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# αβ T Cell-Depleted Haploidentical Hematopoietic Stem Cell Transplantation without Antithymocyte Globulin in Children with Chemorefractory Acute Myelogenous Leukemia

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A B S T R A C T

We evaluated the outcome of αβ T cell-depleted haploidentical hematopoietic stem cell transplantation (HSCT) in a cohort of children with chemorefractory acute myelogenous leukemia (AML). Twenty-two patients with either primary refractory (n = 10) or relapsed refractory (n = 12) AML in active disease status received a transplant from haploidentical donors. The preparative regimen included cytoreduction with fludarabine and cytarabine and subsequent myeloablative conditioning with treosulfan and thiotepa. Antithymocyte globulin was substituted with tocilizumab in all patients and also with abatacept in 10 patients. Grafts were peripheral blood stem cells engineered by αβ T cell and CD19 depletion. Post-transplantation prophylactic therapy included infusion of donor lymphocytes, composed of a CD45RA-depleted fraction with or without a hypomethylating agent. Complete remission was achieved in 21 patients (95%). The cumulative incidence of grade II-IV acute graft-versus-host disease (GVHD) was 18%, and the cumulative incidence of chronic GVHD was 23%. At 2 years, transplantation-related mortality was 9%, relapse rate was 42%, event-free survival was 49%, and overall survival was 53%. Our data suggest that αβ T cell-depleted haploidentical HSCT provides a reasonable chance of long-term survival in a cohort of children with chemorefractory AML and creates a solid basis for further improvement.



## INTRODUCTION

Chemorefractory acute myelogenous leukemia (AML) remains an unresolved problem in pediatric oncology. Children with AML who fail to achieve remission on high-dose chemotherapy have no chance of survival without allogeneic hematopoietic stem cell transplantation (HSCT). The reported survival rate after HSCT in this cohort varies from 9% to 44% [1,2]. Ex vivo depletion of αβ T cells is a robust platform for haploidentical HSCT in acute leukemia [3-5]. However, studies evaluating whether this platform will offer a chance of survival in children with chemorefractory AML are lacking. In the original studies of αβ T cell-depleted HSCT, antithymocyte globulin (ATG) was used to secure engraftment and graft-versus-host disease (GVHD) control [3,5,6]. Circulating ATG at the time of graft infusion may inactivate important populations of immune cells. We hypothesized that substituting ATG with non-lymphodepleting targeted immunomodulation could protect the grafted natural killer cells and γδ T cells and improve the control of leukemia.

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## METHODS

To test this hypothesis, we performed haploidentical HSCT with $\alpha\beta$ T cell depletion in a cohort of 22 pediatric patients with AML with active disease between June 2015 and September 2017. There were 15 female and 7 male patients, and the median age at HSCT was 9.6 years (range, 1 to 18 years). The median interval from diagnosis to transplantation was 85 days (range, 35 to 224 days). Primary refractory disease was registered in 10 patients; refractory relapse, in 12 patients. According to a standard classification [7], 9 patients had an adverse genetic aberration, 11 had an intermediate/unknown genetic aberration, and 2 had a favorable genetic aberrations (Supplementary Table 1). All patients received chemotherapy according to contemporary protocols (Supplementary Figure 1) or according to the Relapsed AML-2001 schema [8].

Chemorefractory disease was defined as a bone marrow blast count >5% (with B cell regeneration excluded by flow cytometry) after at least 2 courses of intensive chemotherapy, including at least 1 course with fludarabine, high-dose cytarabine, and an anthracycline. The median bone marrow blast count before cytoreduction was 39% (range, 5% to 100%). Haploidentical donors were mothers in 10 cases and fathers in 12 cases. Killer cell immunoglobulin-like receptor mismatch was predicted in 6 pairs based on a ligand-ligand model [9]. Baseline patient, donor, and graft characteristics are presented in Table 1.

Patients received a course of cytoreductive chemotherapy with fludarabine 30 mg/m$^2$/day on days +1 to +5 and cytarabine 2000 mg/m$^2$/day on days +1 to +5. The conditioning and GVHD prevention regimen included treosulfan at 14 g/m$^2$/day on days -5, -4, and -3 and either melphalan 140 mg/m$^2$ on day -2 (n = 8) or thiotepa 5 mg/kg on days -6 and -5 (n = 14). The change in the second alkylator was due to temporary withdrawal of melphalan from the market. All patients received rituximab 200 mg/m$^2$ on day -1 and bortezomib 1.3 mg/m$^2$/day on days -5, -2, +2, and +5. Bortezomib was introduced as an agent with potential activity in GVHD prophylaxis and leukemia sensitization to NK cell cytotoxicity [10,11]. ATG was substituted with IL-6 blockade with tocilizumab 8 mg/kg on day -1, based on a published report [12]. Starting in October 2016, costimulation blockade with abatacept 10 mg/kg/day was administered in addition to tocilizumab on days 0, +7, +14, and +28 in 14 patients. The decision to add abatacept to tocilizumab was prompted by a case of fatal grade IV aGVHD, not seen previously among >200 patients, who underwent $\alpha\beta$ T cell-depleted HSCT with ATG serotherapy. The rationale was to use an agent with GVHD activity that may spare the NK cells [13-15]. The overall scheme of the conditioning and GVHD prophylaxis is summarized in Supplementary Table 2.

**Table 1**
Patient, Donor, and Graft Characteristics

| Characteristic | Value |
|---|---|
| Patient characteristics | |
| Age at treatment, yr, median (range) | 9.6 (1-18) |
| Sex, male/female, n | 7/15 |
| Interval from diagnosis to HSCT, d, median (range)* | 85 (35-224) |
| Disease characteristics | |
| Cytogenetics, n | |
| Favorable | 2 |
| Intermediate/unknown | 11 |
| Adverse | 9 |
| Disease stage, n | |
| Primary refractory | 10 |
| Relapsed refractory | 12 |
| BM blasts, %, median (range) | 39 (5-100) |
| Donor characteristics | |
| Mother, n | 14 |
| Father, n | 8 |
| Donor age at treatment, yr, median (range) | 32 (23-47) |
| Graft characteristics | |
| NK cells, 10$^6$/kg, median (range) | 28.9 (1.7-19.6) |
| $\gamma\delta$ T cells, 10$^6$/kg, median (range) | 8 (1.7-19.6) |
| $\alpha\beta$ T cells, 10$^6$/kg, median (range) | .012 (.001-.13) |
| B cells, 10$^6$/kg, median (range) | .1 (.015-3.8) |
| CD34$^+$ cells, 10$^6$/kg, median (range) | 8.5 (6.6-12.6) |
| KIR mismatch, n† | |
| Yes | 6 |
| No | 16 |
| DLI after HSCT, n | |
| No DLI | 5‡ |
| Memory | 17 |

KIR indicates killer cell immunoglobulin-like receptor.
* Interval from relapse to transplantation in cases of refractory relapse.
† KIR mismatch, as predicted according to the ligand-ligand model [9].
‡ Five patients were not eligible for DLI due to early progressive disease (n = 1), death (n = 1), or GVHD (n = 3).

Grafts were derived from peripheral blood after stimulation with granulocyte colony-stimulating factor and, in 12 patients, also with plerixafor. Plerixafor was used in patients with poor CD34$^+$ mobilization, insufficient to guarantee the target dose of CD34$^+$ cells ($10 \times 10^6$/kg patient body weight). Depletion of $\alpha\beta$ T and B cells was performed with a CliniMACS Plus or CliniMACS Prodigy (Miltenyi Biotec, Bergisch Gladbach, Germany). Median cells levels in the grafts were $12 \times 10^3$/kg (range, 1.5 to 135 $\times$ $10^3$/kg) $\alpha\beta$ T cells, $8.5 \times 10^6$/kg (range, 6.6 to $12.6 \times 10^6$/kg) CD34$^+$ cells, $28.9 \times 10^6$/kg (range, 8 to $69 \times 10^6$/kg) NK cells, $8.2 \times 10^6$/kg (range, 1.7 to $19.6 \times 10^6$/kg) $\gamma\delta$ T cells, and $104 \times 10^3$/kg (range, 15 to 3862 $\times$ $10^3$/kg) B cells. Engraftment of neutrophils was registered as described previously [3]. aGVHD and cGVHD were diagnosed and graded according to standard guidelines [16,17].

Patients who achieved complete remission (n = 20), were eligible to receive memory (CD45RA-depleted) T cell donor lymphocyte infusion (DLI), as described previously [18]. In brief, a fraction of the apheresis product was processed on a CliniMACS Prodigy (Miltenyi Biotec). The CD45RA depletion reagent, TS 510 tubing set, and custom software were used according to the manufacturer's instructions. In 3 patients, DLI was not administered owing to aGVHD (n = 3). The median time to first DLI was day +36, and the median number of DLIs per patient was 3. The DLI dose was escalated intraindividually from 25 (DLI 1) to 50 (DLI 2) to 100 (DLI 3) $\times$ $10^3$/kg CD3$^+$ cells at monthly intervals. Between May 2015 and December 2016, 17 patients were eligible to receive prophylactic chemotherapy after engraftment. Chemotherapy included 5-azacytidine 35 mg/m$^2$/day on days +1 to +5 and bortezomib 1.3 mg/m$^2$ on days +1 and +4. Ten patients received a median of 3 courses of chemotherapy. Seven patients did not receive the planned chemotherapy owing to manifestations of either aGVHD (n = 2) or cytopenia/infection (n = 5). The median time to the first course was 40 days (range, 33 to 99 days). In all patients, chemotherapy was followed by memory DLI. To summarize post-transplantation therapy, among 20 eligible patients, 10 received chemotherapy and memory DLI, 7 received memory DLI only, and 3 did not receive any post-transplantation therapy.

The therapeutic protocol and retrospective analysis were approved by the local Ethics Committee of the Dmitriy Rogachev National Medical Research Center of Pediatric Hematology, Oncology, and Immunology, and all patients and/or their legal guardians provided informed consent.

The study endpoints included cumulative incidence of relapse/progression (CIR), transplantation-related mortality (TRM), aGVHD and cGVHD, event-free survival (EFS), and overall survival (OS). Death from any cause, relapse, and persistence of leukemia were considered events in the EFS analysis. TRM and relapse/progression were considered reciprocal competing risks and were calculated according to the competing-risk model. EFS and OS were calculated using the Kaplan-Meier method. The XLSTAT package (Addinsoft, Paris, France) was used for statistical analysis. Surviving patients were censored on January 10, 2018, with a median follow-up in survivors of 2 years (range, 1 to 3.5 years).

## RESULTS

The effect of fludarabine cytoreduction was evaluated in 12 patients. Marrow hypocellularity was noted in all patients, and the blast population was not detectable by microscopy in 5 patients (41%). All patients survived until evaluation of engraftment. One patient died on day +14 from acute lung injury associated with engraftment. Leukemia persisted in 1 patient. Twenty patients engrafted and achieved complete remission by day +30. The median time to engraftment was 13 days for neutrophils and 14 days for platelets. Grade II-IV aGVHD was diagnosed in 4 patients at a median of 38 days, and the cumulative incidence of grade II-IV aGVHD was 18% (95% CI, 7% to 44%). Three patients developed grade III-IV aGVHD. There was 1 case of isolated skin aGVHD, 2 cases of skin and lower gastrointestinal (GI) aGVHD, and 1 case of grade IV skin, liver, and lower GI disease, which was fatal. Signs of cGVHD developed in 5 patients, moderate in 3 patients, and severe in 2 patients, at a median of 128 days after HSCT. The cumulative incidence of cGVHD at 2 years was 23% (95% CI, 11% to 49%). The incidence of GVHD is summarized in Figure 1. One of the 12 survivors required continued therapy with sirolimus to control cGVHD of the liver at the last follow-up.

Two patients died from causes unrelated to leukemia, I patient at day +14 post-HSCT from acute lung injury coincident with engraftment and the other at day +223 owing to

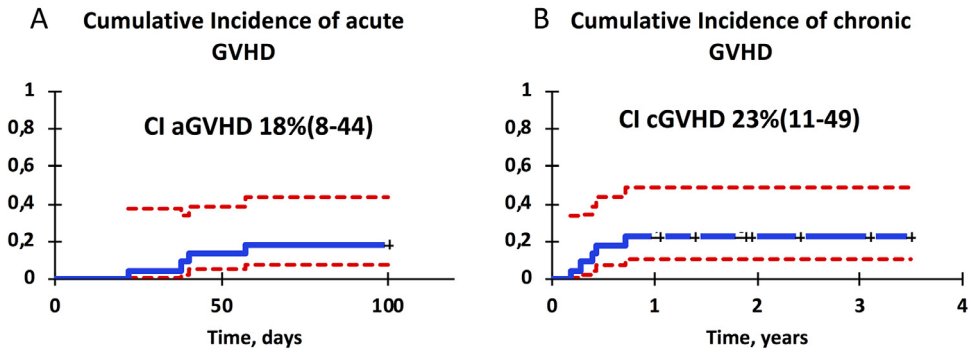


**Figure 1.** Cumulative incidence of acute GVHD grade II-IV (solid line) with 95% CI (dashed line) (A) and of chronic GVHD (solid line) with 95% CI (dashed line) (B).

steroid-refractory grade IV aGVHD. The incidence of TRM was 9% (95% CI, 2% to 34%). Leukemia progression or relapse developed in 10 patients, at a median of 138 days (range, 30 to 454 days). The cumulative incidence of relapse or progression at 2 years was 42% (95% CI, 33% to 88%) in the entire cohort. Two-year EFS was 49% (95% CI, 27% to 70%), and 2-year OS was 53% (95% CI, 31% to 74%). The principal outcomes are summarized in Figure 2. None of the evaluated risk factors was significantly correlated with the principal outcomes (Supplementary Table 3). In particular, the use of post-transplantation chemotherapy was not associated with improved leukemia control in univariate analysis.

## DISCUSSION

Allogeneic HSCT is universally acknowledged as the sole therapy offering a chance of cure for patients with refractory AML. A number of factors were reported to be prognostic of the outcome, including intensity of the conditioning regimen, patient age, leukemia burden before HSCT, aGVHD and cGVHD, baseline cytogenetics, and NK cell alloreactivity for haploidentical donors [1,2,19]. The technology of $\alpha\beta$ T cell depletion was developed with a focus on the antileukemic potential of NK cells and $\gamma\delta$ T cells [20-22]. Early expansion and activation of these cytotoxic subpopulations, in the absence of severe GVHD, potentially compensate for the lack of conventional T cell alloreactivity as a mechanism of leukemia control.

In this retrospective study of a cohort of children with chemorefractory AML who underwent HSCT from a haploidentical donor after treosulfan-based conditioning without any polyclonal serotherapy, uniform graft processing ($\alpha\beta$ T cell depletion) and cell therapy after engraftment

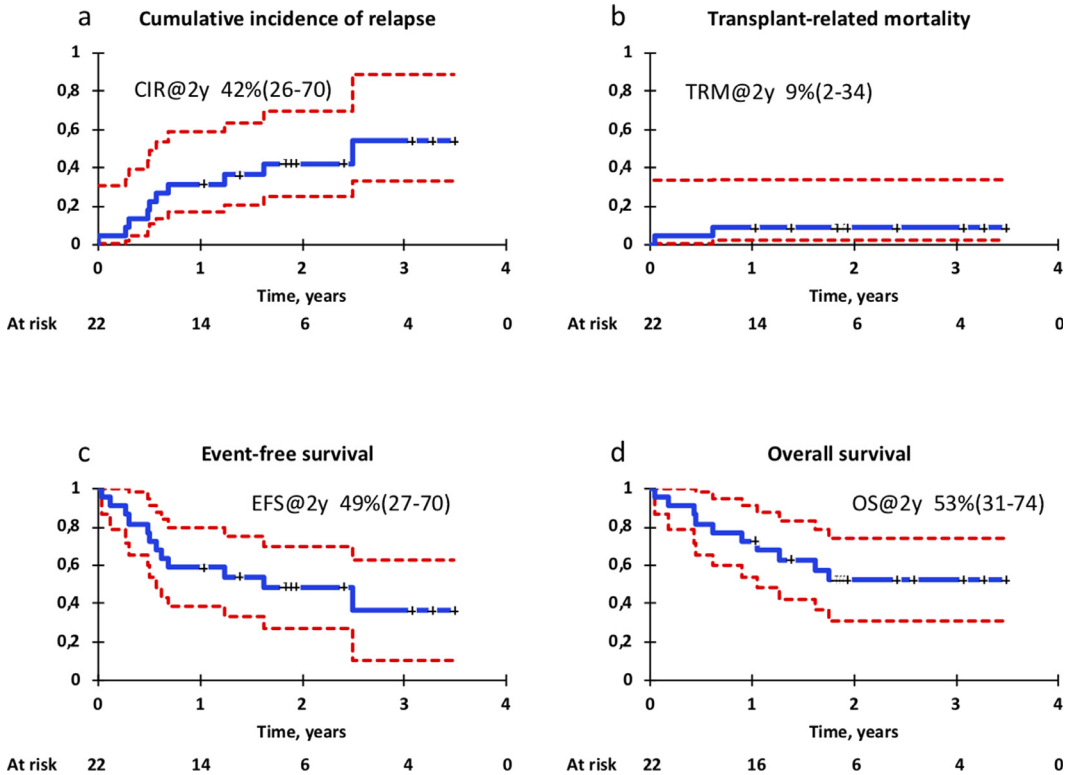


**Figure 2.** Principal outcomes. (A) Cumulative incidence of relapse/progression (solid line) with 95% CI (dashed line). (B) Cumulative incidence of TRM (solid line) with 95% CI (dashed line). (C) EFS (solid line) with 95% CI (dashed line). (D) OS (solid line) with 95% CI (dashed line).

(CD45RA-depleted DLI) were used. One main conclusion that can be drawn from our findings is that the HSCT regimen used may offer a reasonable chance of long-term survival in the cohort of children with chemorefractory AML who underwent transplantation in active disease. Ninety-one percent of the patients achieved complete remission, and 49% remained disease-free at a median follow-up of 2.0 years. The regimen was associated with moderate rates of both aGVHD and cGVHD, which translated into a relatively low nonrelapse mortality. This approach was equally effective among children of different ages, irrespective of baseline cytogenetics and leukemia burden, contrary to some published reports. Several nonconventional elements were used, such as fludarabine cytoreduction, bortezomib as part of the conditioning regimen, and post-transplantation hypomethylating chemotherapy in a proportion of patients. This adds a degree of heterogeneity to a small sample and precludes us from making firm conclusions regarding the importance of particular elements. Currently we do not use post-transplantation chemotherapy, because its value cannot be proven.

An important preliminary finding regarding the described HSCT regimen is that, as much as can be inferred from this limited sample, substitution of ATG with a combination of IL-6 blockade and costimulation blockade is not associated with an increased rate of graft failure or severe GVHD. The choice of replacement agents was based on the published data suggesting activity of tocilizumab and abatacept in GVHD prevention [12,15]. We also reasoned that the IL-6 axis and CD28 signaling are of minor importance for NK cell function. If confirmed in a prospective study, this approach will create an opportunity to deploy NK cell- and/or $\gamma\delta$ T cell-enhancing agents, as well as targeted immunotherapy, immediately after grafting.

To summarize, we have demonstrated that $\alpha\beta$ T cell-depleted transplantation from haploidentical donors results in long-term survival in a significant proportion of children with chemorefractory AML. This promising result can be achieved without an excessive burden of nonrelapse mortality and GVHD. We believe that the addition of personalized targeted therapy, including small-molecule and immunotherapeutic agents, to the safe, timely, and universally available HSCT platform has significant potential to further increase the cure rate of patients with refractory AML.

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## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.01.023.

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