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
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 day. 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/m², days −8 to −5), thiopeta (2 × 5 mg/kg, day −4), and melphalan (70 mg/m², 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 × 10⁹/L. Platelet engraftment was defined as independence from platelet substitution for at least seven consecutive days with a platelet count greater than 20 × 10⁹/L. Primary graft failure was defined as failure to achieve an ANC ≥ 5 × 10⁹/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 (valaciclovir 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 $12.69 \times 10^6$ CD34+ progenitor cells per kg BW. In addition, grafts contained a median number of $4.58 \times 10^6$ per kg BW γδ T-cells. The median number of residual αβ T-cells was $11.72 \times 10^3$ 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.
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.
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 \( \alpha\beta^+ \) T lymphocytes via a biotinylated anti-TcR\( \alpha\beta \) antibody followed by an anti-biotin antibody conjugated to magnetic microbeads while retaining \( \gamma\delta^+ \) T-lymphocytes, NK cells, and other cells in the graft in pediatric patients. It has been suggested that the \( \alpha\beta \) T-cells can cause severe alloreactivity in the mismatched setting, whereas \( \gamma\delta \) T-cells are supposed to be not involved in classical GVHD. Furthermore, \( \gamma\delta \) T-cells can play a 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 \( \alpha\beta \) 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

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

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

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.
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 reconstitu-
tion appears to be faster than that reported in pub-
lished data with CD3/CD19-depleted grafts. In the
pediatric population, T-cell reconstitution except/but CD3+/CD4+ lymphocyte was similar after αβ T-cell-
deployed 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]. Improve-
ments 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 haploi-
dentical transplants with CD3 selections. In our
cohort, all of our patients and donors were

Table 4. Risk factors for OS, DFS.

| Variable                  | OS Univariate HR (95%CI) | OS Multiple HR (95%CI) | DFS Univariate HR (95%CI) | DFS 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.

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.
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

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 QA     | M/19    | ALL, NR, HR                                           | 66                              | Relapse        | 115                           |
| 4 DK     | F/33    | ALL, CR, HR                                           | 120                             | Relapse        | 305                           |
| 5 OE     | F/22    | ALL, CR, HR                                           | –                               | Bacterial infection | 39                  |
| 6 MK     | M/31    | ALL, CR, HR                                           | –                               | Relapse        | 52                            |
| 7 AS     | M/23    | AML, CR, HR                                           | 180                             | Relapse        | 471                           |
| 8 FÅ     | 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 SC    | F/28    | AML, CR2, SR                                          | 115                             | Relapse        | 137                           |
| 13 FC    | M/20    | AML, NR, HR                                           | –                               | Fungal infection | 103             |
| 14 ENÜ   | F/20    | AML, CR, HR                                           | –                               | Fungal infection | 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.

*aBlastoschizomyces capitatus.*

*bMucormycosis.*

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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