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Article

The Effect of Post-Transplant Cyclophosphamide Administration on the Graft-Versus-Host Disease in Allogeneic Bone Marrow Transplantation

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Abstract

Aim. We aimed to compare patients receiving cyclophosphamide treatment post-transplant with those receiving standard graft versus host disease (GVHD) prophylaxis in terms of GVHD development, disease relapse, overall survival, transplant-related mortality and infection development. **Methods:** The data of 78 patients who underwent allogeneic stem cell transplantation (AHSCT) at Medicana Izmir Hospital between January 2022 and June 2024 were retrospectively evaluated. **Results:** Of the patients, 36 (46.2%) were female, and 42 (53.8%) were male. Myeloablative related AHSCT was performed on 38 patients (48.7%), myeloablative unrelated on 26 patients (33.3%), and haploidentical on 14 patients (17.9%). Acute GVHD developed in 42 patients (53.8%). Regarding the clinical and laboratory variables affecting acute GVHD, only ferritin ($p=0.016$) was found to be significantly lower in the group with acute GVHD, and acute GVHD was significantly less observed in the group that received post-transplant cyclophosphamide ($p=0.032$). In 15 patients (19.2%), chronic GVHD developed following acute GVHD. It was found that chronic GVHD developed more frequently in those who did not receive post-transplant cyclophosphamide ($p=0.0001$), in sibling transplants ($p=0.037$), in those without febrile neutropenia ($p=0.021$), and in those with high CMV-DNA levels ($p=0.040$). The median OS (months) was determined as 79.16 months. Median OS (months) was higher in patients with good AML cytogenetic risk group ($p<0.001$) and in patients who underwent transplantation in first remission ($p=0.021$) has been found. **In conclusion;** Cyclophosphamide administration after allogeneic bone marrow transplantation can significantly reduce the development of acute and chronic GVHD.

Keywords: bone marrow transplantation; graft versus host disease; cyclophosphamide

1. Introduction

Allogeneic hematopoietic stem cell transplantation constitutes the consolidation treatment of many hematological diseases. Although significant progress has been made in HLA with increased donor sources, the graft-versus-host disease (GVHD) remains a significant complication that develops after allogeneic transplantation. Despite the routine use of prophylactic immunosuppressive therapies, acute GVHD can be observed in 40% to 50% of transplants, while chronic GVHD can be seen in 10% to 50% of transplants [1–3].

Only 30% of patients have a fully matched sibling donor, while 70% of patients require alternative donors. The increased accessibility of non-relative and haploidentical transplants has been a driving force for the use of alternative treatment regimens to reduce the development of GVHD while preserving the graft-versus-leukemia effect of the transplant.

In 1963, Berenbaum et al. [4] reported longer survival rates in mice with a single dose of cyclophosphamide administered 1-3 days after skin allotransplantation. Subsequent animal

experiments and clinical studies have enhanced our understanding of the immunoregulatory effects of cyclophosphamide and have created a new field for innovation and progress in transplantation.

Cyclophosphamide is an alkylating agent that has been used for many years [5]. It is oxidatively metabolized by hepatic cytochrome P450 into two potent metabolites, namely phosphoramidate mustard and acrolein, and it prevents cell division most prominently in the G1 and S phases by cross-linking DNA strands [6]. Hematopoietic stem cells rich in the enzyme aldehyde dehydrogenase, which is necessary for the conversion of the inactive metabolite of phosphoramidate mustard to carboxycyclophosphamide, are resistant to cyclophosphamide. In conclusion, cyclophosphamide can be administered without disrupting engraftment following allogeneic hematopoietic cell transplantation [7]. The fundamental difference between the effect of cyclophosphamide on T cells and that of other immunosuppressive agents is its ability to induce apoptosis. Strauss et al. [8] reported that only cyclophosphamide and methotrexate induce cell death. Additionally, cyclophosphamide triggers activation-induced cell death by increasing Fas (CD95) expression. None of the other drugs affect CD95 expression.

When we examine the immunosuppressive mechanisms of post-transplant cyclophosphamide, early proliferating alloreactive donor and recipient T cells are selectively eliminated. Increasing T regulatory lymphocytes balance the effect of alloreactive mechanisms. Finally, the delayed but long-term intrathymic clonal deletion of anti-host T cells ensures long-term T cell depletion (9-10).

Most of the early clinical studies on the use of cyclophosphamide post-transplantation for the prevention of GVHD were conducted in haploidentical stem cell transplants. At Johns Hopkins University, O'Donnell et al. [11] published the first study on the use of cyclophosphamide in haploidentical stem cell transplantation. In the initial patients of this study, a single dose of 50 mg/kg/day cyclophosphamide was administered on the 3rd day post-transplantation. Subsequently, due to the high rate of graft rejection, a second dose of cyclophosphamide was added to the treatment.

Later, Munchel et al. at Johns Hopkins University [12] reported the results of a large phase II study involving 210 patients with hematologic malignancies. Haploidentical stem cell transplantation was performed on patients using the group's original non-myeloablative conditioning regimen and GVHD prophylaxis protocol. Permanent engraftment occurred in 87% of the patients. The 5-year transplant-related mortality (TRM) incidence was 18%. The incidence of disease relapse was 55%, while the event-free survival (EFS) and overall survival (OS) rates were 35% and 27%, respectively. The incidence of acute grades II to IV graft-versus-host disease (GVHD) and chronic GVHD was reported as 27% and 13%, respectively. This also indicates that the incidence of chronic GVHD is remarkably low.

Following these studies, Bashey et al. [13] published a retrospective analysis comparing 53 patients who underwent haploidentical allogeneic stem cell transplantation with post-transplant cyclophosphamide to 117 fully matched related donor recipients and 101 fully matched unrelated donor recipients who received standard GVHD prophylaxis at the same center. No difference was found in this study among the three groups in terms of the incidence of GVHD, transplant-related mortality (TRM), relapse, and survival rates.

In light of all these studies, we retrospectively evaluated the data of patients who underwent allogeneic stem cell transplantation at our center between January 2022 and June 2024. We aimed to compare patients receiving cyclophosphamide treatment post-transplant with those receiving standard GVHD prophylaxis in terms of GVHD development, disease relapse, overall survival, transplant-related mortality, and infection development.

Statistical Analysis

Statistical analyses were conducted using "IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)". Descriptive statistics are presented as n and % for categorical variables, and as Mean $\pm$ SD and Median (min-max) for continuous variables. ROC Curve analysis was used in the prediction of morbidity by various indices.

The Kaplan-Meier method was used to compare survival and DFS durations among clinical groups, with $p<0.05$ considered statistically significant.

2. Results

The data of 78 patients who underwent allogeneic stem cell transplantation at Medicana Izmir Hospital between January 2022 and June 2024 were retrospectively evaluated.

The platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR) before and after transplantation, systemic immune-inflammation index (SII) calculated using platelet, neutrophil, and lymphocyte counts, HALP (Hemoglobin, Albumin, Lymphocyte, and Platelet) score, albumin, CRP, ferritin, CMV Ig M, CMV Ig G, hepatitis tests, cardiac ejection fraction, in respiratory function tests FEV1, FVC, DLCO, and KCO ratios were compared.

In cases of hospital admission for transplantation, occurrences of neutropenic fever, subsequent development of acute and chronic GVHD, and CMV infections were evaluated.

Seventy-eight patients who underwent allogeneic transplantation at our center were included in the study. Of the patients, 36 (46.2%) were female, and 42 (53.8%) were male. Myeloablative related allogeneic hematopoietic stem cell transplantation was performed on 38 patients (48.7%), myeloablative unrelated on 26 patients (33.3%), and haploidentical on 14 patients (17.9%). Bone marrow transplants were performed from donors with a 10/10 match for 58 patients, 5/10 match for 11 patients, and a 6/10 match for 3 patients.

The basic characteristics of patients who underwent related (38 patients), unrelated (26 patients), and haploidentical (14 patients) stem cell transplantation are shown in Tables 1–3.

Table 1. Comparison of general characteristics of patients with myeloablative related transplantation before and after transplantation.

Patients undergoing fully matched related donor transplantation			
	Pre-transplant N=38 Median (IQR)	Post-transplant N=38 Median (IQR)	P
Variables			
SII	215.7 (275.6)	297.7 (290.2)	0,151
PLR	126.5 (153.9)	122.5 (69.1)	0,253
NLR	1.4 (2.1)	2.0 (1.5)	0,949
MLR	0.9 (0.2)	0.3 (0.2)	0,431
HALP	53.2 (55.0)	41.2 (48.1)	0,200
Ferritin	863.2 (1986.1)	1409.5 (2605.8)	0,044
CRP	9.1 (10.0)	4.6 (7.7)	0,194
Albumin	4.4 (0.6)	4.3 (0.8)	0,885
CMV-IgM	0.01 (0.9)	0.1 (0.3)	0,166
CMV IgG	46.0 (43.7)	66.0 (113.2)	0,454
HBsAg	0.4 (0.5)	0.2 (0.2)	0,770
Anti-HBC	0.07 (1.5)	0.21 (0.9)	0,858
Anti-HCV	0.04 (0.05)	0.04 (0.04)	0,212
Anti-HIV	0.2 (0.2)	0.15 (0.18)	0,291
Anti-HBS	51.0 (251.5)	18.0 (71.4)	0,151
Covid-IgM	0.06 (0.01)	0.05 (0.12)	0,454
Covid-IgG	29.4 (21.3)	16.1 (33.6)	0,454
EF	60.0 (5.0)	60.0 (5.0)	0,782
FEV1	101.0 (24.5)	87.0 (35.2)	0,161
FVC	98.0 (11.0)	94.5 (18.2)	0,091
FEV1/FVC	106.5 (13.5)	100.5 (17.2)	0,173
Dlco	59.0 (14.5)	56.5 (16.2)	0,207
Kco	82.5 (57.2)	74.0 (39.5)	0,249

Mann Whitney U test, $p<0.05$ is statistically significant.

Table 2. Comparison of the basic characteristics of patients undergoing unrelated donor bone marrow transplantation before and after transplantation.

Variables	Unrelated		P
	Pre-transplant	Post-transplant	
	N=26 Median (min-max)	N=26 Median (min-max)	
SII	162.0 (348.2)	345.2 (337.8)	0,112
PLR	62.2 (181.6)	115.5 (216.8)	0,717
NLR	1.5 (2.5)	2.2 (4.8)	0,117
MLR	0.3 (1.1)	0.4 (0.6)	0,260
HALP	60.0 (54.0)	40.0 (78.9)	0,616
Ferritin	2274.8 (1839.7)	3066.0 (2518.0)	0,420
CRP	12.0 (21.8)	4.2 (14.0)	0,472
Albumin	4.4 (0.6)	4.4 (1.4)	1,000
CMV-IgM	0.02 (0.07)	0.15 (1.3)	0,010
CMV IgG	49.0 (44.0)	60.0 (208.0)	0,306
HBsAg	0.1 (0.5)	0.3 (0.3)	0,679
Anti-HBC	0.17 (1.8)	0.19 (1.44)	0,979
Anti-HCV	0.04 (0.06)	0.04 (0.04)	0,182
Anti-HIV	0.17 (0.2)	0.18 (0.12)	0,191
Anti-HBS	35.0 (95.9)	8.4 (288.4)	0,868
Covid-IgM	0.13 (0.27)	0.04 (0.23)	0,098
Covid-IgG	29.4 (21.3)	16.1 (33.6)	0,064
EF	60.0 (0.0)	60.0 (3.7)	0,298
FEV1	97.0 (21.2)	90.0 (36.5)	0,735
FVC	100.5 (20.7)	91.0 (43.5)	0,866
FEV1/FVC	108.0 (17.5)	100.5 (10.7)	0,108
Dlco	44.5 (5.5)	50.0 (31.2)	0,916
Kco	75.0 (50.5)	60.0 (45.0)	0,028

Mann Whitney U test, p<0.05 is statistically significant.

Table 3. Comparison of the basic characteristics of patients undergoing haploidentical bone marrow transplantation before and after transplantation.

Variables	Pre-transplant		P
	Pre-transplant	Post-transplant	
	N=14 Median (min-max)	N=14 Median (min-max)	
SII	76.9 (545.3)	383.4 (378.2)	0,260
PLR	45.4 (134.0)	145.2 (248.2)	0,038
NLR	1.3 (1.5)	3.1 (9.8)	0,038
MLR	0.2 (0.5)	0.5 (0.4)	0,594
HALP	85.0 (150.0)	32.4 (50.4)	0,038
Ferritin	1744.9 (2122.9)	2211.1 (1890.4)	0,441
CRP	25.0 (46.4)	10.0 (19.8)	0,173
Albumin	4.3 (0.7)	4.5 (0.9)	0,407
CMV-IgM	0.09 (0.1)	0.12 (0.1)	0,262
CMV IgG	63.0 (196.3)	49.0 (101.9)	0,441
HBsAg	0.3 (0.5)	0.2 (0.3)	0,362
Anti-HBC	0.2 (1.4)	0.1 (2.3)	0,263
Anti-HCV	0.04 (0.02)	0.03 (0.02)	0,161
Anti-HIV	0.2 (0.1)	0.17 (0.15)	0,889
Anti-HBS	269.3 (748.1)	97.0 (351.1)	0,161
Covid-IgM	0.09 (0.10)	0.06 (0.24)	1,000

Covid-IgG	38.7 (23.5)	21.5 (27.1)	0,594
EF	60.0 (2.5)	60.0 (5.0)	0,655
FEV1	81.0 (12.5)	90.0 (17.0)	0,141
FVC	91.0 (23.5)	97.0 (23.5)	0,066
FEV1/FVC	102.0 (19.0)	104.0 (12.5)	0,276
Dlco	32.0 (24.5)	43.0 (22.5)	0,144
Kco	77.0 (17.5)	65.0 (20.0)	0,144

Mann Whitney U test, p<0.05 is statistically significant.

The diagnoses of the patients were as follows: 37 patients (45.9%) had AML, 11 patients (14.2%) had ALL, 8 patients (10.3%) had multiple myeloma, and 7 patients (9%) had MDS-RAEB1-2.

Of the patients, 45 (61.6%) had a complete response after the first chemotherapy, while 20 patients (27.4%) were non-responsive to the initial chemotherapy, and 7 patients (9.6%) had a partial response. Before the transplant, 52 patients (66.7%) had an ECOG performance score of 1. In total, 68 patients (88.3%) underwent transplantation in remission, 6 patients in active disease, and 3 patients in partial response.

When patients were classified according to AML risk categories, 8 patients were in the intermediate risk group, and 29 patients were in the poor risk group. No comorbidities were present in 50 (64.1%) of the patients. The most common chronic diseases were HT and DM. Of the patients undergoing transplantation, 50 (71.8%) were in the first remission, 13 patients (18.3%) were in the second remission, and 6 patients (8.5%) were in the third remission. When looking at the transplantation preparation regimens, fludarabine-melphalan was administered to 69 patients (88.5%), and treosulfan-melphalan treatment was given to 9 patients (11.5%). ATG was administered to 27 patients (34.6%) as part of the preparatory regimen (26 patients with unrelated donors, 1 patient with related donor transplants). 49 patients (Total 62.8%: 14 patients haploidentical, 26 patients unrelated donors, 9 patients related donor transplants) received post-transplant cyclophosphamide, while 29 patients (37.2% in total, all related donor transplants) did not receive post-transplant cyclophosphamide treatment.

All patients were started on cyclosporine and mycophenolate mofetil on day +1 for GVHD prophylaxis.

In 72 transplants (92.3%), sufficient stem cells were collected from the donor in a single apheresis for the transplant, while stem cells could only be collected from 5 donors over two days and from 1 donor over three days. Allogeneic stem cell transplantation was performed on 44 patients from their siblings (37 fully matched, 7 haploidentical), on 4 patients from their children (1 fully matched, 3 haploidentical), on 3 patients from their mothers (haploidentical), on 1 patient from their father (haploidentical), and on 26 patients from unrelated donors registered with Türkkök bone marrow bank.

In 28 transplants, the recipient was male and the donor was male, in 23 transplants, the recipient was female and the donor was male, in 15 transplants, the recipient was male and the donor was female, and in 12 transplants, the recipient was female and the donor was female.

In 44 transplants, the blood groups of the patients and donors were the same, while 26 transplants were ABO-incompatible, 3 transplants were Rh-incompatible, and 5 transplants were from donors who were both ABO- and Rh-incompatible. Of the patients with a high VOD risk scale, 38 patients (48.7%) received prophylactic defibrotide post-transplant, while 40 patients did not receive defibrotide (51.3%).

Acute GVHD: According to the IBMTR grading system, acute GVHD did not develop in 36 patients (46.2%), while grade A developed in 20 patients (25.6%), grade B in 9 patients (11.5%), grade C in 3 patients (3.8%), and grade D in 10 patients (12.8%).

In the examination of acute graft-versus-host disease (GVHD) developing post-transplant, skin GVHD did not develop in 39 patients (50%), while grade 1 skin GVHD developed in 27 patients (34.6%), grade 2 in 10 patients (12.8%), and grade 3 skin GVHD developed in 2 patients (2.6%).

Regarding the gastrointestinal system (GIS) GVHD, it did not develop in 58 patients (75.3%), while grade 1 GIS GVHD developed in 5 patients (6.5%), grade 2 in 2 patients (2.6%), grade 3 in 5 patients (6.5%), and grade 4 in 7 patients (9.1%).

An examination of liver GVHD showed that 68 patients (87.2%) did not develop liver GVHD, while it developed in grade 1 in 5 (6.4%) of patients, grade 2 in 4 (5.1%) of patients, and grade 4 in 1 (1.3%) patient.

In 19 patients (24.4%), only skin, in 1 patient (1.3%) GIS, in 1 patient (1.3%) liver, in 13 patients (16.7%) skin and GIS, in 3 patients (3.8%) skin and liver, in 4 patients (5.1%) skin-GIS-liver, and in 1 patient (1.3%) GIS and liver GVHD developed.

All patients received cyclosporine and mycophenolate mofetil treatment for GVHD prophylaxis. When examining the immunosuppressive therapies received by patients who developed acute GVHD, 28 patients received cyclosporine, mycophenolate mofetil, methylprednisolone; 8 patients received tacrolimus, mycophenolate mofetil, methylprednisolone, ruxolitinib; 2 patients received tacrolimus, mycophenolate mofetil, methylprednisolone, cyclophosphamide, ruxolitinib, prolactin C; and 4 patients were given cyclosporine, mycophenolate mofetil, methylprednisolone, cyclophosphamide, ruxolitinib, and mesenchymal stem cells.

Steroid resistance was observed in 14 patients with acute GVHD, and other immunosuppressive treatments were started.

In Table 4, regarding the clinical and laboratory variables affecting acute GVHD, only ferritin ($p=0.016$) was found to be significantly lower in the group with acute GVHD, and acute GVHD was significantly less observed in the group that received post-transplant cyclophosphamide ($p=0.032$).

Chronic GVHD did not develop in 63 patients (80.8%); while in 15 patients (19.2%), chronic GVHD developed following acute GVHD. In 12 patients (15.4%), chronic GVHD developed with skin involvement, in 11 patients (14.1%) eye involvement, in 12 patients (15.4%), mouth involvement, in 3 patients (3.8%), esophageal involvement, in 1 patient (1.3%), serous membrane involvement, in 3 patients (3.8%), liver involvement, in 3 patients (3.8%), bronchiolitis obliterans involvement, and in 6 patients (7.7%), joint fascia involvement.

In Table 5, regarding the clinical and laboratory variables affecting chronic GVHD, it was found that chronic GVHD developed more frequently in those who did not receive post-transplant cyclophosphamide ($p=0.0001$), in sibling transplants ($p=0.037$), in those without febrile neutropenia ($p=0.021$), and in those with high CMV-DNA levels ($p=0.040$).

When examining disease subgroups, patients with AML who received post-transplant cyclophosphamide showed significantly less development of acute and chronic GVHD (respectively, $p=0.036-0.001$).

No significant difference was observed in acute and chronic GVHD among patients who were ALL and received post-transplant cyclophosphamide (respectively, $p=0.54-0.41$).

In patients with other diagnoses, there was no difference in acute GVHD among those who received post-transplant cyclophosphamide, while chronic GVHD developed significantly less. (respectively, $p=0.17-0.005$).

As seen in Table 6, the overall median OS (months) is determined to be 79.16 months (95%CI:24.47-133.85). Median OS (months) was found to be statistically significantly higher in patients with a favorable AML cytogenetic risk group ($p<0.001$) and in those undergoing transplant in the first remission ($p=0.021$).

Age, gender, mode of transplantation, disease, preparative regimens for transplantation, method of antifungal usage, and development of CMV infection did not affect the OAS ($p>0.05$). As seen in Table 7, the median DFS (months) was not reached. Table 8 indicates that the median OS (months) was not reached in patients diagnosed with AML. As the cytogenetic risk category of AML increased ($p<0.001$) and in patients with steroid resistance in acute GVHD ($p=0.026$), the median OS (months) was found to be significantly shorter.

As seen in Table 9, the median OS (months) in patients diagnosed with ALL was 13.66 (95% CI:-). In females (p=0.036) and in patients receiving ATG (p=0.003), the median OS (months) was found to be significantly higher.

Table 4. Comparison of various clinical variables that may affect the development of acute GVHD.

	Acute GVHD		
Variables	None N=36	Present N=42	P
Received ATG, n (%)			
Did not receive	20 (55.6)	31 (73.8)	0.091 ^{mg/L}
Received	16 (44.4)	11 (26.2)	
Post-transplant cycle, n (%)			
Did not receive	9 (25)	20 (47.6)	0.032 ^b
Received	27 (75)	22 (52.4)	
Conditioning regimen, n (%)			
Fludara-melphalan	29 (80.6)	39 (92.9)	0.111 ^b
Fludara-treosulfan	6 (16.7)	2 (4.8)	
Treosulfan+melphalan	1 (2.8)	0 (0)	
Fludara+melphalan+IVIG	0 (0)	1 (2.4)	
Compatibility, median (IQR)	100.0 (7.50)	100.0 (10.0)	0.793 ^c
Stem cell count, median (IQR)	6.7 (3.5)	7.5 (2.9)	0.113 ^c
Donor relation, n (%)			
Sibling	17 (47.2)	26 (61.9)	0.154 ^b
Mother	0 (0)	3 (7.1)	
Child	2 (5.6)	2 (4.8)	
Unrelated	16 (44.4)	11 (26.2)	
Father	1 (2.8)	0 (0)	0.437 ^c
Donor age	43.5 (20-73)	44.5 (20-72)	
Female donor birth	1.0 (0-3)	2.0 (0-8)	
Donor sex, n (%)			
Female patient-female donor	5 (13.9)	7 (16.7)	0.798 ^a
Female patient-male donor	10 (27.8)	13 (31)	
Male patient-male donor	15 (41.7)	13 (31)	
Male patient-female donor	6 (16.7)	9 (21.4)	
Blood Group, n (%)			
Identical	19 (52.8)	25 (59.5)	0.082 ^b
ABO incompatible	11 (30.6)	15 (35.7)	
Rh incompatible	1 (2.8)	2 (4.8)	
ABO-Rh incompatible	5 (13.9)	0 (0)	
Ferritin, median (IQR)	2183.4 (1680.2)	1277.0 (1992.5)	0.016 ^c
Febrile Neutropenia, n (%)			
None	7 (19.4)	16 (38.1)	0.072 ^a
Present	29 (80.6)	26 (61.9)	
CMV DNA, median (IQR)	1161.0 (6822.2)	1584.0 (8320.2)	0.203 ^c
Defibrotide use, n (%)			
Did not receive	16 (44.4)	24 (57.1)	0.263 ^a
Prophylaxis	20 (55.6)	18 (42.9)	

a: Pearson Chi-Square test, b: Fisher's Exact Test, c: Mann Whitney U test, p<0.05 is statistically significant.

Table 5. Comparison of various clinical variables that may affect the development of chronic GVHD.

Chronic GVHD			
Variables	None N=64	Present N=14	P
Received ATG, n (%)			
Did not receive	38 (59.4)	13 (92.9)	0.091 ^{mg/L}
Received	26 (40.6)	1 (7.1)	
Post-transplant cycle, n (%)			
Did not receive	18 (28.1)	11 (78.6)	0.0001 ^b
Received	36 (71.9)	3 (21.4)	
Conditioning regimen, n (%)			
Fludara-melphalan	57 (89.1)	11 (78.6)	0.238 ^b
Fludara-treosulfan	6 (9.4)	2 (14.3)	
Treosulfan+melphalan	1 (1.6)	0 (0)	
Fludara+melphalan+IVIG	0 (0)	1 (7.1)	
Compatibility, median (IQR)	100.0 (10.0)	100.0 (0.0)	0.070 ^c
Stem cell count, median (IQR)	6.9 (3.4)	8.1 (2.3)	0.247 ^c
Donor relation, n (%)			
Sibling	30 (46.9)	13 (92.9)	0.037 ^b
Mother	3 (4.7)	0 (0)	
Child	4 (6.3)	0 (0)	
Unrelated	26 (40.6)	1 (7.1)	
Father	1 (1.6)	0 (0)	0.840 ^c
Donor age	44.0 (20-72)	44.0 (24-73)	
Female donor birth	1.5 (0-4)	2.0 (0-8)	0.231 ^c
Donor sex, n (%)			
Female patient-female donor	9 (14.1)	3 (21.4)	0.420 ^b
Female patient-male donor	21 (32.8)	2 (14.3)	
Male patient-male donor	21 (32.8)	7 (50)	
Male patient-female donor	13 (20.3)	2 (14.3)	
Blood Group, n (%)			
Identical	38 (59.4)	6 (42.9)	0.226 ^b
ABO incompatible	18 (28.1)	8 (57.1)	
Rh incompatible	3 (4.7)	0 (0)	
ABO-Rh incompatible	5 (7.8)	0 (0)	
Ferritin, median (IQR)	1745.7 (2008.2)	1042.6 (2074.8)	0.143 ^c
Febrile neutropenia, n (%)			
None	15 (23.4)	8 (57.1)	0.021
Present	49 (76.6)	6 (42.9)	
CMV DNA, median (IQR)	390.5 (2855.5)	1618 (7832)	0.040 ^c
Defibrotide use, n (%)			
Did not receive	31 (48.4)	9 (64.3)	0.283 ^a
Prophylaxis	33 (51.6)	5 (35.7)	

a: Pearson Chi-Square test, b: Fisher's Exact Test, c: Mann Whitney U test, p<0.05 is statistically significant.

Table 6. Analysis of the Predictive Values of Various Parameter Values in Distinguishing Mortality.

Variables	AUC	95% CI	Cut-off	Sensitivity (%)	Specificity (%)	p
Platelet engraftment	0,650	0.505-0.796	≥13.50	52.4	65.4	0,046

AUC, Area under the curve; %95CI, Confidence interval.

In Table 6, the duration of platelet engraftment (p=0.046) was found to be statistically significant in specifying the presence of mortality.

Table 6. Comparisons of OS in patients (N=78).

OS (months)	2-year %	5-year %	Median (95% CI)	p
Overall	67.6	56.9	79.16 (24.47-133.85)	
Age				
≤60	69.5	55.3	79.16 (39.22-119.11)	0,945
>60	61.7	61.7	114,50 (-)	
Sex				
Female	63.0	48.5	53.46 (0.00-110.60)	0,186
Male	70.5	70.5	114,50 (-)	
Type of transplant				
Myeloablative related	68.5	50.3	106.33 (18.90-193.75)	0,603
Unrelated	59.7	59.7	- (-)	
Haploidentical	82.1	82.1	79.16 (0.00-173.23)	
Diagnosis				
AML	65.3	65.3	- (-)	0,162
ALL	49.9	-	13,66 (-)	
Other	86.2	67.1	106.33 (42.29-170.37)	
AML risk				
Good	-	-	6,70 (-)	<0,001
Moderate	-	-	12.66 (12.61-12.72)	
Poor	69.4	69.4	- (-)	
Remission #				
1	54.2	-	11.65 (8.79-54.47)	0,021
2	84.6	84.6	106,33 (-)	
3 and above	100.0	80.0	114,50 (-)	
Platelet engraftment				
<13.50	78.8	47.3	53.46 (11.25-95.67)	0,894
≥13.50	64.7	64.7	114.50 (12.45-216.55)	

Kaplan-Meier curve, Log-rank test, p<0.05 statistically significant.

Table 7. Comparisons of DFS in patients (N=78).

DFS (months)	2-year %	5-year %	Median (95% CI)	p
Overall	83.1	83.1	- (-)	
Sex				
Female	72.4	72.4	- (-)	0,770
Male	87.4	-	- (-)	
Diagnosis				
AML	92.9		- (-)	0,417
ALL	70.0	-	- (-)	
Other	75.0	75.0	- (-)	

Kaplan-Meier curve, Log-rank test, p<0.05 statistically significant.

As seen in Table 7, the overall median DFS (months) was not reached.

Table 8. Comparisons of OS in AML Patients (N=50).

OS (months)	2-year %	5-year %	Median (95% CI)	p
Overall	65.3	65.3	- (-)	
Age				
≤60	69.9	69.9	- (-)	0,751
>60	53.3	-	- (-)	

Sex				
Female	56.0	56.0	- (-)	0,141
Male	72.5	72.5	- (-)	
Type of transplant				
Myeloablative related	51.9	-	- (-)	0,755
Unrelated	69.3	69.3	- (-)	
Haploidentical	75.0	75.0	- (-)	
AML risk				
Good	-	-	6,70 (-)	<0,001
Moderate	-	-	12.66 (12.61-12.72)	
Poor	69.4	69.4	- (-)	
Acute GVHD steroid resistance				
None	76.5	76.5	-(-)	0,026
Present	33.3	-	12.66 (0.00-27.55)	

Kaplan-Meier curve, Log-rank test, p<0.05 statistically significant.

Table 9. Comparisons of OS in ALL Patients (N=11).

OS (months)	2-year %	Median (95% CI)	p
Overall	49.9	13,66 (-)	
Age			
≤60	56.0	- (-)	0,376
>60	-	11,60 (-)	
Sex			
Female	-	7.93 (5.69-10.17)	0,036
Male	60.0	- (-)	
ATG			
Did not receive	70.0	- (-)	0,003
Received	-	7.93 (5.69-10.17)	

Kaplan-Meier curve, Log-rank test, p<0.05 statistically significant.

All patients were prophylactically started on levofloxacin flk 500 mg 1x1, fluconazole 200 mg IV (6 patients received voriconazole as secondary prophylaxis), and acyclovir IV treatment on day +1 post-transplant. When patients' temperatures were >38 degrees, catheter-peripheral blood cultures were taken, and HRCT was performed. Febrile neutropenia developed in 55 patients (70.5%) during transplantation, while 23 patients (29.5%) did not develop febrile neutropenia. Ground-glass opacities were detected in 10 patients, nodular lesions with surrounding halo sign in 5 patients, and lobar pneumonia in 2 patients on HRCT scans taken during febrile neutropenia. The HRCTs of 38 patients was normal.

At the time of transplant, 72 patients (92.3%) were receiving fluconazole prophylaxis, and 6 patients (7.7%) were receiving voriconazole prophylaxis. During the transplant-associated neutropenic fever, 31 patients received empirical antifungal therapy, 8 patients received preemptive therapy, and 2 patients received proven antifungal treatment.

During treatment, 19 patients received meropenem and teicoplanin, 16 patients received meropenem, teicoplanin, and amikacin, 10 patients received meropenem, tigecycline, and amikacin, 10 patients received meropenem, colistin, tigecycline, and amikacin, and 1 patient received ceftazidime-avibactam, tigecycline, colistin, and amikacin.

In cultures taken during the febrile period, 7 patients had ESBL-negative E. coli, 6 patients had methicillin-resistant coagulase-negative Staphylococcus aureus (MRCNS), 5 patients had Staphylococcus epidermidis, 3 patients had carbapenem-sensitive, 1 patient had carbapenem-resistant Klebsiella pneumoniae, 3 patients had Staphylococcus hominis, 1 patient had Pseudomonas aeruginosa, 1 patient had Enterococcus faecium, 1 patient had Staphylococcus haemolyticus, 1 patient

had Candida parapsilosis, while no growth was observed in the blood cultures of 27 patients. E. Coli was detected in the urine cultures of 4 patients; carbapenem-resistant Klebsiella pneumoniae in 1 patient, carbapenem-sensitive Klebsiella pneumoniae in 1 patient, and Stenotrophomonas maltophilia in 1 patient, while no growth was observed in the urine cultures of 49 patients. In the sputum cultures, Candida glabrata was isolated in 2 patients, Aspergillus fumigatus in 1 patient, Enterococcus faecium in 1 patient, and E. coli in 1 patient.

Primary engraftment failure was observed in one patient and secondary engraftment failure in five patients following transplantation (7.8% in total). Only 2 patients were lost within the first 30 days of transplantation (%2.6). During the first 100 days post-transplant, 12 patients died; and during the first year, 23 patients died.

It was observed that 25 patients died due to various reasons by the last follow-up date. The causes of death of the patients are presented in Table 11. During follow-up, 7 patients experienced relapse, and 5 patients were lost due to disease progression, while non-relapse mortality occurred in 20 patients.

Table 10. OS comparisons for Patients with Other Diagnoses (N=17).

OS (months)	2-year %	5-year %	Median (95% CI)	p
Overall	86.2	67.1	106.33 (42.29-170.37)	
Age				
≤60	82.5	57.8	79.16 (16.88-141.45)	0,274
>60	100.0	100.0	114,50 (-)	
Sex				
Female	88.9	62.2	79.16 (16.01-142.31)	0,731
Male	80.0	80.0	114,50 (-)	
Type of transplant				
Myeloablative related	90.0	64.0	106.33 (49.21-163.45)	0,739
Unrelated	66.7	66.7	- (-)	
Haploidentical	0.0	-	79,16 (-)	
ECOG				
0	100.0	66.7	106,33 (-)	0,049
1	88.9	71.1	29.46 (56.75-172.24)	
2	0.0	-	23,36 (-)	

Kaplan-Meier curve, Log-rank test, p<0.05 statistically significant.

Table 11. Causes of patient mortality.

Cause of death	Patient count	Percentage (%)
Disease progression-pneumonia	5	6.4
Pneumonia	4	5.1
GVHD	4	5.1
Pneumonia-GVHD	4	5.1
Klebsiella pneumonia sepsis	2	2.6
Pseudomonas aeruginosa sepsis	2	2.6
Engraftment failure-pneumonia	1	1.3
Multiple organ failure	2	2.6
Covid pneumonia	1	1.3

3. Discussion

The administration of cyclophosphamide (PTCy) following allogeneic stem cell transplantation contributes to the development of peripheral tolerance by eliminating alloreactive donor T cells through clonal deletion and Treg suppression. Recent studies have shown that alloreactive T cells are not completely eliminated, their proliferation is reduced, and their functions are impaired [14–16].

The initial study on post-transplant cyclophosphamide administration was published by O'Donnell et al. at Johns Hopkins University. Patients who underwent haploidentical stem cell transplantation were treated with cyclophosphamide post-transplantation (11). The use of (PTCy) has led to a significant transformation by expanding haploidentical transplantation practices.

Subsequently, Luznik et al. published a study in which they performed haploidentical stem cell transplantation on 68 patients with advanced hematologic malignancies following a non-myeloablative conditioning regimen. On the 3rd and/or 4th day post-transplant, 28 patients received cyclophosphamide treatment for one day, and 40 patients for two days; all patients received mycophenolate mofetil and tacrolimus for GVHD prophylaxis. Engraftment failure developed in 9 patients (13%), while the median neutrophil engraftment occurred on day 15 and the platelet engraftment occurred on day 24. Transplant-related mortality was 15%, the one-year relapse rate was 51%, two-year overall survival was 36%, two-year event-free survival was 26%, grade 2-4 acute GVHD developed in 34%, and grade 3-4 acute GVHD developed in 6%. In cases of chronic GVHD, 25% of patients who received a single dose of cyclophosphamide developed the condition, whereas it developed in only 5% of patients who received a double dose of cyclophosphamide (14).

Following this success achieved in haploidentical transplants, PTCy-based GVHD prophylaxis regimens have been tested as alternatives to traditional calcineurin inhibitor (CNI)-based regimens and have begun to be administered in fully matched related donor (MRD) and fully matched unrelated donor (MUD) settings.

Luznik et al. at Johns Hopkins University started post-transplant cyclophosphamide administration in fully matched related and unrelated transplants. In a study they conducted (40), they administered 50 mg/kg/day cyclophosphamide treatment on days 3-4 post-transplant to 78 fully matched related and 39 fully matched unrelated transplant patients following a myeloablative conditioning regimen; 63% of patients undergoing fully matched transplants and 54% of unrelated transplants were in remission at the time of transplantation. Transplant-related mortality was observed in 17% of patients, while the 2-year OAS was found to be 55% and EFS was 39%. Acute Grade 2-4 GVHD developed in 43% of patients, while chronic GVHD developed in 10% of patients (17).

In another clinical study conducted by Kanarky et al., 45 fully matched related and 47 fully matched unrelated hematological malignancy patients received 2 days of cyclophosphamide treatment post-transplant following a myeloablative conditioning regimen containing fludarabine and busulfan. Twenty-five patients underwent transplantation with active disease. In the median 1.6-year follow-up, TRM was found to be 16%, OAS 62%, and EFS 67%. Acute Grade 2-4 GVHD developed in 51% of patients, acute Grade 3-4 GVHD in 15% of patients, and chronic GVHD in 14% of patients (18).

Regarding our patients, 78 patients were included in the study. Myeloablative related allogeneic hematopoietic stem cell transplantation was performed on 38 patients (48.7%), myeloablative unrelated on 26 patients (33.3%), and haploidentical on 14 patients (17.9%). Acute GVHD developed in 53.8% (42 patients) of the patients, with grade 2-4 acute GVHD observed in 22 patients (28.1%) and grade 3-4 acute GVHD in 13 patients (16.6%). In 19 patients (24.4%), only skin involvement was observed; in 1 patient (1.3%), GIS involvement; in 1 patient (1.3%), liver involvement; in 13 patients (16.7%), both skin and GIS; in 3 patients (3.8%), both skin and liver; in 4 patients (5.1%), skin-GIS-liver; and in 1 patient (1.3%), both GIS and liver GVHD developed. Steroid resistance was observed in 14 patients with acute GVHD, and other immunosuppressive treatments were started.

Regarding the clinical and laboratory variables affecting acute GVHD, only ferritin ($p=0.016$) was found to be significantly lower in the group with acute GVHD, and acute GVHD was significantly less observed in the group that received post-transplant cyclophosphamide ($p=0.032$).

Chronic GVHD developed after acute GVHD in 15 patients (19.2%). In 12 patients (15.4%), chronic GVHD developed with skin involvement, in 11 patients (14.1%) eye involvement, in 12 patients (15.4%), mouth involvement, in 3 patients (3.8%), esophageal involvement, in 1 patient (1.3%), serous membrane involvement, in 3 patients (3.8%), liver involvement, in 3 patients (3.8%), bronchiolitis obliterans involvement, and in 6 patients (7.7%), joint fascia involvement.

Regarding the clinical and laboratory variables affecting chronic GVHD, it was found that chronic GVHD developed more frequently in those who did not receive post-transplant cyclophosphamide ($p=0.0001$), in sibling transplants ($p=0.037$), and in those with high CMV-DNA levels ($p=0.040$).

In a large-scale retrospective registry study based on CIBMTR data, the use of PTCy was compared in 284 adults undergoing matched unrelated donor (MUD) transplantation with 2036 adult patients receiving haploidentical transplantation. It demonstrates that in haploidentical transplants, higher grades (III-IV) of acute GVHD and rates of chronic GVHD are observed, and that MUD transplantation still remains the gold standard in this context [19].

Finally, another study involving 431 leukemia patients compared the combination of PTCy + Tacrolimus + Mycophenolate Mofetil (MMF) with the standard Tacrolimus-MTX prophylaxis regimen. In patients undergoing reduced-intensity or non-myeloablative 8/8 or 7/8 matched related donor HSCT, the incidence of grade III-IV aGVHD was found to be lower in the PTCy group (6.3%) compared to the standard prophylaxis group (14.7%). Similarly, the 12-month incidence of cGVHD was lower in the PTCy group (21.9%) compared to the standard prophylaxis group (35.1%) [20].

The median OS for our patients was determined to be 79.16 months (95%CI: 24.47-133.85 months). Median OS (months) was found to be statistically significantly higher in patients with a favorable AML cytogenetic risk group ($p<0.001$) and in those undergoing transplant in the first remission ($p=0.021$).

Age, gender, type of transplantation, disease, preparative regimens for transplantation, antifungal usage, and development of CMV infection did not affect the OAS ($p>0.05$). Median DFS (months) was not reached.

As the cytogenetic risk category increased in patients with AML ($p<0.001$) and in patients with steroid resistance in acute GVHD ($p=0.026$), the median OS (months) was found to be significantly shorter.

The overall median OS (months) in patients diagnosed with ALL was determined to be 13.66 month (95% CI:-). The median OS (months) was found to be significantly higher in women ($p=0.036$) and in patients receiving ATG ($p=0.003$). Overall 2-year OAS was determined to be 67.6%, 5-year OAS was 56.9%, with a median of 79.16 months (range 24.47-133.85 months).

Post-transplant cyclophosphamide (PTCy) is a significant immunomodulatory strategy in allogeneic hematopoietic stem cell transplantation that supports the development of tolerance by reducing the incidence of GVHD. Current clinical studies demonstrate that PTCy offers an alternative to traditional GVHD prophylaxis regimens by showcasing its effectiveness in haploidentical and fully matched transplants. However, the use of PTCy needs to be evaluated in more comprehensive prospective studies concerning its impact on the development of acute and chronic GVHD, overall survival, and relapse rates.

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References

1. Welniak LA, Blazar BR, Murohy WJ. Immunobiology of allogeneic hematopoietic stem cell transplantation. *Annu Rev Immunol.* 20
2. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med.* 1986;314:729-735.
3. Cutler C, Li S, Ho VT, et al. Extended follow-up of methotrexate-free immunosuppression using sirolimus and tacrolimus in related and unrelated donor peripheral blood stem cell transplantation. *Blood.* 2007;109:3108-3114. 07;25: 139-170,
4. Berenbaum MC. Prolongation of homograft survival in mice with single doses of cyclophosphamide. *Nature.* 1963;200:84-85.
5. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Therap.* 2012;92: 414-417.
6. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. *Nat Rev Clin Oncol.* 2009;6:638-647. 7. Brodsky RA, Petri M, Smith BD, et al. Immunoablative high-dose cyclophosphamide without stem-cell rescue for refractory, severe autoimmune disease. *Ann Intern Med.* 1998;129:1031-1035.
7. Brodsky RA, Petri M, Smith BD, et al. Immunoablative high-dose cyclophosphamide without stem-cell rescue for refractory, severe autoimmune disease. *Ann Intern Med.* 1998;129:1031-1035.
8. Strauss G, Osen W, Debatin KM. Induction of apoptosis and modulation of activation and effector function in T cells by immunosuppressive drugs. *Clin Exp Immunol.* 2002;128:255-266.
9. Mayumi H, Umesue M, Nomoto K. Cyclophosphamide-induced immunological tolerance: an overview. *Immunobiology.* 1996;195: 129-139.
10. Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical BMT. *Semin Oncol.* 2012;39:683-693
11. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant.* 2002;8:377-386.
12. Munchel A, Kesserwan C, Symons HJ, et al. Nonmyeloablative HLA haploidentical bone marrow transplantation with high dose post transplantation cyclophosphamide. *Pediatr Rep.* 2011;3(Suppl 2):43-47.
13. Bashey A, Zhang X, Sizemore CA, et al. T-cell replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *J Clin Oncol.* 2013;31:1310-1316.
14. Luznik L, O'Donnell P.V.; Symons, H.J.; Chen, A.R.; Leffell, M.S.; Zahurak, M.; Gooley, T.A.; Piantadosi, S.; Kaup, M.; Ambinder, R.F.; et al. HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide. *Biol. Blood Marrow Transplant.* 2008, 14, 641–650.
15. Luznik L, O'Donnell P.V.; Fuchs, E.J. Post-Transplantation Cyclophosphamide for Tolerance Induction in HLA-Haploidentical Bone Marrow Transplantation. *Semin. Oncol.* 2012, 39, 683–693.
16. Nunes, N.S.; Kanakry, C.G. Mechanisms of Graft-versus-Host Disease Prevention by Post-transplantation Cyclophosphamide: An Evolving Understanding. *Front. Immunol.* 2019, 10, 2668.
17. Luznik L, Bolaños-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. *Blood.* 2010;115:3224-3230.
18. Kanakry CG, O'Donnell P, Furlong T, et al. Post-transplantation cyclophosphamide following busulfan and fludarabine myeloablative conditioning prevents severe acute and chronic graft-versus-host disease

and minimizes duration of immunosuppression: results of a multi-institutional trial in patients with high-risk hematologic malignancies. *Blood*. 2013;122:3310.

19. Gooptu, M.; Romee, R.; St. Martin, A.; Arora, M.; Al Malki, M.; Antin, J.H.; Bredeson, C.N.; Brunstein, C.G.; Chhabra, S.; Fuchs, E.J.; et al. HLA-haploidentical vs matched unrelated donor transplants with posttransplant cyclophosphamide-based prophylaxis. *Blood* 2021, 138, 273–282.
20. Bolaños-Meade, J.; Hamadani, M.; Wu, J.; Al Malki, M.M.; Martens, M.J.; Runaas, L.; Elmariah, H.; Rezvani, A.R.; Gooptu, M.; Larkin, K.T.; et al. Post-Transplantation Cyclophosphamide-Based Graft-versus-Host Disease Prophylaxis. *N. Engl. J. Med.* 2023, 388, 2338–2348. [CrossRef]

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