Impact of Replacing Low Dose Cyclophosphamide with Fludarabine in Children with Acute Myeloid Leukemia Undergoing Transplantation During First Complete Remission

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ABSTRACT

Busulfan (BU)-based myeloablative conditioning is a standard conditioning regimen for children with AML; however, it is not clear yet which combination of cyclophosphamide (CY) and fludarabine (FLU) is most effective. We performed a study to compare the results of BUCY120 and BU-FLU in pediatric patients with AML in CR1 undergoing allo-HSCT from matched sibling donors. With the combination of BU, 15 patients were given 120 mg/kg of CY, and 12 patients were given 150 mg/m2 of FLU, respectively, in the condition regimen. Patients treated with BUFLU relapsed less than those treated with BUCY120 (p= 0.03). Moreover, these patients engrafted platelets earlier than the BUCY120 administered patients (p= 0.03). The frequency of complications in both groups was comparable. There was no significant difference in survival analysis between the groups. BUFLU has a low toxicity profile, making it a reasonable choice for children with AML in CR1 with low risk and a lower relapse frequency compared to BUCY120.

Keywords: Children, AML, Conditioning, Fludarabine, Leukemia

INTRODUCTION

The use of hematopoietic stem cell transplantation (HSCT) as a consolidation strategy for pediatric patients with acute myeloid leukemia (AML) in first complete remission (CR1) has been a subject of debate for a long time.[1] Some benefits of using allo-HSCT during the first complete remission in intermediate- and high-risk patients have been previously reported.[2] There is no consensus for conditioning regimens during HSCT, however, because total body irradiation (TBI)-based regimens have no survival benefit in pediatric AML, busulfan-based conditioning regimens are currently the standard.[3] Although the relapse rate decreases as the toxicity of conditioning increases, there are some concerns regarding the increased risk of HSCT-related complications.[3,4] Therefore, the historically recommended dose of BUCY (cyclophosphamide 200 mg/kg) in malignancies[5] was reduced to 120 mg/kg in subsequent studies, with a lower toxicity and the same efficacy profile.[6] BU in combination with fludarabine (FLU) conditioning in myeloid malignancies has less toxicity (reduced GVHD, mucositis, and transfusion requirement) but similar immunosuppressive efficacy (comparable engraftment and chimerism rates) as compared to that of CY in adult patients.[7,8]

However, there has been no comparative study of less toxic conditioning regimens in pediatric patients with AML.

We performed a study to compare the results of BUCY120 and BUFLU in pediatric patients with AML in CR1 undergoing allo-HSCT from matched sibling donors (MSD).

PATIENTS AND METHODS

This study was a single-center retrospective review of 27 pediatric patients (under 18 years of age) who underwent allogeneic HSCT between October 2011 and September 2021, and underwent transplantation for intermediate- or poor-risk AML in CR1 from MSD. All patients' risk stratification and treatment were done according to BFM AML 2004 or 2013 Study recommendations. Briefly, all patients with poor responses or carrying high-risk mutations were included in the high-risk group, and patients without favorable or unfavorable prognostic mutations were included in the intermediate-risk group. Patients with the acute promyelocytic leukemia (M3) subtype and those receiving HSCT from umbilical cord blood were excluded from the study. CR was defined as a condition with < 5% leukemia blast cells in bone marrow with no physical signs of extramedullary leukemia. Patients who reached CR1 after one or two induction courses were included in the study. Patients who had molecular positivity during diagnosis, underwent transplantation if they were in molecular remission before HSCT. Patients who were in remission after induction and who did not carry a mutation with a favorable or unfavorable prognosis were classified as an intermediate-risk group in the BFM AML 2013 Study and transplanted if they had MSD. All patients in the high-risk group, including those with acute erythroid leukemia or biphenotypic leukemia, were transplanted with an MSD or MUD; however, only the HSCTs done by MSD were included in this study.

All patients and donors were genotyped using a low- or high-resolution technique. All patients received BU (parenteral, without therapeutic drug monitoring) and one of the following combinations: (1) CY 60 mg/kg for 2 days, or (2) FLU 30 mg/m^2 for 5 days (150 mg/m^2). None of the patients received any in vivo T-cell depletion. All patients were administered a calcineurin inhibitor for graft-versus-host disease (GVHD) prophylaxis, mostly in combination with short-course methotrexate. Neutrophil engraftment was considered to be the first of three consecutive days with an absolute neutrophil count (ANC) ≥ 0.5 × 10^9/L, and platelet recovery was defined as a platelet count greater than ≥ 20 × 10^9/L for seven days without transfusion support. The diagnosis and grading of acute GVHD (aGVHD) and chronic GVHD (cGVHD) were established according to the modified Glucksberg and NIH criteria, respectively (9, 10). All patients were monitored weekly by galactomannan assay for Aspergillus, as well as weekly and fortnightly for CMV and EBV, respectively, by quantitative PCR assay. PCR screening for BK-virus in the urine was done in cases of hematuria or pollakiuria. Intravenous γ-globulin was given whenever the immunoglobulin G level was < 400 mg/dL. The initial treatment for neutropenic fever was monotherapy with piperacillin-tazobactam or cefepime, which, if fever persisted, was changed to carbapenems, with caspofungin or liposomal amphotericin-B as a substitute for fluconazole for broad-spectrum anti-fungal coverage. In cases of an increased viral load of cytomegalovirus (CMV), preemptive ganciclovir treatment was administered for a minimum of 21 days.

Event-free survival (EFS) was defined as the survival probability without evidence of relapse, or death by any cause. Overall survival (OS) was defined as the time from the date of transplantation to death by any cause.

Ethical approval was obtained from Ethics Board of MedicalPark Antalya Hospital (2023/15).

Statistical Analyses

All statistical analyses were performed using SPSS for Windows (version 16.0.0; SPSS Inc., Chicago, IL, USA). Descriptive analyses were created for all study variables using medians, ranges, and percentages. Patient demographics and transplant-related information were analyzed using the Chi-square test, Fisher's exact test, or Kruskal Wallis test, as appropriate. The OS and EFS probabilities were calculated using the Kaplan-Meier method, and comparisons between the probabilities of the

Table 1. Patient and transplant characteristics

Characteristic	BuCy120 (n= 15)	BuFlu (n= 12)	p
Age at HSCT, yr, median (range)	12.4 (3.6-17.3)	12.4 (3.0-16.9)	0.96
Gender			0.93
Female	9	7	
Male	6	5	
Cytogenetics			
No mutation	10	7	
11q23 rearrangement	0	2	
inv16	2	0	
FLT3-ITD	0	1	
Other	3	2	
Risk group			0.09
Intermediate	11	5	
High	4	7	
Source of stem cells			0.65
PBSC	5	5	
CD34 (x10^6/kg) (median, range)	6.9 (5.9-12.9)	7.5 (7.0-12.7)	0.25
BM	10	7	
CD34 (x10^6/kg) (median, range)	6.5 (2.9-14.3)	10.7 (5.0-18.0)	0.18
GVHD prophylaxis			0.75
CNI+MTX	12	9	
CNI	3	3	
Engraftment (median days, range)			
Neutrophil	13 (10-18)	12 (11-16)	0.61
Platelet	14 (9-36)	11 (8-17)	**0.03**
Complications			
CMV viremia	2	3	
VOD	0	1	
ES	0	1	
aGVHD (only Grade II)	3	2	
cGVHD	2 (1 mild, 1 severe)	4 (2 mild, 1 moderate, 1 severe)	
Relapse	7 (47%)	1 (8%)	**0.03**
Death	8 (53%)	3 (25%)	0.13
Progression	4	1	
Infection	2	1	
GVHD	1	0	
VOD	0	1	
Unknown	1	0	
Follow-up (months, median, range)	24 (5-126)	56 (1-91)	0.92

* CMV: cytomegalovirus; GVHD: Graft-versus-host disease; VOD: veno-occlusive disease; ES: engraftment syndrome

two groups were performed using the log-rank test. Statistical significance was set at $p < 0.05$.

RESULTS

All patients were treated with BFM 2004 or 2013 before transplantation and reached CR1 with a maximum of two induction chemotherapies. Three patients underwent transplantation for erythroid leukemia. The characteristics of the patients and HSCT procedures are summarized in Table 1. The demographic properties regarding gender status and median age were comparable, and most of the patients in the two groups had no cytogenetic abnormalities at the time of diagnosis.

In combination with BU, 15 and 12 patients were administered CY 120 and FLU, respectively, in the conditioning regimen. In general, while BUCY120

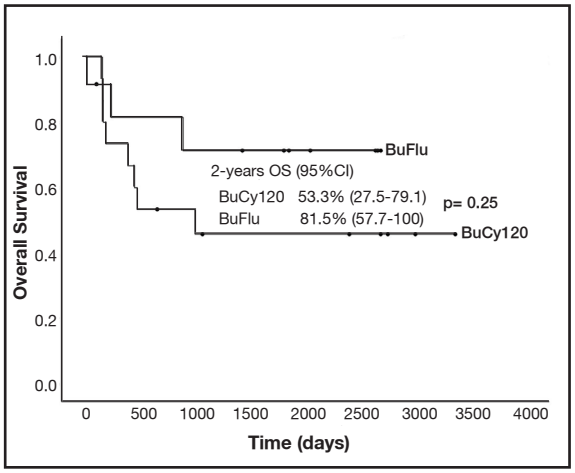

Figure 1. Probability of Overall survival

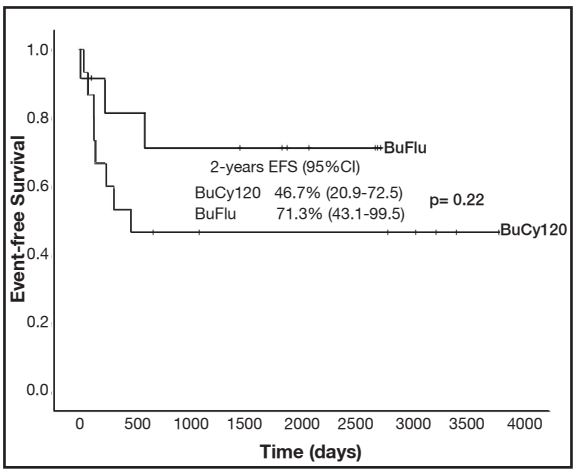

Figure 2. Probability of Event-free survival

was used more frequently before 2014 (n= 11/15), all of BUFLU used HSCTs were performed after 2014. Although there was a trend towards BU-FLU usage in the high-risk groups, this was not statistically significant (Table 1). Patients treated with BUFLU relapsed less than those treated with BUCY120 (p= 0.03). Moreover, these patients engrafted platelets earlier than the BUCY120 administered patients (p= 0.03). Interestingly, there were three patients in the BUCY120 group who were transplanted from singeneic siblings, and none relapsed. Except for these three patients for whom we were not able to check chimerism and two patients who died immediately after HSCT, all patients achieved complete chimerism. No significant difference was detected in the severity of acute or chronic GVHD in either group. The frequency of complications in both groups was comparable.

The median follow-up period of the patients was 24 (5-126) and 56 (1-91) months in the BUCY120 and BUFLU groups, respectively. The cumulative incidence of relapse in BUCY120 and BUFLU patients at 2 years was 46.7 % (95% CI: 27.2%-80.2%) and 12.5% (95% CI: 2.0%-78.0%), respectively (p=0.04). The overall survival (OS) at 2 years was 53.3% (95% CI: 27.5%-79.1%) and 81.5% (95% CI: 57.7%-100%) (p= 0.25), and event-free survival (EFS) at 2 years was 46.7% (95% CI: 20.9%-72.5%) and 71.3% (95% CI: 43.1%-99.5%) (p= 0.22) in the BUCY120 and BUFLU groups, respectively (Figure 1-2). There was no significant difference in survival analysis between the groups.

DISCUSSION

TBI, or busulfan-based conditioning regimens, are usually performed for myeloablation in HSCT of malignant diseases in children. Since regimens administered with TBI have long-term side effects in pediatric AML patients, BU-based conditioning regimens are generally preferred.[3] Recent studies have shown that myeloablation and engraftment problems are not observed in the combination of BU with today's support treatment in pediatric patients with AML.[3,4,11]; however, it is unclear which combinations with BU are optimal for pediatric AML patients. Currently, the goal is to intensify chemotherapy to achieve fewer relapses, however, its toxicity raises some concerns. Lucchini et al. reported that although relapse incidence was decreased in pediatric patients who underwent transplantation with BUCYMEL, there was a tendency for a high incidence of severe GVHD.[4] Bartelnik et al. showed that outcomes were comparable between pediatric patients with myeloid malignancies treated with BUCYMEL and those treated with BUFLU, but less toxicity was documented in the latter patient group.[11] Although BUCY200 has favorable reports of relapse outcomes, the high complication rate has caused some centers to use this regimen less frequently and reduce the CY dose to 120 mg/kg.[3,4,12] BUFLU seems to be an effective regimen in both adults and children owing to its low toxicity profile and favorable relapse rate.[8,11]

Despite serious concerns regarding HSCT in pediatric patients with AML, studies comparing conditioning regimens in children are scarce. Therefore, we aimed to compare the two frequently used regimens, BUCY120 and BUFLU, in considerably homogenous groups of pediatric patients with AML who underwent transplantation during CR1 from an MSD. The two groups had comparable demographic data and transplant characteristics. Although there was a trend favoring the high-risk group in BUFLU-administered patients, they relapsed significantly less frequently than BUCY120 patients.

FLU is one of the most commonly used chemotherapeutic agents with well-known antileukemic effects, even in salvage treatment of refractory leukemias.[13] In conditioning regimens for malignant diseases, when FLU is used in combination with BU (which has an alkylator effect on DNA), it creates a good antileukemic and immunosuppressive effect owing to its synergistic effect on the creation of defective DNA repair.[7,8] However, although there is a need for evaluation of conditioning regimens with high antileukemic effects and low toxicity in children, there are few studies on BUFLU, which has such potential in pediatric AML. Although Harris et al. reported a higher rate of post-transplant relapse failure related to BUFLU in pediatric AML patients[14], Liu et al. reported that BUFLU is not inferior to BUCY120 in terms of relapse and survival outcomes.[7] Our study showed that BUFLU has a comparable toxicity profile, with a lower rate of relapse and faster platelet engraftment than BUCY120, which is a frequently used conditioning regimen in HSCT for pediatric patients with AML.[4] It is difficult to make a comparison between our study and Liu et al.´s study because of the presence of matched unrelated donors and the inclusion of patients only above 12 years of age (range 12-54) in their study.

While the historically recommended dose of CY in HSCT is 200 mg/kg, the dose has been reduced to 120 mg/kg in most diseases owing to its toxicity.[6] Although the efficacy of this dose in HSCT for pediatric AML is controversial, no randomized study has compared these doses. Lucchini et al.[4] reported that there was no difference in the outcome data between patients receiving the two different doses of CY in HSCT in pediatric AML undergoing transplantation during CR1. However, their retrospective observational study had limitations, such as including different kinds of donors and the availability of limited information about the cytogenetic risk. The lack of reliable data related to the 120 mg/kg dose of CY warrants further studies to confirm the efficacy of this dose. However, our study shows that the conditioning regimen BUCY120 is inferior to BUFLU in terms of relapse and engraftment in the HSCT of pediatric AML patients with transplantation from MSDs during CR1.

Our study had a few limitations. First, our study was a retrospective observational study that may carry bias. Second, despite statistically significant differences with regard to fewer relapses and better engraftment in BUFLU patients, better OS and EFS outcomes in that group did not show significance, which may be because there were fewer patients in both groups. An additional study with more patients would increase the significance of these results. Third, the absence of therapeutic drug monitoring for busulfan, and MRD before HSCT may have caused selection bias.

Conclusion

Overall, BUFLU has a low toxicity profile, making it a reasonable choice for children with AML in CR1 with low risk and a lower relapse frequency compared to BUCY120, which is a frequently used alternative. Further studies with more patients should be conducted to compare survival outcomes between these two regimens.

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