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# HLA-Haploidentical T Cell–Depleted Allogeneic Hematopoietic Stem Cell Transplantation in Children with Fanconi Anemia

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A B S T R A C T

We report the outcome of 12 consecutive pediatric patients with Fanconi anemia (FA) who had neither an HLA-identical sibling nor an HLA-matched unrelated donor and who were given T cell–depleted, $CD34^+$ positively selected cells from a haploidentical related donor after a reduced-intensity, fludarabine-based conditioning regimen. Engraftment was achieved in 9 of 12 patients (75%), and the cumulative incidence of graft rejection was 17% (95% confidence interval [CI], 5% to 59%). Cumulative incidences of grades II to IV acute and chronic graft-versus-host disease were 17% (95% CI, 5% to 59%) and 35% (95% CI, 14% to 89%), respectively. The conditioning regimen was well tolerated, with no fatal regimen-related toxicity and 3 cases of grade III regimen-related toxicity. The cumulative incidence of transplant-related mortality was 17% (95% CI, 5% to 59%). The 5-year overall survival, event-free survival, and disease-free survival were 83% (95% CI, 62% to 100%), 67% (95% CI, 40% to 93%), and 83% (95% CI, 62% to 100%), respectively. These data demonstrate that a fludarabine-based conditioning regimen, followed by infusion of high doses of T cell–depleted stem cells, is able to ensure engraftment with good overall survival and disease-free survival, confirming the feasibility of haploidentical hematopoietic stem cell transplantation in FA. To the best of our knowledge, this is the largest series of hematopoietic stem cell transplantation from a haploidentical related donor in FA patients reported to date.



## INTRODUCTION

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous disorder characterized by the variable presence of multiple congenital somatic abnormalities, the gradual onset of bone marrow failure involving one or more hematopoietic cell lineages, and predisposition to develop clonal hematopoietic disorders, as well as solid tumors, mostly head and neck squamous-cell carcinomas [1-5]. On a cellular level, FA is characterized by chromosome fragility and hypersensitivity to DNA interstrand cross-linking agents. With an incidence of approximately 1 out of 100,000 births per year, it is the most common cause of constitutional bone marrow failure [6].

FA is caused by germline mutations, inherited in an autosomal (or rarely X-linked) recessive pattern. To date, 15 genes are known to be involved in FA pathogenesis [7,8], and because patients with mutations in any of these genes share a characteristic cellular and clinical phenotype, they have been postulated to interact in a common cellular pathway involved in DNA repair processes, known as the FA pathway [9]. More recently, using whole-exome and Sanger sequencing on DNA of 2 unclassified FA patients, Bogliolo et al. [10] identified *ERCC4* (Xeroderma pigmentosum group F) as an additional FA-associated gene, thus called *FANCQ*.

Optimized supportive care, including RBC/platelet transfusions, androgen therapy, recombinant growth factors, and prevention/treatment of infectious complications, is a critical aspect of conservative management of FA patients once advanced marrow failure occurs [11]. Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only therapeutic option able to restore normal hematopoiesis in patients with FA, with the potential for definitively correcting the occurrence of marrow failure associated with the disease and for preventing/treating myeloid malignancies, although it does not affect the congenital defects in other tissues and cannot prevent the late occurrence of solid tumors. Initial attempts at HSCT for FA patients yielded poor results, mainly due to the underlying defect in DNA repair and hypersensitivity to treatment with irradiation and cytotoxic agents, such as cyclophosphamide (Cy), leading to excessive regimen-related toxicity and severe acute graft-versus-host disease (GVHD) [12,13]. Pretransplantation conditioning regimens specifically developed for FA patients and based on the use of low-dose Cy and limited-field irradiation (thoracoabdominal irradiation) have met with some success [14].

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Despite significant improvement in outcome for FA patients transplanted from an HLA-identical sibling donor [14-16], results from alternative donor transplantations have been less encouraging [14]. Nonetheless, over the past 2 decades significant improvements in alternative donor HSCT have been achieved, mainly due to the optimization of HLA typing for donor selection, conditioning regimens, with fludarabine (Flu) playing a pivotal role [16], and to the introduction of T cell–depletion techniques for graft manipulation, making indications to transplantation in FA quite consistent regardless of the donor used.

Notwithstanding some encouraging results and potential benefits of allogeneic HSCT from an HLA partially matched related donor in FA patients, current evidence in the medical literature is limited to case reports and small retrospective case series [14,17-26]. The present study describes the outcomes of 12 consecutive pediatric FA patients who underwent T cell–depleted (TCD) HSCT from an HLA partially matched relative, after a Flu-based preparative regimen.

## METHODS

### *Data Collection*

Data concerning patient, disease, graft characteristics, transplantation outcomes, and follow-up were collected by means of standardized data collection forms for each patient.

### *Patients*

Twelve consecutive patients affected by FA received a TCD peripheral blood stem cell allograft from an HLA-haploidentical related donor between July 2001 and June 2012 at 2 Italian pediatric HSCT centers (Pavia and Rome). Details on patient, donor, and transplantation characteristics are reported in Tables 1 and 2. Three of 12 patients (patients 1, 4, and 5, Table 1) have been already described in previously published reports [20,23]. Written informed consent was obtained from all patients or from their parents/legal guardians in accordance with the Declaration of Helsinki.

The patients, 8 girls and 4 boys, ranged in age from 5.9 to 21.7 years (median, 8.6) at time of transplantation. FA diagnosis was confirmed by means of diepoxybutane-induced chromosome breakage assay in all cases. Median age at diagnosis was 6.7 years (range, 2.7 to 12.4), and median time from diagnosis to HSCT was 2.4 years (range .3 to 19.0).

At time of transplantation, all patients were RBC and/or platelet transfusion dependent, but no patient had severe iron overload or was refractory to platelet transfusions. No patient in our cohort had a history of androgen exposure before transplant. Two patients had evidence of clonal evolution at time of first evaluation. Patient 8 was diagnosed with both myelodysplastic syndrome with chromosome 5q31 deletion and chromosome 17p13.1 deletion involving the *TP53* tumor suppressor gene and T cell non-Hodgkin lymphoma (serology showed positive viral capsid antigen and Epstein-Barr nuclear antigen IgG and negative viral capsid antigen IgM, whereas blood Epstein-Barr virus-DNA was negative) and was treated with a chemotherapy regimen consisting of low-dose Flu (25 mg/m$^2$/day for 2 days), L-asparaginase (10,000 U/m$^2$/day for 4 days), and dexamethasone (20 mg/m$^2$/day for 4 days) with a remarkable reduction of the mediastinal mass at time of HSCT. Patient 11 had acute myeloid leukemia with a complex karyotype, namely 46,XX,der(6)t(6;?),der(7)t(7;?),der(15)t(15;?),der(16)t(16;?),der(18)t(18;?), and was successfully induced into remission before HSCT through 2 chemotherapy cycles consisting of Flu, cytosine arabinoside, and liposomal doxorubicine. In all patients, high-resolution molecular typing was performed to characterize HLA class I and II loci.

### *Donors*

Donors were HLA-typed using low-resolution DNA methods for HLA-A and HLA-B loci and high-resolution molecular typing at HLA-C and HLA class II loci. All donors were first-degree relatives, completely matched with the patient at 1 haplotype, with second haplotype mismatches detailed as follows. Eleven of 14 donors were full-haplotype mismatched (HLA-A, -B, -C, and -DR antigen mismatches); in 2 cases 3 mismatches on the antigen level and 1 mismatch on the allele level (HLA-A or -C) were present; in 1 case donor and recipient were different for 3 HLA loci and matched for HLA-DR. Seven of 14 donors were killer immunoglobulin-like receptor (KIR)-ligand mismatched with the respective recipient. Median donor age was 41 years (range, 30 to 58), with mothers and fathers used in 5 and 7 cases, respectively.

Donor selection was based on the following algorithm. First, a natural killer (NK)-alloreactive donor, according to the KIR/KIR ligand mismatch model, was chosen by virtue of the effect of donor NK cell alloreactivity on prevention of GVHD and graft rejection [27]. Second, the mother was selected if no NK-alloreactive donor was available because better outcomes have been reported in parent-to-child transplantation for patients receiving mother-donor grafts [28]. Two patients who rejected their first allograft underwent a second HSCT using the other haploidentical parent as a donor.

Each donor received granulocyte colony-stimulating factor by subcutaneous injection at a dose of 10 μg/kg/day. Donor peripheral blood stem cells were collected via leukapheresis, starting on day 4 of granulocyte colony-stimulating factor administration, and TCD by means of CD34$^+$ cell positive selection, using the CliniMacs one-step procedure (Miltenyi Biotech, Bergisch Gladbach, Germany).

**Table 1**
Details of the 12 Patients, Follow-Ups, and Transplant Outcomes

| Patient No. | Sex | Age at Diagnosis (yr) | Age at HSCT (yr) | Diagnosis HSCT (yr) | Disease Status | No. of HSCT Received | Engraftment | aGVHD | cGVHD | Follow-Up (yr) | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 9.0 | 15.3 | 6.4 | Transfusion dependent | 1<br>2 | Rejection<br>Engraftment | Absent | Absent | 11.5 | Alive and well after 2nd HSCT |
| 2 | M | 4.1 | 8.5 | 4.4 | Transfusion dependent | 1 | Engraftment | Absent | Not assessable | .3 | Died from RSV pneumonia |
| 3 | F | 8.5 | 9.2 | .7 | Transfusion dependent | 1 | Engraftment | Absent | Limited | 1.2 | Alive and well |
| 4 | F | 2.7 | 8.3 | 5.6 | Transfusion dependent | 1 | Engraftment | Absent | Absent | 7.1 | Alive and well |
| 5 | F | 3.0 | 6.3 | 3.3 | Transfusion dependent | 1 | Engraftment | Grade II | Absent | 7.1 | Alive and well |
| 6 | F | 5.8 | 8.0 | 2.2 | Transfusion dependent | 1 | Engraftment | Absent | Absent | 3.3 | Alive and well |
| 7 | F | 7.5 | 8.7 | 1.2 | Transfusion dependent | 1 | Engraftment | Grade I | Limited | 6.9 | Alive, limited cGVHD |
| 8 | M | 4.9 | 5.9 | 1.0 | Transfusion dependent NHL | 1<br>2 | Rejection<br>Engraftment | Absent | Absent | .7 | Alive and well after 2nd HSCT |
| 9 | M | 12.4 | 12.8 | .3 | Transfusion dependent | 1 | Died before engraftment | Not assessable | Not assessable | .03 | Died from *Candida* sepsis |
| 10 | M | 5.9 | 6.7 | .8 | Transfusion dependent | 1 | Engraftment | Absent | Absent | .5 | Alive and well |
| 11 | F | 9.0 | 28.7 | 19.7 | Transfusion dependent AML | 1 | Engraftment | Grade II | Limited | 1.0 | Alive and well |
| 12 | F | 7.4 | 10.0 | 2.6 | Transfusion dependent | 1 | Engraftment | Absent | Absent | 1.3 | Alive and well |

RSV, respiratory syncytial virus; NHL, non-Hodgkin lymphoma; AML, acute myelogenous leukemia.