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Individualized busulfan dosing improves outcomes compared to fixed dose administration in pre-transplant MRD positive AML patients with intermediate risk undergoing allogeneic stem cell transplantation in CR.

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Novelty of the manuscript:

1. **What is the new aspect of your work?**

   Correlation between individualized busulfan administration and lower relapses in AML patients with intermediate risk

2. **What is the central finding of your work?**

   Improved survival due to lower relapse risk in patients with intermediate risk AML after individualized busulfan administration compared to fixed one

3. **What is (or could be) the specific clinical relevance of your work?**

   Using of individualized busulfan as part of conditioning for AML patients with intermediate risk allografted in CR

Abstract

Pre-transplant minimal residual disease (MRD) impacts negatively on post-transplant relapse risk in AML. Therapeutic drug monitoring by calculating area-under-the-curve (AUC) was developed to optimize busulfan exposure. Here, we compared post-transplant outcomes after individualized *versus* fixed busulfan dosage in intermediate-risk AML who achieved CR prior to allograft focusing on pre-transplant flow-MRD.

87 patients (median, 56 years) with intermediate-risk AML and pre-transplant flow-MRD ("different from normal") were included. 32 patients received individualized busulfan ; 54 fixed dosage. Individualized dosage was adjusted in 25/32 patients: increased, n=18/25 (72%); decreased: n=7/25 (28%).

After median follow-up of 27 months, we observed lower 3-year relapses (6%, 2-19% vs 35%, 23-49% p=0.02), improved 3-year LFS (78%, 54-91% vs 55%, 40-70% p=0.009) and -OS (82%, 60-93% vs 69%, 54-81% p=0.05) after individualized compared to fixed Bu. NRM and acute GvHD were not different. In multivariate analysis, fixed Bu showed unfavorable impact on OS (HR 4.6, p=0.044), LFS (HR 3.6, p=0.018) and relapses (HR 3.6, p=0.033). Fixed Bu also had unfavorable impact on LFS (3.6, 1.1-12.6, p=0.041) in pre-transplant MRD-positive patients.

Individualized, AUC-based, busulfan is associated with lower relapses in intermediate-risk AML patients allografted in CR and may overcome pre-transplant MRD-positivity.

Introduction

Busulfan (1,4-dimethanesulphonyloxybutane, Bu) is an alkylating agent with intense myelosuppressive potential which has been widely used in transplant settings since decades.(1) Historicaly, the use of oral Bu was associated with unpredictable and erratic bioavailability due to variable intestinal absorption and a hepatic first-pass extraction effect that led to development of relevant complications such as hepatic sinusoidal obstructive syndrome (SOS) and seizures.(2;3) Several pharmacokinetic (PK) studies were able to demonstrate a relationship of Bu exposure and clinical endpoints such as engraftment and toxicity.(4-7) Introduction of intravenous (i.v.) Bu overcame these issues and led to lower toxicity with at least similar anti-leukemic activity in AML/MDS patients resulting in improved survival.(8;9) Nevertheless, it was found that patient-individual covariates such as age, weight, body surface area or co-medication might affect the clearance or volume of distribution of busulfan leading to inter-individual variability (10-15) and intra-individual change of a pharmacokinetic parameter between different doses (16,17).

PK-guided Bu administration was found to be associated with higher engraftment rates in children(18), lower hepatotoxicity rates(19,20), and lower relapses in patients with previously untreated chronic myeloid leukemia (CML).(21) Further studies reported that toxicity is associated with increased AUC, thus the optimized AUC level led to significantly lower toxicity, lower NRM und higher survival outcomes also in older and/or patients with comorbidities.(22-26) However, its impact on post-transplant relapses remains unclear. Though one large CIBMTR study showed significantly improved relapse rate after i.v. Bu administration(27), a randomized prospective study did not document any impact of PK-guided Bu administration on relapses.(28) Recommendations on the use of PK-guided Bu were published previously by ASBMT.(29)

Pre-transplant minimal/measurable residual disease (MRD) detected by different methods (multicolor flow cytometry (MFC), qPCR and/or next-generation sequencing (NGS)) clearly demonstrated significant unfavorable impact on relapse and survival in patients with AML.(30-32)

The aim of this study was to investigate impact of PK-guided (=individualized) Bu administration on relapses and survival in a homogeneous group of AML patients, focusing on pre-transplant MRD status assessed by multiparameter flow cytometry.

Patients and Methods

Study cohort

Adult (≥18 years) patients were included in this monocentric retrospective study if they had intermediate risk AML, fulfilled the criteria for CR at allo-SCT, underwent allo-SCT after Bu-based myeloablative or reduced intensity conditioning and had available pre-transplant MFC-MRD data. All patients received allo-SCT at the Department for Stem Cell Transplantation of University Cancer Center University of Hamburg in the period 01/2015 to 01/2022. We used the European Leukemia Net (ELN) criteria (2017) to assign disease-dependent risk.(33) Criteria for response to therapy were used as proposed by an International Working Group.(34) For further analysis we augmented the criteria of CR with MRD data. The MRD defined by flow cytometry were subdivided into MRD positive (≥0.1% aberrant myeloid blasts) and MRD negative (<0.1% aberrant myeloid blasts).(33) All patients consented in accordance with the Declaration of Helsinki. Follow-up was current as of February 15, 2022.

Flow-cytometric detection of MRD

Immunophenotypic analysis was done on whole bone marrow specimens after stain-lyse-wash standard techniques.(31) The eight-color based immunostaining analysis was performed according to ELN consensus recommendation.(35) Up to 2,000,000 events per tube (6,000,000 events per sample) were evaluated. All antibodies were obtained from Beckman-Coulter (CA, USA) or Becton Dickinson (BD Biosciences, New Jersey, USA). Analysis of list mode files was performed using Infinicyte™ Flow Cytometry Software (Cytognos, Salamanca, Spain). The assessments were performed using the leukemia-associated phenotype (LAIP) and the "different from normal" strategy in combination. Following ELN guidelines, a threshold of 0.1% or more of aberrant cells in the bone marrow was defining MRD positivity.(35) The sensitivity of our MFC-based approach was 10^{-4} – 10^{-5}.

Conditioning regimens and AUC measurement

Patients received Bu-based myeloablative or reduced intensity conditioning.(36) The AUC goals were determined according to previously published studies.(37,38)

Bu was administered i.v. over 3 hours with an initial dose of 3.2 mg/kg (based on AIBW in overweight patients) once daily. Four consecutive dosages were planned to achieve a cumulative area under the curve (AUC) of 80 mg*h/L for myeloablative Bu and 40-60 mg*h/L for RIC. Therapeutic drug monitoring (TDM) was done on the first or second day of application and the busulfan dose was adjusted based on the results. There was a 24 hours interval between the last Bu administration and graft transfusion. Levetiracetam was used as a seizure prophylaxis.

Bu plasma levels were drawn according to a local sampling schedule at the times 5 min, 1 h, 2 h and 3 h after the end of busulfan infusion, while the exact times of blood sampling were documented. Quantification took place at the Department of Legal Medicine at the University

Medical Centre Hamburg-Eppendorf using a validated gas chromatography with mass spectrometric detection (GC-MS) method. Subsequently, the model-based calculation of the AUC was carried out in the hospital pharmacy by means of Bayesian prediction based on a population pharmacokinetic model using pharmacokinetics software (MW-Pharm, Version 3.60). If the calculated AUC was not within a target range of ± 10 % of the target AUC of 40, 60 or 80 mg*h/l, the busulfan dose was adjusted accordingly. In the case of deviations of > 25%, new level measurements and AUC calculations were carried out after the following dose and, if necessary, a repeated dose adjustment was made. The AUC in the fixed group was nor calculated. All patients received concomitant anticonvulsive prophylaxis with levetiracetam.

Statistical analysis

Unadjusted probabilities of overall survival (OS) and leukemia-free survival (LFS) were estimated by using the Kaplan-Meier and Cox regression methods. Probabilities of NRM and relapse were summarized by using cumulative incidence estimates. NRM was defined as death without relapse and was considered a competing risk for relapse, whereas relapse was a competing risk for NRM. The probability of developing acute (grade II-IV) GVHD and chronic GVHD was depicted by calculating the cumulative incidence with death without GVHD as a competing risk.

Categorical characteristics were compared by Pearson's or Fisher's exact test. Continuous variables were compared using non-parametric Mann-Whitney test. Statistical analysis was performed with IBM SPSS Version 25 (SPSS, Inc.; Chicago, IL, USA) and R software (Version 3.5.1 R Foundation, Vienna, Austria) with competing risks calculated using the package 'cmprsk' (http://CRAN.R-project.org/package=cmprsk).

Results

Patients' characteristics

The characteristics of the study population are summarized in Table1. Eight y-seven patients with intermediate-risk AML (male, n=48) with a median age of 51 years (range, 21-73) were included. There were significantly more females and sex mismatched allografts in the individualized group of patients. The allografts were performed in the majority of cases from matched unrelated donors after myeloablative conditioning (MAC). Conditioning was based on a combination of Bu with fludarabine (Flu) in most of the patients. Before allogeneic transplantation, flow cytometry revealed MRD negativity in 43 patients (49%), whereas 44 (51%) were MRD positive. Fifty-five patients (63%) received non-PK-guided (="fixed"; 12.8 mg/kg bw iv, n=33; 9.6 mg/kg bw iv, n=13, 6.4 mg/kg bw iv, n=9) Bu dosage, while 32 patients received PK-guided (="individualized"; AUC80, n=27, AUC60, n=4, AUC40, n=1) Bu dosage. The median achieved AUC was 75.7. mg*h/L (36.2-87.1). In the individualized group, Bu

dosage was adjusted in 25 of 32 patients: increased in 18/25 (72%) or decreased in 7/25 (28%).

Conditioning regimens

Of the 55 patients from fixed group, 33 (60%) received Bu in combination with cyclophosphamide (Cy, n=2, cumulative Bu dosage 12.8 mg/kg bw i.v.; cumulative Cy dosage, 120 mg/kg bw i.v.) or Flu (n=31; cumulative Bu dosage 12.8 mg/kg bw i.v., n=30; cumulative Bu dosage 9.6 mg/kg bw i.v., n=1; cumulative Flu dosage 150 mg/m^2 i.v.), whereas 18 (33%) patients received Bu (cumulative Bu dosage 9.6 mg/kg i.v., n=12; cumulative Bu dosage 6.4 mg/kg, n=6) in combination with thiotepa (cumulative dosage, 10 mg/kg i.v.). Flu (cumulative dosage 150 mg/m^2 i.v.) was added to thiotepa and Bu in case of mismatched or haploidentical donors. Four (7%) patients received Bu in combination with FLAMSA-protocol (cumulative Bu dosage 12.8 mg/kg i.v., n=1; cumulative Bu dosage 6.4 mg/kg i.v., n=3; Flu).(39) ATG-Fresenius (Grafalon) as immunosuppression was used in 42 patients (cumulative dosage 15-30 mg/kg i.v.), while 12 patients received post-transplant Cy (cumulative dosage 100 mg/kg i.v.). One patient received both due to persistence of anti-donor antibodies.

Of the 32 patients from the individualized group, 27 (84%) received Bu in combination with fludarabine (cumulative Bu AUC80 i.v., n=25; cumulative Bu AUC60 i.v., n=2; cumulative Flu dosage 150 mg/m^2 i.v.); one (3%) patient received Bu (cumulative Bu AUC60 i.v.) in combination with thiotepa (cumulative dosage, 10 mg/kg i.v.) and Flu (cumulative dosage 150 mg/m^2 i.v.) and four (13%) patients in combination with FLAMSA-protocol (cumulative Bu AUC80 i.v., n=2; cumulative Bu AUC60, n=1, cumulative Bu AUC40, n=1). ATG as immunosuppression was used in 27 (84%) patients (cumulative dosage 15-30 mg/kg i.v.), while 5 (16%) patients received post-transplant Cy (cumulative dosage 100 mg/kg i.v.).

Flu was given as a single dose of 30mg/m^2 i.v. over 30 min on 5 consecutive days. There was a 48 hours interval between the last Flu administration and graft transfusion. Thiotepa was given as a single dosage of 5mg/kg bw i.v. over 30 min on two consecutive days immediately before Bu. Cyclophosphamide was given as single dosage of 60mg/kg bw i.v. over 1 hour on two consecutive days 24 hours later after the last Bu administration and 24 hours before the graft transfusion. Urometixan was used for prevention of haemorrhagic cystitis. ATG-Fresenius (Grafalon) was given on four consecutive days (-4, -3, -2 and -1) over 12 hours starting with 200mg absolute on the day -4. Post-transplant cyclophosphamide as GvHD prophylaxis was given as single dosage of 50mg/kg i.v. over 1 hour on two consecutive days (+3 and +4).Pre-transplant MRD positive patients from individualized group received rather thiotepa-based conditioning than those from the fixed Bu group (Table 1S).

Engraftment

All but 2 patients (n=85/87; 98%) showed successful engraftment (leukocytes: median day 11 (8-23); platelets: day 12 (7-117)). Two patients (both from the fixed Bu group) developed graft failure and subsequently underwent a second allograft. There were no differences in the timing of leukocyte and thrombocyte engraftment between fixed and individualized groups.

Relapse, NRM, LFS and OS for all patients

After a median follow up of 33.5 months (2-60) there were 18 deaths, 20 relapses and seven NRM events. The relapses and NRM at 3 years were 26% (18-37%) and 9% (5-17%), respectively. The 3-year OS and LFS were 76% (65-84%) and 65% (55-95%). The results of univariate analysis are represented in Table 2. In the multivariate analysis (Table 3), we observed an independent significant unfavorable impact of fixed vs individualized Bu administration on relapses (HR 3.6, 1.1-11, p=0.033), LFS (HR 3.6 1.3-10.6, p=0.018) and OS (HR 4.6, 1.1-20.5, p=0.044) (Fig.1a-c). The median AUC level (≤923 ng/ml or >923 ng/ml) did not have any impact on survival outcomes. Concerning all patients, thiotepa-based conditioning did not have any impact on 3-year LFS (72%, 46-89% vs 67%, 55-78%, p=0.93) and 3-year OS (80%, 56-93% vs 78%, 67-86%, p=0.97) compared to other regimens.

In the multivariate analysis, fixed Bu administration had a significant unfavorable impact on OS (HR 4.6, 95% CI: 1.1-20.5, p=0.044), LFS (HR 3.6, 95% CI: 1.3-10.6, p=0.018) and relapses (HR 3.6, 95% CI: 1.1-11, p=0.033). Further, we observed a significant favorable impact of pre-transplant MRD negativity on LFS (HR 0.4, 95% CI: 0.2-0.9, p=0.031) and relapses (HR 0.2, 95% CI: 0.1-0.7, p=0.007).

Relapse, NRM, LFS and OS for pre-transplant MRD positive patients

After a median follow up of 30 months (2-59) there were 12 deaths, 26 relapses and three NRM events. The relapses and NRM at 3 years were 42% (26-60%) and 7% (2-19%), respectively. The 3-year OS and LFS were 67% (50-80%) and 51% (34-68%). The results of univariate analysis are represented in the Table 2S. We observed a significant unfavorable impact of fixed Bu administration on LFS (3.6, 1.1-12.6, p=0.041), but not on OS and relapse rate. Due to low number of events the multivariate analysis was not performed.

Relapse, NRM, LFS and OS for pre-transplant MRD negative patients

After a median follow up of 34 months (5-61) there were six deaths, four relapses and four NRM events. The relapses and NRM at 3 years were 11% (5-25%) and 10% (4-24%), respectively. The 3-year OS and LFS were 84% (69-93%) and 79% (63-89%). The results of univariate analysis are represented in the Table 2S. We observed no significant impact of fixed Bu administration on relapses, LFS and OS. Due to low number of events the multivariate analysis was not performed.

Safety

The NRM at 1 year rates did not differ significantly between fixed (10%, 95% CI: 4-20%) and individualized (4%, 95% CI: 1-20%, p=0.22) groups. Seventy-nine of 87 (91%) patients developed mucositis (grade 1, n=3; grade 2, n=37; grade 3, n=38; grade 4, n=1). Sixty-seven (77%) patients developed infections, sepsis was documented in five (6%) patients. Hepatic sinusoidal obstructive syndrome (SOS) developed in two patients (both late onset); both patients received fixed dosage of Bu. Seizures were documented in two patients (fixed, n=1; individualized, n=1). Late onset lung fibrosis was documented in one patient after individualized Bu administration. We did not observe any correlation between individualized Bu concentrations and rates of the mentioned complications.

Graft versus host disease

The incidence of acute severe (grade II-IV) GvHD at 1 year was 13% (7-23%). The rates of acute GvHD did not differ significantly between pre-transplant MRD positive (7%, 2-22%) and MRD negative patients (19%, 10-33%, p=0.10). The rates of acute GvHD did not differ significantly between individualized (13%, 5-29%) and fixed groups (13%, 6-26%, p=0.99).

The incidence of chronic GvHD at 3 year was 48% (39-58%). The rates of chronic GvHD did not differ significantly between pre-transplant MRD positive (36%, 22-52%) and MRD negative patients (60%, 44-74%, p=0.09). The rates of chronic GvHD did not differ significantly between individualized (51%, 33-69%; mild, n=7, moderate, n=10, severe, n=1) and fixed groups (47%, 34-60%; mild, n=10, moderate, n=7, severe, n=3; p=0.57).

Outcomes for patients after BuFlu conditioning according to Bu administration

We performed a separate analysis for the patients (n=60) who received allografts after myeloablative conditioning with BuFlu. Both patients who received BuCy were also included into the analysis. Of all patients, 27 received individualized Bu (pre-transplant MRD positive, n=14; pre-transplant MRD negative, n=13) and 33 received fixed Bu (pre-transplant MRD positive, n=12; pre-transplant MRD negative, n=21). The patients' characteristics were not different between both groups (Table 3S). Engraftment of leukocytes (individualized: median 11 (10-23) vs fixed: median 12 (8-22), p=0.72) and platelets (individualized: median 11 (8-83) vs fixed: median 12 (7-117), p=0.44) was not significantly different between both groups. One patient who received fixed Bu dosage and was pre-transplant MRD negative developed graft failure und underwent second allo-SCT. The frequency of adverse events was similar between both groups. Late-onset SOS developed in two patients from the fixed group.

The 3-year OS, LFS, relapses and NRM were 75% (95% CI: 62-85%), 65% (95% CI: 51-77%), 21% (95% CI: 12-34%) and 10% (95% CI: 5-21%), respectively. In the univariate analysis,

fixed Bu administration was associated with significant unfavourable impact on OS (HR 4.8, 95% CI: 1.1-22, p=0.04), LFS (HR 3.5, 95% CI: 1.2-11, p=0.025), but not on relapses (HR 2.8, 95% CI: 0.8-19, p=0.12) and NRM (HR 1.1, 95% CI: 0.5-34, p=0.18). The multivariate analysis were not performed due low event number.

Discussion

Busulfan is one of the drugs that has been using widely as part of conditioning in the setting of allo-SCT since many years due to its myeloablative potential. Several studies showed that individualized, pharmacokinetic-based administration can improve post-transplant outcomes due to decreased risk of graft failure and low NRM also in older patients.(18;26) However, the role of individualized Bu administration regarding the relapse risk still remains unclear.

In this study, we compared individualized and fixed Bu administration strategies in patients with intermediate risk AML who achieved CR prior to allo-SCT and reported improved survival due to lower relapse risk in the individualized group. Moreover, this impact may be stronger in the pre-transplant flow-MRD positive patients. Further, we observed improved 3-year OS and LFS after individualized Bu administration in patients who received Bu/Flu as a myeloablative conditioning. The goal AUC level after standard myeloablative in our study was 80 mg*h/L.

Increased cumulative Bu dosage was reported to improve survival in AML and MDS patients in myeloablative(40;41) but not in reduced-intensity settings.(40;42) Further, use of individualized Bu administration in patients with hematologic malignancies is associated with better engraftment in children, lower hepatotoxicity in adults and lower relapse risk in patients with CML.(18;19;21) Thus, individualized Bu administration results in lower NRM resulted in improved OS and LFS in patients with AML/MDS and ALL after MAC.(43;44;45) On the other hand, the role of individualized Bu administration regarding the post-transplant relapses remains unclear. The majority of published studies did not show any differences in post-transplant relapse rate after intravenous vs oral Bu administration.(46;47;48) However, a CIBMTR study showed lower relapse rate (>1 year post-transplant) in patients with AML transplanted in first CR after intravenous comparing to oral Bu administration. Unfortunately, this study reported no data on pharmacokinetics.(27) A randomized study published by Popat *et al.* showed no difference in NRM in two groups of transplanted patients with different hematologic malignancies after having achieved “lower” (65.7 mg*h/L) or “higher” (82.1 mg*h/L) cumulative Bu AUC level. Additionally, the authors found no difference in relapses and survival between both groups. The NRM in this study was higher than in our study (20% vs 9%) and 70% of included patients experienced relapsed/refractory disease prior to allo-SCT.(26) This can render the interpretation of the cited study concerning the role of higher Bu exposure in post-transplant disease control difficult.

Furthermore, Andersson *et al.* published results of a prospective randomized study where post-transplant outcomes were compared between individualized and fixed Bu administration after MAC (BuFlu and ATG) in patients with AML/MDS. The patients had different remission status before allo-SCT, including relapsed/refractory patients (48%). Individualized Bu administration was associated with significantly better OS and PFS in all patients with a trend to improved TRM in the PK-guided group. According to the remission status, this difference was seen in patients with non-CR and not in those with CR prior to allograft. Though authors did not observed any significant impact of Bu administration on post-transplant relapses, the TRM in this study was around 20% which together with different pre-transplant remission status may mitigate the favorable impact of individualized Bu administration.(28)

In the present study only AML patients with intermediate risk who achieved CR prior to allo-SCT were included. The NRM was lower (9%) as compared to both randomized studies.(26;28) We suggest, that both low NRM and CR prior to allo-SCT allowed to see significant impact of individualized Bu administration on the relapse rates in all included patients. In contrast to the results of recently published FIGARO study, where augmented RIC conditioning could not improve survival outcomes of the pre-transplant MRD positive AML patients,(49) we showed significantly improved LFS in such patients, but not in MRD negative ones after individualized Bu administration. These results support the idea of the importance of intensive chemotherapy needed to eradicate residual disease in pre-transplant MRD positive patients.(50) To confirm this favorable impact on survival we performed a separate analysis where we excluded patients who received RIC (11%) and those transplanted after thiotepa-based conditioning.(51;52) Here, we showed a significantly favorable impact of individualized Bu administration on OS and PFS, but not on relapses and NRM probably due to the lower number of patients.

In the majority of patients who received individualized Bu administration the Bu dosage was increased, suggesting that the fixed group showed probably lower Bu serum levels. Despite this, in line with Anderson *et al.*, we found no difference in NRM between both groups.(28) We suggest, that optimized Bu exposure together with low NRM was associated with lower relapses in the individualized group. This suggestion may be supported by the observation, that the rate of MRD conversion on day +100 was higher after increased Bu dosage which was associated with significantly higher AUC level compared to the “decreased/no change” group. Moreover, low Bu exposure in the fixed group may have led to graft failure.

Whether there is an association between Bu exposure and development of acute GvHD is controversial.(53;27) In line with a randomized study performed by Andersson *et al.*, we did not observe any difference in rates of acute GvHD regarding the individualized or fixed Bu administration.(28)

In conclusion, this study is the first analyzing the role of individualized Bu administration in a homogeneous patients' population characterized by intermediate risk AML patients who achieved CR prior to allo-SCT. The use of individualized Bu administration can improve the OS for AML patients with intermediate risk transplanted in complete remission due to decreased relapse risk. This impact may be more pronounced in pre-transplant MRD positive patients. A randomized prospective study to evaluate impact of individualized Bu administration on relapses and survival outcomes in larger cohort of patients focusing on MRD studies is warranted.

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Reference list:

1. Santos GW, Tutschka PJ, Brookmeyer R, Saral R, Beschorner WE, Bias WB, Braine HG, Burns WH, Elfenbein GJ, Kaizer H, Mellits, D, Sensenbrenner LL, Stuart RK, Yeager AM. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. N Engl J Med 1983; 309: 1347-1353

2. Peters WP, Henner WD, Grochow LB, Olsen G, Edwards S, Stanbuck H, Stuart A, Gockermann J, Moore J, Jr. Bast RC, Seigler HF, Colvin OM. Clinical and pharmacologic effects of high dose single agent busulfan with autologous bone marrow support in the treatment of solid tumors. Cancer research 1987; 47: 6402-6406.

3. Marcus RE, Goldman JM. Convulsions due to high-dose busulphan. Lancet 1984; 22:1463.

4. Hassan M, Ljungman P, Bolme P, Ringdén O, Syrůcková Z, Békàssy A, Starý J, Wallin I, Kållberg N. Busulfan bioavailability. Blood 1994 ;84:2144-2150.

5. Méresse V, Hartmann O, Vassal G, Benhamou E, Valteau-Couanet D, Brugieres L, Lemerle J. Risk factors for hepatic veno-occlusive disease after high-dose busulfan-containing regimens followed by autologous bone marrow transplantation: a study in 136 children. Bone Marrow Transplant 1992;10:135-141.

6. Slattery JT, Sanders JE, Buckner CD, Schaffer RL, Lambert KW, Langer FP, Anasetti C, Bensinger WI, Fisher LD, Appelbaum FR. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. Bone Marrow Transplant 1995; 16: 31-42.

7. Ljungman P, Hassan M, Békássy AN, Ringdén O, Oberg G. High busulfan concentrations are associated with increased transplant-related mortality in allogeneic bone marrow transplant patients. Bone Marrow Transplant 1997;20:909-913.

8. Kim SE, Lee JH, Choi SJ, Lee JH, Ryu SG, Lee KH. Morbidity and non-relapse mortality after allogeneic bone marrow transplantation in adult leukemia patients conditioned with busulfan plus cyclophosphamide: a retrospective comparison of oral versus intravenous busulfan. Haematologica 2005; 90: 285-286.

9. Andersson BS, de Lima M, Thall PF, Wang X, Couriel D, Korbling M, Roberson S, Giralt S, Pierre B, Russell JA, Shpall EJ, Jones RB, Champlin RE. Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. Biol Blood Marrow Transplant 2008; 14 :672-684.

10. McCune JS, Bemer MJ, Barrett JS, Scott Baker K, Gamis AS, Holford NHG. Busulfan in Infant to Adult Hematopoietic Cell Transplant Recipients: A Population Pharmacokinetic Model for Initial and Bayesian Dose Personalization. Clin Cancer Res 2014; 20: 754–763.

11. Bartelink IH, Boelens JJ, Bredius RGM, Egberts ACG, Wang C, Bierings MB, Shaw PJ, Nath CE, Hempel G, Zwaveling J, Danhof M, Knibbe CAJ. Body Weight-Dependent Pharmacokinetics of Busulfan in Paediatric Haematopoietic Stem Cell Transplantation Patients: Towards Individualized Dosing. Clin Pharmacokinet 2012; 51:331–345.

12.Trame MN, Bergstrand M, Karlsson MO, Boos J, Hempel G. Population Pharmacokinetics of Busulfan in Children: Increased Evidence for Body Surface Area and Allometric Body Weight Dosing of Busulfan in Children. Clin Cancer Res 2011;17:6867–6877.

13. Long-Boyle J, Savic R, Yan S, Bartelink I, Musick L, French D, Law J, Horn B, Cowan MJ, Dvorak CC. Population Pharmacokinetics of Busulfan in Pediatric and Young Adult Patients

Undergoing Hematopoietic Cell Transplant: A Model-Based Dosing Algorithm for Personalized Therapy and Implementation into Routine Clinical Use. Ther Drug Monit 2015; 37: 236-245.

14. Takahashi T, Illamola SM, Jennissen CA, Long SE, Lund TC, Orchard PJ, Gupta AO, Long-Boyle JR. Busulfan dose recommendation in inherited metabolic disorders: Population pharmacokinetic analysis. Transplant Cell Ther 2022;28:104.e1-104.e7.

15. Essmann S, Dadkhah A, Janson D, Wolschke C, Ayuk F, Kröger NM, Langebrake C. Iron Chelation With Deferasirox Increases Busulfan AUC During Conditioning Chemotherapy Prior to Allogeneic Stem Cell Transplantation. Transplant Cell Ther 2022; 28: 115.e1-115.e5.

16. Lee JW, Kang HJ, Lee SH, Yu K-S, Kim NH, Yuk YJ, Jang MK, Han EJ, Kim H, Song SH, Park KD, Shin HY, Jang I-J, Ahn HS. Highly Variable Pharmacokinetics of Once-Daily Intravenous Busulfan When Combined with Fludarabine in Pediatric Patients: Phase I Clinical Study for Determination of Optimal Once-Daily Busulfan Dose Using Pharmacokinetic Modeling. Biol Blood Marrow Transplant 2012; 18: 944–950.

17. Marsit H, Philippe M, Neely M, Rushing T, Bertrand Y, Ducher M, Leclerc V, Guitton J, Bleyzac N, Goutelle S. Intra-individual Pharmacokinetic Variability of Intravenous Busulfan in Hematopoietic Stem Cell-Transplanted Children. Clin Pharmacokinet 2020; 59:1049-1061.

18. Bolinger AM, Zangwill AB, Slattery JT, Risler LJ, Sultan DH, Glidden DV, Norstad D, Cowan MJ. Target dose adjustment of busulfan in pediatric patients undergoing bone marrow transplantation.Bone Marrow Transplant 2001; 28:1013-1018.

19. Grochow LB. Busulfan disposition: the role of therapeutic monitoring in bone marrow transplantation induction regimens. Semin Oncol 1993; 20: 18-25.

20. Feng X, Wu Y, Zhang J, Li J, Zhu G, Fan D, Yang C, Zhao L. Busulfan systemic exposure and its relationship with efficacy and safety in hematopoietic stem cell transplantation in children: a meta-analysis. BMC Pediatr 2020; 20: 176.

21. Radich JP, Gooley T, Bensinger W, Chauncey T, Clift R, Flowers M, Martin P, Slattery J, Sultan D, Appelbaum FR. HLA-matched related hematopoietic cell transplantation for chronic-phase CML using a targeted busulfan and cyclophosphamide preparative regimen. Blood 2003; 102: 31-35.

22. Pidala J, Kim J, Anasetti C, Kharfan-Dabaja MA, Field T, Perkins J, Ayala E, Perez L, Fernandez H. Targeted i.v. BU and fludarabine (t-i.v. BU/Flu) provides effective control of AML in adults with reduced toxicity. Bone Marrow Transplant 2011; 46 :641-649.

23. Perkins JB, Kim J, Anasetti C, Fernandez HF, Perez LE, Ayala E, Kharfan-Dabaja MA, Tomblyn MR, Sullivan DM, Pidala JA, Field TL. Maximally tolerated busulfan systemic exposure in combination with fludarabine as conditioning before allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2012; 18: 1099-1107.

24. Andersson BS, Thall PF, Madden T, Couriel D, Wang X, Tran HT, Anderlini P, de Lima M, Gajewski J, Champlin RE. Busulfan systemic exposure relative to regimen-related toxicity and acute graft-versus-host disease: defining a therapeutic window for i.v. BuCy2 in chronic myelogenous leukemia. Biol Blood Marrow Transplant 2002; 8: 477-485.

25. Russell JA, Kangarloo SB, Williamson T, Chaudhry MA, Savoie ML, Turner AR, Larratt L, Storek J, Bahlis NJ, Shafey M, Brown CB, Yang M, Geddes M, Zacarias N, Yue P, Duggan P, Stewart DA, Daly A. Establishing a target exposure for once-daily intravenous busulfan given with fludarabine and thymoglobulin before allogeneic transplantation. Biol Blood Marrow Transplant 2013; 19: 1381-1386.

26. Popat UR, Mehta RS, Bassett R, Chen J, Valdez BC, Kawedia J, Ahmed S, Alousi AM, Anderlini P, Al-Atrash G, Bashir Q, Ciurea SO, Hosing CM, Im JS, Jones R, Kebriaei P, Khouri I, Marin D, Nieto Y, Olson A, Oran B, Parmar S, Rezvani K, Qazilbash MH, Shah N, Srour SA, Shpall EJ, Champlin RE, Andersson BS. Fludarabine with a higher versus lower dose of myeloablative timed-sequential busulfan in older patients and patients with comorbidities: an open-label, non-stratified, randomised phase 2 trial. Lancet Haematol 2018; 5: e532-e542.

27. Copelan EA, Hamilton BK, Avalos B, Ahn KW, Bolwell BJ, Zhu X, Aljurf M, van Besien K, Bredeson C, Cahn JY, Costa LJ, de Lima M, Gale RP, Hale GA, Halter J, Hamadani M, Inamoto Y, Kamble RT, Litzow MR, Loren AW, Marks DI, Olavarria E, Roy V, Sabloff M, Savani BN, Seftel M, Schouten HC, Ustun C, Waller EK, Weisdorf DJ, Wirk B, Horowitz MM, Arora M, Szer J, Cortes J, Kalaycio ME, Maziarz RT, Saber W. Better leukemia-free and overall survival in AML in first remission following cyclophosphamide in combination with busulfan compared with TBI. Blood 2013; 122: 3863-3870.

28. Andersson BS, Thall PF, Valdez BC, Milton DR, Al-Atrash G, Chen J, Gulbis A, Chu D, Martinez C, Parmar S, Popat U, Nieto Y, Kebriaei P, Alousi A, de Lima M, Rondon G, Meng QH, Myers A, Kawedia J, Worth LL, Fernandez-Vina M, Madden T, Shpall EJ, Jones RB, Champlin RE. Fludarabine with pharmacokinetically guided IV busulfan is superior to fixed-dose delivery in pretransplant conditioning of AML/MDS patients. Bone Marrow Transplant 2017; 52: 580-587.

29. Palmer J, McCune JS, Perales MA, Marks D, Bubalo J, Mohty M, Wingard JR, Paci A, Hassan M, Bredeson C, Pidala J, Shah N, Shaughnessy P, Majhail N, Schriber J, Savani BN, Carpenter PA. Personalizing Busulfan-Based Conditioning: Considerations from the American Society for Blood and Marrow Transplantation Practice Guidelines Committee. Biol Blood Marrow Transplant 2016; 22: 1915-1925.

30. Walter RB, Gooley TA, Wood BL, Milano F, Fang M, Sorror ML, Estey EH, Salter AI, Lansverk E, Chien JW, Gopal AK, Appelbaum FR, Pagel JM. Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. J Clin Oncol. 2011; 29: 1190-1197.

31. Klyuchnikov E, Christopeit M, Badbaran A, Bacher U, Fritzsche-Friedland U, von Pein UM, Wolschke C, Kröger N. Role of pre-transplant MRD level detected by flow cytometry in recipients of allogeneic stem cell transplantation with AML. Eur J Haematol 2021; 106: 606-615.

32. Short NJ, Zhou S, Fu C, Berry DA, Walter RB, Freeman SD, Hourigan CS, Huang X, Nogueras Gonzalez G, Hwang H, Qi X, Kantarjian H, Ravandi F. Association of Measurable Residual Disease With Survival Outcomes in Patients With Acute Myeloid Leukemia: A Systematic Review and Meta-analysis. JAMA Oncol 2020; 6: 1890-1899.

33. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien H-F, Wie AH, Löwenberg B, Bloomfield C. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129: 424-447.

34. Cheson BD, Bennett JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Löwenberg B, Sanz MA, Head DR, Ohno R, Bloomfield CD. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria,

Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 2003; 21: 4642-4649.

35. Schuurhuis GJ, Heuser M, Freeman S, Bene M-C, Buccisano F, Cloos J, Grimwade D, Haferlach T, Hills RK, Hourigan CS, Jorgensen JL, Kern W, Lacombe F, Maurillo L, Preudhomme C, van der Reijden BA, Thiede C, Venditti A, Vyas P, Wood BL, Walter RB, Döhner K, Roboz GJ, Ossenkoppele G. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood 2018; 131: 1275-1291.

36. Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, Apperley J, Slavin S, Pasquini M, Sandmaier BM, Barrett J, Blaise D, Lowski R, Horowitz M. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant 2009; 15: 1628.

37. Bornhauser M, Storer B, Slattery JT, Appelbaum FR, Deeg HJ, Hansen J, Martin PJ, McDonald GB, Nichols WG, Radich J, Woolfrey A, Jenke A, Schleyer E, Thiede C, Ehninger G, Anasetti C. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. Blood 2003; 102 :820-826.

38. Slattery JT, Clift RA, Buckner CD, Radich J, Storer B, Bensinger WI, Soll E, Anasetti C, Bowden R, Bryant E, Chauncey T, Deeg HJ, Doney KC, Flowers M, Gooley T, Hansen JA, Martin PJ, McDonald GB, Nash R, Petersdorf EW, Sanders JE, Schoch G, Stewart P, Storb R, Sullivan KM, Thomas ED, Witherspoon RP, Appelbaum FR. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood 1997;89: 3055-3060.

39. Schmid C, Schleuning M, Ledderose G, Tischer J, Kolb HJ. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. J Clin Oncol 2005; 23: 5675-5687

40. Parmar S, Rondon G, de Lima M, Thall P, Bassett R, Anderlini P, Kebriaei P, Khouri I, Ganesan P, Champlin R, Giralt S. Dose intensification of busulfan in the preparative regimen is associated with improved survival: a phase I/II controlled, randomized study. Biol Blood Marrow Transplant 2013; 19:474-480.

41. Kharfan-Dabaja MA, Labopin M, Bazarbachi A, Socie G, Kroeger N, Blaise D, Veelken H, Bermudez A, Or R, Lioure B, Beelen D, Fegueux N, Hamladji RM, Nagler A, Mohty M. Higher busulfan dose intensity appears to improve leukemia-free and overall survival in AML allografted in CR2: An analysis from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Leuk Res 2015; 39: 933-937.

42. Chen YB, Coughlin E, Kennedy KF, Alyea EP, Armand P, Attar EC, Ballen KK, Cutler C, Dey BR, Koreth J, McAfee SL, Spitzer T4, Antin JH, Soiffer RJ, Ho VT. Busulfan dose intensity and outcomes in reduced-intensity allogeneic peripheral blood stem cell transplantation for myelodysplastic syndrome or acute myeloid leukemia. Biol Blood Marrow Transplant 2013; 19: 981-987.

43. Russell JA, Tran HT, Quinlan D, Chaudhry A, Duggan P, Brown C, Stewart D, Ruether JD, Morris D, Glick S, Gyonyor E, Andersson BS. Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. Biol Blood Marrow Transplant 2002; 8: 468-476.

44. de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R, Shpall EJ, Shahjahan M, Pierre B, Giralt S, Korbling M, Russell JA, Champlin RE, Andersson BS. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative,

reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood 2004; 104: 857-864.

45. Russell JA, Savoie ML, Balogh A, Turner AR, Larratt L, Chaudhry MA, Storek J, Bahlis NJ, Brown CB, Quinlan D, Geddes M, Stewart DA. Allogeneic transplantation for adult acute leukemia in first and second remission with a novel regimen incorporating daily intravenous busulfan, fludarabine, 400 CGY total-body irradiation, and thymoglobulin.Biol Blood Marrow Transplant 2007; 13 :814-821.

46. Sobecks RM, Rybicki L, Yurch M, Kalaycio M, Dean R, Andresen S, Pohlman B, Duong H, Bolwell B, Copelan E. Intravenous compared with oral busulfan as preparation for allogeneic hematopoietic progenitor cell transplantation for AML and MDS. Bone Marrow Transplant 2012; 47: 633-638.

47. Lombardi LR, Kanakry CG, Zahurak M, Durakovic N, Bolaños-Meade J, Kasamon YL, Gladstone DE, Matsui W, Borrello I, Huff CA, Swinnen LJ, Brodsky RA, Ambinder RF, Fuchs EJ, Rosner GL, Jones RJ, Luznik L. Therapeutic drug monitoring for either oral or intravenous busulfan when combined with pre- and post-transplantation cyclophosphamide. Leuk Lymphoma 2016; 57: 666-675.

48. Mamlouk K, Saracino G, Berryman RB, Fay JW, Pineiro LA, Vance EA, White M, Sandler I, Agura ED. Modification of the Bu/Cy myeloablative regimen using daily parenteral busulfan: reduced toxicity without the need for pharmacokinetic monitoring. Bone Marrow Transplant 2005; 35: 747-754.

49. Craddock C, Jackson A, Loke J, Siddique S, Hodgkinson A, Mason J, Andrew G, Nagra S, Malladi R, Peniket A, Gilleece M, Salim R, Tholouli E, Potter V, Crawley C, Wheatley K, Protheroe R, Vyas P, Hunter A, Parker A, Wilson K, Pavlu J, Byrne J, Dillon R, Khan N, McCarthy N, Freeman SD. Augmented Reduced-Intensity Regimen Does Not Improve Postallogeneic Transplant Outcomes in Acute Myeloid Leukemia. J Clin Oncol 2021; 39: 768-778.

50. Hourigan CS, Dillon LW, Gui G, Logan BR, Fei M, Ghannam J, Li Y, Licon A, Alyea EP, Bashey A, Deeg HJ, Devine SM, Fernandez HF, Giralt S, Hamadani M, Howard A, Maziarz RT, Porter DL, Scott BL, Warlick ED, Pasquini MC, Horwitz ME. Impact of Conditioning Intensity of Allogeneic Transplantation for Acute Myeloid Leukemia With Genomic Evidence of Residual Disease. J Clin Oncol 2020; 38: 1273-1283.

51. Sora F, Grazia CD, Chiusolo P, Raiola AM, Bregante S, Mordini N, Olivieri A, Iori AP, Patriarca F, Grisariu S, Terruzzi E, Rambaldi A, Sica S, Bruno B, Angelucci E, Bacigalupo A. Allogeneic Hemopoietic Stem Cell Transplants in Patients with Acute Myeloid Leukemia (AML) Prepared with Busulfan and Fludarabine (BUFLU) or Thiotepa, Busulfan, and Fludarabine (TBF): A Retrospective Study.Biol Blood Marrow Transplant 2020; 26: 698-703.

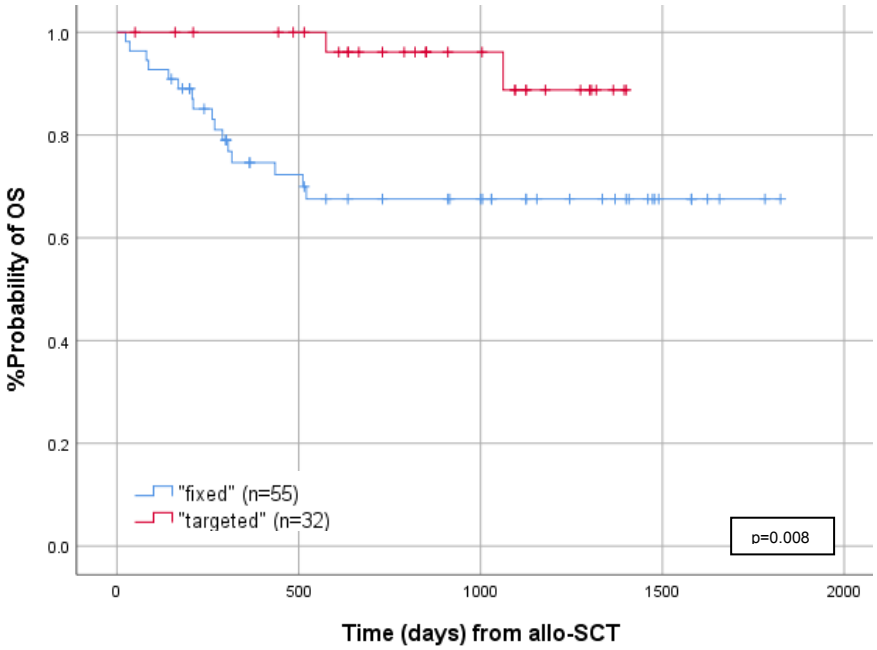
52. Saraceni F, Beohou E, Labopin M, Arcese W, Bonifazi F, Stepensky P, Aljurf M, Bruno B, Pioltelli P, Passweg J, Sociè G, Santarone S, Yakoub-Agha I, Lanza F, Savani BN, Mohty M, Nagler A; Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). Thiotepa, busulfan and fludarabine compared to busulfan and cyclophosphamide as conditioning regimen for allogeneic stem cell transplant from matched siblings and unrelated donors for acute myeloid leukemia. Am J Hematol 2018; 93: 1211-1219.

53. Russell JA, Kangarloo SB, Williamson T, Chaudhry MA, Savoie ML, Turner AR, Larratt L, Storek J, Bahlis NJ, Shafey M, Brown CB, Yang M, Geddes M, Zacarias N, Yue P, Duggan P, Stewart DA, Daly A. Establishing a target exposure for once-daily intravenous busulfan given

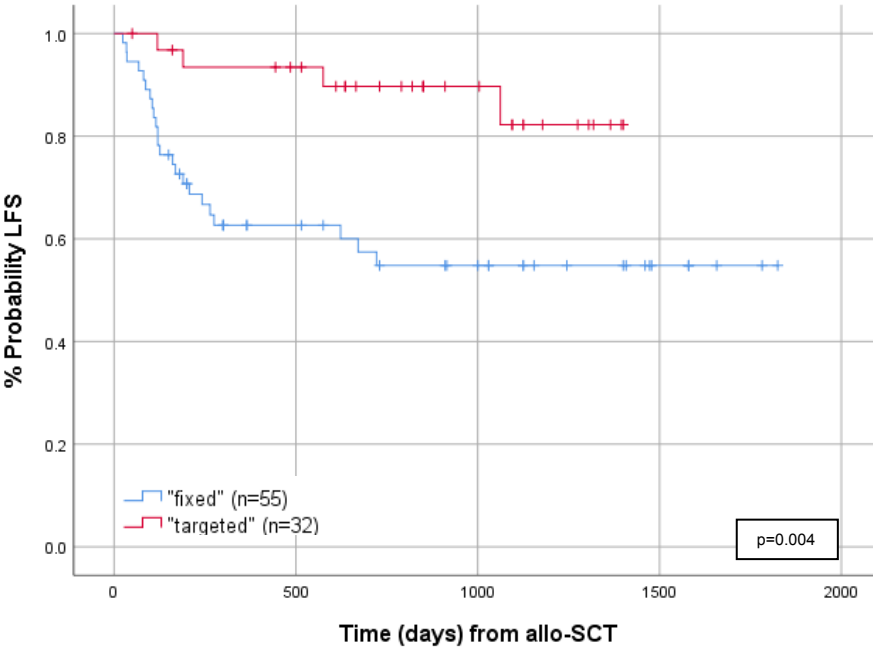
with fludarabine and thymoglobulin before allogeneic transplantation.Biol Blood Marrow Transplant 2013; 19: 1381-1386.

Figure 1. Outcomes for patients according to Bu administration in 87 patients with intermediate risk AML: a) overall survival (OS); b) leukemia-free survival (LFS); c) relapses and non-relapsed mortality (NRM).

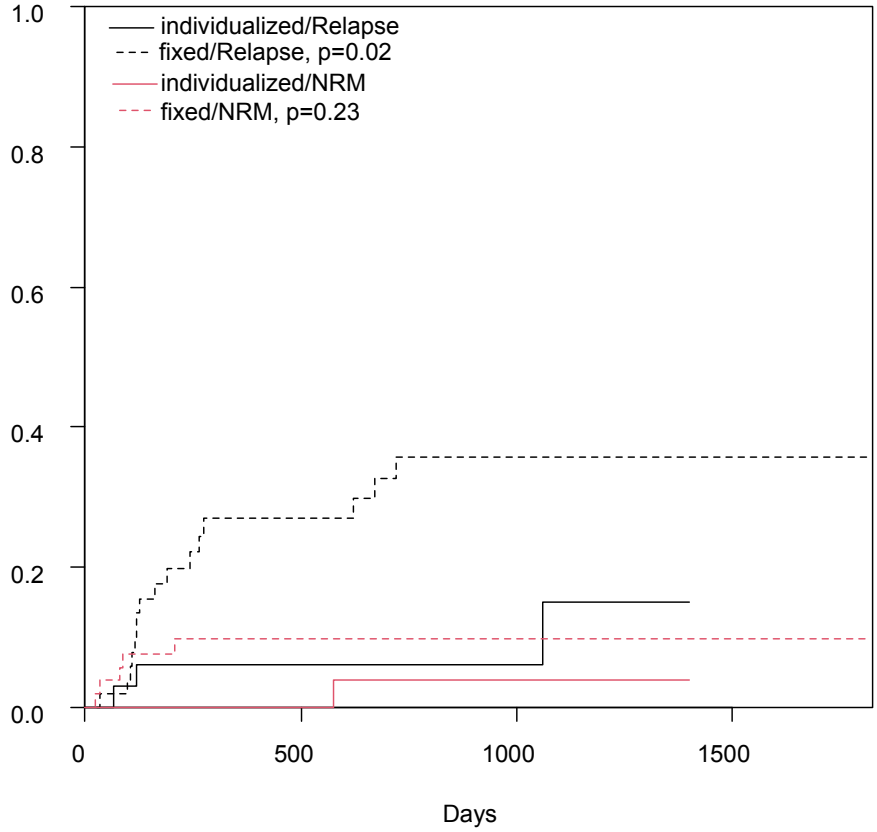
a)



b)



c)



Tables

Table 1. Patient's characteristics

Characteristic	fixed Bu, n=55	target Bu, n=32	P
Patient's sex			0.01
male	36 (66%)	12 (38%)	
female	19 (34%)	20 (62%)	
Patient/Donor sex constellation:			0.043
match	39 (71%)	16 (50%)	
mismatch	16 (29%)	16 (50%)	
Patient's age (median, range)	51 (21-73)	56 (31-72)	0.67
Origin of disease			0.47
de novo	43 (78%)	24 (75%)	
sAML/tAML	12 (22%)	8 (25%)	
Remission status			0.48
1.CR	40 (73%)	23 (72%)	
2.CR	7 (13%)	2 (6%)	
CRi	8 (14%)	7 (22%)	
Cytogenetics at diagnosis:			0.25
normal	27 (51%)	19 (61%)	
abnormal	26 (49%)	12 (39%)	
n.a.		1	
Previous therapy:			0.25
Chemotherapy	37 (69%)	27 (84%)	
Chemotherapy and TKIs	10 (18%)	4 (12%)	
Hypomethylating agents	5 (9%)	-	
Venetoclax-based	2 (3%)	1 (3%)	
Donor type:			0.38
MRD	12 (22%)	8 (25%)	
MUD	23 (42%)	18 (56%)	
MMUD	13 (23%)	4 (13%)	
Haploidentical/cord blood	7 (12%)	2 (6%)	
CMV status (P):			0.35
neg	17 (31%)	12 (38%)	
pos	38 (69%)	20 (62%)	
CMV reactivation	25 (46%)	14 (44%)	0.53
Conditioning:			0.06
MAC	46 (84%)	31 (97%)	
RIC	9 (16%)	1 (3%)	
			0.14
Bu/Cy or Bu/Flu	33 (60%)	27 (84%)	
Thiotepa/Bu/Flu	18 (33%)	1 (3%)	
FLAMSA/Bu	4 (7%)	4 (13%)	
planned Bu AUC, median (range)	-	55.6 (28-71)	
achieved Bu AUC, median (range)	-	74.4 (36.2-87.1)	
Immunosuppression			0.33
ATG	42 (78%)	27 (84%)	
post-Cy	12 (22%)	5 (16%)	
MRD at allo-SCT:			0.38
negative	26 (47%)	17 (53%)	
positive	29 (53%)	15 (47%)	
Engraftment (median, range)			
Leukocytes	11 (8-23)	11 (10-23)	0.86
Platelets	12 (7-117)	11 (8-83)	0.34
Mucositis			0.61
no	6 (11%)	2 (6%)	
grade 1	1 (2%)	2 (7%)	
grade 2	24 (49%)	13 (43%)	
grade 3	23 (47%)	15 (50%)	
grade 4	1 (2%)	-	
			0.84
median (range)	2 (1-4)	3 (1-3)	
VOD			0.40
yes	2 (4%)	-	
Infections			0.27
yes	44 (80%)	23 (72%)	
Sepsis			0.26
yes	2 (4%)	3 (9%)	
Other:	3 (6%)	5 (16%)	0.12
graft failure	2	-	
seizures	1	1	
*hyperbilirubinemia**	.	3	
lung fibrosis	.	1	

* without VOD criteria

Table 2. Results of univariate analysis (n=87)

Characteristic	OS	LFS	RI	NRM
Patient's sex				
male vs female	**3.5 (1.1-10.6), p=0.028**	**2.9 (1.2-6.7), p=0.017**	**2.8 (1.01-7.5), p=0.047**	**2.2 (0.4-10.6), p=0.35**
Patient-Donor sex constellation:				
match vs mismatch	1.6 (0.6-4.6), p=0.36	1.8 (0.8-4.3), p=0.18		
Patient's age (median, range)	1.03 (0.99-1.1), p=0.12	1.0 (0.97-1.0), p=0.91		
Origin of disease				
de novo vs sAML/tAML	1.1 (0.4-3.3), p=0.87	1.1 (0.45-2.8), p=0.81		
Remission status	p=0.61	p=0.66		
1.CR vs CRi	1.4 (0.3-6.4), p=0.63	1.1 (0.4-3.3), p=0.85		
2.CR vs CRi	2.4 (0.4-14.2), p=0.35	1.8 (0.4-7.1), p=0.42		
Cytogenetics at diagnosis:				
normal vs abnormal	**0.46 (0.2-1.2), p=0.11**	**1.2 (0.6-2.5), p=0.68**		
Donor type:	**p=0.038**	**p=0.008**	**p=0.044**	**p=0.26**
MUD vs MRD	**0.7 (0.2-3.0), p=0.64**	**0.7 (0.2-2.3), p=0.60**	**0.7 (0.2-2.3), p=0.55**	**0.9 (0.1-9.9), p=0.96**
MMUD vs MRD	**3.1 (0.8-12), p=0.10**	**3.3 (1.1-9.7), p=0.029**	**3.1 (0.9-10), p=0.065**	**2.4 (0.2-25), p=0.46**
Haploidentical vs MRD	**3.4 (0.7-17), p=0.14**	**2.5 (0.7-9.2), p=0.18**	**1.2 (0.2-5.8), p=0.85**	**5.6 (0.5-62), p=0.15**
matched vs mismatched	**0.25 (0.1-0.6), p=0.004**	**0.3 (0.1-0.6), p=0.001**	**0.3 (0.1-0.8), p=0.014**	**0.3 (0.1-1.2), p=0.09**
CMV status (P):				
neg vs pos	0.8 (0.3-2.1), p=0.59	0.96 (0.4-2.1), p=0.92		
Conditioning:				
MAC vs RIC	**0.3 (0.1-0.9), p=0.037**	**0.4 (0.2-1.1), p=0.069**	**0.4 (0.1-1.1), p=0.07**	**1.2 (0.1-5.4), p=0.68**
fixed vs individualized Bu	**5.8 (1.3-25), p=0.02**	**4.2 (1.5-12), p=0.008**	**3.8 (1.1-12.6), p=0.031**	**3.7 (0.5-28), p=0.21**
In vivo suppression				
ATG vs post-Cy	0.6 (0.2-1.8), p=0.34	0.86 (0.3-2.3), p=0.77		
MRD at allo-SCT:				
negative vs positive	**0.5 (0.2-1.3), p=0.13**	**0.4 (0.2-0.8), p=0.018**	**0.2 (0.1-0.7), p=0.006**	**1.4 (0.3-6.1), p=0.65**
CMV reactivation				
no vs yes	**0.5 (0.2-1.2), p=0.10**	**0.5 (0.2-1.1), p=0.09**	0.5 (0.2-1.3), p=0.14	0.6 (0.2-2.8). p=0.55

Table 3. Results of multivariate analysis.

Factor	OS	LFS	Relapses
Patients' sex male vs female	2.7 (0.9-8.2), 0.089	-	-
Donor type: matched vs mismatched	-	0.3 (0.2-0.7), 0.004	-
Conditioning: Non-AUC vs AUC	4.6 (1.1-20.5), 0.044	3.6 (1.3-10.6), 0.018	3.6 (1.1-11), 0.033
Pre-transplant MRD: neg vs pos	-	0.4 (0.2-0.9), 0.031	0.2 (0.1-0.7), 0.007

Supplemental files

Table 1S. Patient's characteristics according to pre-transplant MRD status.

Characteristic	MRD negative (n=43)			MRD positive (n=44)		
	Non-AUC (n=26)	AUC (n=17)	p	Non-AUC (n=29)	AUC (n=15)	p
Patient's sex			0.16			**0.026**
male	16 (62%)	7 (41%)		**20 (69%)**	**5 (33%)**	
female	10 (38%)	10 (59%)		**9 (31%)**	**10 (67%)**	
Patient/Donor sex constellation:			0.06			0.26
match	21 (81%)	9 (53%)		18 (62%)	7 (47%)	
mismatch	5 (19%)	8 (47%)		11 (38%)	8 (53%)	
Patient's age (median, range)			0.34			0.68
	50 (29-70)	59 (31-68)		53 (21-73)	53 (31-72)	
Origin of disease			0.61			0.38
de novo	21 (81%)	14 (82%)		22 (76%)	10 (67%)	
sAML/tAML	5 (19%)	3 (18%)		7 (24%)	5 (33%)	
Remission status			0.43			0.83
1.CR	22 (85%)	13 (77%)		18 (62%)	10 (67%)	
2.CR	1 (4%)	-		6 (21%)	2 (13%)	
CRi	3 (11%)	4 (23%)		5 (17%)	3 (20%)	
Cytogenetics at diagnosis:			0.07			0.41
normal	11 (46%)	12 (75%)		16 (55%)	7 (46%)	
abnormal	13 (54%)	4 (25%)		13 (45%)	8 (53%)	
n.a.	2	1				
Previous therapy:			0.27			0.61
Chemotherapy	14 (56%)	14 (82%)		23 (79%)	13 (86%)	
Chemotherapy and TKIs	9 (32%)	3 (18%)		2 (7%)	1 (7%)	
Hypomethylating agents	2 (8%)	-		3 (10%)	-	
Venetoclax-based	1 (4%)	-		1 (3%)	1 (7%)	
Donor type:			0.64			0.58
MRD	4 (15%)	4 (23%)		8 (28%)	4 (27%)	
MUD	13 (50%)	10 (58%)		10 (35%)	8 (53%)	
mMUD	7 (27%)	2 (12%)		6 (21%)	2 (13%)	
Haploidentical/cord blood	2 (8%)	1 (6%)		5 (17%)	1 (7%)	
CMV status (P):			0.44			0.13
neg	8 (31%)	4 (24%)		9 (31%)	8 (53%)	
pos	18 (69%)	13 (76%)		20 (69%)	7 (47%)	
CMV reactivation	11 (42%)	7 (41%)	0.60	14 (48%)	7 (47%)	0.59
Conditioning:			0.21			0.23
MAC	23 (89%)	17 (100%)		23 (79%)	14 (93%)	
RIC	3 (11%)	-		6 (21%)	1 (7%)	
			0.24			**0.036**
Bu/Cy or Bu/Flu	21 (92%)	13 (76%)		**12 (41%)**	**14 (93%)**	
Thiotepa/Bu/Flu	5 (8%)	1 (6%)		**13 (45%)**	**-**	
FLAMSA/Bu	-	3 (18%)		**4 (14%)**	**1 (7%)**	
Immunosuppression*			0.14			0.49

ATG	20 (77%)	16 (94%)		22 (79%)	11 (73%)	
post-Cy	6 (23%)	1 (6%)		6 (21%)	4 (27%)	
both*	-	-		1	-	
Engraftment (median, range)						
Leukocytes	12 (8-23)	11 (10-23)	0.18	11 (9-22)	12 (10-18)	0.39
Platelets	12 (9-117)	8 (8-83)	0.84	13 (7-34)	11 (10-25)	0.21
Mucositis			0.77			0.54
no	2 (8%)	1 (5%)		4 (14%)	1 (7%)	
grade 1	1 (4%)	1 (5%)		-	1 (7%)	
grade 2	13 (50%)	8 (42%)		11 (38%)	7 (47%)	
grade 3	10 (39%)	9 (47%)		13 (45%)	6 (40%)	
grade 4	-	-		1 (3%)	-	
			0.44			0.67
median (range)	2 (0-3)	3 (0-3)		2 (0-4)	2 (0-3)	
VOD			0.36			
yes	2 (8%)	-		-	-	
Infections			0.45			0.36
yes	20 (77%)	12 (71%)		24 (83%)	11 (73%)	
Sepsis			0.66			0.11
yes	2 (8%)	1 (6%)		-	2 (13%)	
Other:			0.41			0.44
graft failure	1	-		-	-	
seizures	-	1		1	-	
hyperbilirubinemia**	-	-		-	2	
lung fibrosis	-	1		-	-	

Table S2. Results of univariate analysis according to pre-transplant MRD status.

Characteristic	OS	LFS	RI	NRM
Pre-transplant MRD positive (n=44)				
Patient's sex				
male vs female	**5.1 (1.1-23), p=0.037**	**2.7 (0.97-7.5), p=0.06**	1.9 (0.7-5), p=0.22	-
Patient/Donor sex constellation:				
match vs mismatch	2.6 (0.7-9.6), p=0.16	2.5 (0.9-7.1), p=0.07	2.6 (0.9-7.4), p=0.08	1.4 (0.1-14), p=0.80
Patient's age (median, range)	**1.1 (1.0-1.1), p=0.032**	1.0 (0.98-1.1), p=0.52	**-**	**-**
Origin of disease				
de novo vs sAML/tAML	0.75 (0.2-2.5), p=0.64	0.8 (0.3-2.2), p=0.70	-	-
Remission status	p=0.98	p=0.84		
1.CR vs CRi	1.1 (0.2-5.1), p=0.93	1.4 (0.4-4.8), p=0.62	-	-
2.CR vs CRi	0.9 (0.1-6.5), p=0.93	1.1 (0.2-5.3), p=0.94	-	-
Cytogenetics at diagnosis:				
normal vs abnormal	0.5 (0.2-1.6), p=0.23	0.9 (0.4-2.3), p=0.89	-	-
Donor type:	**p=0.099**	**p=0.094**	p=0.28	
MUD vs MRD	**0.98 (0.2-5.9), p=0.98**	**1.2 (0.3-4.2), p=0.80**	0.93 (0.3-1), p=0.91	-
MMUD vs MRD	**4.8 (0.9-25), p=0.06**	**4.1 (1.1-15), p=0.03**	3.2 (0.8-12.8), p=0.10	-
Haploidentical vs MRD	**2.5 (0.4-17.5), p=0.37**	**1.8 (0.4-8), p=0.45**	0.99 (0.2-4.7), p=0.99	-
CMV status (P):				
neg vs pos	0.8 (0.3-2.7), p=0.72	0.8 (0.3-2.1), p=0.71	-	-
Conditioning:				
MAC vs RIC	**0.3 (0.1-0.98), p=0.047**	**0.4 (0.1-1.1), p=0.086**	0.5 (0.2-1.4), p=0.17	0.3 (0.03-3.7), p=0.38
Non-AUC vs AUC	**7.1 (0.9-55), p=0.06**	**3.6 (1.1-12.6), p=0.041**	**2.7 (0.8-8.9), p=0.11**	-
Immunosuppression				
ATG vs post-Cy	0.6 (0.2-2.2), p=0.46	0.9 (0.3-2.8), p=0.88	-	-
CMV reactivation				
no vs yes	0.7 (0.2-2.3), p=0.58	0.7 (0.3-1.8), p=0.50	-	-
Pre-transplant MRD negative (n=43)				
Patient's sex				
male vs female	1.9 (0.4-10.5), 0.45	**3.1 (0.6-15), 0.17**	-	1.0 (0.2-7), 0.99
Patient/Donor sex constellation:				
match vs mismatch	0.9 (0.2-5), 0.93	1.4 (0.3-7.0), 0.68	-	-
Patient's age (median, range)	0.98 (0.9-1.1), 0.59	0.98 (0.9-1.0), 0.40	-	-
Origin of disease				
de novo vs sAML/tAML	-	-	-	-
Remission status	-	-	-	-
1.CR vs CRi				
2.CR vs CRi				
Cytogenetics at diagnosis:				
normal vs abnormal	0.4 (0.1-2), 0.23	0.7 (0.2-2.9), 0.65	-	-
Donor type:	**0.21**	**0.09**		
MUD vs MRD	**0.6 (0.1-6.5), 0.66**	**0.6 (0.1-6.2), 0.63**	-	-
MMUD vs MRD	**1.9 (0.2-21), 0.61**	**4.0 (0.5-37), 0.21**	-	-
Haploidentical vs MRD	**10 (0.5-200), 0.099**	**5.7 (0.3-94), 0.23**	-	-
CMV status (P):				
neg vs pos	0.5 (0.1-4.4), 0.55	0.8 (0.2-4), 0.80	-	-

Conditioning:				
MAC vs RIC	-	-	-	-
Non-AUC vs AUC	3.8 (0.5-32), 0.22	5.4 (0.7-43), 0.12	-	-
Bu/Cy or Bu/Flu vs others	-	-	-	-
Immunosuppression				
ATG vs post-Cy	0.7 (0.1-5.9), 0.72	1.1 (0.1-8.9), 0.94	-	-
CMV reactivation				
no vs yes	**0.2 (0.02-1.3), 0.09**	**0.2 (0.1-1.2), 0.08**	1.2 (0.03-2.7), 0.27	0.3 (0.03-2.6), 0.26

Table 3S. Patient's characteristics according to of patients who received BuFlu only (n=60).

Characteristic	Non-AUC (n=33)	AUC (n=27)	p
Patient's sex			0.065
male	21 (64%)	11 (41%)	
female	12 (36%)	16 (59%)	
Patient/Donor sex constellation:			0.05
match	26 (79%)	15 (45%)	
mismatch	7 (21%)	12 (55%)	
Patient's age (median, range)	49 (27-73)	56 (31-72)	0.31
Origin of disease			0.37
de novo	29 (88%)	22 (81%)	
sAML/tAML	4 (12%)	5 (19%)	
Remission status			0.34
1.CR	28 (85%)	19 (70%)	
2.CR	2 (6%)	2 (7%)	
CRi	3 (9%)	6 (23%)	
Cytogenetics at diagnosis:			0.20
normal	15 (45%)	16 (59%)	
abnormal	17 (52%)	10 (37%)	
n.a.	1 (3%)	1 (4%)	
Previous therapy:			0.19
Chemotherapy	23 (70%)	23 (85%)	
Chemotherapy and TKIs	8 (24%)	3 (11%)	
Hypomethylating agents	2 (6%)	-	
Venetoclax-based	-	1 (4%)	
Pre-transplant MRD:			0.17
neg	21 (64%)	13 (48%)	
pos	12 (36%)	14 (52%)	
AUC:			
planned Bu AUC, median (range)	-	65.7 (39.6-145.5)	
achieved Bu AUC, median (range)	-	77.8 (49.4-81.9)	
Donor type:			0.42
MRD	6 (18%)	8 (30%)	
MUD	16 (48%)	14 (52%)	
MMUD	9 (27%)	3 (11%)	
Haploidentical/cord blood	2 (6%)	2 (7%)	
CMV status (P):			0.56
neg	13 (39%)	11 (41%)	
pos	20 (61%)	16 (59%)	
CMV reactivation	16 (48%)	12 (44%)	0.48
Immunosuppression			0.50
ATG	28 (85%)	22 (81%)	
post-Cy	5 (15%)	5 (19%)	
Engraftment (median, range)			
Leukocytes	12 (8-22)	11 (10-23)	0.72
Platelets	12 (7-117)	11 (8-83)	0.44

Mucositis			0.86
no	1 (3%)	1 (4%)	
grade 1	1 (3%)	1 (4%)	
grade 2	16 (48%)	11 (41%)	
grade 3	14 (42%)	14 (52%)	
grade 4	1 (3%)	-	
VOD			0.30
yes	2 (6%)	-	
Infections			0.57
yes	24 (73%)	20 (74%)	
Sepsis			0.40
yes	2 (6%)	3 (11%)	
Other:			0.41
graft failure	1 (3%)	-	
seizures	1 (3%)	1 (4%)	
hyperbilirubinemia*	-	2 (8%)	
lung fibrosis	-	1 (4%)	

*without VOD criteria