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CLINICAL RESEARCH

Bone Marrow Transplantation for Fanconi Anemia Using Fludarabine-Based Conditioning

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In the mid-1990s, we introduced a fludarabine (Flu)-based conditioning regimen for hematopoietic stem cell transplantation (HSCT) in patients with Fanconi anemia (FA).The aim of this study is to compare Flu-based conditioning to alternative regimens in patients with FA. Forty-one patients with FA (aged 0.5-31, median, 10.3 years) who underwent allogeneic HSCT were included in this retrospective study. Hospital records were reviewed for conditioning regimens, engraftment data, and toxicity. The median (range) follow-up was 32 (0.5-149) months. Flu-based conditioning regimens were used in 24 patients: 17 patients were treated with alternative conditioning regimens including a radiation-based regimen/cyclophosphamide and busulfan regimen. The disease-free survival (DFS) after Flu-based regimens is 83% (20/24) versus 35% (6/17) for the alternative regimens ($P = .002$). Toxicity was significantly lower in patients who received Flu-based conditioning (modified Bearman toxicity score [$P = .001$]). Seven patients received transplants from matched unrelated donors without irradiation (5 of whom are currently alive and well). All patients who survived are disease free and in good clinical condition. We conclude that a combination of fludarabine with antithymocyte globulin (ATG) and low-dose cyclophosphamide (Cy) and/or busulfan (Bu) is safe, demonstrates low rejection rates, and is well tolerated by FA patients.

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INTRODUCTION

Fanconi anemia (FA) is a part of a group of inherited bone marrow failure disorders that was initially described in 1927 by Guido Fanconi. FA is a heterogenous group of diseases with variable genotypes and phenotypes with defective DNA repair mechanisms. FA usually manifests clinically with congenital malformations and with progressive development of severe aplastic anemia and myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). The diagnosis of FA is confirmed by chromosome breakage tests (diepoxybutane-DEB test) and is supported by identification of a defect in a specific complementation group and by mutation analysis. Hematopoietic stem cell transplantation (HSCT) is the only treatment with a curative potential for the hematologic manifestations in this severe congenital disease [1-3]. Early experience with HSCT in the treatment of FA was unsuccessful because of the basic molecular defect in these patients: an excessive sensitivity to cytotoxic agents that led to severe radiation and chemotherapy-related toxicty, and consequently, a high rate of treatment-related mortality (TRM) as well as unusually severe graft-versus-host disease (GVHD) [4,5]. More recent publications have presented convincing evidence supporting the use of reduced-intensity conditioning (RIC) as a preparative regimen in HSCT of FA patients. More than 10 years ago we introduced a fludarabine (Flu)-based conditioning for HSC and umbilical cord blood transplantation [6,7] for FA. This approach dramatically improved the results of FA treatment. Following initial case reports and a small series [8-10], larger studies were published that demonstrated a marked decrease in transplant-related mortality and a significant increase in disease-free survival (DFS) [11-14].

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Here, we present an update from 3 medical centers on our experience in HSC transplantation for FA focusing on a Flu-containing preparative regimen in which no irradiation or T cell depletion was used.

MATERIALS AND METHODS

Patient Characteristics

Forty-one patients with FA (16 males, 25 females) who underwent 46 HSCTs in the 3 participating medical centers from June 1993 until September 2007 were included in this retrospective study. Clinical diagnosis of FA was confirmed by DEB test, complementation group tests, and molecular analysis in some of the patients. All patients or guardians signed informed consent. The demographic and clinical data are presented in Table 1. All patients but 1 were in the pediatric age group (<21 years). Approximately half of the patients had more than 3 congenital abnormalities. At the time of diagnosis, the majority of patients had severe aplastic anemia (SAA), whereas 6 had clonal disease.

Transplant and Donor Characteristics

We describe 2 groups of patients according to their conditioning regimens. The first group (group 1, n = 17) received Flu-free protocols, the second group (group 2, n = 24) was conditioned with a Flu-based protocol. Details are presented in Table 1.

In group 1 (n = 17) 14 donors were fully matched family members (12/14 were siblings, 2/14 full matched parents). Three patients were transplanted from matched unrelated donors. In group 2 (n = 24), 16 patients were transplanted from matched family donors (MFD) (14 of 16 from matched siblings, 1 of 16 from a full matched parent and 1 of 16 from a matched cousin). Seven patients were transplanted from a matched unrelated donor (MUD), 2 of 7 were mismatched in 1 or 2 loci, and 1 of 7 underwent 2 bone marrow transplantations (BMTs) from 2 different matched unrelated donors. One patient was transplanted from a 2-locus mismatched umbilical cord blood, developed primary graft failure, underwent a second haploidentical transplantation as a rescue, and died from multiorgan failure.

Peripheral blood stem cell (PBSC) donors were injected subcutaneously with granulocyte colony-stimulating factor (G-CSF, filgrastim or Neupogen (Roche, Israel), 5 μg/kg twice daily for 5 days) and mobilized PBSC were collected on days 5 and 6. Bone marrow (BM) harvesting was performed under regional or general anesthesia from the posterior ileum. Unmanipulated PBSC and BM served as the source of stem cells in 6 and 29 patients, respectively; a combination of BM and PBSC was used in 2 cases, 1 patient underwent BMT from unrelated umbilical cord blood (UCB), and in 3 cases, a combination of BM and cord blood from matched sibling donor was the source of cells for transplantation (Table 1). All MFD had a negative DEB test. Both groups were statistically similar

Table 1. Patients, Transplant, and Graft Characteristics; Details in Text Body

	Group 1	Group 2
Number of patients	17	24
Number of BMT	18	28
Age at BMT	10.2	9
	(0.5-17.3)	(3.5-30.8)
M:F	8/9	8/16
Congenital abnormalities (>3)	7	11
Hematology		
AA	6	3
SAA	8	18
MDS	1	2
AML	2	1
Protocols		
Bu8Cy40	3	
Cy10 $_{nf}$TAI5	7	
Cy40 $_{f}$TLI15	1	
Bu8Cy60ATG(Fresenius)40	1	
Cy20TAI400/500±ATG	5	
Bu4Flu150Atgam90		7
Bu2Flu150Atgam90		1
Flu180Cy20ATG(Fresenius)40/C-1H30		8
Flu180Cy20Bu4ATG(Fresenius)40		4
Flu150Cy20ATG(Fresenius)40		3
Flu150TBI200		1
GVHD prophylaxis		
CsA (3 mg\kg) only	16	14
CsA + MMF		2
Daclizumab (1 mg/kg+4,+14,+28)		
CsA (3 mg\kg)+daclizumab (1 mg\kg+4,+8,+14,+28)	1	7
FK506+MMF(30 mg/kg)+daclizumab (1 mg/kg+4,+14,+28)		1
Stem cell source		
BM	14	16
PBSC	1	5
UCB	1	1
BM + PBSC	1	
BM + UCB		2
TNC: average ± SD	6.8 ± 5.0	11.2 ± 13.9
CD34: med (max-min)	8.7 ± 7.6	9.1 ± 8.9

AA indicates aplastic anemia; SAA, severe aplastic anemia; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; Bu8Cy40, busulfan 8 mg/kg; cyclophosphamide, 40 mg/kg; Cy10 $_{nf}$TAI5, cyclophosphamide10 mg/kg, nonfractionated thoracoabdominal irradiation 500 cGy; Cy40 $_{f}$TLI15, cyclophosphamide 40 mg/kg, fractionated total lymphoid irradiation 500 cGy; Bu8Cy60ATG(Fresenius)40, busulfan 8 mg/kg, cyclophosphamide 60 mg/kg, rabbit antithymocyte globulin 40 mg/kg; Cy20TAI400/500 ± ATG, cyclophosphamide 20 mg/kg, non-fractionated thoracoabdominal irradiation 400-500 cGy with or without antithymocyte globulin; Bu4Flu150Atgam90, busulfan 4 mg/kg, fludarabine 150 mg/kg, horse antithymocyte globulin 90 mg/kg; Bu2Flu150Atgam90, busulfan 2 mg/kg, fludarabine 150 mg/kg, horse antithymocyte globulin 90 mg/kg; Flu180Cy20ATG(Fresenius)40/C-1H30, fludarabine 150 mg/kg cyclophosphamide 20 mg/kg, rabbit antithymocyte globulin 40 mg/kg or Campath-1H 30 mg/kg; Flu180Cy20Bu4ATG(Fresenius)40, fludarabine 180 mg/kg cyclophosphamide 20 mg/kg, busulfan 4 mg/kg, rabbit antithymocyte globulin 40 mg/kg; Flu150Cy20ATG(Fresenius)40, fludarabine 150 mg/kg cyclophosphamide 20 mg/kg, rabbit antithymocyte globulin 40 mg/kg; Flu150TBI200, fludarabine 150 mg/kg, total-body irradiation 200 cGy; CsA, cyclosporine A; MMF, mycophenolate mofetil; BM, bone marrow; PBSC, peripheral blood stem cells; UCB, umbilical cord blood; TNC, total nucleated cells.

regarding participant's age at transplantation, the pathology for which the patient was treated, the time elapsed between diagnosis and BMT, the relationship and gender of the donor and the patient, the nuclear cell counts of the graft, and the cell source.

GVHD Prophylaxis and Treatment

The majority of patients (n = 30) received GVHD prophylaxis with cyclosporine A (CsA) 3 mg/kg per day intravenously as a single agent starting on day −1. Two patients received combined treatment with CsA and mycophenolate mofetil (MMF) as a part of GVHD prevention; in 8 cases, daclizumab was given once weekly starting from day +8 four times in combination with CsA; and in 1 case, a combination of FK 506 with daclizumab was used. All but 1 patient treated with a Flu-based preparative regimen received antithymocyte globulin (ATG), either rabbit ATG-Fresenius 40 mg/kg or horse ATG (Atgam) 90 mg/kg total dose (in 1 case, MabCampath-1H (Genzyme, Cambridge, MA, USA) 30 mg/kg was used), whereas non-Flu–based regimens did not include ATG except 3 cases (Table 1).

CsA administration in patients without signs of GVHD was continued for at least 6 months and was gradually reduced thereafter.

Acute and chronic GVHD (aGVHD, cGVHD) were defined using published criteria [15]. Immediately upon the appearance of signs and symptoms of GVHD, methylprednisolone (2 mg/kg) and CsA were administered.

Supportive Treatment

Before transplantation, all patients received trimethoprim/sulfamethoxazole, acyclovir and allopurinol during the conditioning. Patients were isolated in rooms equipped with HEPA filters and received a regular diet. Additional supportive measures, such as total parenteral nutrition and blood component transfusion, were administered as necessary. Cytomegalovirus antigenemia (pp65) or polymerase chain reaction (PCR) were performed weekly. and in case of reactivations, preemptive antiviral treatment was started.

Organ toxicity evaluation was performed using the modified Bearman toxicity score and included 8 organs (central nervous system, heart, lungs, liver, gut, kidneys, bladder, and mucosa). Toxicities were classified into 3 grades—mild, moderate, and severe—and were reported from the beginning of the conditioning regimen until 90 days after transplantation (see Table 2). Events because of infections or aGVHD were not included [16,17].

Chimerism Analysis

To assess engraftment, the degree of chimerism was monitored weekly by donor and host-specific DNA markers, using male and female amelogenin gene PCR bands, and by variable number tandem repeats (VNTR) or Short tandem repeats (STR)-PCR assay [18,19].

Table 2. Toxicity Grading According to Modified Bearman Criteria [16]

CNS	1. easily woken somnolence, mild EEG changes, transient diplopia; 2. heavily somnolence, confusional status, neurologic impairment, optic neuritis; 3. seizures, coma
Heart	1. mild ECG or X-ray changes, arrhythmias, transient hypertension; 2. same abnormalities as above but more severe and requiring treatment; 3. unresponsive cardiac failure.
Lungs	1. asymptomatic X-ray changes; 2. dyspnea and hypoxia (percutaneous O_2 saturation less than 90%) because of disventilation; 3. ARDS, need of mechanical ventilation.
Liver	1. bilirubin 2-5 mg/dL, AST/ALT 80-200 U/L, liver enlargement <1 in, weight gain <10%; 2. bilirubin >5 mg/dL, AST/ALT >200 U/L, liver enlargement >1 in, weight gain >10%, ascites; 3. severe VOD, liver failure, coma.
Gut	1. vomiting, diarrhea less than 30 mL/kg, abdominal pain; 2. hematemezis, melena, diarrhea more than 30 mL/kg and/or persisting >10 days; 3. ileus.
Kidneys	1. increased creatinine less than twice basal value; 2. increased creatinine more than twice basal value; 3. dialysis.
Bladder	1. macrohematuria; 2. hemorrhagic cystitis; 3. severe hemorrhagic cystitis (>10 days).
Mucosa	1. mild stomatitis; 2. severe stomatitis requiring morphine (15 days); 3. intubation.

ARDS indicates acute respiratory distress syndrome; VOD, veno-occulsion disease.

Statistical Methods

Data were analyzed using Microsoft Excel and MedCalc (MedCalc Software, Mariakerke, Belgium).

Assessments of median, average, standard deviation, and Student's *t*-test with 2-tailed distribution were used as parametric criteria, whereas categoric data were compared by chi-square or Fisher exact tests.

The probabilities of engraftment and OS were plotted using the Kaplan-Meier method. The significance was estimated by log-rank test and logistic regression analysis. Chi-square was used to analyze some survival predictors.

RESULTS

Engraftment

Engraftment characteristics are shown in Figure 1. Following the Flu-based conditioning, the time to engraftment was significantly longer: time to an absolute neutrophil count (ANC) $>0.5 \times 10^9$/L for patients from group 1 or group 2 was 12.6 ± 3.6 and 20.3 ± 10 days, respectively ($P = .001$): patients from group 1 reached ANC $>0.5 \times 10^9$/L earlier than group 2

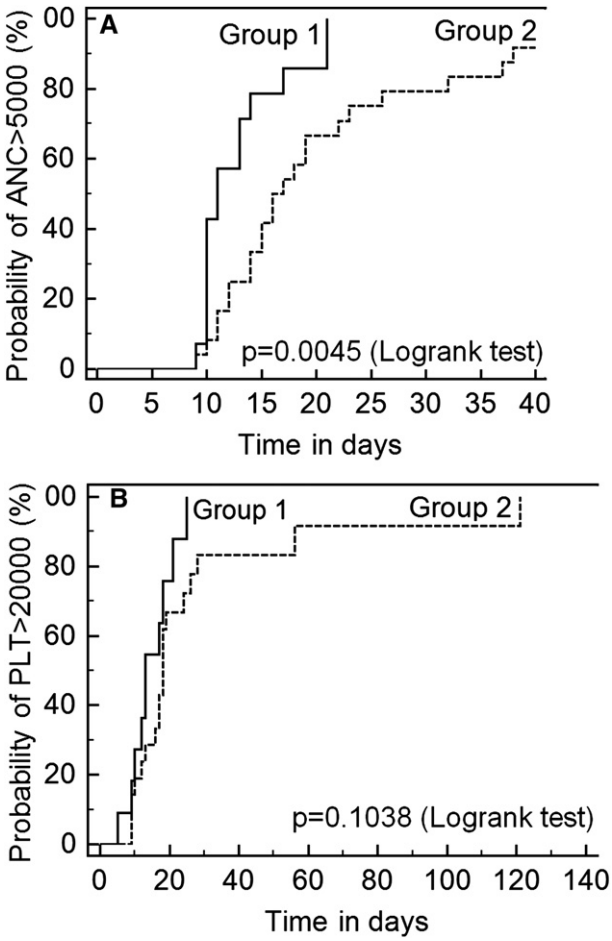

Figure 1. Probability of achieving (A) ANC $>0.5 \times 10^9$/L and (B) platelets $>20 \times 10^9$/L in the 2 treatment groups.

(Figure 1A). Similarly, the average time from engraftment to the presence of platelets $>20 \times 10^9$/L for group 1 and group 2 was 15 ± 5.6 and 24.4 ± 20 days, respectively (Figure 1B). This difference was not found to be statistically significant ($P = .09$).

Two patients in the Flu group demonstrated primary graft failure, both after transplantation from unrelated donors. The first 1 was a 21-year-old woman who was transplanted from a 2 locus mismatched donor. She developed neutropenic fever and sepsis and died 28 days after transplantation. The second was a 3.5-year-old girl who rejected her first graft, restored her own hematopoiesis, and 120 days after the first transplantation was retransplanted from another MUD following a regimen containing Flu, busulfan, and MabCampath. Currently, 120 months after transplantation, she is alive and clinically well. All engrafted patients demonstrate long-term 100% donor chimerism.

Toxicity

Toxicity was related to the type of protocol used. The grade of toxicity was lower among those in group 2: the cumulative toxicity score in group 1, was 10.6 ± 1.4, whereas the average toxicity score among patients from group 2 was 3.9 ± 0.8 ($P = .001$).

This difference was statistically significant for all organs ($P << .05$) except for the central nervous system (CNS) and gut (in which the difference was seen but was not found to be statistically significant). In group 1, the most frequent toxicities were reported to be related to mucosa, kidneys, and bladder (Table 3). The number of proven infections was lower in the second group ($P = .026$ in the *t*-test) despite a later ANC engraftment, probably because of lower mucosal toxicity.

Acute GVHD

In the first group, 14 of 17 patients (82%) developed aGVHD grade 2-4, 8 of them (47%) grade 3-4. In the second group, 11 of 24 patients (46%) developed aGVHD grade 2 or more; of them, only 1 developed grade 3, and none developed grade 4; these differences were statistically significant ($P = .015$).

A strong association was found between the type of conditioning protocol used and both the development of aGVHD, as well as the grade of the GVHD: there was a lower prevalence of severe GVHD in patients who received the Flu-based conditioning ($P = .002$; Table 4).

Comparison of aGVHD prevalence in groups excluding patients who received daclizumab demonstrates that the prevalence and severity ($P = .001$ and $P = .005$ accordingly; chi-square test) of this complication are still lower in group 2. Moreover, when comparing the subsets of patients within group 2 between those who received daclizumab and those who did not, it was found that both the prevalence and the severity of GVHD in the subgroup that received daclizumab was higher ($P = .043$ and $P = .059$, both in the Fisher exact test).

Chronic GVHD

Chronic GVHD was diagnosed in 36.7% patients (extensive in 6.7%). In group 1, 4 of the 6 patients (66%) who survived suffered from cGVHD, 2 of whom suffered from the extensive form. In group 2, development of cGVHD was observed in 8 of the 20 survivors (40%), and only 2 of them experienced extensive disease (NS).

No statistically significant differences were found between the groups, neither in the rate nor in the severity of the cGVHD (30 valuable patients).

Overall survival (OS)

A profound difference in OS (which was equal to DFS) was found between the 2 study groups. In group 1, 6 of the 17 patients (35%) survived compared to 20 of the 24 patients (83%) in group 2 ($P = .002$).

Table 3. Comparative Toxicity by Grade for Each Organ of 2 Treatment Groups

	CNS	Heart	Lungs	Liver	Gut	Kidneys	Bladder	Mucosa	Cumulative Toxicity
Group 1	1.13	1.	1.33	1.50	1.14	1.53	1.33	2.13	10.6
Group 2	0.39	0.27	0.30	0.61	0.87	0.35	0.14	0.83	3.9
***P* (t-test)**	.094	**.050**	**.018**	**.020**	.25	**.002**	**.004**	**.004**	**.001**

The odds ratio (OR) for OS in group 1 was 0.289 (95% confidence interval [CI]: 0.099-0.83) compared to group 2. Kaplan-Meier survival estimates are modeled in Figure 2. GVHD prophylaxis protocol in itself was not associated with increased survival rate and CsA alone or in combination with methotrexate, and anti-CD25 mAb showed similar long-term survival.

Causes of death in group 1 were transplant related in 10 out of 11 patient: in 8 cases death resulted from aGVHD-related causes, 1 death from cGVHD-related cause (bronchiolitis obliterans), 1 patient developed a secondary malignancy, and 1 patient died on day +1, before engraftment, from sepsis and multiorgan failure.

In group 2, two patients died from sepsis, 1 from toxicity, and 1 from cGVHD; these 4 deaths were considered transplant-related mortality.

BMT from MUD

In long-term follow-up posttransplant, 5 of the 7 (71%) patients that underwent BMT from MUD are alive and well with normal blood counts and full donor chimerism. Two died (a 3-year-old girl with multiple congenital anomalies and a 21-year-old woman who was transplanted from a 2-loci mismatched donor).

All these patients underwent BMT after Flu-based conditioning without use of irradiation, and all but 1 were conditioned with a regimen containing Flu, cyclophosphamide, and a relatively small dose of busulfan.

DISCUSSION

Flu-based regimens for HSC transplantation for FA were introduced at the Hadassah-Hebrew University Medical Center in the mid-1990s. The first cases were described by Kapelushnick et al. (successful HSCT) [6] and Aker et al. (successful related UCB) [7] followed by expanding patient series reported both from our center [8,9,20] and others [10,21,22]. Recently, larger series were published [23-26], all of them presenting different aspects of the benefit in the use of Flu-based conditioning for FA patients.

Here, we present a retrospective analysis of our experience with BMT for FA, which clearly demonstrates that Flu-ATG–based protocols give significantly better results. There are three major advantages to this regimen.

These protocols are significantly less toxic than others, in particular compared to irradiation-based treatments. Because of the tendency of FA patients to develop excessive organ toxicities during chemotherapy that could result in death, there are major limitations providing either transplantation or chemotherapy in these patients. A far better tolerance to Flu compared to other immunosuppressive or cytoreductive agents was shown in different BMT settings. Unlike cyclophosphamide or irradiation, Flu does not cause DNA cross-linking, which is not effectively controlled in FA, and therefore has a significant role in conditioning in these patients [27-29]. As shown recently in an in vitro study by Yabe et al. [30], unlike the majority of chemotherapeutic agents, Flu had no influence on induced breakage, even compared to spontaneous breakage. This explains the improved tolerability of Flu by patients with FA, as shown in all recent published series as well as in our current study. The resulting reduced toxicity during the conditioning regimen can explain the improved tolerability of the transplantation with a significantly higher OS and a lower rate of GVHD (an epiphenomenon associated with infectious complications and quality

Table 4. Evidence of Acute and Chronic GVHD in the 2 Treatment Groups

	Group 1 (n = 17)	Group 2 (n = 24)	*P* Value
Time (days) from transplantation to acute GVHD (mean ± standard deviation)	23.6 ± 17.2	34.4 ± 17.5	NS (Student test)
Acute GVHD grade (patients number)			
0	1	13	
1	2	4	.002
2	4	5	(chi-square test)
3	2	1	
4	6	0	
Chronic GVHD grade (patients number)			
No	2	10	NS
Limited	2	6	(chi-square test)
Extended	2	2	

NS indicates nonsignificant; GVHD, graft-versus-host disease.

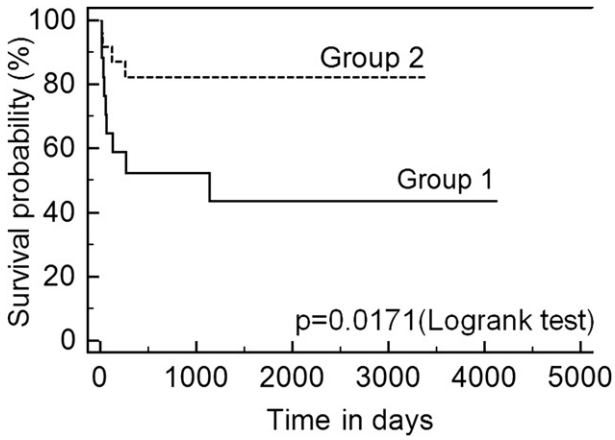

Figure 2. Probability of survival among patients with Fanconi anemia.

of life post-BMT). We suggest that Flu-ATG–based conditioning should be highly considered when BMT is required for other chromosomal fragility syndromes such as dyskeratosis congenita and Nijmegen breakage syndrome.

Patients with FA have a higher rate of aGVHD and cGVHD post-BMT, resulting in increased morbidity and mortality compared to other patients. A review of recent papers shows that T cell–depleted grafts are used for GVHD prophylaxis even in cases of 100% matched donors [10]. Our experience shows that both in the setting of matched family donors as well as in matched unrelated donors, the incidence of GVHD is dramatically lower in patients who received drug-based GVHD prophylaxis without in vitro T cell depletion. In addition to the substitution of higher doses of cyclophosphamide and irradiation with Flu, our protocol incorporated ATG as a part of the conditioning. The role of ATG in RIC regimens is still unclear. On the one hand, ATG causes host immunosuppression, which can improve engraftment, and in FA, can be used even as a single agent for a second BMT [31]. On the other hand, ATG remains in the circulation for several days, therefore causing partial in vivo donor T cell depletion, and possibly providing additional protection from GVHD [32,33]. In addition, we found that engraftment after Flu-based protocols was significantly slower. By definition, this means that there is a lower grade of cytokine production and a lower incidence of cytokine storm and engraftment syndrome, which can trigger aGVHD development [34].This may be an additional explanation to the lower incidence of GVHD compared with the other protocols. Previous studies demonstrated that the addition of anti-CD25 antibodies to standard anti-GVHD prophylaxis does not improve OS and does not decrease the rate of aGVHD, probably by elimination of CD25-positive T-regulatory cells [35]. This is consistent with our current analysis in which we found that the addition of daclizumab to the traditional prophylaxis with CsA does not reduce the rate of GVHD.

Another point worth emphasizing is the ability to eliminate irradiation even in cases of MUD. A few studies have shown that in Flu-based conditioning, stable engraftment can be achieved without the need of radiation [36,37]. Our data supports these findings.

In summary, the combination of Flu with ATG and low-dose cyclophosphamide and/or busulfan results in improved long-term OS and in a lower complication rate in patients transplanted for FA. Regimens containing busulfan (4 mg/kg) or cyclophosphamide (20 mg/kg) demonstrate similar toxicities and engraftment rates, whereas irradiation-based regimens should be avoided in FA.

In conclusion, our study demonstrates three important points. First, use of Flu-ATG–based protocols dramatically improves OS with low rejection rates and excellent toxicity profile, and can therefore be strongly recommended for allogeneic transplantation for FA. Second, elimination of irradiation in the setting of MUD seems feasible when using Flu-ATG–based protocols, thus achieving stable engraftment without excessive toxicity. And finally, it seems that augmentation of GVHD prophylaxis with additional modalities, such as T cell depletion or combination with daclizumab and methotrexate (MTX), gives no advantage over the CsA and ATG regimen, either in the effect on the rate or the severity of GVHD. We therefore suggest that CsA in combination with ATG-containing regimens may be sufficient in prevention of GVHD, especially in cases of transplantation from MFD. Development of Flu-based protocol details, such as comparing busulfan versus cyclophosphamide, details of GVHD prophylaxis, reduction of the Flu dose, and the type and dose of ATG used, is still an actual immediate goal. Additional trials for elimination of irradiation as a part of conditioning in the setting of MUD warrant further investigation in larger groups of patients.

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