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# Results of Allogenic Hematopoietic Stem Cell Transplantation in Fanconi Anemia Caused by Bone Marrow Failure: Single-Regimen, Single-Center Experience of 14 Years

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A B S T R A C T

Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for bone marrow failure (BMF) in patients with Fanconi anemia (FA). We retrospectively analyzed the records of patients with FA who underwent HSCT with a radiation-free, reduced-intensity conditioning regimen (fludarabine, cyclophosphamide, and antithymocyte globulin) along with an unmanipulated graft infusion between 2004 and 2018. A total of 44 patients underwent HSCT during the study period. Median age at transplantation was 121 months. Regarding the donor source, 22 transplants (50%) were collected from matched related donors (MRDs), and 22 transplants (50%) were collected from alternative donors (ADs). The median infused $CD34^+$ cell dose was $4.7 \times 10^6$/kg (range, 0.8 to 23) in bone marrow or peripheral blood stem cell recipients and $1.2 \times 10^5$/kg (range, 1.1 to 3.6) in umbilical cord blood recipients. All but 2 patients achieved primary neutrophil engraftment (95%). In a median follow-up of 36 months (range, 1 to 159), 3-year overall survival was 70.5% in the entire group and 91% in the MRD recipients. Primary causes of death were infections (n = 5), acute grade 3 to 4 graft-versus-host disease (n = 4), and hemorrhagic cystitis (n = 3). All surviving patients have full (n = 29) and acceptable mixed (n = 2) donor chimerism and good clinical status. Our results showed an excellent outcome with unmanipulated grafts using a fludarabine-based, radiation-free preparative regimen for MRD recipients. Even though primary neutrophil engraftment rates were good in AD recipients, intervening complications increased mortality in these patients. In clinics where T cell depletion is not feasible, more effort is warranted to improve outcomes for AD recipients.

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## INTRODUCTION

Fanconi anemia (FA) is an inherited chromosomal instability syndrome that presents clinically with progressive bone marrow failure (BMF) associated with a variety of congenital anomalies and a high predisposition to malignancies [1]. FA is caused by a mutation in 1 of the 22 FA genes that encodes a protein involved in DNA damage repair [2]. Hypersensitivity to cross-linking agents such as mitomycin C or diepoxybutane (DEB) is the diagnostic hallmark of FA [3].

The hematologic abnormalities of FA classically present within the first decade of life. Macrocytosis is usually the first detected abnormality followed by thrombocytopenia and neutropenia. Patients with FA have increased risk of developing myelodysplastic syndrome (MDS) and malignancies, especially acute myeloid leukemia (AML) in childhood and squamous cell carcinoma later in life [4,5].

Allogenic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment for BMF and the only option to prevent hematologic malignancies, although it accelerates and increases the occurrence of late tumors [6,7]. Due to the high sensitivity of FA-affected cells to alkylating agents, conventional conditioning regimens and radiation are associated with a high incidence of toxicity. With the evolution of conditioning regimens, graft-versus-host disease (GVHD) prophylaxis, and better donor availability, the outcomes of HSCT in patients with FA have improved in recent years [8].

Currently, the most recommended and used regimen for HSCT in patients with FA is a radiation-free, reduced-intensity conditioning protocol including fludarabine (FLU) and cyclophosphamide (CY). The reported success of this regimen is around 90% for matched sibling donor (MSD) recipients and highly encouraging for alternative donor (AD) recipients in developed countries [9–13]. However, data from countries with limited sources are scarce, especially regarding AD transplantation. In contrast to developed countries, we are unable

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

| Characteristic | All Patients | MRD | AD | *P* Value |
|---|---|---|---|---|
| Number of patients | 44 | 22 | 22 | 1 |
| Male patients, n (%) | 24 (55) | 13 | 11 | .76 |
| Age at HSCT, median (range), mo | 121 (52-192) | 148 (52-192) | 119 (58-70) | .31 |
| Time from FA diagnosis to HSCT, median (range), mo | 43.5 (4-142) | 47 (6-142) | 47 (8-131) | .66 |
| Previous transfusion therapy, median (range) | 44 (0-220) | 44 (0-220) | 42 (0-68) | .80 |
| Previous androgen use, n (%) | 25 (56) | 13 | 12 | 1 |
| Congenital malformations, median, range (n) | 1.6 (0-5) | 1 (0-3) | 2 (0-5) | **.023** |
| Patient-donor CMV match ($D^-/R^-$ or $D^+/R^+$), n (%) | 27 (61) | 17 | 10 | |
| Patient-donor CMV mismatch ($D^-/R^+$ or $D^+/R^-$), n (%) | 15 (34) | 12 | 3 | **.011** |
| Neutrophil engraftment achieved, n (%) | 41 (93) | 22 | 19 | .23 |
| Time to neutrophil engraftment, median (range), d | 11 (8-33) | 11.5 (8-15) | 11 (8-33) | .91 |
| Platelet engraftment achieved, n (%) | 36 (82) | 22 | 14 | **.004** |
| Time to platelet engraftment, median (range), d | 15 (9-350) | 14.5 (9-60) | 15 (9-350) | .44 |
| Stem cell source, n | | | | NE |
| 10/10 HLA MSD | 15 | 15 | | |
| 10/10 HLA MRD | 7 | 7 | | |
| 10/10 HLA AD BM | 13 | | 13 | |
| 9/10 HLA AD BM | 6 | | 6 | |
| 5/6 HLA AD UCB | 2 | | 2 | |
| 4/6 HLA AD UCB | 1 | | 1 | |
| CD34 ($\times 10^6$/kg), median (range) | | | | |
| BM and PBSC | 4.7 (0.8-23) | 5 (1.98-23) | 4.4 (0.8-9.6) | .27 |
| UCB | 0.12 (0.11-0.36) | | 0.12 (0.11-0.36) | NE |
| GVHD prophylaxis, n (%) | | | | NE |
| CSA alone | 22 | 22 | | |
| CSA + MMF | 19 | | 19 | |
| CSA + MP | 3 | | 3 | |

NE indicates not evaluated; MSD, matched sibling donor; CSA, cyclosporine A; MMF, mycophenolate mofetil; MP, methylprednisolone.
Bold values represent statistical significance at the $P < 0.05$ level.

to manipulate grafts in our clinic because of both technical and financial restrictions. Ex vivo T cell depletion of the graft is one of the main factors contributing to better survival rates by abrogating the risk of GVHD, thus improving outcomes in AD recipients in particular [12]. In this study, we report the results of matched related donor (MRD) and AD HSCT for FA-associated BMF at a single center with limited resources over a period of 14 years.

## MATERIALS AND METHODS

We retrospectively analyzed patients' sex, age at transplantation, number of pretransplant transfusions, incidence of androgen use, extent of congenital malformations, and time from diagnosis to transplantation. Donor type, sex, and age; degree of HLA match between donor and recipient; stem cell source; GVHD prophylaxis; post-transplant complications; chimerism analysis; and mortality were also recorded from the patients' files. All patients or their legal guardians provided informed consent according to the Declaration of Helsinki, and the study was approved by a regulatory board.

### *Patient Information*

Between January 2004 and June 2018, a total of 44 patients with FA with severe BMF underwent HSCT in the Akdeniz University Pediatric Stem Cell Transplantation Unit. FA diagnosis was confirmed by DEB analysis showing increased chromosomal fragility to cross-linking agents. Severe BMF was defined as persistent absolute neutrophil count $<0.5 \times 10^9$, hemoglobin <8 g/dL, and/or platelet count $<20 \times 10^9$. Bone marrow biopsy and aspiration were performed for all patients within 3 months of transplantation to exclude leukemia or coexisting MDS. Cytogenetic analysis before transplantation was normal karyotype for 43 patients. Only 1 patient had 5q deletion with no signs of MDS on bone marrow biopsy. There were 24 boys and 20 girls (male/female ratio of 1.2) in the patient group. The median age at transplantation was 121 months (range, 52 to 192 months), and 19 patients underwent transplantation before the age of 10 years (43%). The median time from FA diagnosis to allogenic HSCT was 43.5 months (range, 4 to 142 months).

Among the 44 patients, 33 (75%) had axial-skeletal abnormalities, 17 (39%) had eye malformations, 10 (23%) had renal-genital malformations, 4 (9%) had ear abnormalities, and 3 patients (7%) had gonadal malformations. Nine (20%) of the patients had 3 or more malformations. Twenty-five patients (56%) had a history of anabolic-androgenic steroid therapy before transplantation to correct cytopenia, but this treatment transiently improved hematologic parameters in only 16 of these patients. At transplant, 13 patients (30%) were untransfused, 12 patients (27%) had received 5 to 20 transfusions, and the remaining 19 (43%) were heavily transfused (>20 transfusions). Cytomegalovirus (CMV) serology was positive for 37 and negative for the other 7 patients. The demographic characteristics of the patients are presented in Table 1.

### *Donor Characteristics*

The median age of the donors was 121 months (range, 52 to 192 months) and the male/female ratio was 1.7. Before transplantation, donors and recipients underwent HLA genotyping using PCR using sequence-specific primers, which showed that the donor was an HLA-identical sibling for 15 patients (34%), HLA-matched related donor (10/10 allele matched) for 7 patients (16%), and unrelated donors for the remaining 22 patients (10/10 allele matched for 13 patients, 9/10 matched for 6 patients [HLA-A single-antigen mismatch], 5/6 HLA-matched umbilical cord blood [UCB] for 2 [HLA-DR allele mismatch], and 4/6 HLA-matched UCB for 1 [HLA-A antigen 2 mismatch]). All related donors were tested for chromosomal instability with DEB analysis and showed normal chromosomal breakage. Patient and donor CMV status was matched in 32, was mismatched in 10, and could not be determined for the donor in 2 of the transplantations. The stem cell source was bone marrow (BM) for 24 patients, peripheral blood stem cell (PBSC) for 17 patients, and UCB for the remaining 3 patients. Harvested stem cells were not manipulated in any of the transplantations. The median infused $CD34^+$ cell dose was $4.7 \times 10^6$/kg (range, 0.8 to 23) for BM and PBSC recipients and $1.2 \times 10^5$ (range, 1.1 to 3.6) for UCB recipients. Details regarding the transplantation are provided in Table 1.

### *Conditioning Regimen, GVHD, and Veno-Occlusive Disease Prophylaxis*

The conditioning regimen consisted of FLU 30 mg/kg/d for 4 days (days −9 through −6) for MRD transplants and 5 days (days −10 through −6) for AD transplants and CY 10 mg/kg/d for 4 days (day −4 through −1) for all

patients, together with antithymocyte globulin (ATG) (Fresenius-Gräfelfing/Germany) for 4 days (day −4 through −1): 30 mg/kg/d for AD transplants and 20 mg/kg/d for MRD transplants.

Prophylactic treatment for GVHD differed according to donor type. MRD recipients received only cyclosporine A (target trough level, 150 to 250 mg/L), whereas AD recipients received additional treatment with mycophenolate mofetil and UCB recipients received methylprednisolone (2 mg/kg/d) plus cyclosporine A. Cyclosporine dosage was tapered after the first 6 months, mycophenolate mofetil after the first 30 days, and steroid after the first 24 days according to chimeric status and signs of GVHD.

Veno-occlusive disease (VOD) prophylaxis included heparin 100 IU/kg/d in a 24-hour continuous infusion from 12 hours before the conditioning regimen until day 30 for all patients.

### *Supportive Care*

All patients were hospitalized in isolated single-occupancy rooms with high-efficiency particulate air filters. All patients had indwelling central venous catheters. For infectious disease prophylaxis, patients received trimethoprim-sulfamethoxazole against *Pneumocystisjirovecii*pneumonia, acyclovir against herpes virus infections, and fluconazole against fungal infections. Patients were monitored for CMV reactivation with weekly CMV PCR DNA testing from the time of neutrophil engraftment until discontinuation of immunosuppressive treatment. In case of clinical suspicion, patients were also checked for Epstein-Barr virus, adenovirus (ADV), and BK virus infections. Despite hyperhydration and prophylactic 2-mercaptoethane sulfonate Na infusion, transplantations in first 10 years of this study resulted in severe hemorrhagic cystitis (HC). Therefore, intravesical sodium hyaluronate (HA) and sodium chondroitin sulfate (CS) treatment was administered routinely after October 2013 to prevent HC. According to age (<1 year: 10 mL, 1 to 4 years: 25 mL, >4 years: 50 mL), a mixed solution of intravesical HA (1.6%) and CS (2%) was administered through the urethral catheter for 120 minutes before the first dose of CY infusion.

### *Engraftment and Chimerism Analysis*

Neutrophil and platelet engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of $>0.5 \times 10^9$/L and the first of 7 consecutive days with platelet counts of $>20 \times 10^9$/L without platelet transfusion. Primary graft failure was defined as failure to achieve an absolute neutrophil count of $>0.5 \times 10^9$/L by 28 days after BM or PBSC transplantation or 42 days after UCB transplantation. Chimerism was documented and monitored by in situ hybridization of the Y-chromosome in blood samples of sex-mismatched donor/recipient pairs and by analysis of a variable number of tandem repeats based on PCR analysis of blood samples in the sex-matched pairs. A donor component of >95% was accepted as full chimera, 10% to 95% as mixed chimera, and <10% as the complete recipient type, indicating primary HSCT failure. For chimerism analysis, peripheral blood samples were collected from the donor and recipient before transplantation and from the recipient on days 21 to 28 (depending on neutrophil engraftment) and at months 3, 6, 9, 12, 18, and 24. Patients who did not achieve full chimerism or developed neutropenia during follow-up underwent more frequent chimerism analysis.

### *Statistical Analysis*

Descriptive statistics were performed on the patients' demographic and clinical data, showing counts and percentages for categorical data and median and range for continuous data. The Shapiro-Wilk test was used to verify normality of the distribution of continuous variables. The independent samples *t* test was used to compare normally distributed numerical variables between 2 independent groups, and the Mann-Whitney *U* test was used to compare 2 independent groups when the dependent variable was not normally distributed. Analysis of categorical variables was performed using the *t* test or chi-squared test as appropriate, depending on the assumptions required for each test. The Kaplan-Meier method was used to estimate survival as a function of time for the entire population, MRD group, and AD group, and survival differences were analyzed with a log-rank test. GraphPad Prism 8.1 (GraphPad Software, La Jolla, California) was used to draw survival graphics. All analyses were performed using SPSS version 23.0 (SPSS, Chicago, Illinois), and $P < .05$ was used as the criterion for statistical significance.

## RESULTS

### *Engraftment and Chimerism*

Of the 44 patients, 40 had full-donor chimerism, 2 had mixed-donor chimerism, and 2 had primary graft failure in the first chimerism analysis. Neutrophil engraftment occurred at a median of 11 days (range, 8 to 33 days) in 41 patients (93%), and platelet engraftment occurred at a median of 15 days (range, 9 to 350 days) in 37 patients (84%). One patient who achieved mixed chimerism did not survive to the time for neutrophil engraftment. Although patients who achieved engraftment showed no significant difference in time to hematopoietic recovery based on donor type, a lower proportion of AD transplant recipients achieved platelet engraftment compared with MRD recipients ($P = .002$). Seven of 8 patients with failed platelet engraftment and all of the patients with failed neutrophil engraftment (n = 3) died of transplantation complications. Platelet and neutrophil engraftment failure was significantly associated with mortality ($P < .001$ and $P = .022$, respectively), as shown in Table 2. We

**Table 2**
Determinants of Mortality

| Variables | Patients, n (%) (n = 44; 100%) | Mortality, n (%) | *P* Value |
|---|---|---|---|
| Sex | | | .54* |
| Female | 20 (45) | 5 (24) | |
| Male | 24 (55) | 8 (33) | |
| Age at transplantation, yr | | | .09† |
| <10 | 22 (50) | 4 (18) | |
| ≥10 | 22 (50) | 9 (40) | |
| Pretransplant transfusion | | | .28* |
| Yes | 31 (70.5) | 5 (35) | |
| No | 13 (29.5) | 8 (15) | |
| Number of congenital abnormalities | | | .70* |
| <3 | 35 (80) | 11 (31) | |
| ≥3 | 9 (20) | 2 (22) | |
| Stem cell source | | | .32† |
| BM | 24 (54) | 6 (25) | |
| PBSC | 17 (39) | 5 (29) | |
| UCB | 3 (7) | 2 (66) | |
| Donor | | | **.007**† |
| MRD | 22 (50) | 2 (9) | |
| AD | 22 (50) | 11 (50) | |
| Neutrophil engraftment | | | **.022*** |
| Achieved | 41 (93) | 10 (24) | |
| Failed | 3 (7) | 3 (100) | |
| Platelet engraftment | | | <**.001*** |
| Achieved | 36 (82) | 6 (17) | |
| Failed | 8 (18) | 7 (87.5) | |
| Acute grade III-IV GVHD | | | **.002*** |
| Presence of acute grade III-IV GVHD | 12 (27) | 8 (67) | |
| Absent acute grade III-IV GVHD | 32 (73) | 5 (16) | |
| Hemorrhagic cystitis | | | .07* |
| Presence of hemorrhagic cystitis | 14 (32) | 7 (50) | |
| Absent hemorrhagic cystitis | 30 (68) | 6 (20) | |
| Grade III-IV hemorrhagic cystitis | | | **.005*** |
| Presence of grade III-IV hemorrhagic cystitis | 8 (18) | 6 (75) | |
| Absent grade III-IV hemorrhagic cystitis | 36 (82) | 7 (19) | |
| Viral reactivation | | | .49* |
| Presence of viral reactivation | 30 (68) | 10 (33) | |
| Absent viral reactivation | 14 (32) | 3 (21) | |
| Patient-donor CMV status | | | .17* |
| CMV matched | 27 (61) | 6 (22) | |
| CMV mismatched | 15 (34) | 7 (47) | |

Bold values represent statistical significance at the $P < 0.05$ level.
* Fisher exact test.
† Chi-square test.

observed no secondary graft failure in our series during a median follow-up of 36.5 months (range, 1 to 159 months).

### *GVHD*

Among the 44 patients, 12 (27%) had developed grade 2 to 4 acute GVHD by day +100. The incidence of grade 2 to 4 acute GVHD was significantly higher in the AD group than in the MRD group (40% versus 13%; $P = .002$). There was no statistically significant difference in the incidence of acute GVHD based on stem cell source (45% for BM, 52% for PBSC, and 33% for UCB; $P = .92$). Only 2 (4%) patients developed chronic GVHD. Because of the very small number of affected patients, we cannot associate any factors with chronic GVHD development.

### *Post-Transplant Infection*

Forty-two of the 44 patients developed neutropenic fever before engraftment. Blood culture revealed bacterial infection in 14 (30%) of these patients. Viremia was observed in 29 patients with CMV (65%), 11 patients (25%) with BK virus, and 1 patient (2.3%) with ADV. The viral reactivation rate was significantly lower in MRD recipients compared with AD recipients (50% versus 86%, $P = .022$). Despite the high CMV reactivation rate, none of our patients experienced any organ involvement, and none of the deaths were attributable to CMV infection. A total of 5 patients died of infection: 3 of invasive fungal infection (2 with invasive pulmonary aspergillosis, 1 with rhinocerebral mucormycosis), 1 of bacterial endocarditis, and 1 of adenoviral encephalitis.

### *Regimen-Related Toxicities, HC, and VOD*

Fourteen patients (32%) developed HC, 7 (16%) of whom had severe disease (grades 3 to 4). Of these 14 patients, stem cell source was AD for 11 (79%) and MRD for 3 (21%) patients, showing a significantly higher risk for AD transplantations ($P = .003$). Although intravesical HA and CS therapy reduced the frequency of HC after October 2013 (before October 2013, 11 of 29 patients [38%] developed HC with grades 3 to 4 in 5 patients; after October 2013, 3 of 15 patients [20%] developed HC with grades 3 to 4 in only 1 patient), the difference between these 2 periods was not statistically significant. VOD occurred in 3 patients. We cannot describe any factors contributing to VOD development because of the limited number of patients.

### *Mortality and Survival Analysis*

The median follow-up time was 36.5 months, and 3-year overall survival for the entire group was 70.5%. A total of 13 patients died, all but 2 of whom were in the AD group. Three patients were lost to follow-up. The 3-year overall survival rates were 91% for the MRD group and 50% for the AD group ($P = .003$) (Figures 1 and 2). Besides donor source, presence of acute grade 3 to 4 GVHD, neutrophil and platelet engraftment failure, and acute severe HC were factors associated with increased mortality ($P = .002$, $P = .022$, $P < .001$, and $P = .005$, respectively) (Table 2). Because of the limited number of patients in our cohort, we could not perform multivariate analysis. Causes of death included infections (n = 5), acute grade 3 to 4 GVHD (n = 4), and HC (n = 3). One patient died at another center of unknown causes. All of the patients who survived and are still under follow-up are in good clinical condition, have full (n = 29) or acceptable mixed chimerism (n = 2), and have been free of any malignancy for a median follow-up of 36.5 months. Only 2 of these survivors are receiving immunosuppressive therapy because of chronic GVHD.

## DISCUSSION

The natural history of FA involves a poor prognosis. Patients may develop hematologic complications, including BMF, MDS, and/or AML later in their lives if not treated with allogenic HSCT [4]. Because of genomic instability, patients with FA have a low tolerance for standard doses of alkylating agents [13]. Therefore, nonmyeloablative HSCT conditioning regimens are recommended for patients with FA undergoing transplantation caused by BMF without MDS or AML [14]. Achieving full-donor chimerism is essential to limit the risk of leukemic transformation of any residual FA-mutated hematopoietic cells in this population [15]. Cancer predisposition is also a challenge for transplantation in patients with FA, which imposes a restriction on the inclusion of radiation in conditioning regimens [16,17]. Therefore, the ideal pretransplant regimen for patients with FA should be potently immunosuppressive and radiation free, as well as show limited toxicity.

After years of rigorous studies investigating conditioning regimens, clinicians have shown that a radiation-free preparative regimen including FLU, CY, and ATG was sufficient for sustained donor engraftment, had limited end-organ toxicity, lowered the risk of acute and/or chronic GVHD, and was associated with better long-term overall survival [8,18,19]. With the evolution of reported conditioning regimens, the

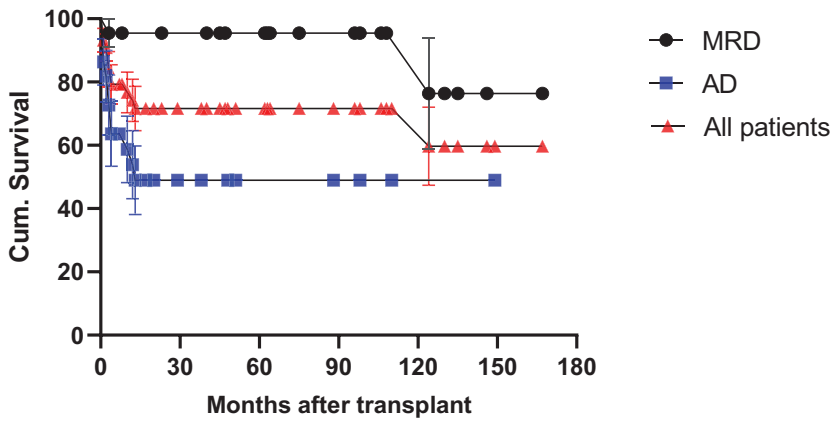



**Figure 1.** Overall survival with a median follow-up of 36.5 months.

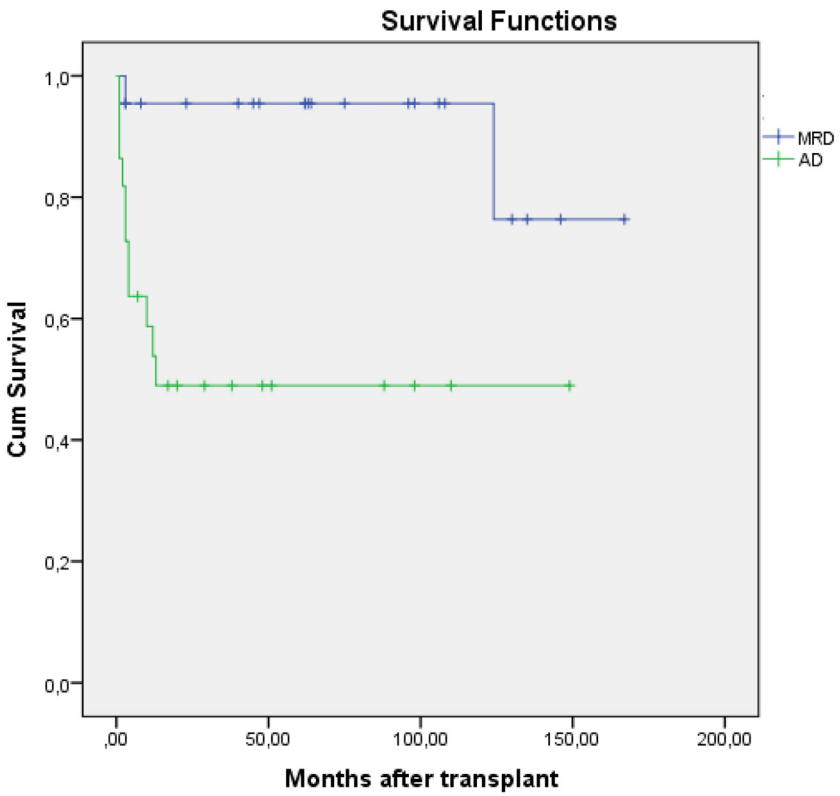



**Figure 2.** Overall survival according to donor types.

abovementioned protocol has been used for HSCT in patients with FA in our clinic since January 2002.

This study presents a retrospective analysis of our 14 years of experience with this regimen for HSCT in patients with FA. Our findings show that both MRD and AD recipients showed a high rate of chimerism in the first chimerism analysis (90% full-donor chimerism, 5% mixed-donor chimerism, and 5% primary graft failure), which demonstrates the potent immunosuppressive effect of this regimen. Even in long-term follow-up, none of our patients needed a second HSCT. Of the 31 surviving patients, all have full chimerism with normal hematopoiesis. Tan et al. [18] reported primary engraftment in all patients with FA (n = 11) who received stem cell transplantation from a genotypically identical donor (T cell depleted graft) caused by BMF. In their report, the preparative regimen of FLU + ATG + CY was sufficient to obtain long-term sustained donor engraftment in the entire group except 2 patients who required a second HSCT. The favorable effects of FLU-based conditioning protocols on engraftment were confirmed by Smetsers et al. [20], who emphasized that the use of FLU-based conditioning regimens reduced graft failure, improved 5-year overall survival, and decreased early mortality in their cohort. Wagner et al. [19] also reported a higher likelihood of neutrophil and platelet recovery and a statistically lower risk of transplantation-related mortality with FLU-containing regimens compared with non-FLU-containing regimens in AD BM transplantations to patients with FA. de Latour et al. [8] reported the results of a multicenter study by the European Society for Blood and Marrow Transplantation in 2013. This 38-year retrospective analysis of 795 FA transplantations showed that the use of a FLU-based conditioning regimen was associated with better engraftment, a lower rate of acute GVHD, and better long-term overall survival.

In this study, we found that donor type significantly affected overall survival, as recipients of stem cells from MRD had better overall survival compared with patients transplanted from ADs. Our overall survival of 92% at 3 years with MRD transplantation is comparable to literature data [21–23]. Ayas et al. [24] reported 10-year overall survival odds of 86% among 94 pediatric patients with FA who underwent HSCT from related donors (86 MSD, 3 matched parents, one 7/8 sibling, and five 7/8 parents). The authors concluded that low-dose CY supplemented with FLU was associated with the best survival.

Transplantation from AD led to higher mortality compared with MRD transplants in our study, which is also consistent with the existing literature. In a retrospective analysis of FA transplantation from the Italian registry, Locatelli et al. [25] reported a significantly lower 8-year estimated overall survival rate for AD (40%) compared with a HLA-identical sibling group (87%). Subsequent GVHD and infectious complications have been reported as the major causes of death. de Latour et al. [8] reported that survival of patients transplanted from AD was clearly lower compared with related donor recipients. Even though previous reports indicate inferiority of AD transplantation, a recent study from Ebens et al. [12] showed comparable outcomes of AD (n = 57) and HLA-matched sibling donor (n = 17) HSCT for children with FA and BMF. In their study, BM grafts underwent T cell depletion for both MSD and AD recipients. Rates of both acute grade 3 to 4 GVHD (6% for MSD and 12% for AD, $P = .44$) and 5-year overall survival (94% for MSD and 86% for AD, $P = .37$) were comparable between MSD and AD recipients. The effectiveness of a T cell-depleted graft in preventing severe acute GVHD among AD recipients was shown in previous studies [15,19]. In our opinion, the inferior results regarding AD transplantations in our series were

mainly caused by the infusion of unmanipulated grafts, which led to higher rates of acute severe GVHD and resulted in lower overall survival rates.

GVHD is one of the most important risk factors for invasive fungal infection in HSCT recipients [26,27]. Of the 3 patients in our cohort who died of invasive fungal infections, 2 had grade 2 to 4 acute GVHD. Like all other patients, these 3 patients were already receiving fluconazole prophylaxis when they were diagnosed with fungal infections. Fluconazole is effective against *Candida*, but it does not protect patients from mold infections [28,29]. Even though a prospective, randomized multicenter study has shown the superiority of micafungin chemoprophylaxis compared with fluconazole in the neutropenic phase of HSCT, fluconazole is still the recommended antifungal prophylactic agent during the pre-engraftment period of HSCT for centers with a low incidence of mold infection [30,31]. However, posaconazole is advised (level A1), for better protection against mold, in patients with GVHD [30]. In Turkey, posaconazole is on the market only as an oral formulation, and therapeutic drug monitoring is not available. Because of concerns regarding efficient trough levels of posaconazole, we administer fluconazole prophylaxis to every patient independent of GVHD status. Itraconazole may be an alternative treatment for patients with GVHD (level B-I), but reported toxicity and limited tolerability of the drug prevented us to prescribe this drug [30]. In our opinion, better antifungal prophylaxis will decrease the incidence of invasive fungal infections, especially for patients with acute GVHD.

Despite a high CMV reactivation rate (65%), routine monitoring with a CMV real-time PCR assay enabled us to detect early antigenemia and treat patients before the onset of any end-organ CMV disease. Because of the relative rarity of ADV disease, we do not conduct routine surveillance; ADV PCR analysis is done only in the presence of clinical symptoms. Unfortunately, one of our patients died at post-transplant 12 months of ADV encephalitis despite antiviral treatment with cidofovir and cessation of immunosuppressive agents. Even though routine monitoring of ADV PCR in T cell replete graft recipients is not recommended [29,32], physicians should be aware of the possibility of an ADV infection, especially in the presence of clinical signs in patients with GVHD.

Stem cell source has been shown to affect the incidence of GVHD as PBSC recipients experienced higher rates of GVHD, which increases the risk of squamous cell carcinoma, compared with BM recipients [8,33,34]. Considering these data, in recent years, we have preferred to obtain BM from donors, but because of volunteer selection and the high number of transplantations performed in the early part of the study period, there was no statistical difference between stem cell sources among our patients. Although stem cell source was not statistically associated with the incidence GVHD in our series, we still believe that BM should be preferred over PBSC for transplantation in patients with FA.

Our attempts to decrease the frequency and severity of HC with intravesical HA and CS did not result in a significant difference between patients who did and did not receive intravesical treatment. This treatment should be tested in larger patient cohorts to determine its efficacy.

Regarding somatic malformations, there was no difference in overall survival between patients with 3 or more congenital abnormalities (n = 7, 16%) and those with fewer malformations ($P = .70$). In addition, pretransplant transfusions and older age at transplantation (>10 years) did not affect survival in our patients, in contrast to previous studies showing poorer survival outcomes in patients with extensive malformations, more pretransplant transfusions, and advanced age [8,24,35,36].

## CONCLUSION

Our study shows that a radiation-free, FLU-based conditioning regimen with unmanipulated graft infusion can be used safely for transplantation for FA-associated BMF in MRD recipients. For AD recipients, however, rates of acute GVHD and infection are quite high and lead to increased mortality rates. This suggests that better anti-infectious disease prophylaxis and further improvements in acute GVHD treatment could eventually enable better transplant outcomes in centers where T cell depletion of graft is not possible. More effort is warranted to improve outcomes for AD transplantations in countries with limited resources.

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