Title page

**Retrospective cohort study comparing outcomes of intravenous busulfan versus total body irradiation after single cord blood transplantation**

**Running title:** IV-Bu versus TBI for cord blood transplantation

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**Abstract:**

Limited to inadequate stem cell doses, cord blood transplantation(CBT) is accompanied by increased graft failure and delayed hematopoietic recovery. The conditioning regimen is critically important for engfratment, but there’s still no consensus for CBT. Numerous trials have been undertaken on the outcomes between IV Bu and TBI, but there are no comparative data for CBT. We conducted a retrospective multicentre study to analyze the outcomes of IV Bu and TBI in CBT patients with hematologic malignancies. From May 1, 2008 to Mar 31, 2018, a total of 331 patients from the China Umbilical Cord Blood Transplantation Cooperation (IV Bu, N=131; TBI, N=200) were involved. The cumulative incidence of neutrophil engraftment were 91.6% in IV Bu/Cy cohort and 98.0% in Cy/TBI cohort(P<0.001), respectively. The median times to neutrophil engraftment were 16 and 19 days(P<0.001). Multivariate analysis showed there was not statistically different for nonrelapse mortality(hazard ratio [HR], 1.11; 95% confidence interval [CI], 0.66 to 1.86; P=0.695), relapse(HR, 0.90; 95% CI, 0.50 to 1.60; P=0.713), overall survival(HR, 0.94; 95% CI, 0.61 to 1.44; P=0.763), disease-free survival(HR, 1.08; 95% CI, 0.73 to 1.59; P=0.714) and GVHD-free relapse-free survival(HR, 0.77; 95% CI, 0.54 to 1.09; P=0.136)between the two conditioning regimens. Our results show that both IV Bu and TBI are valid myeloablative conditioning regimens for hematologic malignancies patients treated with CBT.

**Introduction**

Cyclophosphamide (Cy) combined with either busulfan (Bu) or total-body irradiation (TBI) have been widely used as standard myeloablative preparative regimens for allogeneic hematopoietic stem-cell transplantation(allo-SCT)[1-3]. Researches of two regimens ocurred largely in parallel. Those two regimens can effectively eradiate tumor and offer sufficient immunosuppress to facilitating allogeneic engraftment. In the oral Bu formulation era, because of variability in absorption and metabolism may lead to 3-fold or more differences in Bu plasma levels, many conflicting results and toxicity have been obtained with two regimens[4-9]. At that time, Cy plus TBI was usually regarded as a standard preparative regimen for eligible patients with acute lymphoblastic leukemia and advanced or refractory acute myeloid leukaemia[10,11]. TBI based regimens seem to show a little superior compare with oral Bu based regimens in specific types of diseases such as AML and ALL [8,10,12].

Therefore, with the consistent pharmacokinetics and reliable dosing, improved clinical outcomes were achieved in patients transplanted with IV Bu regimens. A retrospective registry-based study comparing IV Bu and TBI in combination with Cy as preparative regimens was reported by Acute Leukemia Working Party of European Group for Blood and Marrow Transplantation, 1,659 AML patients received allo-SCT from sibling donors were involved[1]. Although multivariable analysis showed IV BU group had lower incidence of nonrelapse mortality(NRM), acute and chronic graft-versus-host disease (GVHD), there was no statistically different of leukemia free survival(LFS) was found between the two conditioning regimens(61%±2% for IV Bu and 64%±2% for TBI; P=0.27). Two consecutive studies from CIBMTR together indicated superior survival in patients with myeloid malignancies receiving IV Bu as preparative regimen compared with TBI[13,14]. In another recently retrospective study for adult ALL patients from CIBMTR, despite of higher risk of relapse, IV Bu confered equivalent survival with TBI-based preparative regimens due to decreased rates of NRM[15]. These results suggest that intronevous formulation of Bu has becoming an attactive alternitive of TBI with improved outcome.

Due to less restrictions of human leukocyte antigen (HLA), rapid graft availability, and low incidence of relapse and chronic GVHD, Unrelated donor umbilical cord blood has been widely accepted as an extension of allograft access for hematologic malignancies[16-20]. The clinical usefulness of umbilical cord blood transplantation(CBT) has been restricted by the relatively low number of stem cell doses. Delayed engraftment of neutrophils and platelets or graft failure with CBT is always accompanied by increased risk of transplant-related complications or death. Besides of include cell dose, degree of HLA matching and post-grafting immunosuppression, the conditioning regimen is critically important for engraftment in CBT. Currently there are no specialized research aim to compare the outcome of IV Bu/Cy with CyTBI/ in CBT. To evaluate the efficacy of these two myeloablative preparative regimens in CBT, we conduct a retrospective cohort study in patients with hematologic malignancies undergoing unrelated single CBT in China.

**Patients and methods**

**Study Design and Patients**

This was a retrospective, multicentre, registry-based analysis. Data were provided by the China Umbilical Cord Blood Transplantation Cooperation. From May 1, 2008 to Mar 31, 2016, a total of 331 consecutive patients with hematologic malignancies received singe unrelated CBT were involved in the study. Eligibility criteria for this analysis included:(1)Weigh ≥35 kilograms and age ≤ 60 years; (2)All patients received a single unit CBT but not a double units CBT; (3)Karnofsky performance status must be ≥70%;(4)No HLA-compatible related donors available. Patients who has received a previous autologous or allogeneic transplantation was excluded in the study. Informed consent for CBT was obtained in accordance with the Declaration of Helsinki.

**Conditioning Regimen and GVHD Prophylaxis**

Consensus criteria preparative regimens were based on full dose (total 12.8 mg/kg, 0.8mg/kg every 6 h for 4 days) or TBI(total 12 Gy, 4 fractions) combined with Cy(60mg/kg daily for 2 days). High-dose cytarabine (2.0 g/m2 every 12 h for 2 days) was added to all the Cy/TBI regimen(n=200) and 21.4% of IV Bu regimen(n=28); fludarabine (30 mg/m2 daily for 4 days) was added to 78.6% of IV Bu regimen(n=103).

GVHD prophylaxis regimens include cyclosporine(CSA) and mycophenolate mofetil(MMF) without Antithymocyte Globulin(ATG).

**Cord blood selection and HLA typing**

Cord blood selection and HLA typing have been previously described[21]. Briefly, HLA typing of cord blood and patients was determined using molecular techniques with a minimum of antigen split-level resolution for HLA-A and -B and allele-level resolution at DRB1. Cord blood units from Chinese cord blood banks that were serologically matched for ≥4 of 6 HLA antigens and that contained at least 3 × 107/kg of recipient body weight total nucleated cells and 1.2 × 105/kg CD34+ cells before freezing were chosen for transplantation. In our cohort, the median dose of infused total nucleated cells were 4.11(range, 2.14 to 12.06 )× 107/kg, and the median number of CD34+ cells was 2.32(range, 0.91 to 9.64) ×105/kg.

**Study end points and definitions**

The primary endpoint was disease-free survival (DFS), Other endpoints included neutrophil and platelet engraftment, overall survival (OS), GVHD-free, relapse-free survival(GRFS), NRM, relapse, incidence and severity of veno-occlusive disease/sinusoidal obstruction syndrome(VOD/SOS) according to the McDonald scale[22], incidence and severity of acute GVHD and chronic GVHD[23,24], and cause of death.

**Statistical Analysis**

All surviving patients were followed until March 31, 2018. Median values and ranges were used for continuous parameters and percentages for categorical parameters. The two preparative regimens were compared using X2 method for qualitative variables and Mann-Whitney test for continuous variables. The probabilities of 3-year OS, DFS and GRFS were analyzed according to the Kaplan-Meier method and tested univariately using the log-rank test. The cumulative incidence of neutrophil and platelet engraftment, 100-day acute GVHD, chronic GVHD, 3-year NRM, and relapse rate were estimated using cumulative incidence curves to accommodate competing risks and were compared using Gray’s test. Competing risk for neutrophil and platelet engraftment is death without neutrophil and platelet engraftment. Competing risks for the occurrence of GVHD are death without GVHD and relapse without GVHD. Competing risk for NRM is death with relapse. When relapse of the disease is the outcome of interest among patients, death without relapse is a competing risk event.

Statistical significance was assessed at the P<0.05 level (2-sided). Univariate and multivariate analyses were performed using the Cox proportional hazards regression model. The following parameters were examined in the regression model: type of conditioning regimen; age at transplantation; patient gender; donor-recipient gender; ABO compatibility ; number of HLA(A,B,DR) disparities; total nucleated cell doses and total CD34+ cell doses; year of UCBT and disease risk index(DRI). First, a univariate model was calculated for all parameters. The factors with P<0.15 in the univariate analysis were included in a multivariate regression, and P<0.05 in the multivariate analysis was considered statistically significant. For each analysis, hazard ratios (HRs) with a 95% confidence intervals (CIs) were calculated with P values for either the corresponding category or the overall test. Correlations were evaluated by Spearman’s rank correlation test. Most analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Cumulative incidences of neutrophil and platelet engraftment, GVHD, NRM, and relapse were performed with R software packages(R Foundation for Statistical Computing, Vienna, Austria, version 3.5.0).

**Results**

**Patient Characteristics**

The median follow-up time in IV Bu/Cy and Cy/TBI cohorts was 28.7(range, 12.2 to 91.3) months and 55.5(range, 13.1 to 117.1) months, respectively(P<0.001). Of them, 200 patients received Cy/TBI, and 131 patients received IV Bu/Cy. Patients, diseases, and transplantation related characteristics are described in Table 1. In the IV Bu/Cy cohort were younger than those in the Cy/TBI cohort (P<0.001). And Patients in the IV Bu/Cy cohort had less weight than that in the Cy/TBI cohort(P<0.001). The median dose of infused total nucleated cells were 4.51(range, 2.19 to 12.06 )× 107/kg and 3.77(range, 2.14 to 9.05)(P<0.001), respectively. And the median number of CD34+ cells was 2.61(range, 0.91 to 9.64) ×105/kg and 2.01(range, 1.12 to 8.71)(P<0.001), respectively. No difference was noted in disease type ABO compatibility and number of HLA(A, B, DR) disparities between two groups.

**Engraftmemt**

The cumulative incidence of neutrophil engraftment by day 42 were 91.6%(95% confidence interval [CI], 85.1% to 95.3%) in IV Bu/Cy cohort and 98.0%(95% CI, 62.9% to 99.9%) in Cy/TBI cohort (P<0.001). The median times to neutrophil engraftment were 16 days (range, 11–41 days) and 19 days (range, 13–42 days)(P<0.001), respectively(Table 2; Figure 1A). The cumulative incidence of platelet engraftment by day 100 in each group were 82.1%(95% CI, 74.2% to 84.8%) and 83.6%(95% CI, 77.5% to 88.2%) (P=0.656). The median times to platelet engraftment were 37 days (range, 14-216 days) and 39 days (range, 17-196 days) (P=0.121), respectively(Table 2; Figure 1B).

**Toxicity ,NRM and Cause of death**

The 100-day cumulative incidences for VOD/SOS were 6.9% in the IV Bu/Cy cohort and 7.0% in the Cy/TBI cohort(P=0.958; Table 2). The median interval from transplantation to occurrence of SOS was 11 days and 10 days in each group. No patient was diagnosed with severe SOS/VOD. Hemorrhagic cystitis was observed in 28 patients in IV Bu/Cy cohort (21.37%) and 19 patients (9.50%) in Cy/TBI cohort(P=0.002; Table 2). Univariate analysis showed that IV Bu/Cy was associated with a lower risk of 180 days and 3 years NRM than Cy/TBI(Table 3; Figure 2A). However, we noted no difference in 3 years NRM between those two conditioning regimens in multivariable analysis(Table 4). While there was a higher risk of 3 years NRM with female recipients in multivariable analysis (HR, 1.74; 95% CI, 1.15 to 2.63; P=0.009; Table 4). The main causes of NRM in order were: acute GVHD(15.9% vs.31.7%), infectious complications(25.0% vs.14.6%) and organ failure(11.4% vs.9.8%) in IV Bu/Cy and Cy/TBI protocols. There was no patient died of SOS/VOD.

**Acute GVHD, chronic GVHD and Relapse**

At day 100 after UCBT, the cumulative incidence of II-IV acute GVHD was similar between IV Bu/Cy and Cy/TBI cohorts(Tables 2 and 3). III-IV acute GVHD is more frequency in IV Bu/Cy cohort than that in Cy/TBI cohort(19.1% vs. 11.0%, p=0.033; Tables 2 and 3). But after adjustment with multivariable analysis, the cumulative incidence of III-IV acute GVHD did not differ in two conditioning regimens(Table 4). The cumulative incidence of chronic graft-versus-host disease was also similar between the two regimens (Tables 3 and 4). No difference in 3 years relapse incidence was found between those two conditioning regimens in both univariate analysis and multivariable analysis (Tables 3 and 4; Figure 2B).

**DFS, OS and GRFS**

The estimated DFS at 3 years were not statistically different between the conditioning regimens in univariate analysis, 63.9%±4.21% in patients undergoing conditioning with IV Bu/Cy and 54.4%±3.57% in patients undergoing conditioning with Cy/TBI(P=0.21; Tables 2 and 3; Figure 3B). These results were similar in multivariable analysis (HR, 1.08; 95% CI, 0.73 to 1.59; P=0.714; Table 4). Univariate analysis and multivariable analysis demonstrated that estimated OS and GRFS at 3 years were also not different between those two conditioning regimens (Tables 3 and 4; figure 3A and 3C).

**Discussion**

A number of retrospective and prospective studies revealed that Cy plus Bu or TBI can be regarded as cornerstone of myeloablative preparative regimens[13-15]. Those two schemes are also extensively applied in myeloablative conditioning for CBT patients, but few literatures are available on the comparison of them previously[16,18-20]. The present study was performed to retrospectively analysis the superiority following conditioning with IV Bu versus TBI in CBT recipients.

Due to heterogeneous patient characteristics and disease types, so much as the different resourse of stem cells and variation in dose of TBI and Bu, the available data of the superiority of those two regimens is still controversial. To minimize the variation, all patients eligibled to our study recieved fixed full-dose IV Bu or TBI combined with Cy as conditioning regimens. And the GVHD prophylaxis regimens were exactly the same. Because there’s still no consensus on target exposure lever and extent dose-adjustment of IV Bu, especially for pediatric patients[13,25]. Even the recommendation of dose-adjustment for pediatric are different in dispensatory from U.S. Food and Drug Administration (FDA) and European Medicinal Agency(EMA). So patients weigh less than 35 kilograms were excluded from the present study. Controversies have been raised about the outcomes of single and double CBT, patients received double CBT were also excluded from the present study[26-28].

In this study, we observed that the median dose of infused total nucleated cells and CD34+ cells was significantly higher in the IV Bu cohort than that in the TBI cohort. However, the cumulative incidence of neutrophil engraftment was lower in IV Bu cohort by univariate analysis and multivariable analysis. Despite of high potential of engraftment, TBI based regimen always correlated with intensive myelosuppression. Consequently, delayed neutropil engraftment may result in fatal infection. Results of our study indicated that the median times to neutrophil engraftment were delayed in TBI cohort, and infection was the leading cause of death in TBI based regimen. Ultimately, although patients who received IV Bu/Cy had a lower incidence of neutrophil engraftment, there were no statistical difference in OS and DFS between two regimens. Different from other types of transplantation, limited to inadequate stem cell doses, CBT is accompanied by high frequency of graft failure and delayed hematopoietic engraftment[18,19,29,30]. Originally, to overcome the HLA barrier and provide sufficient immunosuppressive and anti-malignancy activity, majority of CBT patients received conditioning with fractionated TBI[31]. Our results suggested that IV Bu/Cy can also be efficiently used in patients undergoing CBT.

Limited to the short follow-up period, we can’t make a comprehensive assessment of the long-term side effects of these two schemes. As for transplantation-related toxicity, in univariate analysis, there was a tendency toward lower NRM in IV Bu/Cy. But adjusted for differences between both groups, multivariable analysis in our analysis showed similar NRM. The incidence of [sinusoidal obstruction syndrome/veno-occlusive disease](https://www.ncbi.nlm.nih.gov/pubmed/29519713)

(SOS/VOD) was similar between two conditioning regimens in this study, and no patient developed severe SOS/VOD. The low incidence of severe SOS/VOD might be attributed to our prophylaxis protocol with low-dose heparin and PGE1[32,33]. There was also no consistent conclusion about the incidence of acute GVHD and chronic GVHD between two regimens[1,14,15]. In our patients undergoing CBT, no different frequencies of acute GVHD and chronic GVHD were observed in our study.

Previous comparative researches showed similar anti-malignancy effect of IV Bu and TBI in patients transplanted with other sources of stem cells[1,13-15]. Identical with those conclusions, there were no differences in relapse between those two conditioning regimens. Remarkably, we can find that 60(45.8%) patients in the Bu cohort and 83(41.5%) patients in the TBI cohort were diagnosised refactory or relapse acute leukemia at transplantation, the estimated 3 years relapse incidence were 15.5%(95% CI, 9.9%-22.3%) and 13.7%(95% CI, 9.3%-18.9%) in two cohorts, respectively.

Although many factors influence the outcome of HCT, disease type and status at the time of transplantation are the strongest determinants of post-HCT survival. It is therefore essential to categorize patients by disease risk in HCT studies. This is already feasible for other variables, such as donor HLA match or comorbidity burden.The Disease Risk Index (DRI) was developed as a tool to assign patients into one of 4 OS risk groups based on disease type and status at the time of transplantation.This index was developed by using a single-institution patient cohort and has since been successfully applied in other studies[34].In our cohort, we also found that DRI was strongly associated with 3-year relapse rate, OS,DFS and GRFS both in univariate and multivariable modeling.

The low incidence of relapse in the study may not only be attributable to the myeloablative preparative regimens, the intrinsic graft versus leukemia effect of cord blood may play an important role in it.

There was several limitations of the present study should be taken into consideration. Firstly, it is a retrospective study with inherent biases for patients selection and lack of randomization. Further more, in China, more than 40% of the umbilical cord blood transplants were treated at a single center. And some centers used only one regimen bacause of lack of considerable technical requirements of TBI. Secondly, patients in IV Bu/Cy cohort was younger with less weigh than Cy/TBI cohort, accompany with higher infused TNC, multivariate analyses have been performed to correct for these differences. But data on HCT-CI score of recipients was not available, therefore we can’t know if there was an imbalance between those two regimens. Third, since BU was approved by SFDA of China later than many other countries and regions,the Bu based cohort has significantly shorter follow up than the TBI based regimen. In order to know whether year of UCBT had the impact on transplantation outcome, we compared transplantation results before 2014 and 2014 and beyond. Univariate analysis showed that the cumulative incidence of 3-year NRM was significantly lower in the year 2014 and beyond than before 2014(22.2% vs 33.3%, P=0.0484). But multivariate analysis did not show statistical difference.

Finally, all patients were treated with identical full dose Bu or TBI and unified GVHD prophylaxis. However, in Cy/TBI cohort, all patients received high-dose cytarabine as a third agent for conditioning. While in Bu/Cy cohort, 103(78.6%) and 28(21.4%) patients individually received fludarabine and high-dose cytarabine.

In the absence of existing literature for direct comparison of BU and TBI-based myeloablative conditioning in patients undergoing CBT, our study may provide useful information to them. Our results demonstrates that, compared with TBI, IV Bu regimen was associated with a higher incidence of graft rejection in CBT. But there was no difference in survival with no increased risk for NRM or relapse between two regimens. For those centers lack of radiation facilities, IV Bu may be a valid and efficient alternative to TBI. With the restriction of a retrospective registry analysis and limited patient munbers, rigorously designed prospective randomized controlled trials are needed to further investigated the availability of IV Bu and TBI for CBT.

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**Compliance with ethical standards**

**Conflict-of-interest disclosure**

The authors declare no competing financial interests.