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TcRαβ-depleted haploidentical transplantation results in adult acute leukemia patients

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ABSTRACT

Introduction: The use of αβ+ T-cell-depleted grafts is a novel approach to prevent graft failure, graft-versus-host disease (GVHD), and non-relapse mortality (NRM) in patients undergoing haploidentical hematopoietic stem cell transplantation.

Patient and method: Thirty-four patients with acute leukemia and lacking a match donor were treated with αβ T-cell-depleted allografts from haploidentical family donors. A total of 24 patients had acute myeloid leukemia (AML) and 10 had acute lymphoblastic leukemia. 84.4% of patients were in the high-risk group, and 55.9% were not in remission. The preparative regimen included thiotepa, melphalan, fludarabine, and anti-thymocyte globulin-Fresenius. Grafts were peripheral blood stem cells engineered by TcR-alpha/beta depletion.

Results: Neutrophil and platelet engraftment was achieved on days +12 (range, 10.5–15) and +11 (range, 10–12). All but three patients were engrafted with full donor chimerism. Grade III-IV acute GVHD occurred in two (5.9%) patients and chronic GVHD in two (6.1%). Disease-free survival and overall survival were 42 and 54% at 1 year, respectively. AML as disease type (HR: 4.87, 95% CI: 1.50–15.87) and mother as donor (HR: 1.05, 95% CI: 1.00–1.11) were found to be independent risk factors on patient survival. Mortality and NRM in the first 100 days were 5 of 34 (14.7%) and 4 of 34 (11.7%). Relapse was the main cause of death (56.3%). T-cell reconstitution appears to be faster than that reported in published data with CD3/CD19-depleted grafts.

Conclusion: αβ T-cell-depleted haploidentical transplantation may be a good alternative for high-risk patients if there are no human leukocyte antigen matched donors.

KEYWORDS
αβ T cell depletion; haploidentical transplantation; immune reconstitution

Introduction

Hematopoietic stem cell transplantation (HSCT) is an effective treatment for a lot of malignant and non-malignant conditions [1,2]. However, finding suitable donors for patients who would benefit from HSCT still remains a major challenge. Human leukocyte antigen (HLA)-haploidentical HSCT (haplo-HSCT) is an alternative transplantation option.

A partially HLA mismatched or HLA haploidentical, first-degree relative can be identified rapidly for any patients without HLA-matched donors [3].

Initial results from the use of haploidentical allografts were disappointing. Historically, haplo-HSCT resulted in high rates of graft failure, mortal graft-versus-host disease (GVHD), relapse and non-relapse mortality (NRM) [4–6].

Several approaches have been tried to obviate such results, including *ex vivo* graft manipulation procedures, *in vivo* lymphocyte depletion with post-transplant cyclophosphamide or intensive immune suppression and various conditioning regimens [7–9]. So the aim of haplo-HSCT approaches is to minimize graft-versus-host responses and host-versus-graft while preserving of graft-versus-tumor effect and immune responses to infection [10].

Unfortunately, serious late infections and disease relapse resulting from slow immune reconstitution remain the two most frequent causes of mortality in patients undergoing haplo-HSCT [11]. To improve the immune recovery, Lang et al. in pediatric patients have established a new T-cell depletion method that removes αβ+ T lymphocytes via a biotinylated anti T-cell receptor (TcR) αβ+ Ab followed by an anti-biotin Ab conjugated to magnetic microbeads while retaining γδ+ T lymphocytes, natural killer (NK) cells, and other cells in the graft. In addition, CD19+ B

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lymphocytes were concomitantly depleted for the prevention of post-transplant Epstein–Barr–virus (EBV)-associated lymphoproliferative disease [12].

We performed a TcR αβ depleted but CD19 haploidentical transplantation with a myeloablative conditioning regimen in 34 acute leukemia patients. In this retrospective analysis, we report the outcomes of adult acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients after TcRαβ-depleted haplo-HSCT.

Patients and methods

We carried out a retrospective analysis of 34 adult acute leukemia patients who received TcRαβ-depleted haploidentical peripheral allografts after myeloablative conditioning regimen.

Patients were referred for allogeneic stem cell transplantation and lacked an HLA-matched sibling or unrelated donors. All donors were at least two HLA loci-mismatched first-degree relatives. Patients who would receive TcRαβ-depleted haplo-HSCT between dates June 2012 and June 2015 were included in the study, and data recording was stopped on 01 July 2015.

We collected patient's data retrospectively from hospital registry. All the patients and donors provided written informed consent for the treatment protocol.

All the patients signed written informed consent allowing the use of their medical data in clinical research. All HSCT and data collection protocols were reviewed and approved by Erciyes University Ethic Committee.

Patients and donors

A total of 24 patients had AML and 10 had ALL. Two patients progressed from myelodysplastic syndrome, and one progressed from chronic myeloid leukemia to AML. Patients were evaluated in two groups as AML and ALL.

All donors were at least two HLA loci-mismatched first-degree relatives. Donors were prioritized on the basis of HLA matching, age, and ABO compatibility.

Stem cell mobilization and depletion of αβ+ T-cells

Peripheral blood mononuclear cells were mobilized with human G-CSF at a dose of 2×5 μg/kg/day for 5 days, and stem cells were collected every 1 or 2 days. The αβ+ T-cells were depleted by using anti-TcRαβ-coated microbeads and the automated CliniMACS device (Miltenyi Biotec, Bergisch Gladbach, Germany). Method of Schumm et al. was used but B-cell depletion was not performed.

Conditioning regimens and GVHD prophylaxis

The conditioning regimen consisted of fludarabine (40 mg/m^2, days −8 to −5), thiotepa (2×5 mg/kg, day −4), and melphalan (70 mg/m^2, days −3, −2). In addition to chemotherapy, anti-thymocyte globulin (ATG-Fresenius, Graefelfing, Germany) 30 mg/kg starting on days −12 to −9 was used to deplete remaining host T-cells, avoiding graft rejection. Methylprednisolone was administered on days −12 to −9 (2 mg/kg) to avoid the side effects of ATG. The used preparation regimen was evaluated as myeloablative [12]. Mycophenolate sodium was given as prophylactic immune suppression (2×720 mg) until the day 60 where residual T-cells in the graft exceeded 25×104/kg body weight (BW). G-CSF was not given routinely.

Definitions

Assessment of disease risk, engraftment, graft failure, GVHD, chimerism, and immune reconstitution.

The day of neutrophil engraftment was defined as the first of three consecutive days on which the absolute neutrophil count (ANC) was $>0.5 \times 10^9$/L. Platelet engraftment was defined as independence from platelet substitution for at least seven consecutive days with a platelet count greater than 20×10^9/L. Primary graft failure was defined as failure to achieve an ANC $\geq 5 \times 10^9$/L before day 28. Secondary graft failure was defined as a recurrent neutropenia less than 500/μL after initial recovery and/or a decline of platelet counts below 20,000/μL for seven consecutive days or requiring transfusion support after achieving sustained platelet counts > or = 20,000/μL without transfusions for seven consecutive days after hematopoietic SCT. Acute and chronic GVHD were defined and graded according to the Glucksberg criteria and National Institutes of Health consensus criteria [13,14]. Chimerism was assessed in peripheral blood by polymerase chain reaction (PCR) analysis of STR regions. Reconstitution of lymphocyte subsets were evaluated by flow cytometry at 1, 3, 6, and 12 months (range ± 10 days) post-HSCT.

Disease-free survival (DFS) was defined as the time from transplantation to relapse of leukemia and overall survival (OS) was defined as the time from transplantation to death.

Patients who were induced remission after standard induction treatments, but by salvage treatments, and patients who were complete remission (CR) 3 and above without remission were considered as high-risk patients for recurrence. Patients who were induced and were at CR 1 or CR 2 after standard treatments were considered as patients with standard risks.

Supportive care

All the patients were treated in high-efficiency particulate air-filtered inpatient bone marrow transplantation unit. Patients received bacterial (levofloxacin 1×400 mg, metronidazole 3×500 mg), fungal (flucanozole 2×200 mg), and herpes zoster (valasiclovir 4×500 mg) prophylactic treatment at the time of transplantation. Twelve of 34 patients were given secondary fungal profilaxis with voriconazole, amphotericin B or posaconazole due previous fungal infection.

Following engraftment, *Pneumocystis jiroveci* prophylaxis (trimethoprim-sulfamethoxazole) was given at least 12 months.

Immunoglobulins were given intravenously each month if immunoglobulin G level is lower than 500 mg/dL. All patients and their donors were CMV seropositive.

The pre-emptive strategy for CMV reactivation was used. PCR screening for CMV in blood was performed at least weekly until +100 days and one time in 10–15 days from +100 to +180 days. In case of viremia, ganciclovir, valganciclovir, or foscarnet was started.

EBV PCR was performed during febrile episodes or monthly. Additional PCR tests for ADV, BK virus, HHV6, and the other respiratory viruses were performed during febrile episodes or if there were any related symptoms or signs (diarrhea, dysuria, etc.).

Statistical analysis

Histogram and *q*–*q* plots were examined and Shapiro–Wilk's test was performed to assess the data normality. To compare the differences between groups, Fisher's exact test and Pearson chi-square analysis were applied for categorical variables while the Mann–Whitney *U* test was applied for continuous variables. The Kaplan–Meier method was used to estimate the survival probabilities and the log-rank test was used for comparisons. The Pepe and Mori test was applied to compare disease groups while taking into account the competing risks using the cumulative incidence rates. Univariate and multiple Cox proportional hazards regression method was used to determine the most significant independent factors. Significant variables at $p < 0.20$ level on univariate analysis were taken into a multiple model and backward stepwise selection was performed using Wald statistic at $p < 0.10$ stringency level to identify the independent risk factors. Hazard ratios and their 95% confidence intervals are also calculated. Analyses were performed using R 3.2.0 (www.r-project.org). A *p*-value less than 5% was considered as statistically significant.

Results

Patients characteristics

The demographic characteristics and relevant transplantation data for the patients and donors are shown in Table 1. The characteristics of the patients who were diagnosed with either AML or ALL were not significantly different. Majority of patients (84.4%) were in the high-risk group, and 55.9% of them were not in remission.

Graft composition

All the patients received HSCT with αβ+ T-cell-depleted haploidentical grafts. The patients received a median number of 12.69×10^6 CD 34+ progenitor cells per kg BW. In addition, grafts contained a median number of 4.58×10^6 per kg BW γδ T-cells. The median number of residual αβ T-cells was 11.72×10^3 cells per kg BW (Table 2). There was no difference between groups.

Engraftment time and engraftment failure

Median time to neutrophil engraftment without G-CSF stimulation was 12 (range 10.5–15) days and to platelet engraftment was 11.00 (10.00–12.00) days. Neutrophil engraftment days of ALL patients were later than the AML patients (11 vs. 14 days, $p = 0.045$). Other than this, there was no difference between two groups.

All but three patients engrafted with full donor chimerism. One of these patients died due to bacterial infection. The second patient was re-transplanted with alpha-beta T-cell-depleted stem cells from a second (another) haploidentical-related donor and achieved donor-cell engraftment. Reconditioning regimen comprised total body irradiation (2×200 cGy), thiotepa, fludarabine, and ATG. Engraftment eventually occurred in 31 patients (91.2%) with TcRαβ-depleted transplantation.

Third patient who experienced engraftment failure was re-transplanted with different haploidentical transplantation protocol.

Another patient who had secondary graft failure because of CMV reactivation was successfully re-transplanted from another haploidentical donor. Conditioning regimen consisted of total body irradiation (2×200 cGy), thiotepa, fludarabine, and ATG.

GVHD

Eleven (32.4%) patients developed aGVHD after the transplantation. Grade I–II aGVHD occurred in nine (26.5%) patients and grade III–IV in two (5.9%) patients (Table 3). One patient died because of GVHD progression.

Table 1. Patient and donor characteristics.

Variable	Groups		Total (34)	*p*
	AML (*n* = 24)	ALL (*n* = 10)		
Gender (male/female)	14/10 (58.3/41.7)	6/4 (60.0/40.0)	20 (58.8)	1.000
Median age years	30 (21–38)	30 (19–50)	28 (21–38)	1.000
CR at HSCT	12 (50.0)	7 (70.0)	19 (55.9)	0.451
High-risk patients	21 (87.5)	7 (70.0)	28 (82.4)	0.328
Donor				
Mother	6 (25.0)	4 (40.0)	10 (29.4)	0.698
Father	5 (20.8)	2 (20.0)	7 (20.6)	
Sibling	11 (45.8)	4 (40.0)	15 (44.1)	
Children	2 (8.3)	0 (0.0)	2 (5.9)	

Notes: Values are expressed as *n* (%) or median (1st–3rd quartiles). AML; acute myeloid leukemia: ALL; acute lymphoblastic leukemia: HSCT; hematopoietic stem cell.

Only two (6.1%) patients had chronic GVHD. One of them was classified as limited and the other was extensive.

Clinical outcome

By 1 July 2015, the median follow-up time was 176 days (ranging from 35 to 933 days). DFS and OS is 42 and 54% at 1 year and 33 and 36% at 2 years, respectively (Figure 1). When AML and ALL patients were evaluated in two different groups, OS rate had a tendency to be better in AML patients when compared with ALL ones. (1 year OS 65 vs. 22%, $p = 0.084$). When risk factors which might affect OS were evaluated, disease type and donor identity were determined as factors affecting survival in multiple analysis (Table 4).

Although 1-year DFS seemed to be better in the AML group than the ALL group, the difference was not statistically significant (46 vs. 22%, $p = 0.29$). Factors affecting DFS were determined in multiple analyses as disease type, and γδ T-cell amount in the graft. None of the patients developed veno-occlusive disease during the post-transplant follow-up.

None of the patients developed high levels of EBV viremia (>5000 copies/mL) during the follow-up after engraftment, and no transplantation-related PTLD was observed.

The cumulative incidence of CMV DNAemia was 73.5%. No patient developed CMV disease or died. Excluding two patients who were receiving steroid, cyclosporine, and sodium mycofenolate for GVHD treatment, none of the patients developed CMV reactivation after the third month.

BK-virus DNA in urine was detected in 25%. Cidofovir treatment was started in BK-virus positive patients.

Veno-occlusive disease was not observed in any of the patients.

There was no mortality in first 30 days. Mortality and NRM in the first 100 days were 5 of 34 (14.7%) and 4 of 34 (11.7%) (Figure 2). Sixteen patients died. Causes of death were relapse (*n* = 9/16, 56.3%), bacterial infection (*n* = 3/16, 18.8%), fungal infection (*n* = 2/16, 12.5%), GVHD (*n* = 1/16, 6.3%), and engraftment failure (*n* = 1/16, 6.3%) (Table 4). No mortality was observed due to toxicity of preparation regimen.

Mortality related to post-transplant relapse was more common among ALL patients than AML patients ($p = 0.022$). A univariate and multiple analyses of risk factors for OS and DFS are given (Table 5).

Immune reconstitution

Patients who experienced graft failure and were subsequently performed re-transplantation were excluded from analysis (Figure 3). Data were available in 16 patients.

T-cells regenerated with a median of 262 CD3+ cells/μL (range 84–957) on day 90. On day 90 a median of 121 CD8+ cells/μL (range 12–675) vs. 82 CD4+ cells/μL (range 8–252), and on day 365 a median of 498 CD8+ cells/μL (range 175–1356) vs. 180 CD4+ cells/μL (range 133–555) were observed. On day 30, a median of 309 CD16+56+CD3−NK- cells/μL (range 257–917), 130 γδ T-cell/μL (range 83–325) was observed. B-cell reconstitution reached a median of 162 (range 19–363) CD19+ cells/μL on day 90 and 179 (range 23–577) CD19+ cells/μL on day 180.

Discussion

We presented haplo-HSCT results of adult patients with αβ T-cell selection without CD19 positive cell selection. Although our study is retrospective in nature, we believe that it is a significant study in the literature,

Table 2. Graft composition.

Variable (cells per kg BW)	Groups		Total (34)	*p*
	AML (*n* = 25)	ALL (*n* = 9)		
CD34 × 10^6	12.80 (10.72–16.31)	12.58 (9.96–12.84)	12.69 (10.34–16.01)	0.727
Alfa/beta T cell × 10^3	13.46 (5.60–27.46)	9.98 (7.32–21.10)	11.72 (7.25–24.98)	1.000
Gamma/delta T cell × 10^6	4.28 (1.29–7.66)	5.44 (4.49–7.70)	4.58 (1.43–7.70)	0.284

Note: Values are expressed as *n* (%) or median (1st–3rd quartiles).

Table 3. Engraftment days, GVHD status, and viral activations.

Variable	Groups		Total (33)	*p*
	AML (*n* = 25)	ALL (*n* = 8)		
Neutrophile engraftment (day)	11.00 (10.00–15.00)	14.00 (12.00–17.50)	12.00 (10.50–15.00)	0.045
Platelet engraftment (day)	11.00 (10.00–12.00)	12.00 (11.00–22.00)	11.00 (10.00–12.00)	0.308
Acute GVHD (I–IV)	9 (37.5)	1 (11.1)	10 (30.3)	0.217
Acute GVHD (III–IV)	2 (8.3)	0 (0.0)	2 (6.1)	1.000
Chronic GVHD	2 (8.3)	0 (0.0)	2 (6.1)	1.000
BK virus reactivation	5 (20.8)	4 (40.0)	9 (26.5)	0.395
CMV reactivation	16 (66.7)	9 (90.0)	25 (73.5)	0.225

Notes: Values are expressed as *n* (%) or median (1st–3rd quartiles). AML; acute myeloid leukemia: ALL; acute lymphoblastic leukemia: HSCT; hematopoetic stem cell transplantation: GVHD; graft-versus-host disease.

because it is presenting the first haploidentical transplant results in adults without CD19 selection and with alpha-beta T-selection as well it is a comparative study between AML and ALL patient groups.

In vitro T-cell depletion of the graft is an effective method to prevent GVHD in haplo-HSCT. *In vitro* T-cell depletion history, CD34+ cell selection, later CD3/CD19 depletion has been successfully used prior to haplo-HSCT [15].

In order to increase the T-cell depletion efficacy while maintaining the anti-tumor and anti-infectious properties of the graft, Lang et al. have used T-cell depletion method which removes αβ+ T lymphocytes via a biotinylated anti-TcRαβ antibody followed by an anti-biotin antibody conjugated to magnetic microbeads while retaining γδ+ T-lymphocytes, NK cells, and other cells in the graft in pediatric patients. It has been suggested that the αβ T-cells can cause severe alloreactivity in the mismatched setting, whereas γδ T-cells are supposed to be not involved in classical GVHD. Furthermore, γδ T-cells can play in role graft-versus-tumor activity and anti-infection immunity [12].

In addition, CD19+ B-lymphocytes have been generally concomitantly depleted for the prevention of post-transplant EBV-associated lymphoproliferative disease [16]. For haplo-HSCTs, we used αβ T-cell-depleted allograft without CD19+ B-lymphocytes depletion in our study. No progressive increase was observed in EBV copy numbers in engrafted patients, and no

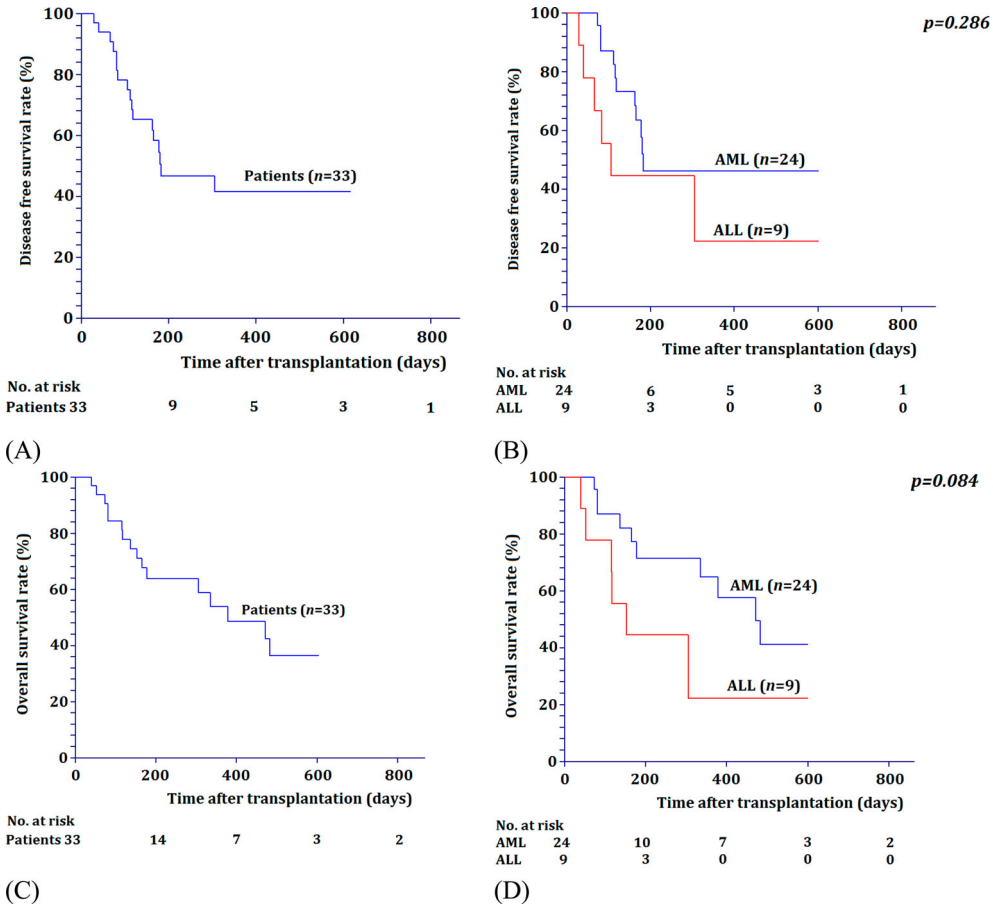

Figure 1. OS and DFS; (a) DFS of the whole patient group; (b) DFS of patients in ALL vs. AML patients; (c) OS of the whole patient group; (d) OS of patients in ALL vs. AML patients.

Table 4. Risk factors for OS, DFS.

	OS		DFS	
Variable	Univariate HR (95%CI)	Multiple HR (95%CI)	Univariate HR (95%CI)	Multiple HR (95%CI)
Gender (female/male)	1.68 (0.64–4.41)	–	1.90 (0.74–4.87)	–
Age (>30 years old /<30 years old)	0.91 (0.34–2.47)	–	1.06 (0.41–2.75)	–
Remission status at HSCT	1.03 (0.37–2.85)	–	1.21 (0.46–3.20)	–
Risk status (high/standard)	1.44 (0.33–6.35)	–	1.54 (0.35–6.75)	–
Group (ALL/AML)	2.42 (0.86–6.78)	3.00 (1.00–9.13)	2.17 (0.79–5.97)	4.87 (1.50–15.87)
Donor				
Mother	1.00	1.00	1.00	–
Father	5.20 (0.97–27.85)	5.55 (1.01–30.35)	5.10 (0.96–27.18)	–
Sibling	4.58 (0.98–21.49)	5.54 (1.14–26.81)	5.14 (1.11–23.76)	–
Children	2.71 (0.24–30.12)	3.95 (0.34–46.24)	2.75 (0.25–30.53)	–
Graft composition				
CD34+ cell count	1.00 (0.88–1.14)	–	1.01 (0.89–1.14)	–
αβ− T cell count	1.00 (0.98–1.01)	–	1.00 (0.98–1.01)	–
γδ− T cell count	1.01 (0.95–1.09)	–	1.04 (0.99–1.09)	1.05 (1.00–1.11)

Notes: HR: hazard ratio; CI: confidence interval; OS: overall survival; DFS: disease free survival; ALL: acute lymphoblastic leukemia; AML; acute myeloid leukemia.

post-transplant lymphoproliferative disease developed. As CD19+ depletion was not performed, early elevations of B lymphocytes were observed in receivers when compared with CD+19 depleted transplants. The median value of 113 cells/μL was reached on day 60. It seemed that performing no CD19 depletion in the early phase with B-cell engraftment did not increase post-transplant lymphoproliferative disease possibility.

Using αβ T-cell-depleted grafts, T-cell reconstitution appears to be faster than that reported in published data with CD3/CD19-depleted grafts. In the pediatric population, T-cell reconstitution except/but CD3+/CD4+ lymphocyte was similar after αβ T-cell-depleted haplo-HSCT [12]. On 365th day, median CD3+/CD4+ lymphocyte count was 180 cells/μL in our adult patients vs. 538 cells/μL in pediatric patients. This may be explained by the presence of a still functional thymus in children [17]. Improvements of B, NK, and CD8+ lymphocyte counts in the early transplant period were noteworthy in the picture of immune structure. CMV related deaths in late post-transplant periods were reported in haploidentical transplants with CD3 selections. In our cohort, all of our patients and donors were

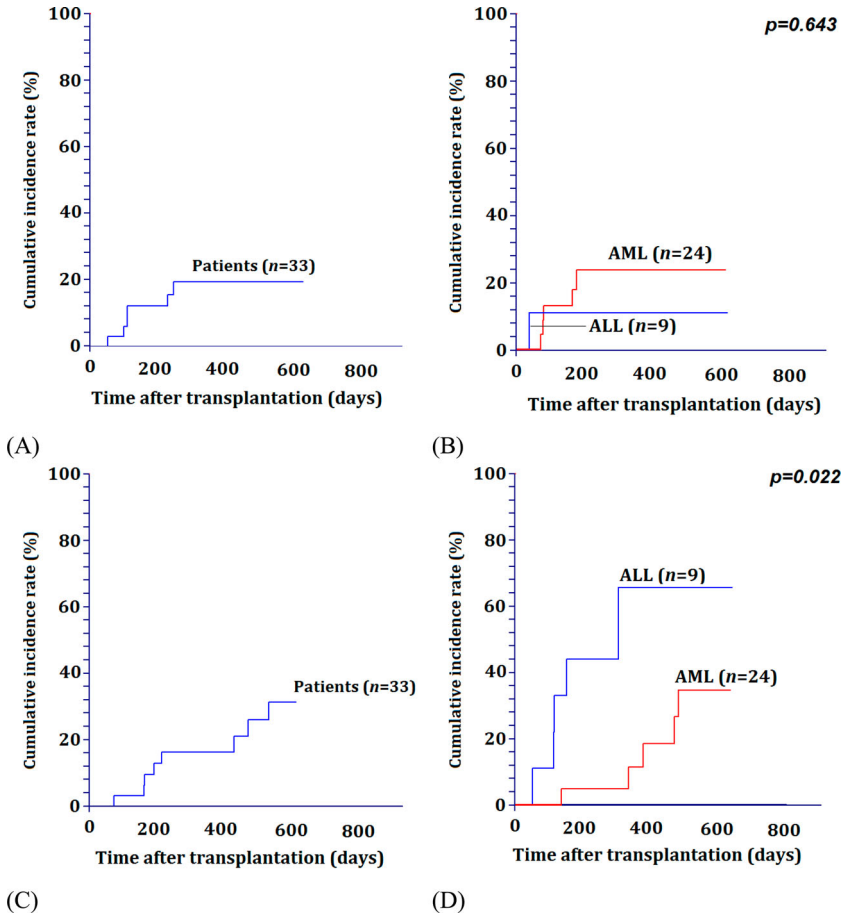

Figure 2. NRM and relapse-related mortality (RRM). (a) NRM of the whole patient group; (b) NRM of patients in ALL vs. AML patients; (c) RRM of the whole patient group; (d) RRM of patients in ALL vs. AML patients.

Table 5. Causes and timing of mortality.

	Patients	Sex/age	Diagnosis, disease status and risk status at HSCT time	Time of relapse after HSCT (day)	Cause of death	Time of death after HSCT (day)
1	AB	M/18	ALL, CR, HR	83	Relapse	152
2	NB	F/52	ALL, CR,HR	105	Relapse	117
3	ÖA	M/19	ALL, NR, HR	66	Relapse	115
4	DK	F/33	ALL, CR, HR	120	Relapse	305
5	ÖE	F/22	ALL, CR, HR	–	Bacterial infection	39
6	MK	M/31	ALL, CR, HR	–	Relapse	52
7	AŞ	M/23	AML, NR, HR,	180	Relapse	471
8	FA	M/23	AML, CR, HR	182	Relapse	379
9	HK	F/35	AML, CR, HR	–	Engraftment failure	81
10	MBÖ	M/51	AML, NR, HR	112	Relapse	483
11	DA	F/41	AML, CR2, HR	–	Bacterial infection	165
12	SÇ	F/28	AML, CR2, SR	115	Relapse	137
13	FÇ	M/20	AML, NR, HR	–	Fungal infection[a]	103
14	ENÜ	F/20	AML, CR, HR		Fungal infection[b]	335
15	ED	F/21	AML, CR1, SR		GVHD	80
16	MM	M/21	AML. CR2, SR	–	Bacterial infection	177

Notes: NR: not in remission; HR: high risk; SR: standard risk; CR: complete remission.
[a]*Blastoschizomyces capitatus*.
[b]*Mucormycosis*.

seropositive for CMV, and CMV reactivation frequencies of patients were high within the first 90 days, but none of the patients developed CMV. Moreover, except two patients who were receiving intense immunosuppressive treatment due to chronic GVHD, CMV reactivation was not observed after day 90. This might be interpreted as the improvement of CD8+ cells being more prominent after day 90.

One of the main concerns with haplo-HSCT is GVHD. We observed a lower incidence of grade II–IV acute GVHD after haplo-HSCT with αβ T-cell-depleted grafts compared to the reported 46% in patients receiving haplo-HSCT with CD3/CD19-depleted grafts. Ten (30.3%) patients developed grade I–IV aGVHD and 6.6% of chronic cGVHD after the transplantation. Grade I–II aGVHD occurred in nine (23.8%) patients

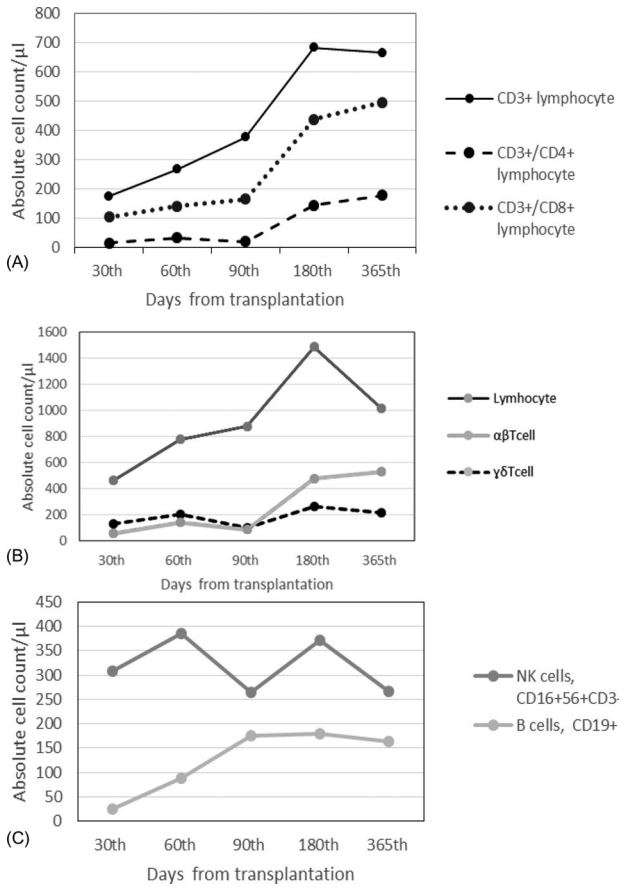

Figure 3. Immune reconstitution after transplantation of TcRαβ-depleted allografts: (a) reconstitution of CD3+, CD3+CD4+, CD3+CD8+cells, (b) reconstitution of lymphocyte, TcRαβ+ and TcRγδ+ cell, (c) CD19+ B-cell and CD16+CD56+CD3−NK cells.

and grade III–IV in two (6.1%) patients. The manipulation of αβ T-cells resulted in effective prevention of both acute and chronic GVHD. While GVHD rates in αβ T-cells depleted haploidentical transplants among sibling allogeneic transplantations were similar in the literature, they were also associated with lower chronic GVHD rates.

There was no death case related to transplantation protocol. The most significant cause of mortality was relapse. Relapse-related death rates were higher among ALL patients. In general, the main cause of mortality was relapsing among ALL patients after allogeneic stem cell transplantations [18]. In our cohort, frequency of relapse rates in post-transplant ALL patients might be due to the general opinion of weak graft-versus-malignancy effect, but also [19] it might be due to early relapsing of patients before development of graft-versus-leukemia effect. However, it might also have been caused by the fact that reparation regimen for transplantation had a limited anti-leukemia efficacy in ALL patients [20].

In our study, estimated OS in overall patients was determined as 54%, but OS was determined better (65%) in AML patients. However, statistical significance was determined at the borderline in the Kaplan–Meier method ($p = 0.084$). This difference may have been significant if higher number of patients had been included in the study. For OS, ALL was determined as an independent prognostic factor in multiple analysis (hazard ratio, ALL vs. ALL = 3).

Again according to multiple Cox regression analysis results, the other independent factor affecting OS results was the identity of donor. It was confirmed that when the mother was the donor, it was an independent prognostic factor for survival for transplantation (hazard ratio, father vs. mother = 5.55, and sibling vs. mother = 5.54).

In contrast, patients who received transplants from haploidentical siblings had no influence on outcome. Similarly, Stern et al. showed that if the donor was mother, then it had a positive effect on OS in T-cell-depleted haplo-HSCT. This may be explained as maternal immune system exposure to fetal antigens during pregnancy and it is capable of being sensitized by paternal histocompatibility antigens. Therefore, maternal grafts exerted a more potent alloreactive effect that was active preferentially against leukemia cells [21].

Gamma delta T-cells, NK, and T regulatory cells are present in αβ+ T-cell-depleted grafts. It is known that these cells may cause graft-versus-leukemia effect without specifically causing GVHD [22–24]. Therefore, anti-leukemic effect is expected at the maximum level after transplantations performed by αβ+ T-cell-depleted grafts, because immunosuppressive treatments are not used post-transplantation. In our study cohort, on the contrary to many haplo-HSCT outcomes, there was no effects of relapse on the survival rates whether patients were in the high-risk group, and patients were in the remission at the time of transplantation [25,26]. Gamma delta T-cells, NK, and T regulatory cells are present in αβ+ T-cell-depleted graft. It is known that specifically these cells may have anti-leukemia effect without causing GVHD. Therefore, anti-leukemia effect is expected at the maximum level after transplantations performed with αβ T-cell-depleted grafts, because post-transplant immunosuppressive treatment is not given. Graft-versus-leukemia effect caused by cells in the graft may contribute to response maintenance obtained after myeloproliferative preparation protocol especially in AML patients who are not in remission.

The advantages of haplo-HSCTs performed by αβ+ T-cell-depleted grafts are low rates of early period mortality related to transplantation and low rate of graft failure risk, absence of intense immunosuppressive treatment requirement after transplantation, better improved immune reconstitution when compared with other transplantations with T cell selection, low incidence of both acute and chronic GVHD, and no increase in EBV-related lymphoproliferative disease rates despite no CD19 depletion.

It is important to note some limitations of our study, such as the small number of patients included and its' retrospective nature. However, the present study is still a substantial one, because it presents the first results of the applied protocol in adult patients, and the outcomes presented are better than *in vitro* T-cell-depleted transplants which have been previously published in the literature.

In conclusion, we believe that αβ T-cell-depleted haplo-HSCT may be a good alternative for high-risk patients if there are no HLA-matched donors as well as for patients, especially with AML, who also cannot have remission despite salvage treatments.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Thomas ED, Buckner CD, Banaji M, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood. 1977;49:511–533.
[2] Copelan EA. Hematopoietic stem-cell transplantation. N Engl J Med. 2006;354:1813–1826.
[3] Locatelli F, Vinti L, Palumbo G, et al. Strategies to optimize the outcome of children given T-cell depleted HLA-haploidentical hematopoietic stem cell transplantation. Best Pract Res Clin Haematol. 2011;24:339–349.
[4] Szydlo R, Goldman JM, Klein JP, et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. J Clin Oncol. 1997;15:1767–1777.
[5] Beatty PG, Clift RA, Mickelson EM, et al. Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med. 1985;13:765–771.
[6] Koh LP, Chao N. Haploidentical hematopoietic cell transplantation. Bone Marrow Transplant. 2008;42:S60–S63.
[7] Munchel AT, Kasamon YL, Fuchs EJ. Treatment of hematological malignancies with nonmyeloablative, HLA-haploidentical bone marrow transplantation and high dose, post-transplantation cyclophosphamide. Best Pract Res Clin Haematol. 2011;24:359–368.
[8] Liu H, Rich ES, Godley L, et al. Reduced-intensity conditioning with combined haploidentical and cord blood transplantation results in rapid engraftment, low GVHD, and durable remissions. Blood. 2011;118:6438–6445.
[9] Locatelli F, Vinti L, Palumbo G, et al. Strategies to optimize the outcome of children given T-cell depleted HLA-haploidentical hematopoietic stem cell transplantation. Best Pract Res Clin Haematol. 2011;24:339–349.
[10] Fuchs EJ. Haploidentical transplantation for hematologic malignancies: where do we stand? Hematology Am Soc Hematol Educ Program. 2012;2012:230–236.
[11] Chang Y-J, Zhao X-Y, Huang X-Y. Immune reconstitution after haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2014;20:440–449.
[12] Lang P, Feuchtinger T, Teltschik HM, et al. Improved immune recovery after transplantation of TCRαβ/CD19-depleted allografts from haploidentical donors in pediatric patients. Bone Marrow Transplant. 2015;50 (Suppl. 2):S6–S10.
[13] Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304.
[14] Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005;11:945–956.
[15] Reisner Y, Hagin D, Martelli MF. Haploidentical hematopoietic transplantation: current status and future perspectives. Blood. 2011;118:6006–6017.
[16] Feuchtinger T, Teltschik HM, Schumm M, et al. Transplantation of TcRαβ/CD19 depleted stem cells from haploidentical donors in children: current results. Blood. 2013;122:692.
[17] Federmann B, Bornhauser M, Meisner C, et al. Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: a phase II study. Haematologica. 2012;97:1523–1531.
[18] Chen R, Campbell JL, Chen B. Prophylaxis and treatment of acute lymphoblastic leukemia relapse after allogeneic hematopoietic stem cell transplantation. Onco Targets Ther. 2015;8:405–412.
[19] Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood. 2008;112:4371–4383.
[20] Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75:555–562.
[21] Stern M, Ruggeri L, Mancusi A, et al. Survival after T cell–depleted haploidentical stem cell transplantation is improved using the mother as donor. Blood. 2008;112:2990–2995.
[22] Alpdogan O, Grosso D, Flomenberg N. Recent advances in haploidentical stem cell transplantation. Discov Med. 2013;16:159–165.
[23] Verneris MR. Natural killer cells and regulatory T cells: how to manipulate a graft for optimal GVL. Hematol Am Soc Hematol Educ Program. 2013;2013:335–341.
[24] Bayraktar UD, Ciurea SO. Strategies in haploidentical stem cell transplantation in adults. Turk J Hematol. 2013;30:342–350.
[25] Solomon SR, Sizemore C, Sanacore M, et al. Total Body Irradiation-based myeloablative haploidentical stem cell transplantation is a safe and effective alternative to unrelated donor transplantation in patients without matched sibling donors. Blood. 2015;21:1299–1307.
[26] Piemontese S, Ciceri F, Labopin M, et al. Acute Leukemia Working Party (ALWP) of the European Group for Blood and Marrow Transplantation (EBMT). A survey on unmanipulated haploidentical hematopoietic stem cell transplantation in adults with acute leukemia. Leukemia. 2015;29:1069–1075.