

Long-term results of allogeneic hematopoietic stem cell transplantation after reduced-intensity conditioning with busulfan, fludarabine, and antithymocyte globulin

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Reduced-intensity conditioning (RIC) is widely used for allogeneic stem cell transplantation (SCT). Here we present our long-term experience with RIC regimen consisting of fludarabine (30 mg/m²/day on days -10 to -5), busulfan (4mg/kg/day on days -6 and -5) and antithymocyte globulin (ATG Fresenius, 10 mg/kg/day on days -4 to -1) (Flu-Bu-ATG) in a cohort of 71 patients with various hematological malignancies including chronic myeloid leukemia (24 patients), acute myeloid leukemia (19 patients), lymphoma (20 patients), multiple myeloma (3 patients), myelodysplastic syndrome (3 patients), and myelofibrosis (2 patients). The median age was 50 years.

The overall response rate was 87%, including 83% CR and 4% PR. The incidence of acute and chronic GVHD was 35% and 52% and the cumulative incidence of non-relapse mortality at 1 year and 4 years was 8% and 14%. With the median follow-up of 55.0 months, the 2- and 4-year event-free survival (EFS) was 49.0% and 40.3%, and the overall survival (OS) was 73.2% and 62.6%, respectively. Gender, age at SCT, type of donor, disease status at SCT, previous autologous transplantation, and complete chimerism by day +100 did not significantly influence EFS and OS. In a multivariate analysis, no presence of chronic GVHD (p=0.029, HR: 2.5), and diagnosis other than CML (p=0.018, HR: 4.6), and CD34+ dose < 5x10⁶/kg (p=0.010, HR: 2.8) were significant predictors of poor OS.

Flu-Bu-ATG protocol is a RIC regimen that combines effective disease control with low non-relapse mortality and acceptable toxicity profile.

Key words: reduced-intensity conditioning, fludarabine, busulfan, antithymocyte globulin

Allogeneic hematopoietic stem cell transplantation is a potentially curative option for a wide variety of malignant and nonmalignant diseases. However, relapses remain a significant problem, especially if the procedure is carried out in advanced stages of malignant disease [1]. Attempts to increase the intensity of conditioning have reduced the relapse rate but augmented the toxicity of the procedure [2]. One reason for the failure of this strategy is that clinically achievable radiation doses are not able to kill all residual leukemic cells [3]. Immunity-based approach has proved more successful and studies showing that transplanted immune cells can eliminate cancer cells eventually led to the introduction of donor lymphocyte infusion (DLI) [4-6]. Better understanding of graft-versus-tumor biology finally enabled the development of reduced-intensity conditioning (RIC) in the late 1990s [7-9]. Unlike traditional myeloablative

conditioning regimens, RIC is primarily immunosuppressive and depends on the graft to eradicate cancer.

Many RIC regimens have been published recently [10-11]. They include low- or intermediate-dose total body irradiation or chemotherapy, both with or without T-cell depletion. RIC regimens have rarely been compared in prospective clinical trials and the evidence for their use is usually based on retrospective studies or registry-based data [12-14]. However, there are some indications that clinically significant differences might exist between different RIC regimens. In addition, efficacy and toxicity data are available only for a few RIC regimens [15-17]. Fludarabine, busulfan, and antithymocyte globulin (Flu-Bu-ATG) regimen was first published in 1998 [8] and is one of the most popular RIC regimens [10, 13]. Surprisingly, comprehensive studies on its long-term efficacy and toxicity are lacking.

In this report, we present a detailed analysis of mature data on 71 patients treated with this protocol, focusing on acute toxicity, chimerism, opportunistic infections, GVHD, relapse rate, long-term disease control, and survivorship issues.

Patients and methods

Patients. The cohort included 71 patients (median age 50 years; range 15-65 years) with various hematological malignancies who received allogeneic stem cell transplantation (SCT) using RIC with Flu-Bu-ATG conditioning from 1998 to 2008. All patients were treated at the Department of Internal Medicine, Hematology and Oncology, University Hospital Brno for malignant hematological diseases including chronic myeloid leukemia (CML, n = 24), acute myeloid leukemia (AML, n = 19), non-Hodgkin's lymphoma (NHL, n = 17), Hodgkin's lymphoma (HL, n = 3), multiple myeloma (MM, n = 3), myelodysplastic syndrome (MDS, n = 3), and myelofibrosis (n = 2).

At the time of SCT, many of the patients (49%, n = 35) were in advanced stages of the underlying disease, defined as primary treatment-refractory disease, relapsing disease greater than or equal to CR2/CP2, or untreated disease. Early disease was defined as CR1/CP1 [18] and was present in 51% of patients (n = 36).

The reasons for performing RIC SCT rather than conventional myeloablative SCT were as follows: age > 50 years old (40 cases, 56%), presence of significant comorbidities (12 cases), extensive previous therapy such as failed autologous SCT (12 cases), and patient's choice after an extensive explanation of the differences between RIC and myeloablative SCT (7 cases). Exclusion criteria for RIC SCT were infection with human immunodeficiency virus, active hepatitis B or C, severe impairment of renal, cardiac or hepatic function, and Karnofsky performance status lower than 60%. All patients signed an informed consent before commencing treatment.

The median interval from diagnosis to transplant was 201 days (range 61-4520). The patients received peripheral blood stem cells (PBSC) (n = 68) or bone marrow (n = 3) from matched related (n = 65), matched unrelated (n = 3), or minor HLA-mismatched unrelated (n = 3) donors. Baseline characteristics of the patients are summarized in Tables 1 and 2.

Conditioning regimen, and graft-versus-host disease (GVHD) prophylaxis and management. The conditioning regimen consisted of intravenous fludarabine 30 mg/m²/day on days -10 to -5, oral busulfan (4 mg/kg/day) for two consecutive days (-6 and -5), and ATG Fresenius at 10 mg/kg/day for four consecutive days (-4 to -1) as published previously [8,19].

Cyclosporin A (CsA) (n = 65) or CsA plus mycophenolate mofetil (n = 6) were used for graft-versus-host disease prophylaxis. CsA was tapered from the second or third month after transplantation taking into account the chimerism [20], disease status, and any evidence of GVHD. Supportive care, antimicrobial prophylaxis and treatment of infections were guided by standard good clinical practice procedures. Acute GVHD

Table 1. Underlying hematological malignancies in the cohort of 71 patients

| Diagnosis | Number |
|----------------------------|--------|
| CML | 24 |
| CP1 | 21 |
| AP or CP2 | 3 |
| AML | 19 |
| CR1 | 15 |
| CR2 or greater | 2 |
| Relapsed/refractory | 2 |
| MDS | 3 |
| Other MDS than RA/RARS | 3 |
| CLL | 8 |
| All relapsed/refractory/PR | 8 |
| Myeloma | 3 |
| All relapsed/refractory/PR | 3 |
| NHL | 9 |
| CR2 | 3 |
| Relapsed/refractory/PR | 6 |
| HL | 3 |
| All relapsed/refractory/PR | 3 |
| Myelofibrosis | 2 |
| Refractory/untreated | 2 |

Table 2. Characteristics of patients and stem cell grafts

| Parameter | Total number |
|-------------------------------------|--|
| Patients | 71 |
| Male | 48 |
| Female | 23 |
| Median age in years, range | 50, (15-65) |
| Status of disease at transplant | |
| Early (CR1/CP1) | 38 |
| Advanced (≥ CR2/relapse/refractory) | 33 |
| Donor type | |
| Related, fully matched | 65 |
| Unrelated, fully matched | 3 |
| Unrelated, minor HLA-mismatched | 3 |
| Donor sex match | |
| Patient male/donor female | 20 |
| Patient female/donor male | 15 |
| Match | 36 |
| CMV status | |
| Patient neg/donor neg | 8 |
| Patient pos/donor neg | 13 |
| Patient neg/donor pos | 7 |
| Patient pos/donor pos | 43 |
| Donor age | Median age in years, range 49, (20-73) |
| GVHD prophylaxis | |
| CsA alone | 65 |
| CsA plus MMF | 6 |

was assessed using the criteria developed by Przepiorka [21]. Chronic GVHD was defined as GVHD occurring at day +100 or later and graded as none, limited, or extensive [22].

Acute GVHD was initially treated with corticosteroids (prednisone 2 mg/kg/day). In non-responsive cases, pulse cyclophosphamide, ATG, mycophenolate mofetil, or other second-line treatment was administered. Chronic GVHD

was managed by CsA with or without corticosteroids and/or mycophenolate mofetil [23].

Chimerism analysis and quantification were performed on peripheral blood samples before transplantation and in regular intervals after SCT.

Statistical analysis. Data were analyzed as of September 30, 2010. Overall survival (OS) was calculated as time from transplantation until death from any cause. Event-free survival (EFS) was defined as time from transplantation to relapse or death from any cause. Any patients dying without evidence of relapse or progression were considered as cases of non-relapse mortality (NRM). The correlation between the infused dose of CD34+ cells and time to engraftment of neutrophils and platelets was assessed by Pearson and Spearman correlation coefficients.

The Kaplan-Meier method was used to estimate EFS and OS probabilities and differences between subgroups were compared by the log-rank test. All statistical tests were two-sided and performed at the 5% significance level. Multivariate analysis was performed for significant univariate variables using Cox regression modeling.

Results

Hematopoietic recovery, transplant-related toxicity, and chimerism. The median dose of infused CD34+ cells was $6.59 \times 10^6/\text{kg}$ (range: $1.62\text{--}28.78 \times 10^6/\text{kg}$). The median time to platelet recovery ($>20 \times 10^9/\text{l}$) was 13 days (range: 8–27 days), while the neutrophil engraftment ($>0.5 \times 10^9/\text{l}$) occurred after a median of 16 days (range: 9–28 days). Engraftment was achieved in 69 patients (97%). Two patients died before engraftment could have occurred of lung hemorrhage and fulminant progression of malignancy on days +2 and +15, respectively. Three secondary graft failures were observed and treated with autologous PBSC graft to reconstitute hematopoiesis. We observed a statistically significant association between the quantity of infused CD34+ cells and engraftment of neutrophils ($p = 0.003$) as well as that of platelets ($p < 0.001$).

The conditioning regimen was well tolerated. Grade 4 non-hematological toxicity was observed in one patient case only (pulmonary toxicity). Grade 3 non-hematological toxicity was observed in 6 cases (mucositis = 1, cardiac toxicity = 1, gastrointestinal and liver toxicity = 2, pulmonary toxicity = 2). The most frequent non-hematological grade 1/2 toxicities

were mucositis (43 cases), gastrointestinal toxicity (20 cases), liver toxicity (22 cases), and renal toxicity (15 cases).

The mortality to day +100 was 10% (7/71 patients). The causes of death were sepsis (1 patient), hemorrhage (1 patient), GvHD (1 patient), and the progression of underlying malignancy (4 patients).

Complete chimerism was achieved by 19 (27%), 23 (32%), and 31 (44%) of patients on days +30, +60, and +100, respectively. Complete donor chimerism was observed in 57 (80%) patients after a median interval of 118 days after SCT.

Disease response and survival. Of 69 patients who achieved hematopoietic recovery after RIC SCT, complete remission of underlying disease was observed in 57 patients (83%). Three additional patients attained partial remission, and the overall response rate was 87% (60/69).

Stable disease was present in two patients (3%), and progression of disease occurred in seven patients (10%). The cumulative incidence of NRM was 4%, 8%, 10%, and 14% after 100 days, 1 year, 2 years, and 4 years, respectively.

After the median follow-up of 55.0 months from SCT, forty (56%) patients were alive and disease-free and four (6%) patients were alive with progression or relapse of disease. Seventeen (24%) patients died of progression of their hematological malignancy and 10 (14%) patients died as a result of NRM. The causes of death after SCT are summarized in Table 3.

In total, in thirty-two (46%) patients occurred progression or relapse after SCT (AML = 13, CML = 9, other diagnoses = 10). Next remission after relapse treatment was achieved in 12 patients (AML = 2, CML = 8, other diagnoses = 2).

The median EFS from transplant was 23.0 months. Median OS had not been reached by the data cut-off date with the median follow-up 55.0 months (Figure 1). The entire study cohort had 1-, 2-, and 4-year overall survival rates of 74.7%, 73.2% and 62.6%, respectively. EFS rates at these time points were 63.4%, 49.0% and 40.3%, respectively.

Univariate and multivariate analysis of factors associated with EFS and OS.

Results of univariate analysis are summarized in Table 4. Gender, age at SCT, type of donor, disease status at SCT, previous autologous transplantation, or achievement of complete chimerism by day +100 did not have significant impact on EFS or OS in our group of patients.

When comparing EFS and OS for AML patients versus others, patients with AML had significantly shorter OS (median OS 46.0 months versus not yet reached, $p = 0.023$), but there was no significant difference in EFS (median EFS 21.4 months versus 27.7 months, $p = 0.301$). EFS and OS for AML patients are shown in Figure 2.

The patients with CML had significantly longer OS than those with other diagnoses (median not yet been achieved versus 49.0 months, $p = 0.002$). Median EFS for CML patients was 45.4 months and median EFS for non-CML patients was 20.9 months. There was a trend to longer EFS in CML patients, but the difference was not statistically significant ($p = 0.112$). Event-free survival and OS for CML patients is shown in Figure 3.

Table 3. Causes of mortality after SCT

| | All patients, n = 71 |
|--------------|----------------------|
| Total deaths | 27 |
| Relapses | 17 |
| NRM | 10 |
| Infection | 5 |
| GVHD | 3 |
| Hemorrhage | 1 |
| Other | 1 |

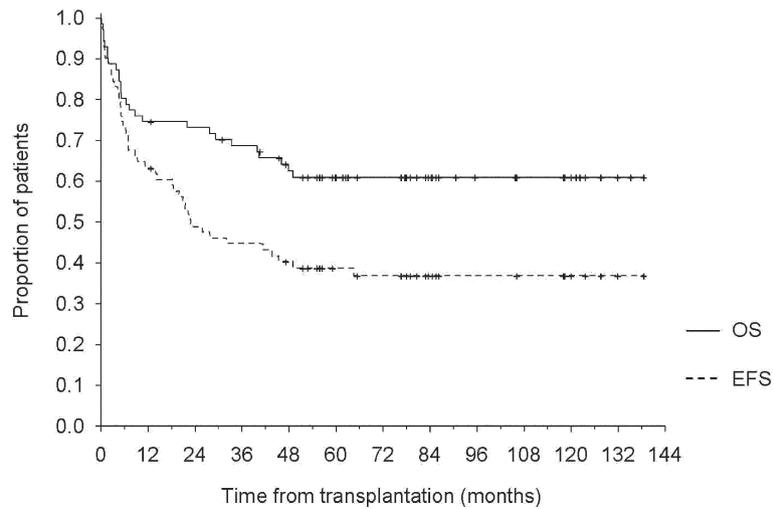


Figure 1. EFS and OS in a cohort of 71 patients treated with Flu-Bu-ATG and SCT

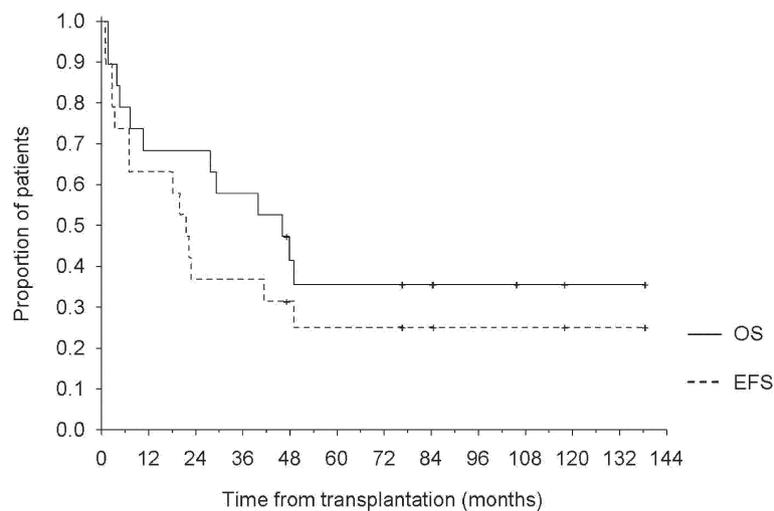


Figure 2. EFS and OS in 19 AML patients

Table 4. Impact of selected variables on EFS and OS: univariate analysis

| | EFS (P value) | OS (P value) |
|--|------------------|-----------------|
| Gender (male versus female) | 0.672 | 0.270 |
| Age at SCT (over 50 versus others) | 0.347 | 0.444 |
| Age at SCT (over 55 versus others) | 0.808 | 0.870 |
| Type of donor (related versus unrelated) | 0.174 | 0.175 |
| Disease status at SCT (early versus advanced) | 0.549 | 0.489 |
| Type of diagnosis (AML versus others) | 0.301 | 0.023 |
| Type of diagnosis (CML versus others) | 0.112 | 0.002 |
| Achievement of complete chimerism on day +100 (yes versus no) | 0.854 | 0.384 |
| Previous autologous transplantation (yes versus no) | 0.325 | 0.196 |
| Presence of acute GVHD (yes versus no) | 0.131 | 0.124 |
| Presence of chronic GVHD (yes versus no) | 0.035 | 0.003 |
| CD34+ cell dose: $\geq 5 \times 10^6/\text{kg}$ versus $< 5 \times 10^6/\text{kg}$ | 0.123 | 0.002 |

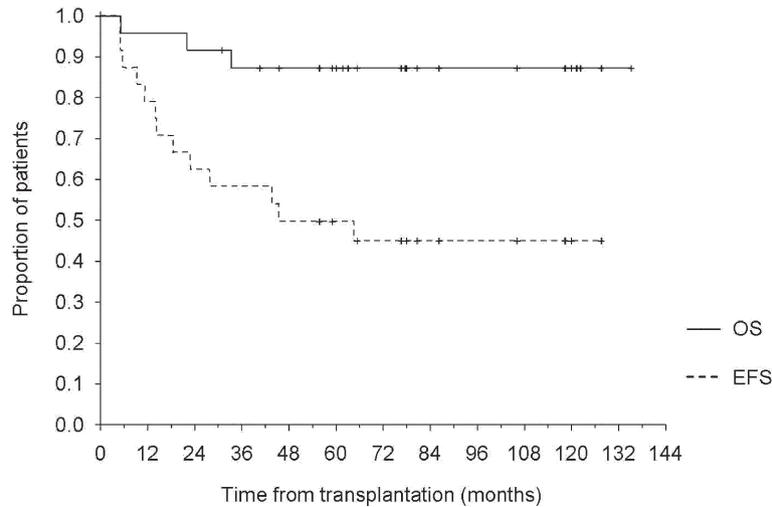


Figure 3. EFS and OS in 24 CML patients

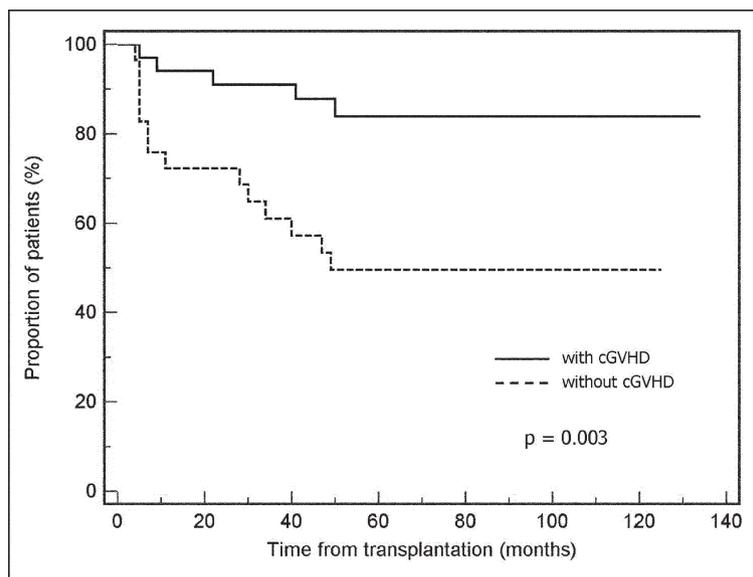


Figure 4. Impact of chronic GvHD on overall survival after SCT

No statistically significant differences in EFS or OS between aGVHD and non-aGVHD group were detected ($p = 0.131$ and $p = 0.124$, respectively).

The presence of chronic GVHD was associated with significant prolongation of both EFS and OS. Median EFS for

cGVHD patients has not yet been reached, while median EFS for non-cGVHD patients was 23.2 months ($p = 0.035$). Similarly, median OS for cGVHD cases has not been reached, contrasting with median OS of 49.3 months for non-cGVHD patients ($p = 0.003$, Figure 4).

The presence of complete chimerism on days +30, +60 and +100 had no significant impact on EFS or OS.

Patients with infused CD34(+) cells $\geq 5 \times 10^6/\text{kg}$ had significantly longer OS than those with CD34(+) cell dose $< 5 \times 10^6/\text{kg}$ (median not reached versus 33.3 months, $p = 0.002$). Differences in EFS were not statistically significant ($p = 0.123$).

Results of multivariate analysis are shown in Table 5. The following factors were associated with significantly shorter

Table 5. Multivariate analysis of factors associated with mortality after RIC SCT

| Parameter | P value | Hazard ratio | 95% CI |
|---|---------|--------------|----------|
| No presence of chronic GVHD | 0.029 | 2.5 | 1.2-5.9 |
| Diagnosis other than CML | 0.018 | 4.6 | 1.3-16.4 |
| Dose of CD34+ cells $< 5 \times 10^6/\text{kg}$ | 0.010 | 2.8 | 1.3-6.1 |

overall survival according to the multivariate analysis: absence of chronic GVHD, diagnosis other than CML, and dose of CD34(+) cells < 5x10⁶/kg. No statistically significant factors influencing EFS were identified by multivariate analysis.

GVHD and infections. The occurrence and clinical course of acute GVHD (aGVHD) was evaluated in 69 patients. The incidence of aGVHD was 35% (24 patients), with grades I+II and III+IV occurring in 20 patients (29%), and four patients (6%), respectively. Chronic GVHD (cGVHD) developed in 33 (52%) of 64 patients with evaluable data. Of these, twenty-five had limited cGVHD (39%) and eight patients had extensive cGVHD (13%). Extensive chronic GVHD often leads to poor quality of life, especially in long-term SCT survivors. Of eight patients with extensive cGVHD, three patients died (two of GVHD, one of progression of underlying hematological malignancy) and five are alive including three with complete and two with partial resolution of their GVHD and Karnofsky performance status of 80% or higher. Three patients with steroid-refractory GVHD did not respond to any GVHD treatment and died. In all other cases, treatment of GVHD was successful.

Infectious complications by day +100 occurred in 50 patients (70%), in some even repeatedly. Febrile neutropenia was diagnosed in 32 (45%) patients with the median duration of 2 days (range 1-15). In all cases, febrile neutropenia resolved after antimicrobial therapy and appropriate supportive measures. PCR-CMV reactivation was observed in 17 (24%) patients, all of whom were successfully treated with ganciclovir. Other infectious complications included sepsis (10 cases), bronchopneumonia (7 cases), and macroscopic hematuria with BK virus positivity in the urine (2 cases occurring during immunosuppressive therapy for GVHD). By day +100, only one patient died of sepsis with multiorgan failure. All other infectious episodes resolved after antimicrobial therapy.

After day +100 after SCT, no infections occurred in 29 of 64 evaluable patients (45%). Thirty-five (55%) had various infectious complications after day +100, in some cases repeatedly. These included pneumonia (7 cases), CNS toxoplasmosis (1 case), herpes zoster infection (8 cases), bronchitis (15 cases), meningitis (1 case), and sepsis (8 cases). Four patients died of sepsis with multiorgan failure after day +100 after SCT (2 patients with coincident relapse of underlying malignant disease and 2 patients with coincident GvHD); all other infectious episodes resolved after antimicrobial therapy.

Discussion

In this report, we describe a cohort of 71 patients with various hematological malignancies who underwent allogeneic SCT with Flu-Bu-ATG conditioning. Encouraging results with acceptable toxicity profiles and reduction of NRM [8,19] have been reported for Flu-Bu-ATG-based allogeneic SCT, predominantly in CML patients. We confirm these findings as the toxicity of Flu-Bu-ATG regimen was low in our cohort

of patients. NRM by day +100 was 4%, non-hematological toxicities grade 3+4 were observed in seven patients (10%), and only one death occurred due to acute organ toxicity.

Some authors have reported that infections are a frequent and serious complication after RIC SCT [24,25]. On the other hand, it has also been published that RIC reduces the risk of severe infections after SCT compared to myeloablative SCT [26,27]. In our cohort of patients, the incidence of infections was 70% by day +100 (50/71 patients) and 55% after day +100 (35/64) and we observed 5 deaths due to serious infections (7%, 5/71). According to our findings, infections remain a frequent complication after RIC SCT but infection-related mortality is relatively low.

The influence of graft parameters on the outcome of RIC SCT remains controversial. Some authors have reported that CD34(+) cell dose is associated with survival [28], but others have not confirmed this hypothesis [29]. In our cohort of patients, CD 34(+) cell dose over 5x10⁶/kg was associated with significantly longer survival. This observation merits further investigation.

Although the Flu-Bu-ATG regimen has been widely used [30-32] the dose and type of ATG was variable and the median follow-up from SCT was relatively short in most studies, ranging from 21 to 37 months [30,31]. Furthermore, various RIC regimens were often analyzed together [13,30,31].

In our hands, this protocol results in high CR rates with acceptable toxicity even in older patients, heavily pretreated patients, and patients with comorbidities.

The overall response rate was 87% and conditioning with Flu-Bu-ATG was effective also in the presence of advanced disease. We did not observe significant differences in EFS and OS between groups with early versus advanced disease in our cohort of patients, despite the fact that advanced disease was identified as negative prognostic factor in some RIC trials [13,30]. It must be pointed out, however, that treatment options in patients with advanced disease are limited and SCT may improve their prognosis [18].

Source, dose and timing of ATG may also significantly influence the final outcome of allogeneic transplantation [33]. We have used ATG Fresenius at the dose of 10mg/kg/day as published previously [8,19]. In a study of 465 patients treated with RIC with fludarabine, busulfan and rabbit ATG (Thymoglobuline) conditioning, doses of ATG exceeding 10 mg/kg were associated with decreased EFS and OS [30]. Improved non-relapse mortality and infection rate with lower doses of ATG in patients undergoing RIC-SCT has been reported by Hamadani [32]. According to Finke et al [34], the addition of ATG to GVHD prophylaxis resulted in decreased incidence of acute and chronic GVHD without increase in relapse rate, non-relapse mortality, or overall mortality.

A high proportion of our patients achieved complete chimerism (80% of patients) and the incidence of graft failure was relatively low (3/71, 4%). Chimerism assessment early after SCT might help identify patients at risk for graft rejection, GVHD and relapse/progression [35]. However, no association

has been found between early complete chimerism and the occurrence of aGVHD or disease progression after SCT [36]. In agreement with this report, we have observed no association between complete chimerism and EFS or OS.

GVHD remains a significant problem after allogeneic SCT and is one of the major causes of SCT-related morbidity and mortality [10,19]. The incidence of aGVHD and cGVHD in our group of patients was similar to that seen in other published RIC SCT trials [13,19]. The majority of our patients had mild forms of GVHD. Chronic GVHD has been identified as a major factor associated with low relapse rate and improved EFS and OS [38]. Accordingly, our patients with cGVHD had significantly longer EFS and OS compared to patients without cGVHD.

In our study as in most other reports on RIC SCT [37,38] relapse was the main cause of treatment failure, with 32/69 (46%) patients experiencing progression or relapse after SCT after a median follow-up of 55 months. Patients with AML (19/71 patients in our cohort, 27%) had the worst outcome after SCT with Flu-Bu-ATG and 13 (68%) relapsed or progressed. Representative clinical trials using fludarabine and busulfan in similar patients with myeloid malignancies have reported 2-year EFS of 43% to 49% and 2-year OS of 47% to 49%.³⁷ On the other hand conventional chemotherapy results in 3-year OS of 18% for standard risk AML.³⁹ RIC SCT could potentially improve these treatment results and prolong survival and is feasible even for older patients [18,37,39].

A significant proportion of our cases had CML (24/71, 34%). A similar protocol although with lower ATG dose in some patients was used by Or et al [19] in 24 CML patients achieving excellent results with disease-free survival of 85% at 5 years. Our CML patients had the median EFS of 45.4 months and the median OS has not yet been reached. Nine CML patients of 24 (37%) relapsed after RIC-SCT but a next molecular remission after DLI or/and imatinib was achieved in eight of them.

In conclusion, the Flu-Bu-ATG protocol is a RIC regimen that combines effective disease control with low non-relapse mortality and acceptable toxicity profile. This protocol is a feasible option even for older or pretreated patients and those with significant comorbidities. Nevertheless, relapses do occur after Flu-Bu-ATG regimen, especially in patients with AML. In our group of patients, favorable overall survival was associated with the diagnosis of CML, dose of CD34(+) cells exceeding $5 \times 10^6/\text{kg}$, and the occurrence of chronic GVHD.

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