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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/m$^2$), very low dose cyclophosphamide (1200 mg/m$^2$) 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.



## 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/m$^2$) 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/m$^2$/day over 4 days (−6, −5, −4 and −3), intravenous cyclophosphamide 300 mg/m$^2$/day over 4 days (−6, −5, −4 and −3) and ATG (Genzyme rabbit) 3.75 mg/m$^2$/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/m$^2$ on day + 1 and 10 mg/m$^2$/day on days + 3 and + 6) for 9 patients or mycophenolate mofetil (600 mg/m$^2$/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/μL 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 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).

**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 × 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.

### 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 × 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.

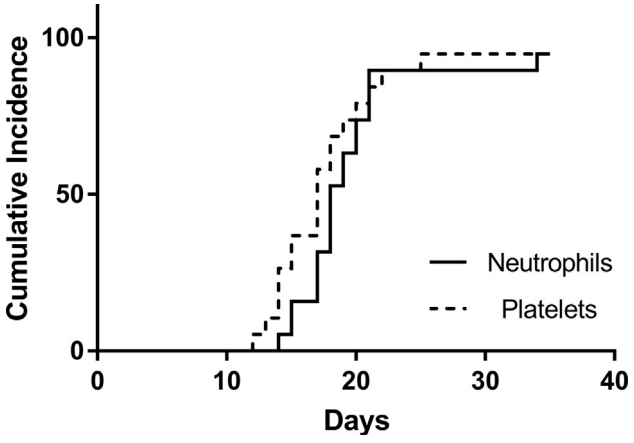


**Fig. 1.** Cumulative incidence of neutrophil and platelet engraftment.

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