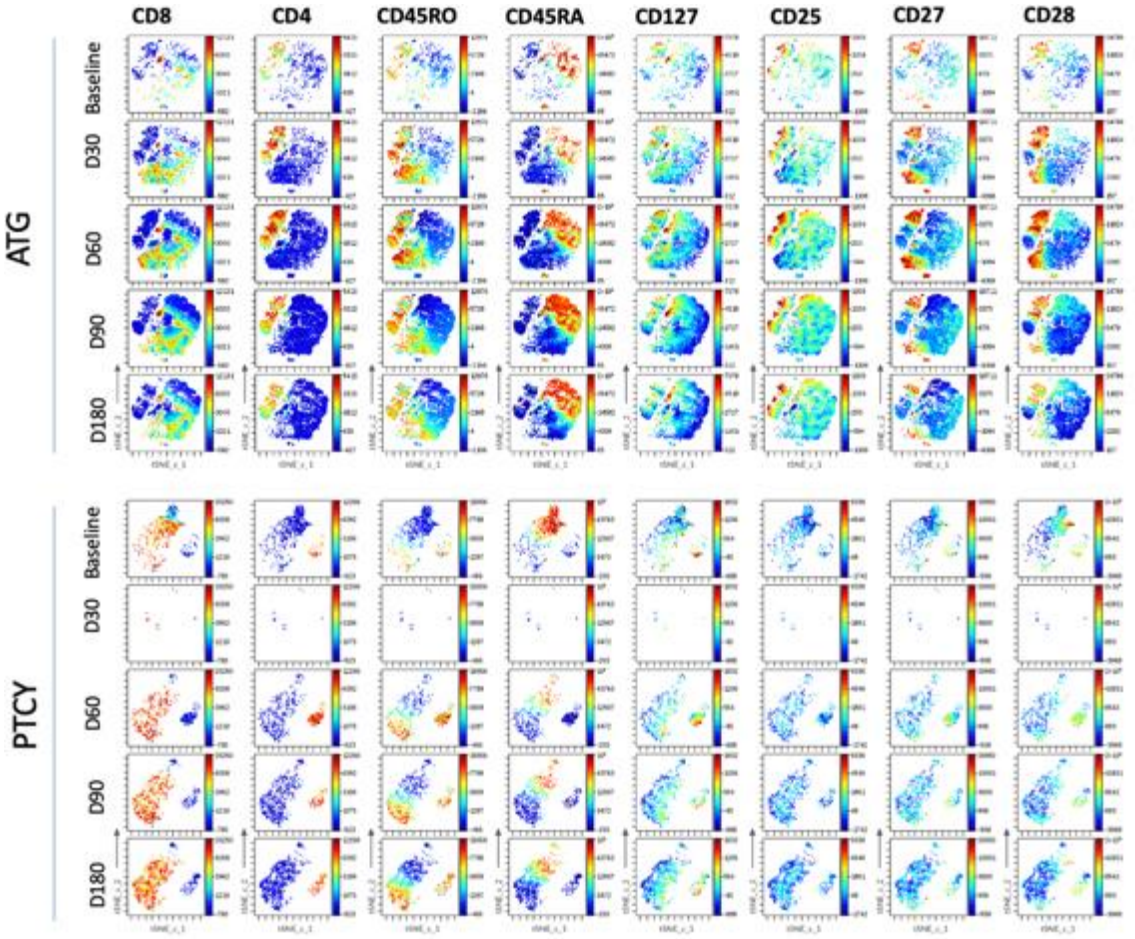
Supplementary Figure 7. Flow cytometry results for T lymphocytes of patients undergoing hematopoietic stem cell transplantation (HTSC) according to the type of immunosupression used: anti-thymocyte globulin (ATG) or post-transplant cyclophosphamide (PTCy) on 30 (D+30) or 180 (D+180) days after transplant

Cases of T lymphocyte profiling cytometry from patients who received PTCy or ATG demonstrate a lower number of T lymphocytes in both groups on D+30 compared to D+180, with a predominance of memory CD4+ T lymphocytes and a higher number of naive CD8+ T lymphocytes in both groups.



Supplementary Figure 8. Cell clusters from the Cytobank platform showing differences between 2 patients, one who received anti-thymocyte globulin (ATG) and another who received post-transplant cyclophosphamide (PTCy) regarding markers of subsets of T cells on the days + 30, 60, 90 and 180

There is a predominance of CD8+ T lymphocytes in the early stages up to D+180, with a predominance of CD4+ cells with memory characteristics (CD45RO+) and higher number of transition and naive cells (CD45RA+) in CD8+ cells



Supplementary Table 1. T lymphocytes subsets

Variable		ATG	PTCy	P
		Cells/uL - mean [range]	**Cells/uL - mean [range]**	
Pre	CD4+ memory	114[77-148]	66[29-253]	0.579
	CD4+ naive	40[16-95]	27[5-50]	0.119
	CD4+ transitional	31[18-47]	31[14-72]	0.972
	CD8+ memory	47[35-82]	37[20-171]	0.703
	CD8+ naive	128[77-242]	139[59-204]	0.652
	CD8+ transitional	44 [38-110]	81[81-131]	0.945
D+30	CD4+ memory	100[36.2-157]	78[21-168]	0.795
	CD4+ naive	3[0.7-13]	3[0.8-5]	0.728
	CD4+ transitional	9[2-31]	8[4-25]	0.972
	CD8+ memory	108[79-233]	120[34-315]	0.959
	CD8+ naive	103[38-145]	41[25-100]	0.188
	CD8+ transitional	54[28-90]	79[23-129]	0.488
D+60	CD4+ memory	61[43-82]	42[24-167]	0.945
	CD4+ naive	3[2-15]	5[0.6-10]	0.781
	CD4+ transitional	8[4-17]	18[4-36]	0.386
	CD8+ memory	98[62-165]	97[60-257]	0.677
	CD8+ naive	108[48-209]	77[39-153]	0.405
	CD8+ transitional	106[84-162]	108[42-241]	0.945
D+90	CD4+ memory	98[73-119]	91[34-172]	0.970
	CD4+ naive	5[0.7-21]	7[1-12]	0.623
	CD4+ transitional	12[6-35]	18[9-50]	0.650
	CD8+memoy	97[53-148]	67[53-166]	0.880
	CD8+ naive	106[73-259]	82[52-154]	0.257
	CD8+ transitional	114[78-130]	167[74-214]	0.257
D+180	CD4+ memory	122[48-160]	57[25-120]	0.320
	CD4+ naive	12[0.8-27]	7[2-15]	0.698
	CD4+ transitional	8[4-27]	12[7-18]	1.000
	CD8+ memory	63[46-124]	108[38-243]	0.512
	CD8+ naive	133[72-408]	117[66-152]	0.903
	CD8+ transitional	112[69-167]	93[33-254]	0.903

ATG: anti-thymocyte globulin; PTCy: post-transplant cyclophosphamide.

Supplementary Table 2. Effects of acute GVHD on immune reconstitution

Variable	Without DECH Cells/uL (mean)	With DECH Cells/uL (mean)	p-value
Lymphocytes (D+60)	939.95	476.0	**0.009**
T cells (D+60)	307.0	186.0	0.157
CD4 (D+60)	34.0	33.0	0.715
CD8 (D+60)	246.0	50.0	0.135
CD4CD8 (D+60)	18.0	18.0	0.64
B cells (D+60)	44.0	10.0	0.104
NK cells (D+60)	260.0	163.0	0.268
CD56high (D+60)	124.0	118.0	0.903
CD56dim (D+60)	74.0	49.0	0.066
CD4 memory (D+60)	31.0	26.0	0.715
CD4 transitional (D+60)	4.0	11.0	0.448
CD4 naive (D+60)	2.0	3.0	0.94
CD8 memory (D+60)	78.0	11.0	0.157
CD8 transitional (D+60)	50.0	18.0	0.19
CD8 naive (D+60)	36.0	24.0	419
Lymphocytes (D+90)	1155.6	739.8	0.19
T cells (D+90)	404.0	289.0	0.237
CD4 (D+90)	74.0	44.0	0.124
CD8 (D+90)	280.0	164.0	0.295
CD4CD8 (D+90)	28.0	5.0	0.061
B cells (D+90)	79.0	20.0	**0.037**
NK cells (D+90)	267.0	259.0	1.0
CD56 (D+90)	90.0	147.0	0.508
CD56dim (D+90)	88.0	79.0	0.623
CD56 neg (D+90)	22.0	12.0	0.064
Monocytes (D+90)	170.0	180.0	0.404
CD4 memory (D+90)	76.0	37.0	**0.026**
CD4 naive (D+90)	4.0	1.0	0.057
CD4 transitional (D+90)	12.0	3.0	0.057
CD8 memory (D+90)	67.0	51.0	0.508
CD8 transitional (D+90)	90.0	45.0	0.273
CD8 naive (D+90)	93.0	53.0	0.404

GVHD: graft versus host disease