The outcome of children with Fanconi anemia given hematopoietic stem cell transplantation and the influence of fludarabine in the conditioning regimen: a report from the Italian pediatric group

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ABSTRACT

Background and Objectives

Hematopoietic stem cell transplantation (HSCT) still represents the only treatment potentially able to prevent/rescue the development of marrow failure and myeloid malignancies in patients with Fanconi anemia (FA). While in the past HSCT from an HLA-identical sibling was proven to cure many patients, a higher incidence of treatment failure has been reported in recipients of an unrelated donor (UD) or HLA-partially matched related allograft.

Design and Methods

We analyzed the outcome of 64 FA patients (age range, 2-20 years) who underwent HSCT between January 1989 and December 2005. Patients were transplanted from either an HLA-identical sibling (n=31), an UD (n=26), or an HLA-partially matched relative (n=7). T-cell depletion of the graft was performed in patients transplanted from an HLA-disparate relative.

Results

The 8-year estimate of overall survival (OS) for the whole cohort was 67%; it was 87%, 40% and 69% when the donor was an HLA-identical sibling, an UD and a mismatched relative, respectively ($p<0.01$). The outcome of recipients of grafts from an UD improved over time, the probability of survival being 10% and 72% for patients transplanted before and after 1998, respectively ($p<0.05$). The OS probability of children who did or did not receive fludarabine in preparation for the allograft was 86% and 59%, respectively ($p<0.05$).

Interpretation and Conclusions

These data, useful for counselling, provide support to the concept that a relevant proportion of FA patients undergoing HSCT can now be successfully cured, even in the absence of an HLA-identical sibling, especially if the conditioning regimen includes fludarabine.

Key words: Fanconi anemia, unrelated donor, fludarabine, hematopoietic stem cell transplantation.

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Fanconi anemia (FA) is a rare, genetically heterogeneous, inherited disorder clinically characterised by congenital abnormalities, progressive bone marrow failure and a predisposition to develop malignancies, especially acute myeloid leukemia (AML) and squamous cell carcinoma (SCC).[1-4] Hematopoietic stem cell transplantation (HSCT) still represents the only option able to definitively cure the marrow failure associated with this disease, as well as to prevent/treat myeloid malignancies, although it does not prevent the occurrence of solid tumors, mostly head and neck SCC.[5-7] The use of conditioning regimens based on reduced doses of cyclophosphamide, either alone or together with limited field radiotherapy, has cured a large proportion of patients transplanted from an HLA-identical sibling.[5,8,9] Results of unrelated donor (UD) HSCT have been less encouraging, mainly due to increased difficulties with engraftment and higher incidence of both acute and chronic graft-versus-host disease (GVHD).[10,11] Better results were recently reported in a retrospective study including both pediatric and adult FA patients transplanted from an UD.[12] A significant contribution to this improved outcome was provided by the use of fludarabine as part of the conditioning regimen,[12] this finding confirming previous reports on HSCT from alternative donors including either anecdotal cases or a limited number of patients.[13-16] We here report the outcome of 64 pediatric FA patients transplanted in Italy from either an HLA-identical sibling, an UD, or an HLA-partially matched relative.

Design and Methods

Data concerning patient and disease characteristics, as well as transplantation outcome, were collected by means of a standardized questionnaire of the AIEOP (Associazione Italiana di Ematologia e Oncologia Pediatrica) Registry for each patient enrolled into this study. This study includes patients with a diagnosis of FA, confirmed by the presence of multiple chromosome breaks, enhanced by incubation with cross-linking agents, who received an allogeneic HSCT between January 1989 and December 2005. A total of 64 children with FA transplanted from an HLA-identical sibling (n=31), an unrelated volunteer (n=26), or an HLA-partially matched relative (n=7) were included in the analysis. Fifteen of these 64 patients, all transplanted from an HLA-identical sibling, have been previously reported.[17] UD were located through a network of international bone marrow donor Registries.[18] In all donor-recipient pairs, histocompatibility was determined by serology for HLA-A and -B antigens and by DNA typing for HLA-DRB1 locus (conventional typing). In all patients transplanted from an UD, HLA-DRB1 typing was performed using a high-resolution allelic technique. After January 1998, high-resolution molecular typing was also performed to characterize HLA class I loci of both donors and patients in UD transplants. Bone marrow was used as the source of stem cells in 52 patients, whereas six patients were transplanted with cord blood cells. All patients transplanted from an HLA-partially matched relative but one, transplanted with bone marrow cells, were given T-cell-depleted peripheral blood progenitor cells. $CD34^+$ cells were positively selected using the CliniMacs one-step procedure (Miltenyi Biotech, Bergisch Gladbach, Germany). After transplantation, the seven children given a T-cell-depleted allograft received either cyclosporine A (CsA) or tacrolimus for 40-60 days in order to minimize the risk of graft rejection. Details on the patients' and donors' characteristics, conditioning regimen, GVHD prophylaxis, as well as on the median number of bone marrow and cord blood nucleated cells infused, are reported in Table 1. Preparative regimens varied, mainly according to the patients' age and transplant Center protocols (Table 1). Twenty-five patients received a preparative regimen including the combination of fludarabine (30 mg/m^2/day for 4 consecutive days) and cyclophosphamide (300 mg/m^2/day for 4 consecutive days).[19] Thirty children received a conditioning regimen based on the use of cyclophosphamide, either alone or in combination with radiotherapy (mainly involving limited field of irradiation). The remaining nine patients were transplanted after heterogeneous pre-transplantation regimens. As GVHD prophylaxis, most of the HLA-identical sibling HSCT recipients received CsA alone, while children transplanted from an UD were given CsA together with a short course of methotrexate. Pre-transplant antithymocyte globulin was used in all recipients of a T-cell depleted allograft, in 21 out of the 26 patients transplanted from an unrelated volunteer and in 12 children given HSCT from an HLA-compatible sibling (see also Table 1 for further details).

Definition of outcomes

The primary outcomes were: (i) transplantation-related mortality (TRM), defined as all causes of death related to the transplantation procedure; (ii) overall survival (OS), which was measured as the time interval between the date of transplantation and the date of death from any cause or the date of last follow-up for survivors. Other outcomes were: (iii) hematopoietic recovery: neutrophil and platelet recoveries being analyzed separately, and defined, respectively, by a neutrophil count greater than 0.5×10^9/L for 3 consecutive days and an unsupported platelet count greater than 50×10^9/L for 7 consecutive days. Absence of hematopoietic recovery at day 60, second transplantation or autologous hematopoietic reconstitution were considered as failure of engraftment. iv) graft-versus-host disease: acute and chronic GVHD were diagnosed and graded at each transplant Center according to the Seattle criteria.[20,21] Patients surviving for more than 14 and 100 days post-transplantation were evaluated for acute and chronic GVHD occurrence, respectively.

Statistical analysis

Analysis used December 31, 2005 as the report date, i.e., the day at which all Centers locked data on patients' out-

comes. Patients were censored at the time of death or at last follow-up. Univariate and multivariate proportional hazard regression models were used to identify independent patient-, donor- and transplantation-related variables influencing the different outcomes by means of log-rank tests and Cox proportional hazard models. Since hematopoietic recovery and development of GVHD are considered events which compete with death, estimations of the incidence of these events relied on the non-parametric estimator of cumulative incidence curves, while predictive analyses were based on the proportional hazards model for the subdistribution of competing risks.[22,23] All results are expressed as 8-year probability or 8-year cumulative incidence (%) with a 95% confidence interval (95% CI). p values >0.1 are reported as non-significant (N.S.); p values between 0.05 and 0.1 were also considered non-significant, but are reported in detail; p values <0.05 were considered statistically significant and are reported in detail.

Results

Neutrophil and platelet engraftment, and graft rejection

Graft failure occurred in four patients at 20, 25, 29 and 30 days after HSCT; two had primary graft failure, while two children experienced secondary loss of the graft after transient engraftment of donor cells. All these four patients (two given HSCT from an HLA-identical sibling, one from a partially matched family donor and one from an UD) were re-transplanted from the same donor, and one of the patients, transplanted from an HLA-identical sibling and who experienced secondary graft failure, reached sustained engraftment of donor hematopoiesis after a third allograft. This patient is currently alive with normal blood counts and without any sign of GVHD. The cumulative incidence of primary and secondary graft failure was 6% (95% CI, 2-16%). For patients who engrafted, the median time to neutrophil recovery was 13 days (range, 8-46), whereas the median time to platelet recovery was 21 days (range, 7-107). The only factor which negatively influenced neutrophil and platelet engraftment in multivariate analysis was the use of methotrexate for GVHD prophylaxis (*data not shown*).

Acute and chronic GVHD

Sixty-three out of the 64 patient enrolled in the study, surviving more than 14 days after HSCT, were evaluated for the occurrence of acute GVHD. Twenty-three of them experienced grade II-IV acute GVHD; the cumulative probability of developing grade II-IV acute GVHD was 37% (95% CI, 26-51%), whereas that of developing grade III-IV acute GVHD was 13% (95% CI, 7-24%). Multivariate analysis did not identify any variable predicting the occurrence of acute GVHD. Chronic GVHD developed in 13 out of the 51 patients surviving more than 100 days after the allograft and, thus, at risk of this complication. All patients with chronic GVHD had previously had acute GVHD. Five out of these 13 patients had extensive chronic GVHD and eight had limited chronic GVHD. The overall cumulative probability of developing chronic GVHD was 26% (95% CI, 16-42%), while that of developing the extensive form was 11% (CI 5-26%). Only the occurrence of grade II-IV acute GVHD predicted the development of chronic GVHD in multivariate analysis (71% vs. 3% for patients who did or did not have acute GVHD, respectively, p<0.0001).

Table 1. Patient, donor and transplantation characteristics of the 64 FA patients included in the study.

Number of patients	64 (100%)
Gender	
Males	39 (61%)
Females	25 (39%)
Median age at diagnosis and range (years)	6 (1–13)
Median age at HSCT and range (years)	9 (2–20)
Median interval from diagnosis to HSCT (months)	20 (2–190)
Type of donor employed	
Matched family donor	31 (48%)
Matched unrelated donor	26 (41%)
HLA-partially matched family donor	7 (11%)
Number of cells infused	
Bone marrow recipients (nucleated cells×10^8/kg)	4.5 (0.9–13.7)
Cord blood recipients (nucleated cells×10^7/kg)	3.1 (2.5–9.1)
Peripheral blood recipients ($CD34^+$ cells×10^6/kg)	15.1 (9-30)
Conditioning regimen	
CY-based	
CY alone	6 (9%)
CY + irradiation ± other	22 (34%)
CY + thiotepa	2 (3%)
Fludarabine based	
Fludarabine +CY	25 (40%)
Other regimens	9 (14%)
Serotherapy (ATG)	40 (62%)
GVHD prophylaxis	
CsA	17 (27%)
CsA + ATG	6 (14%)
CsA + MTX	6 (9%)
CsA + MTX + ATG	25 (39%)
CsA + Steroids	1 (2%)
CsA + Steroids + ATG	2 (3%)
T-cell-depletion	7 (6%)
Use of G-CSF after HSCT	
Yes	42 (66%)
No	22 (34%)

HSCT: hematopoietic stem cell transplantation; CY: cyclophosphamide; ATG: anti-thymocyte globulin; GVHD: graft-versus-host disease; CsA: cyclosporine A; MTX, methotrexate; G-CSF: granulocyte colony-stimulating factor.

Transplantation-related mortality

Nineteen patients died of transplantation-related complications at a median time of 88 days after the allograft (range, 8 days–4.5 years). The 8-year cumulative incidence of TRM was 33% (95% CI, 23-49%, see also Figure 1 for further details). It was 14% (95%, CI 6-35) for patients receiving HCST from an HLA-compatible sibling, 60% (95% CI, 40-89) for patients transplanted from an UD and 29% (95% CI, 9-92) for those given transplantation from an HLA disparate relative (p<0.01). Details on 6-month

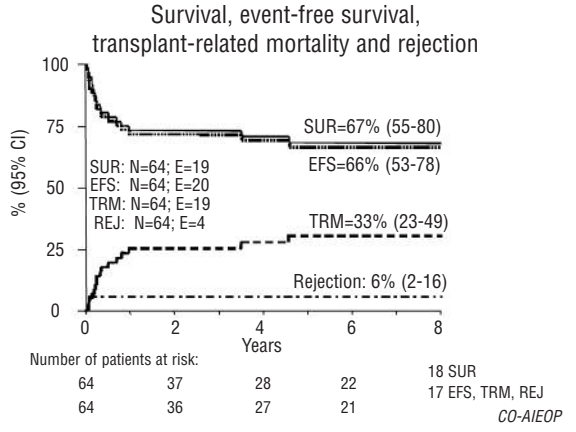

Figure 1. Eight-year Kaplan-Meier estimate of survival (SUR) and event-free survival (EFS) as well as 8-year cumulative incidence of transplant-related mortality (TRM) and graft rejection (REJ) for the whole cohort of patients. In the calculation of EFS, both death and graft failure were considered events.

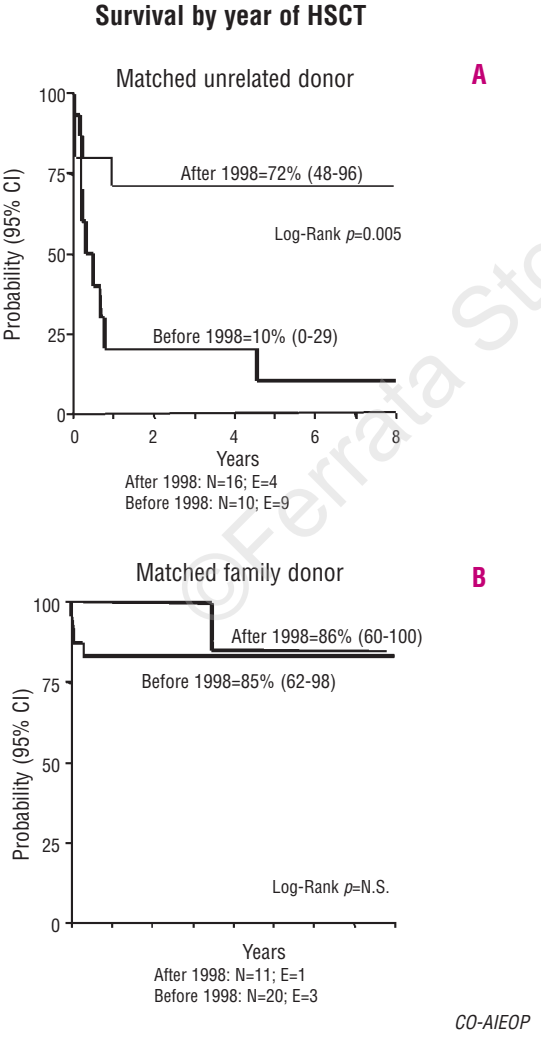

Figure 3. Probability of survival for patients transplanted from either an unrelated donor (A) or an HLA-identical sibling (B) according to the year of transplantation.

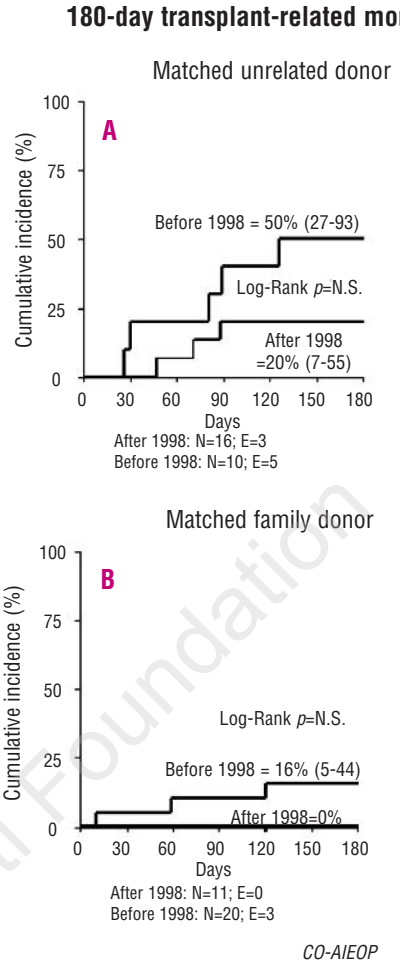

Figure 2. Probability of 180-day transplant-related mortality for patients transplanted from either an unrelated donor (A) or an HLA-identical sibling (B) according to the year of transplantation.

TRM for patients transplanted from either a relative or an UD according to the transplant period are reported in Figure 2. In univariate analysis, also the following factors were associated with increased risk of transplant-related death: ≥20 months between diagnosis and transplantation (p=0.05), a conditioning regimen not including fludarabine (p=0.04), the administration of granulocyte colony-stimulating factor (G-CSF) (p<0.01) and the occurrence of grade II-IV acute GVHD (p<0.01) or of chronic GVHD (p=0.02). However, in multivariate analysis only the occurrence of grade II-IV acute GVHD (relative risk=3.7; p=0.028) and a graft from an UD (relative risk=5.3; p=0.019) remained associated with an increased TRM.

One patient, transplanted from an HLA-compatible sibling after a conditioning regimen including total body irradiation and cyclophosphamide and who had developed both grade II acute GVHD, requiring prolonged steroid treatment, and limited chronic GVHD, involving oral mucosa, died 4.5 years after transplantation due to a tongue carcinoma.

Table 2. Univariate analysis of overall survival.

Variable	*N. of patients*	*Events*	*Probability of overall survival at 8 years*	*(95% CI)*	*P*
Gender					
Male	39	12	68%	(53-83)	N.S.
Female	25	7	65%	(42-88)	
Age at diagnosis					
<6 years	33	11	63%	(46-81)	N.S.
≥6 years	31	8	71%	(54-89)	
Year of transplantation					
Before 1998	31	13	58%	(41-75)	0.083
After 1998	33	6	76%	(59-94)	
Age at transplantation					
<9 years	33	9	70%	(53-87)	N.S.
≥9 years	31	10	65%	(47-83)	
Interval diagnosis – HSCT					
<20 months	32	6	79%	(64-94))	0.053
≥20 months	32	13	55%	(36-74)	
Donor					
Matched family donor	31	4	87%	(74-99)	0.003
Matched unrelated donor	26	13	40%	(16-64)	
Partially matched family donor	7	2	69%	(32-100)	
Stem cell source					
Bone marrow	52	15	64%	(55-82)	N.S.
Peripheral blood	6	1	80%	(45-100)	
Cord blood	6	3	50%	(10-90)	
Cell dose (bone marrow only)					
<4.5 x 10^8/Kg	21	8	61%	(40-82)	N.S.
≥4.5 x 10^8/Kg	24	6	67%	(44-90)	
Use of fludarabine					
No	39	16	59%	(43-74)	0.04
Yes	25	3	86%	(71-100)	
Use of G-CSF					
No	22	1	85%	(70-100)	0.02
Yes	42	18	59%	(31-76)	
Use of methotrexate					
No	33	7	78%	(63-92)	0.074
Yes	31	12	53%	(31-75)	
Use of serotherapy					
No	24	6	75%	(57-92)	N.S.
Yes	40	13	62%	(45-79)	
Acute GVHD grade*					
0 – I	40	6	85%	(73-96)	0.003
II – IV	23	12	43%	(21-65)	
Chronic GVHD**					
Absent	38	3	92%	(83-100)	0.011
Limited	8	2	71%	(38-100)	
Extensive	5	3	30%	(0-77)	

**Only the 63 patients surviving at least 14 days after transplantation were evaluated for acute GVHD occurrence. **Only the 51 patients surviving at least 100 days after transplantation were evaluated for chronic GVHD occurrence. HSCT: hematopoietic stem cell transplantation; GVHD: graft-versus-host disease; G-CSF: granulocyte colony-stimulating factor.*

Overall survival

Forty-five patients are alive with sustained donor engraftment, the median follow-up of surviving patients being 6 years (range, 3 months–16 years). The 8-year Kaplan-Meier estimate of OS is 67% (95% CI, 55-80, see also Figure 1). In patients transplanted from an HLA-identical sibling, an UD and a mismatched relative, the OS probability at 8 years is 87% (95% CI, 74-99%), 40% (95% CI, 16-64) and 69% (95% CI, 32-100), respectively (p=0.003) (see also Table 2). The outcome of patients transplanted from an unrelated donor improved significantly over time, the probability of OS being 10% (95% CI, 0-29), and 72% (95% CI, 48-96) for children transplanted before and after 1998, respectively (p= 0.005; see Figure 3A). By contrast, there was no significant difference in the outcome of children transplanted from an HLA-identical sibling before and after 1998 (Figure 3B). Patients who did or did not receive fludarabine in the conditioning regimen had a probability of OS at 8 years of 86% (95% CI, 71-100%) and 59% (95% CI, 43-74%), respectively (p=0.04, Figure 4). Children who did not receive G-CSF after HSCT had a better OS than that of patients who received G-CSF, the OS being 85% (95% CI, 70-100) and 59% (95% CI, 31-76), respectively (p=0.02) (*see also Table 2*). The 8-year OS was 43% (95% CI, 21-65%) and 85% (95% CI, 73-96%) for children who did or did not experience grade II-IV acute GVHD, respectively (p=0.003). The estimated probability of OS in patients without chronic GVHD was 92% (95% CI, 83-100%), while it was 71% (95% CI, 38-100%) for patients with limited chronic GVHD and 30% (95% CI, 0-77) for patients with extensive disease (p=0.011) (see also Table 2).

Table 3. Multivariate analysis for overall survival.

Variable	*Relative risk of treatment failure*	*(95% CI)*	*P*
Interval diagnosis - transplantation			
≥20 vs. <20 months	1.66	(0.60-4.60)	N.S.
Year of transplantation			
Before 1998 vs. after 1998	1.05	(0.22-4.97)	N.S.
Donor			
UD vs. MFD	7.65	(1.24-47.15)	0.0283
PMFD vs. MFD	2.16	(0.74-16.20)	N.S.
Use of Fludarabine			
Yes vs. no	0.16	(0.02-0.99)	0.0495
Use of MTX in GVHD prophylaxis			
Yes vs. no	1.09	(0.26-4.59)	N.S.
Use of G-CSF			
Yes vs. no	4.74	(0.50-45.00)	N.S.
Acute GVHD			
Grade II-IV vs. grade 0-I	2.24	(0.61-8.18)	N.S.

HSCT: hematopoietic stem cell transplantation; UD: unrelated donor; MFD: matched family donor; PMFD: partially matched family donor; MTX: methotrexate; GVHD: graft-versus-host disease; G-CSF: granulocyte colony-stimulating factor.

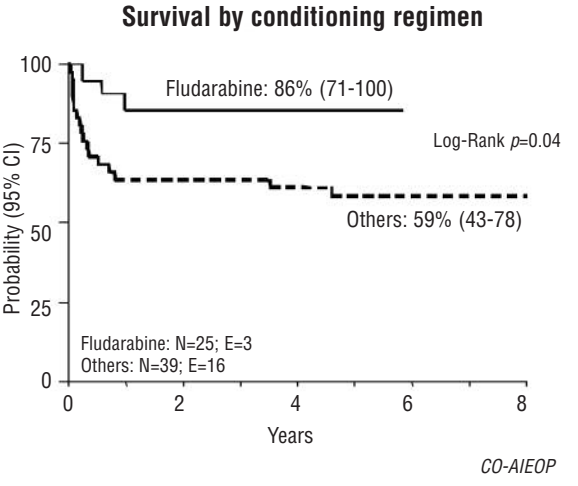

Figure 4. Influence of fludarabine in the conditioning regimen on the probability of survival at 8 years for the whole cohort of patients. Patients receiving fludarabine as preparation for the allograft did significantly better than those who did not receive fludarabine.

In multivariate analysis, only two factors were associated with a better probability of OS: use of fludarabine in the conditioning regimen and an HLA-identical sibling as the donor (see also Table 3). All patients surviving with sustained engraftment, except the two with extensive chronic GVHD, have a Karnofsky/Lansky score of 100%.

Discussion

This retrospective, multicenter analysis evaluated the outcome of 64 Italian pediatric patients with FA given HSCT in the last 17 years. We found that the type of donor significantly influenced the outcome, as recipients of allograft from an HLA-identical sibling had a better probability of survival in comparison to children transplanted from an UD. The 87% probability of OS at 8 years after the allograft observed in our 31 patients transplanted from an HLA-identical sibling is comparable to that reported in another, previously described cohort of Italian patients[17] and only marginally improved over time, suggesting that it will be difficult to further ameliorate the outcome of patients transplanted from an HLA-identical sibling.

The 8-year Kaplan-Meier estimate of OS in our 26 patients transplanted from an UD was 40%, which is comparable to that in the other few studies which analyzed the outcome of FA patients given HSCT from an unrelated volunteer.[5,10,12,24] Graft rejection, regimen-related toxicity and high risk of GVHD have been reported to be the greatest obstacles to successful outcome in patients with FA given HSCT from an UD.[5,10,12,24] Only one patient of our cohort transplanted from an unrelated volunteer experienced graft failure. GVHD and infectious complications were the main contributors to the deaths that occurred in our patients transplanted from an UD. The outcome of recipients of grafts from UD improved remarkably over time. Several factors related to the optimization of the preparative regimens and GVHD prophylaxis may have contributed to the improved outcome of our children transplanted in the last years. Moreover, the possibility of selecting an UD using high-resolution molecular typing for both class I and class II HLA-alleles has been demonstrated to decrease the risk of GVHD and TRM.[25,26] Indeed, the probability of survival of our FA patients transplanted from an UD after 1998, the year in which high-resolution molecular typing for both HLA class I and II antigens became available in most Centers, was comparable to that of patients receiving transplantation in the same time period from an HLA-compatible sibling. A patient's age less than 10 years predicted a better outcome in the experience on UD-HSCT recently reported by the Center for International Blood and Marrow Transplantation Research.[12] We were unable to confirm this finding, probably because most of our patients were transplanted early, the median age at time of allograft being only 9 years.

Although employed in a limited number of patients, fludarabine-containing regimens were found to be associated with better outcome than other regimens. Fludarabine-based conditioning regimens, capable of intense T-cell immunosuppression, have been reported to lead to early, stable engraftment with minimal toxicity in patients with several malignant and non-malignant diseases unable to tolerate conventional myeloablative therapy.[27,28] FA patients are natural candidates for non-myeloablative stem cell transplantation after a fludara-

bine-containing regimen because of the hypersensitivity of their cells to DNA cross-linking. The favorable role played by fludarabine in FA patients has been described in reports from single centers[13–16] and, more recently, in a large cohort of matched or one-antigen mismatched unrelated bone marrow donor recipients.[12] In this latter study, the use of fludarabine improved neutrophil recovery, decreased 100-day TRM, and improved 3-year adjusted OS rate, which was 52% and 13% in patients who did or did not receive fludarabine, respectively.

Grade II-IV acute GVHD had a detrimental effect in univariate analysis on the probability of OS in our patients and there was also a negative trend for chronic GVHD. Previous grade II-IV acute GVHD was the only factor predicting the occurrence of chronic GVHD in our cohort. Patients with FA have been documented to be at high risk of developing solid tumors, in particular SCC of the head and neck.[3,29-31] Chronic GVHD has also been known to render non-FA patients at higher risk of SCC of the oral mucosa and the skin, when these areas are involved by GVHD.[29] The correlation between GVHD and subsequent occurrence of secondary SCC in patients with FA, already at risk of this complication, has been recently demonstrated.[31] Consequently, as adequate *in vivo* depletion or modulation of T cells can decrease the risk of GVHD significantly, it may also decrease, in the long-term, the risk of SCC.

Our FA patients given G-CSF after transplantation were found to have a worse outcome in univariate analysis. A previously published study on acute leukemia patients has shown that the use of this cytokine after allografting is associated with an increased risk of TRM, translating into reduced survival and leukemia-free survival rates.[32] Several biological mechanisms can be hypothesized to explain the detrimental effect played by G-CSF administered after HSCT. Production of interleukin-12 was found to be profoundly impaired in patients given either an unmanipulated or a T-cell-depleted graft and who received G-CSF after the allograft.[33,34] Several studies in human volunteers and animals also showed that G-CSF increases monocyte production of interleukin-10,[35] which is known to inhibit both the ability to produce interleukin-12 and the stimulatory capacity of dendritic cells, and to promote Th2 immune deviation.[36] Since a high level of production of interleukin-12 by dendritic cells is a key factor in the initiation of protective Th1 immunity against fungi, bacteria, and viruses,[37,38] it is reasonable to hypothesize that G-CSF administration after transplantation may, at least partly, contribute to the post-graft immune deficiency syndrome and to the risk of dying from transplantation-related complications.

Only two of the seven patients given a T-cell-depleted allograft from an HLA-disparate relative died; the remaining patients benefited from sustained engraftment of donor hematopoiesis which led to normalization of blood counts, without any evidence of GVHD. This result suggests that transplantation of megadoses of T-cell-depleted CD34$^+$ cells may be considered in patients lacking a suitable related or unrelated HLA-compatible donor, especially if patients are transplanted in an experienced center able to offer adoptive immunotherapy for the prevention/treatment of infectious complications.[16,39] It is reasonable to speculate that high doses of normal hematopoietic progenitors, having a growth advantage in comparison to progenitors of FA patients, contributed significantly to the favorable outcome of our patients.

In conclusion, the results of the present study indicate that the outcome of FA patients given HSCT from an UD have become comparable in recent years to that of patients transplanted from an HLA-identical sibling. Moreover, they suggest that there is an advantage from using fludarabine in the preparation for the allograft. Although obtained in a limited number of patients, the results of patients given a T-cell-depleted HSCT from an HLA-partially matched relative are also encouraging and deserve further investigation.

Appendix

The following AIEOP-HSCT centers and investigators reporting data on the patients included in this analysis (the number of patients transplanted in each single Center is given in brackets):

Oncoematologia Pediatrica, Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Franco Locatelli, Marco Zecca, Giovanna Giorgiani, Maria Ester Bernardo (28 patients) Dipartimento di Ematologia e Oncologia, Ospedale Giannina Gaslini, Genova, Giorgio Dini, Edoardo Lanino, Giuseppe Morreale, Carlo Dufour (9 patients) Divisione di Ematologia, Ospedale di Pescara, Paolo Di Bartolomeo (6 patients) Clinica Pediatrica, Università di Milano-Bicocca, Ospedale Nuovo San Gerardo, Monza, Cornelio Uderzo, Adriana Balduzzi, Daniela Longoni, Giuseppe Masera (5 patients) Clinica Pediatrica, Spedali Civili, Università di Brescia, Fulvio Porta, Alessandro Plebani (4 patients) Clinica Pediatrica Università di Padova, Chiara Messina, Simone Cesaro, Modesto Carli (4 patients) Clinica Pediatrica, Ospedale Regina Margherita, Università di Torino, Franca Fagioli, Enrico Madon (3 patients) Clinica Pediatrica, Università di Cagliari, Franca Argiolu, Renzo Galanello (2 patients) Oncoematologia Pediatrica, Ospedale Santa Chiara, Pisa, Claudio Favre, Piergiorgio Macchia (2 patients) Oncoematologia Pediatrica, Ospedale Pausilipon, Napoli, Mimmo Ripaldi, Vincenzo Poggi (1 patient).

Authors' contribution

FL performed the HSCT, designed the study and wrote the manuscript. AP, MZ and MEB collected the data and performed the statistical analysis. MZ, GM, PDB, DL, FP, MEB and CM performed the HSCT. BN contributed to the design of the study. All authors read and approved the definitive version of the manuscript.

Conflicts of Interest

The authors reported no potential conflicts of interest.

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