Myeloablative conditioning using timed-sequential busulfan plus fludarabine in older patients with acute myeloid leukemia: long-term results of a prospective phase II clinical trial

Myeloablative hematopoietic cell transplantation (HCT) reduces relapse, but is precluded in older patients and those with high comorbidities in whom outcomes remain disappointing. After demonstrating the safety of timed-sequential administration of myeloablative busulfan with fludarabine (Bu-Flu) in older patients and those with high comorbidities,[1] we evaluated its efficacy in acute myeloid leukemia (AML). Our original study (*clinicaltrials.gov identifier: 01572662*) was an equally randomized Phase II trial that compared two myeloablative timed-sequential Bu-Flu conditioning regimens: lower dose busulfan [area under the curve (AUC) =16,000± 12% µmol/min, the 16K arm] *versus* higher dose (AUC=20,000 ± 12% µmol/min, the 20K arm), as described previously.[1] Graft-*versus*-host disease (GvHD) prophylaxis included tacrolimus and methotrexate.[1] All patients who met the eligibility criteria and were deemed suitable for the trial by treating physicians were enrolled. After 98 patients were enrolled, the randomization was stopped because the 20K arm was demonstrated to be as safe as the 16K arm. The outcomes of those patients were reported.[1] To estimate the efficacy of this regimen, the trial was extended and 101 additional patients with hematologic malignancies were enrolled onto the higher dose (20K) arm with the approval of the institutional review board. Herein, we report the outcomes of AML patients (n=71). The research was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent.

The primary outcome was efficacy, as determined by the rates of relapse, progression-free survival (PFS; defined as the time from HCT to relapse/progression or death) and overall survival (OS; defined as the time from HCT to death from any cause). Categorical variables were compared between treatment arms using Fisher's exact test. Continuous variables were compared using the Wilcoxon rank sum test. The rate of non-relapse mortality (NRM) was estimated in a competing risks framework, with relapse as the competing risk. Acute and chronic GvHD were assessed with competing risks of relapse and death. Kaplan-Meier curves were used to estimate OS and PFS, and the log-rank test was used to test differences between groups based on variables of interest. Cox proportional hazards regression models were fit to model the association between OS, PFS, relapse, and NRM and co-variates of interest. Results were analyzed by pre-HCT disease status as that is one of the most important factors predicting post-HCT outcomes. All statistical analyses were performed using R version 3.5.1.

Seventy-one patients with AML were enrolled. Table 1 summarizes the patients' characteristics. Median age was 64 years (range 29-73 years). A majority had matched unrelated donors (n=45, 63%) and received peripheral blood grafts (n=46, 65%). Most patients (n=59, 83%) received busulfan 20K (median, 12.7 mg/kg intravenously; interquartile range (IQR) 10.79-14.02]; the rest received 16K (median, 9.7 mg/kg intravenously; IQR 8.53-10.74). Over 60% had primary induction failure (PIF; n=32) or relapsed disease (n=11). Nearly half (n=33, 47%) had adverse-risk disease as per the European LeukemiaNet (ELN) revised classification.[2] Fifty-five percent had HCT comorbidity index (HCT-CI) score >3.3 The median follow up was 40 months (IQR: 34.11-48.69) among survivors.

There were no graft failures. All patients engrafted neutrophils (absolute neutrophil count of >0.5x10^9/L for 3 consecutive days) at a median of 12 days (IQR: 11-15). Of the 71 patients, 66 engrafted platelets (>20x10^9/L without transfusion for 7 consecutive days) at a median of 14 days (IQR: 11-19). At day 30, whole-blood chimerism analysis showed a median of 100% (IQR: 98-100%) cells of donor origin. The median T-cell chimerism was 85% (IQR: 70-100%) at day 30, which increased to 96% (IQR: 82-100%) at day 100, and 100% (IQR: 100-100%) by one year. In the myeloid compartment, we observed 100% donor cells throughout the study period. The cumulative incidences of grade II-IV and III-IV acute GvHD at day 100 were 39% [95% confidence interval (CI): 28-51%] and 10% (95%CI:

Table 1. Baseline patients' characteristics.

Characteristic	Number of patients n=71
Age at HCT in years, median (IQR)	64 (57-69)
Males, n (%)	43 (61)
Race, n (%)	
White	59 (83)
Other	12 (17)
Diagnosis, n (%)	
Primary AML	51 (72)
Secondary AML (MDS/MPD)	20 (28)
Donor, n (%)	
HLA-matched, unrelated	45 (63)
HLA-matched sibling	26 (37)
Graft source, n (%)	
PB	46 (65)
BM	25 (35)
Busulfan dose, n (%)	
AUC 20K dose	59 (83)
AUC 16K	12 (17)
Disease status,* n (%)	
CR 1/2 (MRD negative)	18 (25)
CR 1/2 (MRD positive)	9 (13)
Primary induction failure	32 (45)
Relapsed disease	11 (16)
Revised ELN classification, n (%)	
Favorable	8 (11)
Intermediate	30 (42)
Adverse	33 (47)
HCT-CI, n (%)	
0-2	32 (45)
≥ 3	39 (55)
Follow-up in months, median (IQR)	40 (34-49)

*One patient in complete remission (CR) did not have minimal residual disease (MRD) analysis performed before hematopoietic cell transplantation (HCT). MRD assessment was performed by either multi-parameter flow cytometry (MFC) or quantitative polymerase chain reaction (PCR). Of the 27 MRD positive patients, 18 were positive by both MFC and PCR, and nine were positive only by PCR. Among 18 patients who were MRD positive by MFC, 11 patients were 1% or higher, two patients were <0.1% and the rest were between 0.1-1%. CR is defined as the morphological complete remission with <5% blasts. *All patients were retrospectively categorized as per the Revised European LeukemiaNet (ELN) classification. AML: acute myeloid leukemia; AUC: area under the curve; BM: bone marrow; CR1: first complete remission; CR2: second complete remission; HCT: hematopoietic cell transplantation; HCT-CI: hematopoietic cell transplantation-specific comorbidity index; HLA: human leukocyte antigen; IQR: interquartile range; MDS: myelodysplastic syndromes; MPD: myeloproliferative disorder; MRD: measurable residual disease; PB: peripheral blood.

Table 2. Results of multivariate analyses.

	OS			PFS			Relapse			NRM		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Busulfan dose												
16K	1			1			1			1		
20K	0.89	0.35-2.24	0.79	0.79	0.33-1.94	0.62	0.39	0.18-0.86	0.02	1.98	0.49-7.89	0.33
Age at HCT												
<60 years	1			1			1			1		
>60 years	1.24	0.63-2.45	0.53	1.04	0.55-1.98	0.90	0.88	0.41-1.87	0.73	2.26	0.79-6.47	0.13
Disease status at HCT												
CR/MRD-negative	1			1			1					
Others	2.67	0.99-7.23	0.05	3.22	1.27-8.15	0.01	2.29	0.77-6.85	0.14	–	–	–
ELN disease risk												
Favorable	1			1			1					
Intermediate	1.14	0.32-4.11	0.84	0.79	0.25-2.49	0.69	1.94	0.23-16.28	0.54	–	–	–
Adverse	1.74	0.49-6.10	0.39	1.36	0.45-4.11	0.58	4.9	0.57-41.65	0.15	–	–	–
HCT-CI												
0-2	1			1						1		
>3	1.98	1.00-3.91	0.05	1.29	0.69-2.42	0.42	–	–	–	3.97	1.41-11.14	0.009

CI: Confidence Interval; ELN: European LeukemiaNet; HCT: hematopoietic cell transplantation; HCT-CI: hematopoietic cell transplantation-specific comorbidity index; HR: Hazard Ratio; OS: overall survival; PFS: progression-free survival; CR: complete remission; NRM: non-relapse mortality. MRD: minimal residual disease.

3-17%), respectively. The cumulative incidences of overall chronic GvHD and systemic therapy-requiring chronic GvHD were both 20% (95%CI: 10-29%) at one year.

The cumulative incidence of relapse was 34% (95%CI: 23-45%) at two years; it was 11% (95%CI: 0-26%) in those who were in complete remission (CR) with undetectable measurable residual disease (MRD) *versus* 42% (95%CI: 29-56%) in others. In multivariate analysis, the receipt of higher-dose (20K) *versus* lower-dose (16K) busulfan was the only factor associated with a significantly lower risk of relapse [hazard ratio (HR) 0.39; 95%CI: 0.18-0.86; *P*=0.02] (Table 2). The cumulative incidence of NRM was 6% (95%CI: 0-11%) at day 100 and 24% (95%CI: 14-34%) at two years. In multivariate analysis, HCT-CI >3 was the only significant predictor of NRM (HR, 3.97; 95%CI: 1.41-11.14; *P*=0.009) (Table 2). The cumulative incidence of NRM at two years was 33% (95%CI: 18-49%) in patients with HCT-CI >3 *versus* 13% (95%CI: 8-24%) in those with HCT-CI <3; *P*=0.03.

At two years, the estimated OS was 45% (95%CI: 35-58%); it was 78% (95%CI: 61-100%) in the CR/MRD-negative group *versus* 35% (95%CI: 24-50%) in others (Figure 1A). In multivariate analysis, not being in a CR/MRD-negative state (HR: 2.67; 95%CI: 0.99-7.23; *P*=0.05) and having an HCT-CI >3 (HR, 1.98; 95%CI: 1.00-3.91; *P*=0.05) were independently associated with a higher risk of mortality (Table 2). At two years, the estimated PFS was 42% (95%CI: 32-55%); it was 78% (95%CI: 61-100%) in the CR/MRD-negative group *versus* 31% (95%CI: 21-46%) in others (Figure 1B). In multivariate analysis, not being in CR/MRD-negative state at the time of HCT was the only factor associated with a significantly inferior PFS (HR, 3.22; 95%CI: 1.27-8.15; *P*=0.01) (Table 2). Sixteen patients received maintenance with azacytidine (n=14) or FLT-3 inhibitor (n=2). We found no difference in OS among those who received maintenance (median 43 months; 95%CI: 18-non-analyzable) and those who did not (median 16 months; 95%CI: 11- non-analyzable), *P*=0.23.

We demonstrated that myeloablative HCT can be safely and effectively performed in older AML patients and those with significant comorbidities by administering busulfan in a timed-sequential manner and closely monitoring the dose through pharmacokinetic analysis. Prior registry study of older AML patients (>60 years) in CR1 showed 2-year PFS and OS of approximately 30-35%,[4] and a prospective Phase II trial of AML patients in CR1 (>60 years) showed somewhat better outcomes with a PFS of 42% and OS of 48% at two years.[5] In our study, CR patients (CR1 and CR2) had a 2-year PFS of 59% and OS of 67%. Patients who attained MRD-negative CR before HCT had a striking 3-year OS of 78%, which is similar to that of previous reports for younger patients (<50 years) with CR/MRD-negative disease status (2-3 year OS, 73-80%).[6-9] Patients with persistent disease at HCT (MRD-positive CR, relapsed disease, or PIF) were analyzed as a single group as they have similarly poor outcomes, as shown by others[6] and noted by us. The 3-year OS of this group was 33% (95%CI: 22-48%), which is harmonious with prior reports that showed a 2-3-year OS of approximately 25-30%, regardless of the conditioning intensity,[6,9-13] and which is comparable to the OS of younger patients (median age <50 years) with active disease.[13]

In multivariate analyses, we found that higher myeloablative dose of busulfan (20K) was associated with an approximately 60% reduced risk of relapse compared to lower dose (16K) but was not a significant predictor of NRM. Notably, age (<60 *vs.* >60 years) was also not a predictor of NRM suggesting that myeloablative doses of busulfan could be safely delivered in a timed-sequential manner across the study population. The only factor associated with high NRM (almost 4-times greater risk) and poor survival (about 2-times greater risk) was an HCT-CI >3. However, even in that subgroup, NRM (33% at 3 years) was not higher than the reported NRM in younger patients with high comorbidities (32-46% at 2 years) with either myeloablative[14] or reduced intensity conditioning (RIC).[15]

Our study is limited by a relatively fewer number of patients in CR pre-HCT and a predominance of patients with PIF and relapsed disease. Next, although we demonstrated that higher-dose busulfan was well tolerated and led to lower relapse risk than lower-dose busulfan, how it contrasts directly against other regimens is unknown. Also, we

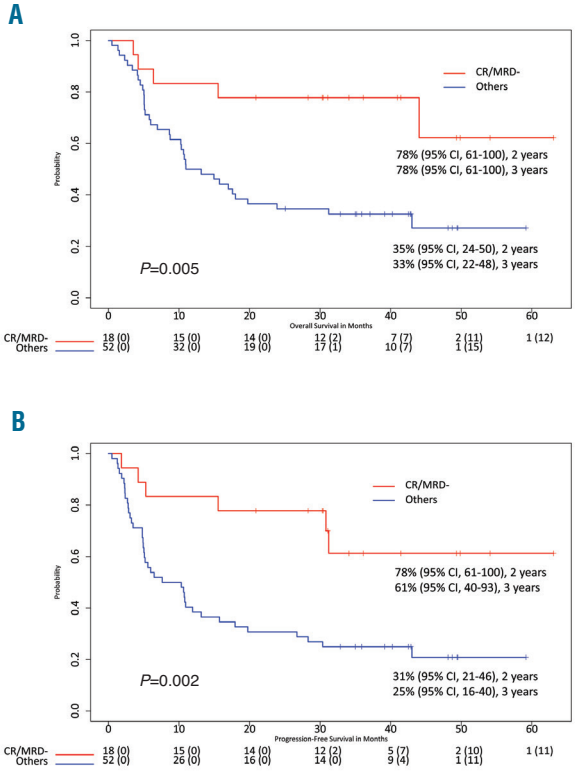

Figure 1. Kaplan-Meier curves showing the impact of pre-hematopoietic cell transplantation (HCT) disease status on survival. (A) Overall survival and (B) progression-free survival; CI: Confidence Interval; CR: complete remission; MRD: measurable residual disease.

categorized patients in CR by the presence or absence of MRD, a well-recognized prognostic factor, but analyzed CR1 and CR2 patients together due to small numbers, which may have deflated our outcomes, as CR2 patients have worse outcomes than those in CR1.[12] Lastly, the MRD assessment was conducted using either multi-parameter flow cytometry or quantitative polymerase chain reaction.

Myeloablative conditioning using timed-sequential delivery of busulfan along with fludarabine is safe and effective in older AML patients up to age 73 years and in those with high comorbidities. In an extremely high-risk population, in which over 60% had PIF or relapsed disease without achieving CR prior to HCT, 55% had HCT-CI >3 and around half had adverse risk disease, our outcomes are encouraging, especially in those who are in remission at the time of HCT. This regimen merits further investigation and comparison to other regimens in older patients with AML.

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