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Original article

Fludarabine-based reduced intensity regimen for matched related donor hematopoietic stem cell transplantation in acquired severe aplastic anemia



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ABSTRACT

Different conditioning regimens have been evaluated in matched-related donor allogeneic hematopoietic stem cell transplantation (allo-HSCT) for acquired severe aplastic anemia (SAA) with varying results. In this manuscript, we report our experience with fludarabine (120 mg/m²), very low dose cyclophosphamide (1200 mg/m²) and antithymocyte globulin (7.5 mg/kg). Low dose total body irradiation (2 Gy) was added to the conditioning regimen for patients older than 15 years. Nineteen patients (median age 23 years) underwent transplant between 2008 and 2015. The majority (89%) were younger than 40 years. Stem cell source was BM ($n = 11$) or PBSC ($n = 8$). GvHD prophylaxis consisted of cyclosporine and either a short course of methotrexate ($n = 9$) or mycophenolate mofetil ($n = 10$). Eighteen (94.7%) patients achieved sustained engraftment. The median times to neutrophil and platelet engraftments were 19 (range: 14–34) and 17.1 (range: 12–25) days, respectively. The day-30 cumulative incidence of neutrophil and platelet engraftment was 89.4% and 94.7%, respectively. No secondary graft rejection was observed. The 1-year cumulative incidence of aGvHD (grade II–IV) and cGvHD was 11.7% and 0%, respectively. The 2-year GvHD-free survival rate was 78.6% (95% CI: 52.5–91.4%). Fludarabine-based reduced intensity regimen for MRD allo-HSCT in SAA compares favorably to other available regimens. This regimen deserves further investigations with larger cohort of patients.

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1. Introduction

Outcome of severe acquired aplastic anemia (SAA) has improved dramatically over the past two decades due to better regimens for allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1,2]. Different conditioning regimens have been evaluated in matched-related donor (MRD) allografts with varying results. Before 2008, we encountered low incidence of day-30 engraftment in 3 patients and one death due to graft failure when using the standard conditioning regimen of high dose cyclophosphamide (200 mg/kg) and antithymocyte globulin (ATG) [3]. Srinivasan et al described outcomes following fludarabine in

combination with cyclophosphamide (120 mg/kg) with encouraging engraftment results; however, a high incidence of acute and chronic GvHD was reported [4]. Fludarabine, very low dose cyclophosphamide (1200 mg/m²) and ATG was pioneered by Bacigalupo et al. for alternative donor transplants with resulting graft failure rate of up to 18% and a 2-year overall survival of 73% [5]. Since 2008, we adopted this regimen in addition to low dose total-body irradiation (TBI) for patients older than 15 years for MRD transplants aiming at improving engraftment and ultimately achieving better survival.

2. Methods

This is a retrospective review of all consecutive patients diagnosed with acquired SAA who underwent allo-HSCT between August 2008 and December 2015 at the American University of Beirut Medical Center (AUBMC). Patients transplanted for other forms of marrow failure (Fanconi anemia, pure red cell aplasia, dyskeratosis congenita) were excluded. This study was approved by our Institutional Review Board and was conducted in accordance with the declaration of Helsinki.

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2.1. Conditioning regimen

The conditioning regimen consisted of fludarabine 30 mg/m²/day over 4 days (–6, –5, –4 and –3), intravenous cyclophosphamide 300 mg/m²/day over 4 days (–6, –5, –4 and –3) and ATG (Genzyme rabbit) 3.75 mg/m²/day over 2 days (–4 and –3). TBI at a dose of 2 Gy was added on day –1 for patients older than 15 years.

2.2. GvHD prophylaxis

GvHD prophylaxis consisted of cyclosporine and either a short course of intravenous methotrexate (15 mg/m² on day +1 and 10 mg/m²/day on days +3 and +6) for 9 patients or mycophenolate mofetil (600 mg/m²/dose) twice daily from day +1 until day +30 for 10 patients. Weaning of cyclosporine was started on day +100 whenever feasible.

2.3. Stem cells source

Stem cell source was either bone marrow (BM) or G-CSF mobilized peripheral blood stem cells (PBSC). The decision to use PBSC or BM was at the discretion of the primary transplant physician.

2.4. Supportive care

All patients were confined to HEPA-filters rooms adhering to isolation criteria. Prophylaxis for herpes simplex and fungal infections using acyclovir and voriconazole or fluconazole was provided to all patients until day 30. Prophylaxis for *Pneumocystis jirovecii* using trimethoprim-sulfamethoxazole was prescribed until day –1 then resumed on day +30 (if hematologic parameters allowed) for a minimum of 6 months. CMV and EBV screening by PCR were performed once weekly until day +100. Prophylactic transfusion with leukofiltered irradiated blood products was prescribed for hemoglobin level below 8 mg/dl and platelet count of less than 20,000/ μ L or if there was evidence of bleeding regardless of the platelet counts.

2.5. Response criteria and definitions

Neutrophil engraftment was defined as the first of three consecutive days with an absolute neutrophil count (ANC) of over 0.5×10^9 /L. Platelet engraftment was defined as the first of five consecutive days of untransfused platelets with a count higher than 20×10^9 /L. Overall engraftment rate is defined as the percentage of patients who have both neutrophil and platelet engraftment at any time post-transplant. Late neutrophil/platelet engraftment is defined as an engraftment requiring more than the median time of neutrophil/platelet engraftment for the whole cohort of patients. Chimerism studies were available for all engrafted patients using multiplex PCR amplification of 15 individual STR loci and the amelogenin gender determining marker locus followed by fragment analysis using capillary electrophoresis and fluorescence detection. Regimen-related toxicity was graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 4 [6]. GVHD was graded according to the modified Seattle criteria [7].

2.6. Statistical analysis

Overall survival analysis was performed using the Kaplan-Meier method. Cumulative incidence of engraftment and GvHD were estimated using competing risk model. Univariate and multivariate analysis of factors affecting survival was performed with Cox-regression model. Univariate and multivariate analysis of factors affecting early/late engraftment was performed using logistic regression model. The required *P*-value for entering into the multivariate analysis was < 0.05. A two-sided *P*-value of less than 0.05 was considered statistically significant. All tests were performed using SPSS version 24 for Windows, except cumulative incidence which was done with EZR, version 2.3 for Windows. Graphs were drawn with GraphPad Prism for Windows, version 7.03.

3. Results

3.1. Patients

A total of 19 consecutive patients underwent allo-HSCT from related HLA-matched donors. The median age of patients was 23 years (range: 1.7–64 years). The majority (17/19) were younger than 40 years of age. Male to female ratio was (2.8:1). All donors were siblings except one who was an HLA high-resolution-fully-matched cousin. Median duration between diagnosis and allo-HSCT was 12.2 months (range: 1–83 months). Information about

Table 1
Patient characteristics.

Number of patients	19
Age by years, median (range)	23 (1.7–65)
Age < 40	17 (89%)
Male/female	2.8/1
Interval between diagnosis and transplant by months, median (range)	12.2 (1–83)
> 12 months	7 (36%)
Failed previous IST	5 (26%)
Donor-recipient sex-mismatch	8 (42%)
Female to male	5 (26%)
Graft CD34 dose $\times 10^6$ /kg, median (range)	5.8 (1–19.5)
Stem cell source (PBSC/BM)	8/11
Age > 15	12 (63%)
TBI	12 (63%)

BM: bone marrow; PBSC: G-CSF mobilized peripheral blood stem cells; TBI: total body irradiation; IST: immunosuppressive therapy.

prior transfusion exposure was not available because most patients were referred from other medical facilities. Five (26%) had failed immunosuppressive therapy (IST) prior to allo-HSCT. Four received cyclosporine as a single IST agent, and one had received cyclosporine in combination with rabbit ATG for 8 cycles. None of the patients had undergone a prior allo-HSCT. Patient characteristics are summarized in (Table 1).

3.2. Stem cell dose, engraftment and chimerism

The stem cell source was BM for 11 patients (58%) and PBSC for the remaining 8 patients (42%). The median CD34 cell dose infused was 5.8×10^6 /kg (range: $1–19.5 \times 10^6$ /kg). The median time for neutrophil engraftment was 19 days (range: 14–34 days); and the median time for platelet engraftment was 17.1 days (range: 12–25 days) (Fig. 1). The day-30 cumulative incidence of neutrophil and platelet engraftment was 89.4% (95% CI: 60.9–97.2%) and 94.7% (64.5–99.2%), respectively. Late neutrophil and platelet engraftment (see definition in methods) occurred in 7/19 and 8/19 patients, respectively. Factors associated with late engraftment were analyzed. Results are shown in (Table 2). None of the factors reached statistical significance for late neutrophil engraftment. The only statistically significant factor for late platelet engraftment was the use of BM as the source of stem cells compared to PBSC. The overall engraftment rate was 94.7%. One patient with severe invasive aspergillus infection at the time of allo-HSCT died before engraftment. No secondary graft rejection was observed. Two patients had mixed donor chimerisms (89% and 76%) while the remaining 16 engrafted patients had complete donor chimerism (> 95%) at a median of 214 days (range: 30–1080 days) from transplant.

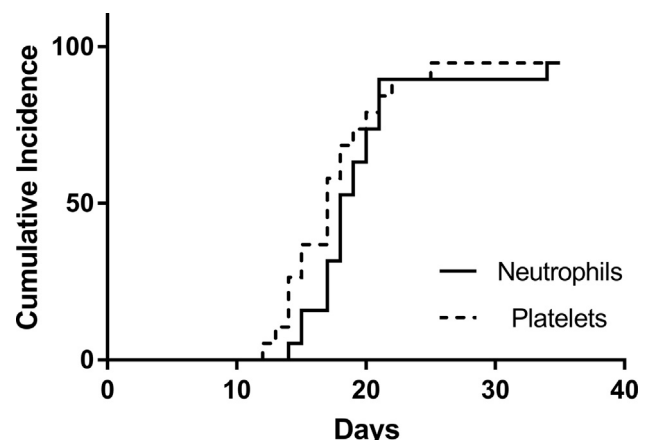


Fig. 1. Cumulative incidence of neutrophil and platelet engraftment.

Table 2
Univariate logistic regression for late engraftment.

Variables	Neutrophils		Platelets	
	OR (95% CI)	P-value	OR (95% CI)	P-value
<i>Prior IST</i>				
No	Reference	0.17	Reference	0.96
Yes	4 (0.54–29.2)		1.05 (0.15–6.92)	
<i>TBI</i>				
No	Reference	0.67	Reference	0.31
Yes	0.66 (0.09–4.54)		0.37 (0.05–2.55)	
<i>Time to transplant^a</i>				
< 1 year	Reference	0.17	Reference	0.08
> 1 year	4 (0.54–29.2)		0.11 (0.01–1.32)	
<i>Stem cell source</i>				
PBSC	Reference	0.36	Reference	0.04
BM	2.5 (0.34–18.3)		12.2 (1.08–139)	
<i>CD34 cell dose (10⁶/kg)</i>				
> 5.8	Reference	0.22	Reference	0.84
< 5.8	3.5 (0.47–25.9)		0.83 (0.13–5.17)	
<i>Sex mismatch^b</i>				
No	Reference	0.96	Reference	0.55
Yes	1.05 (0.15–6.92)		1.75 (0.27–11.2)	

IST: immunosuppressive therapy; TBI: total body irradiation; BM: bone marrow; PBSC: G-CSF mobilized peripheral blood stem cells.

^a Interval between diagnosis and transplant.

^b Between donor and recipient.

3.3. Toxicity

Febrile neutropenia complicated the transplant course in 11 patients (58%). Severe mucositis (grade III–IV) was observed in 7 patients (37%). CMV reactivation occurred in 3 patients while CMV disease was observed in one case. BK virus hemorrhagic cystitis occurred in one patient. Post-transplant EBV reactivation was not observed in our cohort. One patient developed donor cell precursor B-cell acute lymphoblastic leukemia (Pre-B ALL) 23 months after allo-HSCT. No constitutional bone marrow failure was identified in the concerned family, and the donor is still free of leukemia until the last follow-up. At the last follow-up, none of the alive patients developed cardiac, pulmonary or neurologic toxicities, in the TBI and the TBI-free groups.

3.4. Acute and chronic GVHD

The 1-year cumulative incidence of aGVHD (grade II–IV) was 11.7% (95%: 0–25.8%) (Fig. 2). Two patients developed grade II aGVHD at days 65 and 95 from transplant. There were no patients with mild aGVHD that did not require therapy. None of the patients developed chronic GVHD at the time of the last follow-up.

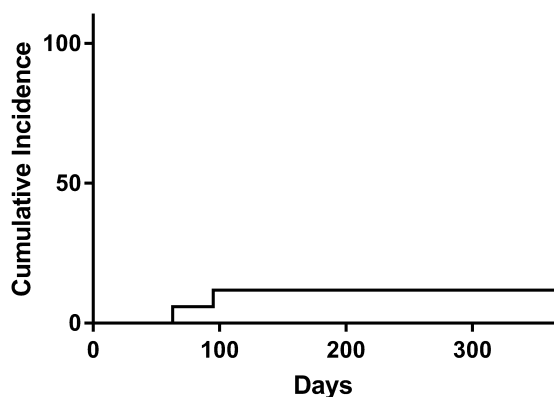


Fig. 2. Cumulative incidence of grade II–IV acute graft-versus-host disease.

3.5. Survival

At a median follow-up of 47 months (range: 19–95 months), the 2-year GvHD-free survival was 78.6% (95% CI: 52.5–91.4%) (Fig. 3). One mortality occurred at day +36 due to sepsis in the setting of graft failure. The second death was due to vancomycin resistant enterococcus (VRE) sepsis at day +40 after engraftment. Two patients died beyond day +100. One patient died at day +193 while receiving immunosuppressive therapy for aGVHD that occurred at day +95. He had CMV disease and severe sepsis that caused acute deterioration, and subsequently multi-organ failure and death. One patient died at 23 months after she developed donor cell pre-B ALL. The death was secondary to leukemia treatment-related toxicity. Using univariate analysis with Cox-regression model considering the previous failure of IST, the use of TBI, donor-recipient sex-mismatch, the long interval between diagnosis and transplant (> 1 year) and the use of PBSC as the source of stem cells, none of previous variables were associated with statistically significant worse survival (Table 3). None of the variables reached statistical significance needed for multivariate analysis.

4. Discussion

Allo-HSCT is the treatment of choice for SAA if matched-related donor is available [1]. Outcomes of allo-HSCT for SAA have improved remarkably over the past years. Decrease in graft rejection rates using more effective conditioning regimens have improved outcomes; however, other short- and long-term transplant-related complications remain important concerns for limiting the success of the procedure.

We adopted fludarabine-based reduced intensity regimen favored for alternative donor transplants in published studies [5] for MRD transplants for SAA in order to overcome low engraftment rate and delayed engraftment observed at our center prior to 2008.

Fludarabine had been an important part of many preparative regimens for transplant in SAA, in combination with different doses of cyclophosphamide (ranging from 1200 mg/m² to 120 mg/kg) [4,8–16]. Anderlini et al have evaluated the use of reduced cyclophosphamide dose (50 mg/kg) in a prospective phase I–II study in unrelated transplants. The reduced dose yielded an engraftment rate of 92% with minimal toxicity [17]. Our approach

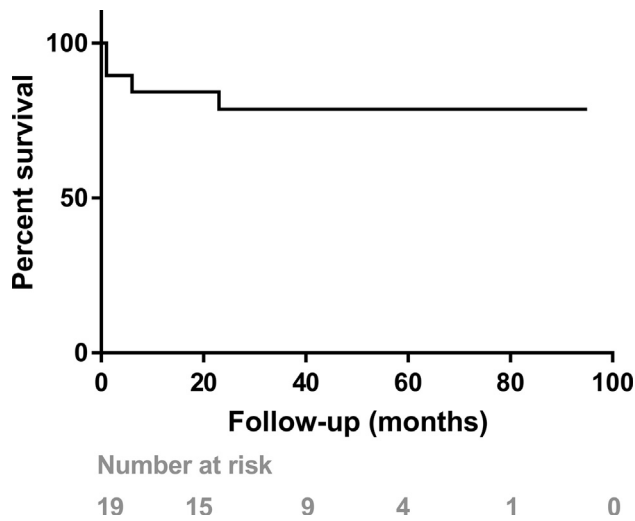


Fig. 3. GvHD-free survival for all patients.

Table 3
Univariate survival analysis.

Variables	HR (95% CI)	P-value
<i>Prior IST</i>		
No	Reference	0.56
Yes	0.51 (0.05–4.96)	
<i>TBI</i>		
No	Reference	0.57
Yes	0.57 (0.08–4.06)	
<i>Time to transplant^a</i>		
< 1 year	Reference	0.56
> 1 year	0.51 (0.05–4.96)	
<i>Stem cell source</i>		
BM	Reference	0.74
PBSC	1.38 (0.19–9.84)	
<i>Sex-mismatch^b</i>		
No	Reference	0.16
Yes	5.05 (0.52–48.8)	

IST: immunosuppressive therapy; TBI: total body irradiation; BM: bone marrow; PBSC: G-CSF mobilized peripheral blood stem cells.

^a Interval between diagnosis and transplant.

^b Between donor and recipient.

of using a low dose cyclophosphamide (1200 mg/m²) was reported in limited number of studies for MRD transplant (Table 4).

Compared to the study of Maury et al. [11], 70% of their patients were older than 40 years whereas 90% of our patients were younger than 40 years. The fact that only 2 patients in our cohort

were older than 40 years did not allow us to assess the effect of older age on transplant outcomes. Sixty-three percent of our patients received TBI whereas none of their patients received TBI. The main goal of the use of TBI in the conditioning regimen is to enhance engraftment. We avoided the use of TBI in patients younger than 15 years to prevent growth impairment and other long-term adverse effects that might complicate TBI therapy in very young patients. In addition, the rate of graft rejection was very low (5%) in very young patients (less than 15 years) using a similar TBI-free regimen with a higher ATG dose for alternative donors [5]. The rate of graft rejection appears to be similar between our current study and the report by Maury et al. [11]; however, the low number of patients prevents an accurate assessment. Bacigalupo and colleagues have studied the effect of low dose TBI on the engraftment rate using a similar conditioning regimen (with a higher ATG dose for the TBI-free group) in the setting of alternative donor transplants. Apparently, there was no difference in graft rejection between the TBI and the TBI-free groups (17% vs 17%, *P*: 0.53) [18]. Further trials are needed to study the role of TBI in this specific regimen in MRD allo-HSCT for SAA.

Notwithstanding limitations associated with retrospective analysis, the burden of GvHD observed in our study appears to be among the lowest in the literature. This could be largely explained by the fact that all donors were matched related as well as by the low intensity of the preparative regimen used. In our cohort, almost half of the patients received MMF instead of MTX in the GvHD prophylaxis regimen. This was due to the preference of the transplant physician without specific indication. Due to the low incidence of GvHD, we could not perform a valid analysis of the difference in outcome between the two regimens. Other studies have used alemtuzumab in an effort to reduce GvHD incidence and facilitate durable engraftment [19,20].

The source of stem cells (BM vs PBSC) did not affect survival in our cohort, but we acknowledge the relatively small numbers. Previous reports from the Center for International Blood and Marrow Transplant Research (CIBMTR) and European Group for Blood and Marrow Transplantation (EBMT) demonstrated worse outcomes with PBSC owing to excessive GVHD [21,22].

We did not observe any difference in overall survival between patients who underwent upfront allo-HSCT or allo-HSCT after prior IST failure. In a larger analysis, no significant difference in survival, but a higher rate of GvHD, was reported in the post IST failure group [1]. This could probably be explained by the higher proportion of unrelated or mismatched donor transplants in patients who failed IST beforehand. Other studies had reported worse outcomes in patients with delayed allo-HSCT. The inferior outcome might be explained in part by the higher rates of rejection due to the higher frequency of transfusions. The same studies had

Table 4
Previous trials with the same conditioning regimen.

	Current	Maury [11]	Anderlini [14]
Number of patients	19	30	7
Age by years, median (range)	23 (1.7–64)	46 (31–66)	49 (33–57)
Median Time-to-transplant (months)	12 (1–83)	9 (1–250)	43 (2–244)
BM/PBSC	11/8	20/10	6/1
GvHD Prophylaxis	CsA/MTX (47%) CsA/MMF (53%)	CsA/MTX (70%) Others (30%)	Not mentioned
TBI	2 Gy for > 15 yrs	None	None
Rejection ^a	5%	3%	0%
Overall survival	2-year 78.6%	5-year 77%	2-year 71%
aGvHD ^a	11.7%	10%	14%
cGvHD ^a	0%	26%	28%

CY: cyclophosphamide; BM: bone marrow; PBSC: G-CSF mobilized peripheral blood stem cells; ATG: antithymocyte globulin; Flu: fludarabine; TBI: total body irradiation; aGvHD: acute graft versus host disease; cGvHD: chronic graft versus host disease.

^a Percentage of patients at the last follow-up.

linked the worse outcome to higher number of previous transfusions [23,24]. In our analysis, the time interval between diagnosis and transplant did not appear to affect overall survival. We speculate that this regimen might be suitable for delayed transplantation in SAA.

Our study has many limitations, including the small sample size, the variability in the stem cell source, CD34 cell dose, and the GvHD prophylaxis regimen, which could alter risk, toxicity and complications of allo-HSCT. Accordingly, we acknowledge that comparing our results to those in the literature might be challenging. Our results might provide the basis for future studies aiming at optimizing allo-HSCT conditioning regimens for SAA when MRD is used as the donor source.

5. Conclusion

Fludarabine-based reduced intensity regimen for MRD allo-HSCT in SAA compares favorably to other available regimens. This regimen deserves further investigations with larger cohort of patients.

Disclosure of interest

The authors declare that they have no competing interest.

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