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Comparison of alpha/beta T cell depletion with posttransplant cyclophosphamide in haploidentical transplantation

Mehmet Ozen, Mehmet Gunduz, Ilknur Aksoyoglu, Ahmet Ifran, Ali Ugur Ural

Hematology and Bone Marrow Tranplantation Unit, Bayındır Söğütözü Hospital, Ankara, Türkiye

ABSTRACT

Aim: To compare alpha/beta T-cell depletion with posttransplant Cyclophosphamide (PTCy) in haploidentical allogeneic transplantation in adult hematological patients, this is the first study.

Method: In our study, we reported 36 haploidentical allogeneic stem cell transplants which were performed in our clinic.

Results: Twenty-six of these haploidentical transplants received standard treatment and transplanted either with PTCy (n=21, 81%) or with alpha/beta T-cell depletion (n=5, 19%). Less CD34+ stem cells were administered in the T-cell depletion group. When the two groups were compared in terms of survival, no difference was found in relapse-free and overall survival in each group. Acute GVHD cases developed in the PTCy group mostly developed after CMV infection, whereas acute GVHD did not develop in the T-cell depletion group. In the PTCy group, 8 cases developed graft failure and relapse; 4 cases developed graft failure or relapse in the T-cell depletion group, and 2 of them developed graft failure or relapse following EBV infection and 1 of them following CMV infection.

Conclusion: We have a small number of patients in the T cell depletion group. Due to our long follow-up period, we believe that our patients with T cell depletion can be compared with those who underwent PTCy, and similar survival results can be achieved in adult patients having hematologic malignancies.

Key words: Malignant hematological disease, haploidentical allogeneic transplantation, cyclophosphamide, alpha/beta T-cell depletion.

✉ *Dr. Mehmet Özen*
Kızılırmak Mahallesi, 1443. Cd. No: 17, 06250, Söğütözü Çankaya /Ankara / Türkiye
E-mail: kanbilimci@gmail.com
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Introduction

Allogeneic stem cell transplantation (allo-SCT) is one of the well-known life-saving treatment options for malignant hematological diseases. The first choice in allo-SCT is a full or well-matched sibling donor. However, in cases in which a suitable sibling donor cannot be found, allo-SCT from alternative donors is performed. Alternative donors include unrelated Human Leukocyte Antigen (HLA)-matched individuals and haploidentical related donors.

Many different conditioning regimens are performed for transplants from haploidentical related donors with an HLA match of 5-8/10. Historically, because of very high rates of graft-versus-host disease (GVHD) and graft rejection observed in initial haploidentical transplants,

various methods have been evaluated to reduce GVHD [1]. To decrease graft rejection and GVHD in haploidentical transplants, some centers use intense conditioning regimens and posttransplant immune suppression; others manipulate the graft [2]. In the first studies, CD34-positive selection was applied to prevent graft failure [3]. However, graft failure, GVHD, and mortality rates were high, and to improve outcomes, selection methods were developed. Initially, in pediatric patients, TCR alpha/beta CD19-depleted allografts were used for haploidentical transplantation to reduce graft rejection and GVHD rates [4,5]. This approach has recently been performed in adult patients [6,7].

In subsequent years, it was observed that posttransplant cyclophosphamide (PTCy) application produced better outcomes than CD34 selection in haploidentical transplantations [8]. There is no study comparing TCR alpha/beta CD19-depleted haploidentical allo-SCTs with PTCy. Therefore, we present results comparing T cell depletion and PTCy in haploidentical transplantation in this study.

Materials and metods

Between 27.04.2012 and 30.04.2022, 36 haploidentical allo-SCTs were performed in our clinic. In this article, our patients who underwent haploidentical allo-SCT will be summarized. Our institutional ethical committee approved the study. The approval number is BTEDK-08/23.

We have firstly included and given demographic features of all haploidentical allo-SCT cases in our clinic. Secondly, we have excluded nonstandard transplants and compared standard transplants according to their results and demographic features. Median follow-up time was 6.6 years for all patients.

Nonstandard transplants are defined as haploidentical transplants in patients with active disease, patients with a donor change after the start of the conditioning regimen, patients without both PTCy and T-cell depletion groups, and nonmalignant patients.

Conditioning regimens

The alpha/beta T-cell depletion conditioning regimen consisted of 140 mg/m^2 melphalan (day −9), 2 × 5 mg/kg thiotepa (day −7), 40 mg/m^2/day fludarabine (days −7 to −3), 30 mg/kg/day antithymocyte globulin (ATG-Fresenius, Graefelfing, Germany) (days −6 to −2), and 2 mg/kg/day methylprednisolone (days −6 to −2). Rituximab (375 mg/m^2/day, Day +1) was given to reduce B cells. Ganciclovir (5 mg/kg/day, daily, Days +5 to +20; 5 days/week, Days +21 to +100; 36 days/week, Days +100 to +210) was given to reduce CMV infection. No immune suppression was given after transplantation.

PTCy conditioning regimens include TBI-fludarabine-based or fludarabine-cyclophosphamide-based protocols. TBI-fludarabine-based regimens included TBI (2x200 cGy/day, Days −8 to −6) and 30 mg/m^2/day fludarabine (days −6 to −2). Additionally, methotrexate (MTX) (On day +1 15 mg/m^2/day, on days +3 and +6 10 mg/m^2) and 50 mg/kg/day cyclophosphamide (days +3 and +4) were administered. Mesna (100 mg/kg/day, Days +3 and +4; 75 mg/kg/day, Day +5) and tacrolimus (0.03 mg/kg/day, Days +5 to +100/180) were given as immunosuppressant. The fludarabine-cyclophosphamide-based regimen included 30 mg/m^2/day fludarabine (days −4 to −2) and 14.5 mg/kg/day cyclophosphamide (days -4 and -3). Cyclophosphamide (50 mg/kg/day, on days +3 and +4), Mesna (100 mg/kg/day, Days +3 and +4), tacrolimus (0.03 mg/kg/day, Days +5 to +100/180 days) and mycophenolate sodium PO

(15 mg/kg, Days +5 to +60) were given as immunosuppressant.

Alpha/beta T-cell depletion

The single-day apheresis was performed via the apheresis system of Spectra Optia following stem cell mobilization of the donor who received daily 10 μg/kg G-CSF for 4 days. Leukapheresis collection material containing <60x10^9 nucleated cells was washed by centrifugation at 300 × g for 5 minutes with PBS-EDTA-0.5% buffer supplemented with human serum albumin and treated with γ-globulins to minimize nonspecific antibody binding to Fc receptors before the addition of the fluorochrome-conjugated, anti-TCR-alpha/beta antibody. Cells were then incubated with magnetic beads conjugated to antibiotin, resuspended at <300 x 10^6/mL and applied to a fully automated Clini-MACS device using the Depletion 3.1 program (Miltenyi Biotec) as described previously and according to standard operating procedures [9]. For quality control, the original fraction, target, and nontarget fractions were analyzed via flow cytometry. Characterizations for target product booster comprised <5x10^4 alpha/beta T cells/kg and >4x10^6 CD34+ cells/kg.

Statistics

We present numeric variables as medians. We compared the categorical variables through the chi-square test or Fisher's exact test. The nonparametric Mann–Whitney U test was used for noncategorical variables. We calculated Overall survival (OS) from the beginning of the date of allo-SCT to the end of the date of the last follow-up or the date of the death. We calculated also Relapse-free survival (RFS) from the date of allo-SCT to the date of the last non-relapse follow-up or the date of relapse. The distributions of OS and RFS durations in the two groups were estimated using the Kaplan–Meier method and compared using the log-rank test. In our study, all the data distributed nonhomogeneously. Therefore, only median (and the lowest and highest) values were given. All reported *p* values <0.05 was considered significant. We performed statistical analyses using SPSS 16.0.

Results

Of the 36 patients who underwent haploidentical allo-SCT, 23 (64%) were male and 13 (36%) were female. Demographic data of the patients are shown in Table 1. Relapse following CMV infection was observed in 9 patients (30%), and acute GVHD following CMV infection was observed in 6 (20%) patients. Fourteen of the patients (39%) survived, and 22 patients (61%) died. The causes of death of the patients in the all groups are shown in Table 1. Additionally, the median relapse-free survival of patients who underwent haploidentical transplant was 4.63 months (1.68-7.58, 95% CI). The median overall survival was 6.6 months (0.46-12.74, 95% CI). Total and event-free survival graphs of the patients are given in Figures 1 and 2. In addition, the survival data of all patients are summarized in Table 2.

Twenty-six patients who underwent haploidentical transplants received standard treatment and were transplanted either with PTCy (n=21, 81%) or with alpha/beta T-cell depletion (n=5, 19%). Ten haploidentical transplants did not receive standard treatment; therefore, they were not included in the comparison.

As shown in Table 3, the PTCy group and the T-cell depletion group were compared according to their demographic characteristics. Fewer CD34+ stem cells were administered in the T-cell depletion group. No differences were found in terms of event-free and overall survival between the two groups (Figures 3 and 4 and Table 4). Five acute GVHD cases developed in the PTCy group and three of 5 acute GVHD cases

Table 1. Demographic characteristics of all patients.

Gender, n (%)	• Female 13 (%36) • Male 23 (%64)
Age, median (range)	• 37 (20-68)
Treatment, n (%)	• Standard 10 (%28) • Non-Standard 26 (%72)
Diagnosis, n (%)	• AML 20 (%56) • ALL 10 (%28) • AA 2 (%5) • HD 2 (%5) • CML 1 (%3) • MDS 1 (%3)
Stem cell source, n (%)	• 34 patients (%94) peripheral stem cell • 2 patients (%6) bone marrow
CD34 count, median (range)	• 7,83 (3.32-12.1) peripheral stem cell • 1,5 (1,29-1,72) bone marrow
Engraftment, n (%)	• Yes 30 (%83) • No 6 (%17)
Engraftment day, median (range)	• 15 (10-21)
Secondary engraftment failure, n (%)	• Yes 13 (%50) • No 13 (%50)
Acute GVHD developing, n (%)	• Yes 8 (%27) • No 22 (%73)
Chronic GVHD developing (Among >100 days survived patients), n (%)	• Yes 3 (%14) • No 19 (%86)
Ex cause (n=22), n (%)	• Graft failure n=11 (%50) • Relapse 5 (%23) • GVHD 4 (%18) • Infection 2 (%9)

AA: Aplastic anemia, ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, CML: Chronic Myeloid Leukemia, HD: Hodgkin's Disease, GVHD: Graft versus host disease, MDS: Myelodysplastic Syndrome

Table 2. Survival characteristics in all patients.

Variables	**Overall**	**Relapse-free**
1 year	%71.6	%71.6
2 years	%41.6	%40.8
Median, years (%95CI)	4.6 (1.7-7.6)	6,6 (0,46-12.7)

CI: confidence interval.

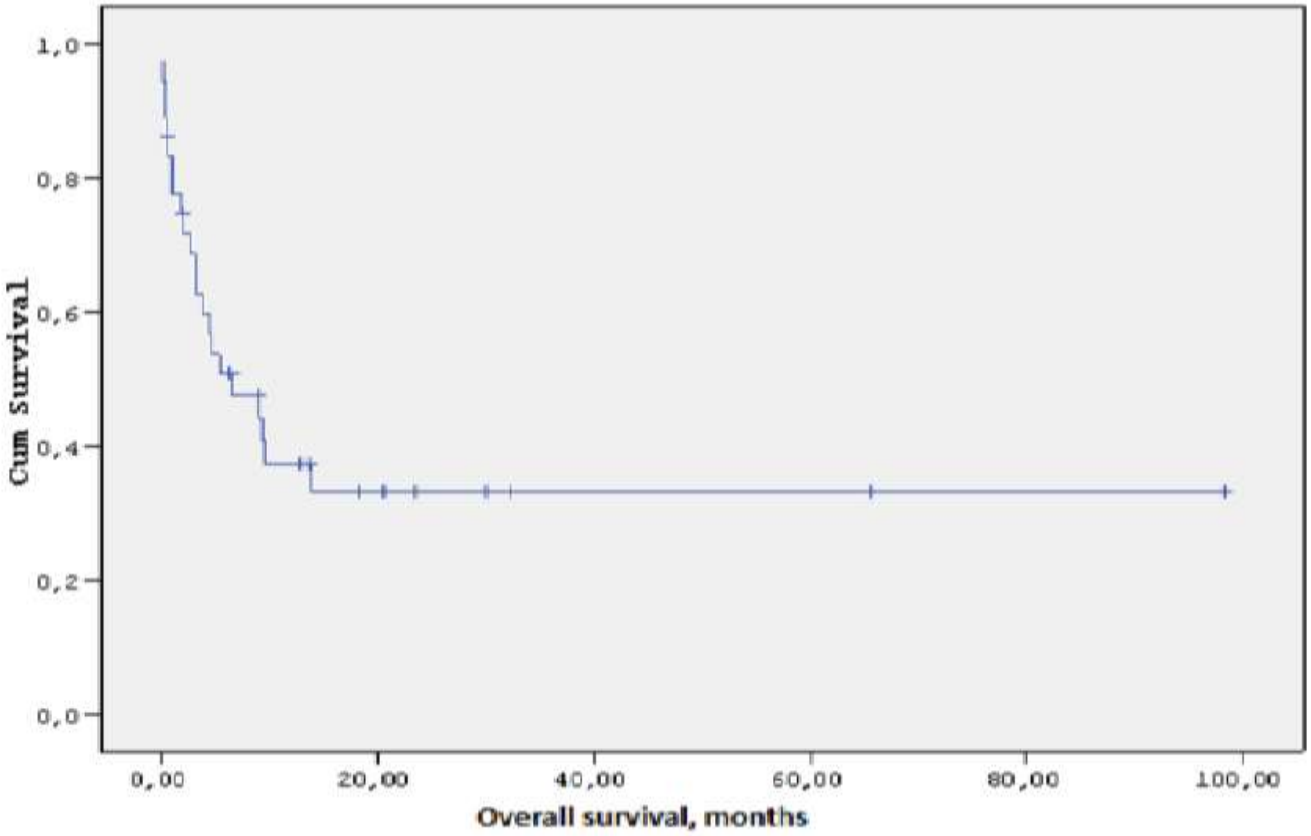

Figure 1. Overall survival in all patients.

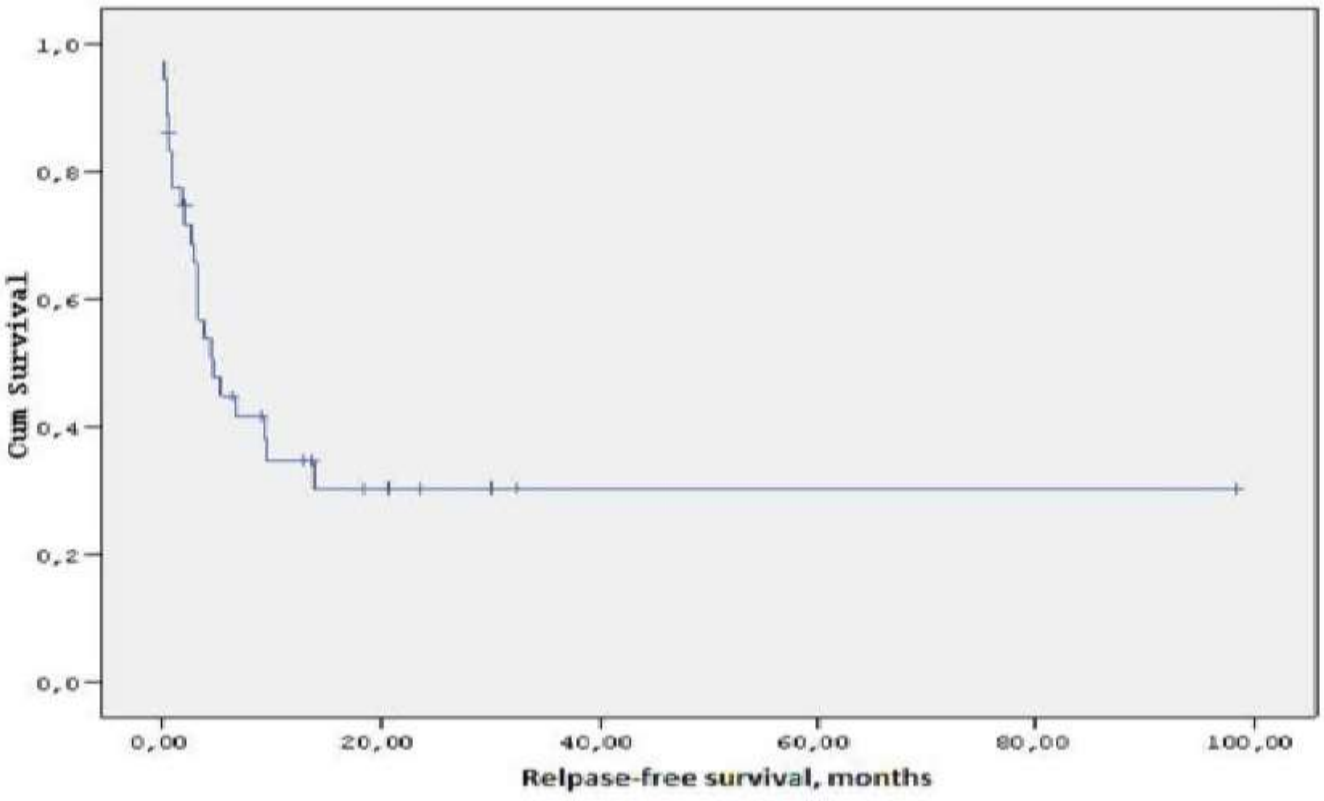

Figure 2. Relapse-free survival in all patients.

Table 3. Demographic characteristics of both groups.

Parameters	PTCy group	T-cell depletion group	*P value*
Gender, n (%)	• Female 9 (%43) • Male 12 (%57)	• Female 0 (%0) • Male 5 (%100)	0.13
Age, median (range)	• 41 (20-68)	• 37 (25-45)	0.63
Diagnosis, n (%)	• AML 12 (%57) • ALL 7 (%33) • HD 1 (%5) • CML 0 (%0) • MDS 1 (%5)	• AML 2 (%40) • ALL 1 (%20) • HD 1 (%20) • CML 1 (%20) • MDS 0 (%0)	0.27
Stem cell source, n (%)	• 20 patients (%95) peripheral stem cell • 1 patient (%5) bone marrow	• 5 patients (%100) peripheral stem cell • 0 patient (%0) bone marrow	1.0
CD34 count, median (range)	• 10 (5.78-12.1) peripheral stem cell • 1,29 (1,29) bone marrow	• 5,11 (4.92-8.88) peripheral stem cell • 0 bone marrow	0.01*
Primary Engraftment, n (%)	• Yes 20 (%95) • No 1 (%5)	• Yes 5 (%100) • No 0 (%100)	1.0
Engraftment, days , median (range)	• 16.5 (13-21)	• 15 (10-21)	0.21
Secondary engraftmant failure, n (%)	• Yes 8 (%42) • No 11 (%58)	• Yes 4 (%80) • No 1 (%20)	0.31
Acute GVHD developing, , n (%)	• Yes 5 (%25) • No 15 (%75)	• Yes 0 (%0) • No 5 (%100)	0.54
Chronic GVHD developing (Among >100 days survived patients) , n (%)	• Yes 2 (%12) • No 14 (%88)	• Yes 1 (%20) • No 4 (%80)	1.0
Ex cause (n=22) , n (%)	• Graft failure n=3 (%33.5) • Relapse 3 (%33.5) • GVHD 2 (%22) • Infection 1 (%11)	• Graft failure n=1 (%33) • Relapse 2 (%67) • GVHD 0 (%0) • Infection 0 (%0)	0.66

ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, CML: Chronic Myeloid Leukemia, HD: Hodgkin's Disease, GVHD: Graft versus host disease, MDS: Myelodysplastic Syndrome, PTCy: posttransplant cyclophosphamide.

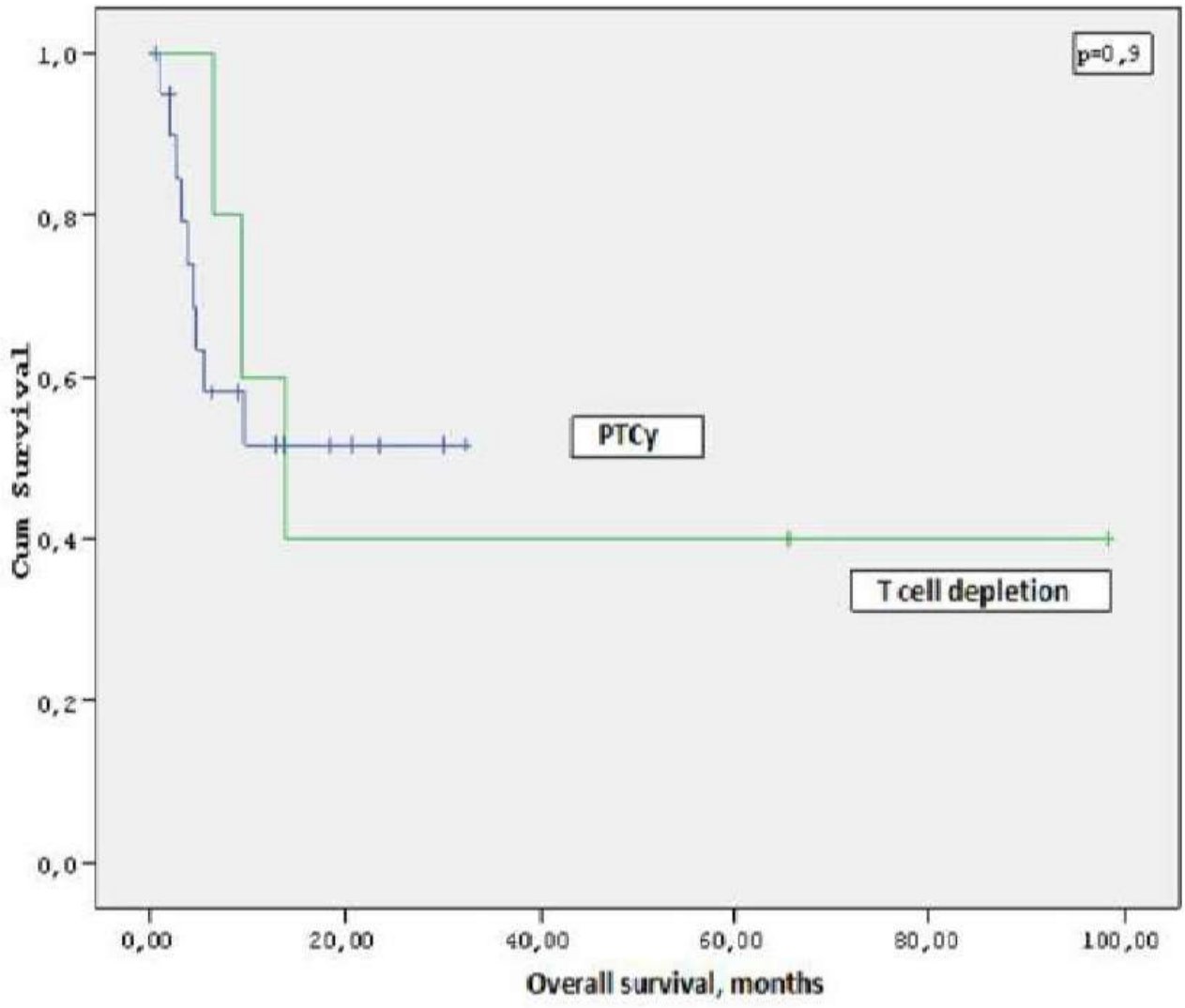

Figure 3. Comparison of PTCy and T-cell depletion in terms of overall survival.

developed after CMV infection, whereas acute GVHD did not develop in the T-cell depletion group. In the PTCy group, 8 cases developed graft failure and relapse; 4 cases developed graft failure or relapse in the T-cell depletion group, and 2 of them developed graft failure or relapse following EBV infection and 1 of them following CMV infection.

Table 4. Relapse-free and overall survival in both groups.

Variables	PTCy overall	T-cell depletion overall	*P*	PTCy relapse-free	T-cell depletion relapse-free	*P*
1 year	%58.1	%60.0		%58.1	%40.0	
2 years	%51.6	%40.0		%51.6	%20.0	
Median, months (%95CI)	18.7 (12.3-25.1)	13,9 (4,2-23,5)	0.9	18.6 (12.1-25.0)	9.4 (3,9-14,9)	0.60

CI: confidence interval, PTCy: posttransplant cyclophosphamide.

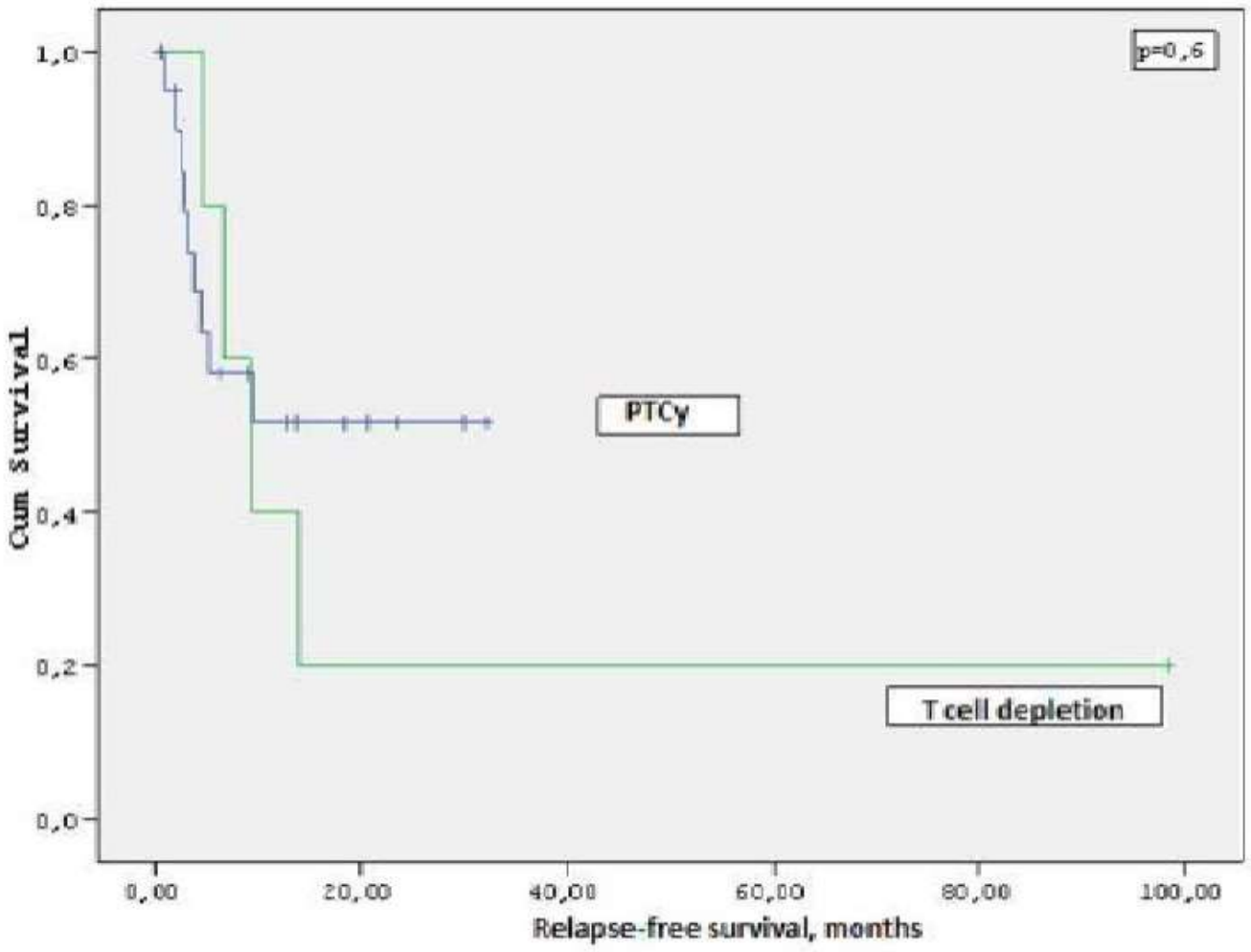

Figure 4. Comparison of PTCy and T-cell depletion in terms of relapse-free survival.

Discussion

Allo-SCT is a treatment option in patients with malignant hematological diseases or immunodeficiency. However, the transplant expectations and approaches for patients with immunodeficiency and those of patients with malignant hematological disease are different due to the absence of GVHD in patients with immunodeficiency, tolerating graft failure, and not needing graft-versus-leukemia effects [10]. Therefore, patients without malignant hematological diseases were excluded from this study. Allo-SCT in patients with active disease is associated with poor survival rates, which persist in both T-cell depletion and other transplants [11,12]. These patients were excluded from the study because their outcomes were different from those of other patients.

We have not found a study comparing T-cell depletion with PTCy in haploidentical transplantation. Therefore, our study is the first to compare haploidentical transplant methods. Alpha/beta T-cell depletion was first applied in children [5]. Indeed, a review summarizing studies in children concluded that there is no clear difference in survival between PTCy and T-cell depletion in haploidentical transplants due to heterogeneity in trials, including patient populations, methodologies and supportive care approaches [13]. In our study in adult patients, we could not detect any significant difference in

overall or relapse-free survival between the PTCy and T-cell depletion groups. Engraftment kinetics, acute and chronic GVHD rates and causes of death were also similar between the PTCy and T-cell depletion groups.

In a meta-analysis, patients having sickle cell disease who underwent haploidentical transplantation using T-cell depletion and PTCy were compared [14]. The meta-analysis included only two studies that used T-cell depletion methods [15,16]. In vitro T-cell depletion was associated with low aGVHD rates, high infection rates, increased transplantation-related mortality and slightly more favorable OS. However, as our study included only patients with malignant hematological disease, our results may not be similar to the results of this meta-analysis.

In a review, although T-cell depletion subgroups, including TCR alpha/beta and CD19 depletion, showed less GVHD and improved GF, infection, and relapse rates, the economically expensive and time-consuming technology was a disadvantage [17]. We also did not apply the in vitro T-cell depletion method in our patients due to its increasing cost after 2018; this is the main reason for the decrease in the number of T-cell depletion patients in our study.

Cell depletion methods to prevent GvHD are quite diverse, and the most recently preferred method is TCR alpha/beta depletion, which we also used. However, as in all depletion methods, there is some loss of CD34+ cells in this method [18,19]. The main reason for the low number of CD34+ cells in the T-cell depletion group, which was the only significant data point in our study, is the loss of CD34+ cells during depletion.

The results of patients who underwent haploidentical allo-SCT with TCR alpha/beta depletion were reported in a single descriptive study conducted in our country [7]. This study did not have a cyclophosphamide arm and only reported TCR alpha/beta depletion results in 24 patients. In this study, neutrophil engraftment was achieved on Day +12, 21 patients were engrafted, Grade III or IV acute GVHD detected in two patients, and chronic GVHD occurred in two patients. These findings are similar to our findings. Disease-free survival and overall survival were reported as 42 and 54% at 1 year, respectively. In our study, the survival rates were 40 and 60% at 1 year. They also reported that relapse was the main reason of death (56.3%), similar to our results.

Limitations of the study

In our study the T-cell depletion group had a small number of patients. Therefore, our statistical results may be misleading. This is the main limitation of our study. Its retrospective design is one of the other limitations. However, due to our long follow-up period, we believe that our patients with T-cell depletion can be compared with those who underwent PTCy.

Conclusions

In haploidentical allogeneic stem cell transplantations T-cell depletion is not obviously superior to PTCy and similar survival results can be achieved in adult patients, which may be an important factor limiting its application, given its cost.

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