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Comparison of Cyclophosphamide-Based Graft Versus Host Disease Prophylaxis after "Allogeneic Stem Cell Transplantation from 9/10 HLA Matched Unrelated Donor" with Standard Graft Versus Host Disease Prophylaxis after "10/10 HLA Matched Relative Donor" Transplant

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ABSTRACT

Background: Graft Versus Host Disease (GvHD), which can be observed at a rate of 30-80% after allogeneic stem cell transplantation (ASCT) is an important complication that adversely affects the survival and quality of the life of patients**.** Posttransplant cyclophosphamide (PTCy) effectively prevents GvHD after HLA-haploidentical ASCT. In our study, the use of PTCy in 1-antigen HLA-mismatched unrelated donor (9/10MMUD) ASCT was compared with standard GvHD prophylaxis in HLA-identical related donor (MRD) ASCT.

Materials and Methods: We conducted a retrospective study of the comparison of 42 patients with 9/10 MMUD ASCT receiving PTCy+Methotrexate (MTX)+Calcineurin Inhibitor (CNI) and 37 patients with HLA-identical MRD who received MTX+CNI in 3 bone marrow transplantation centers.

Results: Cumulative incidences of grade I-II (64.6% vs 45.4%, p=0.187) or grade III to IV acute GvHD (35.4% vs54.6%, p=0.187) and chronic GvHD (11.9% vs 29.7%, p=0.096) were similar in the PTCy group and control group. No statistically significant differences were observed between PTCy and the control group in overall survival rate (52.4% vs 62.2%, p=0.381), progression-free survival (1483.97 vs 1200.70 days, p=0.502), relapsed-related mortality rate (21.4% vs 16.2%, p=0.556) and treatment-related mortality rate (16.7% vs 21.6%, p=0.575).

Conclusion: With the addition of PTCy to standard GvHD prophylaxis in 9/10MMUD ASCT, the risk of GvHD due to incompatibility and unrelated transplantation is eliminated, and transplantation success is achieved with MRD ASCT. PTCy-based prophylaxis is an effective and safe strategy to prevent GvHD in 9/10 MMUD ASCT without increasing the risk of relapse and treatment-related mortality.

Keywords: Cyclophosmaide; Graft-versus-host disease; Post-transplant; Prophylaxis; Unrelated-donor

INTRODUCTION

According to the EBMT 2019 activity survey, the graft source was HLA matched unrelated donor (MUD) in 51% and HLA matched sibling/related donor (MRD) in 31.1% of all allogeneic stem cell transplantation (ASCT)[1]. 9/10 HLA matched sibling

donor (MMRD), 10/10 HLA matched unrelated donor (MUD), 9/10 HLA matched unrelated donor (MMUD) ASCTs are associated with increased risk of Graft Versus Host Disease (GvHD), non-relapse mortality (NRM), short progression-free survival (PFS), and short overall survival (OS)[2-4].

GvHD, which can be observed with a rate of 30-80% after ASCT according to the presence of various risk factors, is an important complication that adversely affects the survival and quality of the life of patients. GvHD prophylaxis regimens mainly target allo-reactive T cells that proliferate in the early post-transplantation period. Since the use of a combination of methotrexate (MTX) and cyclosporine A (CsA) in the 1980s, the standard GvHD prophylaxis regimen in MRD ASCT has been based on the combined use of MTX or Mycophenolate Mofetil (MMF) with calcineurin inhibitors (CNI)[5].

T-cell depletion (T-CD) of stem cell grafts can be done ''*in-vivo*'' with anti-thymocyte globulin (ATG), alemtuzumab and PTCy, ''*ex-vivo*'' with CliniMACS device ((CD34 positive selection (Aversa, Perugia), CD3/CD19 negative selection (Handgretinger, Tuebingen) TCR-αβ/CD-19 negative) selection (Handgretinger, Tuebingen))[6].

GvHD prophylaxis regimens used in ASCT from unrelated haploidentical or MUD/MMUD consist of ATG+MTX+CsA/MMF or ex-vivo αβ T-CD combinations. The fact that ATG and the ex-vivo T-CD methods are both expensive and T-CD can be performed in a few centers limits its use[7].

PTCy is an in-vivo T-CD method developed by Johns Hopkins University primarily to increase survival in haploidentical ASCT [8]. When it was first used, the risk of damaging the stem cell was suspected, but after demonstration that stem cells express Cy-inactivating ALDH1 enzyme at high levels, while lymphocytes express it at low levels, alleviated the concerns[9].

Different groups have reported encouraging results on the use of PTCy in Haplo-ASCT using PBSC grafts and myeloablative conditioning (MAC) regimens. Luznik et al. reported grades II-IV 43% and grade III-IV 10% acute (aGvHD), and 10% chronic GvHD (cGvHD) in 117 patients who underwent ASCT from MRD using the MAC regimen and then PTCy+low-dose immunosuppressive (IS)[10-12].

Although 87% of ASCTs are from non-haploidentical donors, there are limited publications on the use of PTCy application in MRD, MMRD, MUD, and MMUD ASCT.

PTCy as a single agent in GvHD prophylaxis in PBSC graft-MMUD-ASCT resulted in the development of severe grade III-IV GvHD[13].

When only CsA was added to PTCy, the incidence of grade III-IV aGvHD decreased, but Grade II aGvHD was observed at very high rates such as 77% and Grade IV aGvHD was observed at 30%[14].

In a study using PTCy+CsA+MMF/Tacrolimus combination options in PBSC graft- MRD/MUD-ASCT, aGvHD was observed at 19% and cGvHD was 16%, and they were shown to increase survival [15].

Materials and Methods

Study Design, Patients Population and Donors

This study was designed as a retrospective registry-based analysis of three Hematopoietic Stem Cell Transplantation centers in Ankara, between 2016 and 2022. We analyzed 79 consecutive adult patients in the PTCy and control groups. The PTCy group included all patients with hematological malignancies who underwent ASCT from a single-HLA-MMUD graft after MAC regimen (n=42). In the control group, 37 patients underwent MRD graft ASCT with conventional GvHD prophylaxis after MAC regimen.

The criteria for inclusion in this study were the following; patients with AML, ALL, High-Grade Lymphoma in Complete Remission at the time of ASCT, and high-risk MDS. Only patients who were over the age of 18; transplanted with MAC regimen; no prior history of ASCT were included in the study. Patients using immune checkpoint inhibitors previously excluded.

The primary endpoint of the study was to compare the incidences of aGvHD and cGvHD requiring treatment (cumulative incidence of aGvHD and cGvHD in the first year).

The secondary endpoints were to compare the incidences of PFS, TRM, OS, relapse, toxicity, and rates of infectious complications.

Conditioning Regimens and GvHD Prophylaxis

The transplantation conditioning regimen was myeloablative intensity for all patients and the control group. Two most commonly used MAC regimens in PTCy groups included Fludarabine (Flu) (Flu: 30 mg/m^2 per day, -7th to -4th days)+Total Body Irradiation (TBI) (400 cGY/day, -3rd to -1st days totally:1200 cGy) and Cyclophosphamide (Cy) (Cy:60 mg/kg per day, -5th to -4th days)+TBI (400 cGY/day, -3rd to -1st days totaly:1200 cGy).

The other conditioning regimens used are shown in Table-2. GvHD prophylaxis in PTCy group was performed with Cy at a daily dose of 50 mg/kg at +3rd, +4th days plus MTX at days +1st (15 mg/m^2) +3rd, +6th (10 mg/m^2), and tacrolimus with a target concentration of 5 to 15 ng/mL, or CsA at daily doses of 3 mg/kg/day initiated on day +5th continuing to day +90th. Dose reduction initiated due to toxicity or for the aim of discontinuation around +180th days in the absence of any significant GvHD.

Patients in the control group received CsA (3 mg/kg from days −1st to day +270th in the absence of GvHD) plus MTX at days +1st (15 mg/m^2), +3rd, +6th, +11th (10 mg/m^2).

PBSC was the primary choice, used for both groups as the graft source, and an average of 5x10^6 CD34/kg cells was targeted for transplantation. However, bone marrow-derived stem cells were used in very few of the patients (PTCy:4, Control:2).

Definitions of Clinical Outcomes

Neutrophil engraftment time: Number of days from ASCT to the first day when the absolute neutrophil count was above 0.5x10^9/L for 3 consecutive days.

Platelet engraftment time; Number of days, from ASCT to the first of a sustained platelet count >20×10^9/L without transfusion.

OS: Time from the first day of ASCT to death from any cause. PFS: Time from the first day of ASCT to disease relapse or progression. TRM: Death due to complications of ASCT in the absence of relapse, or progression. Patients who did not reach outcome events were censored at the last follow-up date.

Post-transplantation Complications:

Post-ASCT complications were evaluated, including acute GvHD(aGvHD) and chronic GvHD(cGvHD), and organ toxicities. The modified Keystone criteria were used for aGvHD grading and the National Institutes of Health (NIH) Consensus criteria were used to determine cGvHD severity [16]. GvHD scoring was done by two different clinicians. Veno-occlusive disease (VOD) was diagnosed with clinical findings and imaging and graded according to the modified Seattle criteria [17].

The study was approved by each center's ethical committee, and all patients or legal guardians provided written informed consent authorizing the use of clinical information for research purposes (2022/01-2021-405).

Statistical analysis

As descriptive statistics, Mean and Standard deviation were used for continuous data and frequency and percentage were used for categorical data. The compatibility of the variables with the normal distribution was checked with the Kolmogorov-Smirnov test, and the homogeneity of the variances was checked with Levene's test. The distribution of categorical variables in the groups was evaluated with Pearson Chi-square and Fisher's exact test. Group means of normally distributed continuous variables were compared with Student's t-test, and those that did not match were compared with Mann-Whitney U test. The correlation between categorical variables was evaluated with the Phi and Cramér V correlation coefficients. Survival rates at the end of transplant and relapse of patients in the PTCy and control groups were calculated using Kaplan-Meier survival analysis. Survival of the PTCy or control groups was evaluated by Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon), and Tarone-Ware methods. Data were analyzed with the IBM SPSS 21 (IBM SPSS Inc, Chicago, IL) package program. The statistical significance level was considered as 0.05.

Theory/calculation

In this study, we aimed to compare the post-transplant morbidity and mortality effects of using PTCy+Tacrolimus +/- MTX, PTCy + CsA+/- MTX as a GvHD prophylaxis regimen in a very specific transplant arm (9/10 MMUD graft-ASCT). For this purpose, Acute/Chronic GvHD rate, engraftment

time, CMV reactivation, overall survival (OS), progression-free survival (PFS), and disease recurrence were investigated.

RESULTS

Patient Characteristics

Our study included 49 (62%) male and 30 (38%) female totally 79 patients (n: PTCy: 42 Control:37). The mean age of the participants was 40.48±15.04 (18-68) years. Other characteristic features were given at Table-1. There was no statistically significant difference between the PTCy and control groups in terms of gender, age, disease distribution, number of treatment lines, number of previous autologous transplants and disease remission status before transplantation.

However, the distribution of disease stage in the PTCy and control group was statistically significant ($\chi2$=4.411 P=0.036). It was found that 'Intermediate risk' (26.2%, n:11) in the PTCy group was higher than the control (8.1%, n=3). On the contrary, the "High risk" group was found to be higher in the control group (Control 91.9% n:34, PTCy 73.8%, n=31).

Table 1: Demographic data of the groups and comparison of distributions

Patient Characteristics		Post-Cy (n=42)	Control (n=37)	Test, Statistics
Gender	Female	16(38.1%)	14(37.8%)	$\chi2$=0.001*
	Male	26(61.9%)	23(62.2%)	p=0.981
Age (years) (Mean± SS)		41.19±15.44	39.67±14.75	t=0.444** p=0.658
Diagnosis	*AML*	24(57.1%)	22(59.4%)	$\chi2$=5.824* P=0.443
	ALL	11(26.2%)	12(32.4%)	
	MDS	2(4.8%)	1(2.7%)	
	Lymphomas	5(11.9%)	2(5.4%)	
Number of Treatment Lines up to Transplantation	*1*	13(31.0%)	8(21.6%)	$\chi2$=5.259* P=0.511
	2	16(38.1%)	17(45.9%)	
	3	9(21.4%)	9(24.3%)	
	≥4	4(9.6%)	3(8.1%)	
Previous Autologous Transplant History	*1*	3(7.1%)	2 (5.4%)	$\chi2$=2.242* P=0.326
Pre-Transplant Disease Status	*CR1*	4(9.5%)	4(10.8%)	$\chi2$=0.156* P=0.984
	CR2	29(69.0%)	24(64.9%)	
	CR3	8(9.0%)	8(21.6%)	
	CR4	1(2.4%)	1(2.7%)	
Disease Stage	*Intermediate Risk*	11(26.2%)	3(8.1%)	$\chi2$=4.411 P=0.036****
	High Risk	31(73.8%)	34(91.9%)	
Disease Remission Status	*Active Disease*	4(9.5%)	6(16.2%)	P=0.290***

* Pearson Chi-square test statistic value.
**Student-t test statistical value.
*** Fisher's Exact test stat value.
****Significant difference was found at 0.05 Significance Level

Comparison of Transplantation Data

Table-2 shows that there is no statistically significant difference between the mean CD34+ cell count of PTCy (5.28±1.43) and control group (5.38±1.52) (U=764.50, p=0.902). The mean TNC value of PTCy group (7.82±4.78) was significantly lower than control (10.74±4.34) (U=449.50, p=0.003). HLA incompatibility regions were HLA-A (38.1%), HLA-B (21.4%), HLA-DR (16.7%), HLA-C (11.9%) HLA-DQ (11.9%) in order of frequency the mismatch regions in PTCy group. The distribution of blood group incompatibility in the PTCy and control groups was statistically significant ($\chi2$=18.232, p<0.001). It was observed that most of the people in the control group did not have any incompatibility n:32 (86.5%). In the PTCy group, the blood group compatibility group was n:32 (42.9%), while the most incompatibility was ''major'' (n:13 (31.0%)). Stem cell source distributions found to be similar ($\chi2$=0.479, P=0.787) and "peripheral" stem cell source was the majority in both groups (PTCy; n:37 88.1%) and control; n:34(91.9%)).

Table 2: Comparison of Transplantation Data

		Post-Cy (n:42)	**Control (n:37)**	**Test, Statistics**
CD34 (Mean ± SS)		5.28±1.43	5.38±1.52	U=764.50* p=0.902
TNC (Mean± SS)		7.82±4.78	10.74±4.34	U=449.50* p=0.003***
HLA Mismatch Region	HLA-A	16(38.1%)		
	HLA-B	9(21.4%)		
	HLA-C	5(11.9%)		
	HLA-DR	7(16.7%)		
	HLA-DQ	5(11.9%)		
Blood Group Incompatibility (ABO-Mismatch)	Compatible/No Mismatch	18(42.9%)	32(86.5%)	$\chi2$=18.232** P<0.001***
	Minor Mismatch	8(19.0%)	0(0.0%)	
	Major Mismatch	13 (31.0%)	5(13.5%)	
	Major+Minor Mismatch	3(7.1%)	0(0.0%)	
Conditioning Regimen	TBI (800-1200cGy) +Flu (30mg/m^2x5)	36(85.7%)		
	Cy (60mg/kgx2) +TBI (1200cGy)	5(11.9%)	21(%56.7)	
	Treosulfan (10 gr/m^2x3) +Flu (40mg/m^2x4)	1 (2.4%)		
	Cy(60mg/kgx2) +Busulphan (3.2 mg/kgx4)		16(%43.3)	
Stem Cell Source	Bone Marrow (BM)	4(9.5%)	2 (5.4%)	$\chi2$=0.479** P=0.787
	Peripheric Blood (PB)	37(88.1%)	34(91.9%)	
	PB+BM	1 (2.4%)	1 (2.7%)	

* Mann-Whitney U test statistics value.
**Pearson Chi-square test statistical value.
***Significant difference was found at 0.05 significance level

Data after transplantation and GvHD

Table-3 shows that the mean neutrophil and platelet engraftment time in PTCy group (16.97±5.71, 20.23±11.51 respectively) was significantly longer than the control group (12.62±6.02, 12.02±8.80 respectively) (U=416.00, U=386.00 respectively, P<0.001). aGvHD grade distributions in both groups were similar (χ2=6.172, p=0.187), also the rate of patients who did not have aGvHD in both groups were in the majority (PTCy; 25 (59.5%) and control; 26 (70.3%)). aGvHD rates in the PTCy group (n:17(45%)) were higher than control(n:11(40.5%)), but there was no statistical difference. When the organ involvement of the patients who developed aGvHD was evaluated, more skin aGvHD (100% vs. 27.2%) in the PTCy group and more GIS aGvHD (0% vs 10.8%) in the control group observed (χ2=17.574, p=0.004). The distributions of cGvHD were similar in both groups (χ2=4.690, p=0.096) but distribution of cGvHD organ/system involvement was statistically significant (χ2=17.574, p=0.004). Also, it was seen that patients without cGvHD were in the majority in both groups (PTCy; n:37 (88.1%) and control; n:26 (70.3%)).

Table 3: Comparison of data after transplantation

		Post-Cy (n:42)	Control (n:37)	Test, Statistics
Neutrophile engraftment time (day) (Mean ± SD)		16.97±5.71	12.62±6.02	U=416.00* P<0.001****
Thrombocyte engraftment time (day) (Mean± SD)		20.23±11.51	12.02±8.80	U=386.00 P<0.001****
Acute GvHD	No	25(59.5%)	26(70.3%)	χ2=6.172**
	Yes	17(40.5%)	11(29.7%)	P=0.187
Acute GvHD Grade	1-2	11(64.6%)	5(45.4%)	χ2=6.172
	3-4	6(35.4%)	6 (54.6%)	P=0.187
Acute GvHD Involved Organ/System	Skin	17(100%)	3(27.2%)	χ2=17.574
	GIS	0(0.0%)	2 (18.2%)	P=0.004****
	Liver	0(0.0%)	4(36.4%)	
	Skin+Liver	0(0.0%)	1 (9,1%)	
	GIS+Liver	0(0.0%)	1 (9,1%)	
Chronic GvHD	No	37(88.1%)	26(70.3%)	χ2=4.690
	Yes	5(11.9%)	11(29.7%)	P=0.096
Chronic GvHD Involved organ/System	Skin	0(0.0%)	2 (18,2%)	χ2=12.856
	GIS	3(60%)	2 (18,2%)	P=0.045
	Liver	0(0.0%)	4(36,4%)	
	GIS+Liver	2 (40%)	0(0.0%)	
	Skin+Liver	0(0.0%)	2 (18,2%)	
	Skin+Liver+GIS	0(0.0%)	1 (9,1%)	
Overall Survival	Exitus	20(47.6%)	14(37.8%)	χ2=0.768
	Alive	22(52.4%)	23(62.2%)	p=0.381
1st Month Survival	Exitus	2 (4.8%)	4(10.8%)	P=0.411***
	Alive	40(95.2%)	33(89.2%)	
3rd Month Survival	Exitus	7(16.7%)	5(13.5%)	χ2=0.152
	Alive	35(83.3%)	32(86.5%)	P=0.697
6th Month Survival	Exitus	14(33.3%)	10 (27.0%)	χ2=0.370
	Alive	28(66.7%)	27(73.0%)	P=0.543
1 Year Survival	Exitus	17(40.5%)	11(29.7%)	χ2=0.993
	Alive	25(59.5%)	26(70.3%)	P=0.319
Relapse Related Mortality	Exitus	9(21.4%)	6(16.2%)	χ2=0.347
	Alive	33(78.6%)	31(83.8%)	P=0.556
Transplant Related Mortality	Exitus	7(16.7%)	8(21.6%)	χ2=0.314
	Alive	35(83.3%)	29(78.4%)	P=0.575

* Mann-Whitney U test statistics value.
** Pearson Chi-square test statistic value.
*** Fisher's exact test statistic value.
****A significant difference was found at the 0.05 Significance Level

Examination of the factors affecting PTCy GvHD

There was positive correlation (moderate, not statistically significant) between HLA Incompatibility and total GvHD rate (0.256, p=0.757), ABO-Incompatibility and aGvHD rate (0.286, p=0.331).

A weak correlation (all not statistically significant) was found between CMV infection between days 101-365 and aGvHD (0.189, p=0.220), cGvHD rate (-0.057, p=0.710), donor/recipient CMV serology status (0.087, p=0.572).

The only comparative data that we found statistically significant was between HLA Incompatibility and cGvHD rate. There was a positive and moderately significant correlation (0.527, p=0.040) according to the Cramér V correlation coefficient.

ABO-Incompatibility had a weak negative not statistically significant correlation with aGvHD (-0.084, p=0.609), cGvHD (-0.257, p=0.118) and total GvHD rate (-0.145, p=0.377) in the control group (Table 4).

Table 4: Correlations Between Variables in the Post-Cy and Control Group

	Acute GvHD rate		Chronic GvHD rate		Total GvHD rate	
	r**	p	r**	P	r**	P
HLA Incompatibility in the PTCy Group	0.224	0.833	0.527	0.040	0.256	0.737
ABO Incompatibility in the PTCy Group	0.286	0.331	0.227	0.540	0.227	0.539
ABO Incompatibility in the Control Group	-0.084	0.609	-0,257	0.118	-0.145	0.377

*Phi correlation coefficient.
**Cramér V correlation coefficient

Survival Outcomes, Relapse, and TRM

Overall Survival (OS) rates were found to be similar ($\chi2$=0.768, p=0.381) and survivors were the majority in both groups (PTCy; n:22 (52.4%), control; n:23 (62,2%)).

In addition, 1st, 3rd, 6th month and first year survival rates in the PTCy and control groups were similar (p=0.411, p=0.697, p=0.543, p=0.319 respectively) and survivors were majority in both groups.

The rates of Relapse Related Mortality (RRM) were similar in both groups ($\chi2$=0.347, P=0.556), and found 21.4% vs 16.2% in PTCy and control groups respectively.

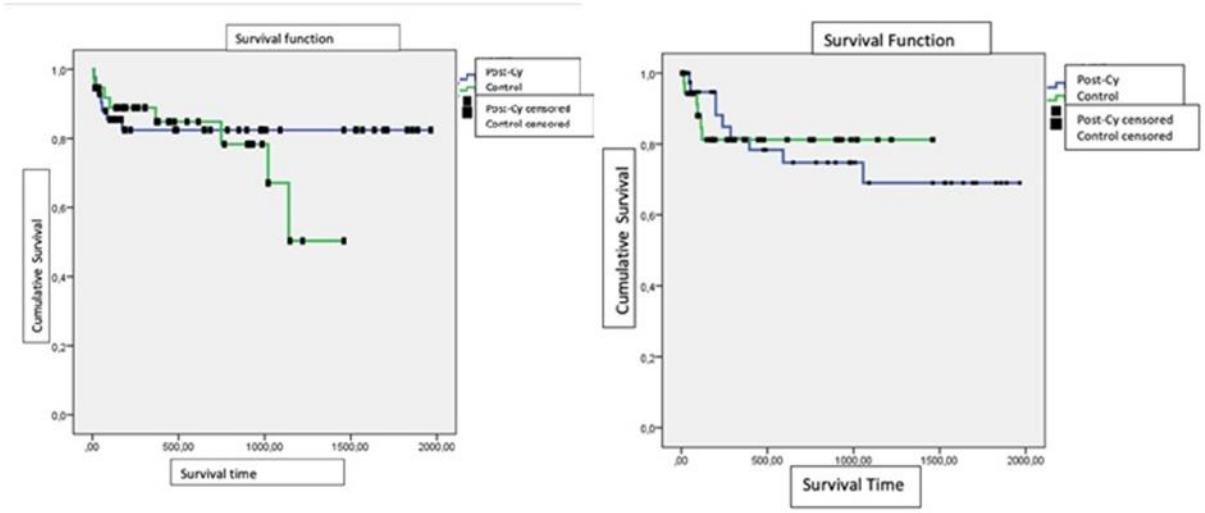
TRM rates were similar in both groups ($\chi2$=0.314, P=0.575), and found 16.7% vs 21.6% in PTCy and control groups respectively (Table-3).

The average of OS in the PTCy group after transplantation was 54.4 months, and the average OS in the control group was 36.9 months.

A total of 79 patients were transplanted, 15 of these patients died, and 81.0% (64) of the individuals constituted the censored data part. The mean OS in transplants was found to be 46.2 months. The difference between the OS distributions in both groups according to the three calculated test statistics was not statistically significant (Log Rank (Mantel – Cox) p=0.502, Breslow (Generalized Wilcoxon) p=0.922, Tarone–Ware p=0.849 (graphic).

PFS in the PTCy group was 49.5 months, and 40 months in control. 15 of 79 patients died in total, 81.0% (n:64) of the individuals constituted the censored data part. The mean PFS was found to be 45 months. According to the three test statistics calculated, the difference between PFS of PTCy and control groups was not statistically significant (Log Rank (Mantel–Cox) p=0.906, Breslow (Generalized Wilcoxon) p2=0.703, Tarone–Ware p=0.879) (graphic).

Graphic: Overall Survival and Progression Free Survival Chart



Overall Survival **Progression Free Survival**

DISCUSSION

The main problems facing ASCT are treatment/transplant-related complications, and disease recurrence. Although there are more recent approaches there is still a 15-20% risk of death due to ASCT complications[18,19]. The main goal of clinicians is to keep patients in balance between GvHD and the disease recurrence. In this regard, there is always concern on concentration of conditioning regimens and prophylaxis drugs for fear of disease recurrence. Although there are numerous studies on to improve GvHD prophylaxis protocols, a standard approach has not been established yet. Nowadays, PTCy administration is a successful and widely used method for the prevention of GvHD after HLA-haplotype matched transplants [20, 21]. Although data on PTCy and CNI use for MRD/MUD-ASCT are limited, there is increasing interest in the use of these agents. In the first clinical studies, PTCy application started to be used in especially bone marrow-derived grafts ASCT[22,23]. In these studies, Tacrolimus and MMF were used together with PTCy. It attracted attention with the reported 5% incidence of Grade III-IV aGvHD, using bone marrow-derived graft and RIC conditioning regimen.

With the improvements observed as a result of these studies, PTCy use was not only limited to MMUD or MUD, but also used with MSD as a single IS or in combination [13, 22, 24].

There is sufficient data that PTCy prophylaxis alone can be used for 10/10 HLA-matched MRD/MUD bone marrow grafts [14, 22-27]. However, it has been understood that it isn't sufficient alone, especially in PBSC grafts. There are lots of data on adding one or two IS therapy to PTCy (13, 28), but currently insufficient for choosing which combination should be used.

For unrelated grafts, there is data suggesting that the combination of MMF and tacrolimus provides effective control of GvHD[29]. In our study, the combined use of CNI and MTX in addition to PTCy was predominantly preferred. Our post-transplant outcome data were similar to studies using MMF and Tacrolimus.

A few studies have evaluated the effect of standard CNI-based GvHD prophylaxis on single-antigen MMUD-ASCT[30-36]. Overall, these studies have shown that standard CNI±MTX-based GvHD prophylaxis after MMUD-ASCT is associated with an increased incidence of graft failure, NRM, and GvHD, resulting in worse OS outcomes.

Based on these studies, in the absence of MRD/MUD various strategies attempted on in-vivo T-CD for

MMUD graft-ASCT. However, due to the insufficient number of prospectively designed studies, these new agents were frequently used for MRD or MUD graft derived ASCT, as in our study.

In a study with a similar design to us, PTCy+Tacrolimus+MMF was used for GvHD prophylaxis in MMUD graft-ASCT.

When PBSC graft MMUD and MUD ASCT compared, no statistically significant difference was found between the groups in the incidence of Grade II to IV or III to IV aGVHD, cGVHD, NRM, recurrence rate, PFS or OS. Unlike our study here, MMF was used instead of MTX in the MMUD patient group, and the control group was chosen as MUD instead of MRD[37].

In another retrospective study with a similar design, Tacrolimus+MMF+PTCy used as a GvHD prophylaxis strategy for MMUD (9/10-HLA compatible) ASCT. ATG was added to classical GvHD prophylaxis (MTX-Tacrolimus) in the control group[29]. In general, rates of aGvDH or cGvHD were similar between both groups, as in our study. However, contrary to us, aGvHD rate was lower, and grade II-IV acute GvHD mas not detected in the first 30 days, while it was 15% (p=0.01) in the control group. The incidence of grade II–IV (37% vs 36%, P=0.8) and grade III–IV (17% vs 12%, P=0.5) aGVHD in first 100 days was similar to our study. The median neutrophil (18 days vs. 12 days, P<0.001) and platelet (25.5 days vs. 18 days, P=0.05) engraftment time was prolonged in the PTCy group, also similar to our study.

Similar results were obtained between groups when patients were analyzed by graft source (BM vs. PB) or by HLA class mismatch (class I versus class II) (excluding isolated HLA-DQ mismatch). These data also support our study, PTCy is safe regardless of HLA class mismatch region compared to traditional GvHD prophylaxis.

We observed similar prolonged neutrophil engraftment duration after PTCy+CNI+MTX prophylaxis as previous studies, with a median of 16.9 days in PTCy and 12.6 days in the control group[22, 23, 29, 38]. In one of these studies using bone marrow graft, it has been shown that neutrophil and platelet recovery times are prolonged in the PTCy arm compared to the control group who received standard GvHD prophylaxis[29,37] and our study also support these findings. Since it didn't cause pre-engraftment infection-related mortality increase, this situation considered as a negligible.

As in our study, the data obtained from studies in general showed that the rate of aGvHD with PTCy application is two-three times the rate of chronic GvHD. This indicates that alloreactive T cells persist after PTCy and are not anergic, but even if these alloreactive T cells persist, it does not cause ongoing clinical alloreactivity in the future.

In a study of 43 patients with high-risk hematological neoplasm who received PTCy and CsA after PBSCT from HLA-matched MRD (N=12) or MUD (N=31) with a MAC regimen (fludarabine, busulfan, TBI), aGvHD (grade II-III) was reported as 77%, while OS was 70% and relapse rate was 17%. Although aGvHD rate was higher than in our study, the cGvHD rate was 16%, aGvHD grade III and above was not reported. In addition, the frequency of relapse and cGvHD were reported at a similar rate as our study (Relapse 21.4%, cGvHD 11.9% in our study)[39].

The impact of ABO-mismatch on ASCT outcomes is controversial, however, our data suggest that major-ABO-mismatched transplantation is associated with higher rates of GvHD. Similar results were obtained in a study, in which ABO-mismatch increased the risk of transplant-related mortality[3]. The distribution of ABO-mismatch in our study was similar in both patient and control groups.

The addition of immunosuppressive (IS) drugs has been shown to reduce the rate of significant GvHD in the PBSC and PTCy setting and the use of two IS can further improve outcomes compared to alone[11,23]. PBSC grafts appear to offer higher rates of cGvHD compared to BM grafts, even with the use of additional IS. Until strategies are modified in the PBSC transplant setting, BM grafts should be considered for patients older and at high risk of developing GvHD.

The protective effect of PTCy on grade III to IV aGvHD is known to differ depending on the hematopoietic stem cell source as with other Conditioning regimens[40]. PBSC grafts were used mostly in our study, but there was no statistically significant difference between both groups in terms of PB and BM graft use. However, it is known that the incidence of aGvHD would be much higher in MMUD

recipients given PBSC graft if PTCy was not used as part of GvHD prophylaxis [41].

A major concern with the PTCy protocol is the high frequency of disease recurrence after transplantation. There was no difference between the treatment arms of various PTCy-containing GvHD prophylaxis regimens with other PTCy-free regimens, in terms of disease recurrence and PFS rates in the randomized large number-patient "Blood and Marrow Transplant Clinical Trials Network Report" data[42]. Although the follow-up period of our study was relatively short, the majority of the patients were with high-risk AML, ALL, and/or MDS, and it is noteworthy that PTCy and the control group were similar in terms of disease relapse (21.4 vs 26.3%) [42].

A more specific patient group was included in our study because the majority of previously reported PTCy studies didn't include a control group and included different diagnoses, disease states, donor types, and conditioning regimens. Although it is a retrospective study and includes limited number of patient groups, our study achieved the goal, and it has been shown that the negative effects related to MMUD graft can be eliminated by PTCy. What it has in common with other studies is that PTCy has been shown to reduce the risk of GvHD without increasing the risk of relapse[22, 23, 26, 27, 39]. The general conclusion to be obtained from these studies is that PTCy alone may be sufficient in bone marrow-derived stem cell transplantation, while combining it with 2-3 different IS treatment affects the results more positively in PBSC transplantation and RIC-ASCT[15, 22, 23, 26-28, 43].

Although the mean follow-up period was short in our study, PTCy-related secondary primary cancer was not observed in any of our patients. However, there are studies showing that PTCy administration in long-term follow-ups is not associated with an increased risk of secondary primary cancer, unlike other immunosuppressive treatments [44].

One of the limitations of our study is that it is a retrospective study and consists of a heterogeneous and limited patient cohort. The second is that the follow-up period is limited. Additionally, due to the nature of all multi-center retrospective studies, potential discrepancies in the diagnosis and grading of GvHD must be considered. Different hematological disease groups were included in our study. This situation prevented homogeneous distribution of risk groups and it was not possible to standardize the high risk group for a single disease group. Since there was no intermediate risk group in ALL and lymphoma patients, all of them were in the high risk group. The intermediate risk group consisted of MDS and AML patients. It is thought that this situation may affect relapse-related mortality and overall survival results rather than GVHD results. When evaluated in this respect, the statistically significant higher number of patients in the high risk group in the control group was reflected in relapse-related mortality rates, although no statistically significant difference was found.

CONCLUSION

Our study demonstrates that the use of PTCy+Tacrolimus/Cyclosporine+MTX as a GvHD prophylaxis in single antigen MMUD PBSC graft-ASCT with MAC regimen is safe and at effective as conventional GvHD prophylaxis regimens of MRD/MUD graft ASCT.

PTCy use didn't contribute to additional toxicities; however, it is obvious that recipients of PBSC grafts show a delayed engraftment time with PTCy.

In addition, our study supports the findings of other studies that PTCy in MMUD ASCT doesn't prevent the graft-versus-leukemia effect. Prospective studies with larger patient numbers are needed to confirm our study results.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Baldemoro H, Passweg J. 30 Years EBMT Activity Survey and 2019 Annual Report. Bone Marrow Transplantation 2021; e-pub ahead of print Jan 2021(33);

2. Bashey ZA, Zhang X, Brown S, Jackson K, Morris LE, Holland HK et al. Comparison of outcomes following transplantation with T-replete HLA-haploidentical donors using post-transplant cyclophosphamide to matched related and unrelated donors for patients with AML and MDS aged 60 years or older. Bone Marrow Transplant. 2018; 53(6): 756-763.

3. Barkhordar M, Kasaeian A, Tavakoli S, Vaezi M, Foumani HK, Bahri T et al. Selection of Suitable Alternative Donor in the Absence of Matched Sibling Donor: A

Retrospective Single-Center Study to Compare between Haploidentical, 10/10 and 9/10 Unrelated Donor Transplantation. Int J Hematol Oncol Stem Cell Res. 2021; 15(1): 51-60.
4. Greco R, Lorentino F, Albanese S, Lupo Stanghellini MT, Giglio F, Piemontese S et al. Posttransplantation Cyclophosphamide- and Sirolimus-Based Graft-Versus-Host-Disease Prophylaxis in Allogeneic Stem Cell Transplant. Transplant Cell Ther. 2021; 27(9): 776.e1-776.e13.
5. Chhabra S, Liu Y, Hemmer MT, Costa L, Pidala JA, Couriel DR et al. Comparative Analysis of Calcineurin Inhibitor-Based Methotrexate and Mycophenolate Mofetil-Containing Regimens for Prevention of Graft-versus-Host Disease after Reduced-Intensity Conditioning Allogeneic Transplantation. Biol Blood Marrow Transplant. 2019; 25(1): 73-85.
6. Aversa F, Pierini A, Ruggeri L, Martelli MF, Velardi A. The Evolution of T Cell Depleted Haploidentical Transplantation. Front Immunol. 2019; 10: 2769.
7. de Witte M, Daenen LGM, van der Wagen L, van Rhenen A, Raymakers R, Westinga K et al. Allogeneic Stem Cell Transplantation Platforms With Ex Vivo and In Vivo Immune Manipulations: Count and Adjust. Hemasphere. 2021; 5(6): e580.
8. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008; 14(6): 641-650.
9. Kanakry CG, Ganguly S, Zahurak M, Bolaños-Meade J, Thoburn C, Perkins B et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. Sci Transl Med. 2013; 5(211): 211ra157.
10. Williams L, Cirrone F, Cole K, Abdul-Hay M, Luznik L, Al-Homsi AS. Post-transplantation Cyclophosphamide: From HLA-Haploidentical to Matched-Related and Matched-Unrelated Donor Blood and Marrow Transplantation. Front Immunol. 2020: 11: 636.
11. Ruggeri A, Labopin M, Bacigalupo A, Afanasyev B, Cornelissen JJ, Elmaagacli A et al. Post-transplant cyclophosphamide for graft-versus-host disease prophylaxis in HLA matched sibling or matched unrelated donor transplant for patients with acute leukemia, on behalf of ALWP-EBMT. J Hematol Oncol. 2018; 11(1): 40.
12. Carnevale-Schianca F, Caravelli D, Gallo S, Becco P, Paruzzo L, Poletto S et al. Post-Transplant Cyclophosphamide and Tacrolimus-Mycophenolate Mofetil Combination Governs GvHD and Immunosuppression Need, Reducing Late Toxicities in Allogeneic Peripheral Blood Hematopoietic Cell Transplantation from HLA-Matched Donors. J Clin Med. 2021; 10(6): 1173.
13. Bradstock KF, Bilmon I, Kwan J, Micklethwaite K, Blyth E, Deren S et al. Single-Agent High-Dose Cyclophosphamide for Graft-versus-Host Disease Prophylaxis in Human Leukocyte Antigen-Matched Reduced-Intensity Peripheral Blood Stem Cell Transplantation Results in an Unacceptably High Rate of Severe Acute Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2015; 21(5): 941-944.
14. Moiseev IS, Pirogova OV, Alyanski AL, Babenko EV, Gindina TL, Darskaya EI et al. Risk-adapted GVHD prophylaxis with post-transplantation cyclophosphamide in adults after related, unrelated, and haploidentical transplantations. Eur J Haematol. 2018; 100(5): 395-402.
15. Moiseev IS, Pirogova OV, Alyanski AL, Babenko EV, Gindina TL, Darskaya EI et al. Graft-versus-Host Disease Prophylaxis in Unrelated Peripheral Blood Stem Cell Transplantation with Post-Transplantation Cyclophosphamide, Tacrolimus, and Mycophenolate Mofetil. Biol Blood Marrow Transplant. 2016; 22(6): 1037-1042.
16. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015; 21(3): 389-401.
17. Lee SJ. Classification systems for chronic graft-versus-host disease. Blood. 2017; 129(1): 30-37.
18. Boyiadzis M, Arora M, Klein JP, Hassebroek A, Hemmer M, Urbano-Ispizua A et al. Impact of chronic graftversus-host disease on late relapse and survival on 7,489 patients after myeloablative allogeneic hematopoietic cell transplantation for leukemia. Clin Cancer Res. 2015; 21(9): 2020–2028.
19. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010; 363(22): 2091–2101.
20. Ostronoff F, Ostronoff M, Souto-Maior AP, Domingues M, Sucupira A, Manso DA et al. Prospective trial of mycophenolate mofetilcyclosporine A prophylaxis for acute GVHD after G-CSF stimulated allogeneic bone marrow transplantation with HLA-identical sibling donors in patients with severe aplastic anemia and hematological malignancies. Clin Transplant. 2009; 23(1): 33–38.
21. Ruutu T, van Biezen A, Hertenstein B, Henseler A, Garderet L, Passweg J et al. Prophylaxis and treatment of GVHD after allogeneic haematopoietic SCT: a survey of centre strategies by the European Group for Blood and

Marrow Transplantation. Bone Marrow Transplant. 2012; 47(11): 1459–1464.
22. Luznik L, Bolanos-Meade J, Zahurak M, Chen AR, Smith BD, Brodsky R et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood. 2010; 115(16): 3224–3230.
23. Kanakry CG, O'Donnell PV, Furlong T, de Lima MJ, Wei W, Medeot M et al. Multi-institutional study of posttransplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. J Clin Oncol. 2014; 32(31): 3497–3505.
24. Alousi AM, Brammer JE, Saliba RM, Andersson B, Popat U, Hosing C et al. Phase II trial of graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide after reduced-intensity busulfan/fludarabine conditioning for hematological malignancies. Biol Blood Marrow Transplant. 2015; 21(5): 906–912.
25. McCurdy SR, Kasamon YL, Kanakry CG, Bolaños -Meade J, Tsai HL, Showel MM et al. Comparable composite endpoints after HLA -matched and HLA -haploidentical transplantation with post -transplantation cyclophosphamide. Haematologica. 2017; 102(2): 391-400.
26. Kanakry CG, Tsai HL, Bolaños-Meade J, Smith BD, Gojo I, Kanakry JA et al. Single-agent GVHD prophylaxis with posttransplantation cyclophosphamide after myeloablative, HLA-matched BMT for AML, ALL, and MDS. Blood. 2014; 124(25): 3817–3827.
27. Jacoby E, Chen A, Loeb DM, Gamper CJ, Zambidis E, Llosa NJ et al. Single-agent post-transplantation cyclophosphamide as graftversus-host disease prophylaxis after human leukocyte antigen-matched related bone marrow transplantation for pediatric and young adult patients with hematologic malignancies. Biol Blood Marrow Transplant. 2016; 22(1): 112–118.
28. Holtick U, Chemnitz JM, Shimabukuro-Vornhagen A, Theurich S, Chakupurakal G, Krause A et al. OCTET - CY: a phase II study to investigate the efficacy of post - transplant cyclophosphamide as sole graft -versus -host prophylaxis after allogeneic peripheral blood stem cell transplantation. Eur J Haematol. 2016; 96(1): 27-35.
29. Mehta RS, Saliba RM, Chen J, Rondon G, Hammerstrom AE, Alousi A et al. Post-transplantation cyclophosphamide versus conventional graft-versus-host disease prophylaxis in mismatched unrelated donor haematopoietic cell transplantation. Br J Haematol. 2016; 173(3): 444-455.
30. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. Blood. 2007; 110(13): 4576-4583.
31. Petersdorf EW, Anasetti C, Martin PJ, Gooley T, Radich J, Malkki M et al. Limits of HLA mismatching in unrelated hematopoietic cell transplantation. Blood. 2004; 104(9): 2976-2980.
32. Woolfrey A, Klein JP, Haagenson M, Spellman S, Petersdorf E, Oudshoorn M et al. HLA-C antigen mismatch is associated with worse outcome in unrelated donor peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2011; 17(6): 885-892.
33. Crocchiolo R, Ciceri F, Fleischhauer K, Oneto R, Bruno B, Pollichieni S et al. HLA matching affects clinical outcome of adult patients undergoing haematopoietic SCT from unrelated donors: a study from the Gruppo Italiano Trapianto di Midollo Osseo and Italian Bone Marrow Donor Registry. Bone Marrow Transplant. 2009; 44(9): 571-577.
34. Flomenberg N, Baxter-Lowe LA, Confer D, Fernandez-vina M, Filipovich A, Horowitz M et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood. 2004; 104(7): 1923-1930.
35. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med. 1998; 339(17): 1177-1185.
36. Michallet M, Sobh M, Serrier C, Morisset S, Labussiere H, Ducastelle S et al. Allogeneic hematopoietic stem cell transplant for hematological malignancies from mismatched 9/10 human leukocyte antigen unrelated donors: comparison with transplants from 10/10 unrelated donors and human leukocyte antigen identical siblings. Leuk Lymphoma. 2015; 56(4): 999-1003.
37. Jorge AS, Lledó MS, Pereira A, et al. Single Antigen–Mismatched Unrelated Hematopoietic Stem Cell Transplantation Using High-Dose Post-Transplantation Cyclophosphamide Is a Suitable Alternative for Patients Lacking HLA-Matched Donors. Biol Blood Marrow Transplant. 2018;24(6):1196–1202.
38. Binkert L, Medinger M, Halter JP, Heim D, Gerull S, Holbro A et al. Lower dose anti-thymocyte globulin for GvHD prophylaxis results in improved survival after allogeneic stem cell transplantation. Bone Marrow Transplant. 2015; 50(10): 1331–1336.
39. Mielcarek M, Furlong T, O'Donnell PV, Storer BE, McCune JS, Storb R et al. Posttransplantation cyclophosphamide for prevention of graft-versus-host

disease after HLA-matched mobilized blood cell transplantation. Blood. 2016; 127(11): 1502–1508.
40. Oran B, Manero GG, Saliba RM, Alfayez M, Al-Atrash G, Ciurea SO et al. Posttransplantation Cyclophosphamide Improves Transplantation Outcomes in Patients With AML/MDS Who Are Treated With Checkpoint Inhibitors. Cancer. 2020; 126(10): 2193-2205.
41. Schoch LK, Cooke KR, Wagner-Johnston ND, Gojo I, Swinnen LJ, Imus P et al. Immune checkpoint inhibitors as a bridge to allogeneic transplantation with posttransplant cyclophosphamide. Blood Adv. 2018; 2(17): 2226-2229.
42. Pasquini MC, Logan B, Jones RJ, Alousi AM, Appelbaum FR, Bolaños-Meade J et al. Blood and Marrow Transplant Clinical Trials Network Report on the Development of Novel Endpoints and Selection of Promising Approaches for Graft-versus-Host Disease Prevention Trials. Biol Blood Marrow Transplant. 2018; 24(6): 1274-1280.
43. Solomon SR, Sanacore M, Zhang X, Brown S, Holland K, Morris LE et al. Calcineurin inhibitor-free graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide and brief-course sirolimus following reduced-intensity peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2014; 20(11): 1828–1834.
44. Majzner RG, Mogri H, Varadhan R, Brown P, Cooke KR, Meade JB et al. Post-Transplant Cyclophosphamide after Bone Marrow Transplantation is not Associated with an Increased Risk of Donor Derived Malignancy. Biol Blood Marrow Transplant. 2017; 23(4): 612–617.