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Original article

Impact of the rest period between sequential chemotherapy and conditioning regimen prior to allogeneic hematopoietic stem cell transplantation for high-risk myeloid malignancies

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative treatment for relapsed/refractory acute myeloid leukemia (r/rAML), high-risk myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN). Sequential conditioning, combining cytoreductive chemotherapy with reduced-intensity conditioning, was designed to reduce toxicity while preserving efficacy in frail patients. While numerous studies have evaluated different sequential regimens, the impact of rest period duration between the two phases remains unexplored. This bicentric retrospective study analyzed 82 allo-HSCT between 2013 and 2021, in patients with high-risk myeloid malignancies (median age 58 years). Short-bridge-to-transplant regimens (SBTT, n=44) with rest periods of 7 days or less, including the well-known FLAMSA-RIC, were compared to long-bridge-to-transplant regimens (LBTT, n=38) with rest periods longer than 7 days. After a median follow-up of 33 months, rest period duration did not significantly affect progression-free survival (PFS), overall survival, relapse incidence or non-relapse mortality (NRM). Two-year PFS was 35.1% for SBTT versus 57.2% for LBTT (*aHR* 1.62; *P*=0.13). However, measurable residual disease-free survival (MRD-FS) was significantly improved with LBTT (*aHR* 2.15; *P*=0.02). Despite longer median aplasia, LBTT showed comparable complications and enabled more intensive chemotherapy without increased NRM. LBTT appears feasible and not inferior to SBTT, with a signal of improved MRD control that may reflect better temporal separation and management of treatment-related toxicities, safe delivery of higher-dose or more intensive chemotherapy, and potential leukemic cell-cycle synchronization effects. While center-specific practices and regimen heterogeneity limit definitive conclusions, these hypothesis-generating findings highlight an underexplored dimension of sequential conditioning and warrant further investigation in larger prospective studies.

Introduction

In high-risk myeloid malignancies, including relapsed or refractory acute myeloid leukemia (r/rAML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN), allogeneic hematopoietic stem cell transplantation (allo-HSCT) still represents the only treatment approach with curative potential [1,2]. The well-known FLAMSA-RIC (fludarabine - amasacrine - cytarabine – reduced-intensity conditioning regimen) protocol, a sequential strategy developed by Schmid and Kolb's team in 2005 [3], led to the development of numerous other sequential regimens designed to offer the benefits of allo-HSCT while reducing its toxicity in elderly or comorbid patients suffering from high-risk myeloid malignancies, usually with active disease. These particular regimens consist of a rapid succession of a cytoreductive chemotherapy followed by a reduced-intensity conditioning (RIC) regimen and allo-HSCT, without waiting for complete remission or

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blood count recovery. This allows frail and refractory patients previously ineligible to transplant to be brought to allo-HSCT while avoiding the high toxicity associated with myeloablative conditioning regimen (MAC) and the high relapse rate observed after RIC alone [4,5].

Recent studies confirmed the benefits of sequential regimens in active myeloid malignancies in comparison with RIC alone, by reducing relapse incidence after allo-HSCT while showing comparable toxicity, resulting in better overall survival [6,7]. Even in young patients with r/rAML, a FLAMSA-RIC approach may offer higher probability of survival compared to MAC [8]. However, this benefit remains unclear for patients in complete remission, as shown by the FIGARO trial where no difference was observed in relapse and survival rates [9]. More recently, the ASAP trial has further supported the value of sequential conditioning regimen. This trial found no benefit of salvage chemotherapy for r/rAML over careful observation and upfront transplantation with sequential conditioning regimen [10].

To date, numerous studies have discussed which agents should be included in the sequential regimen to improve its antileukemic efficacy and attenuate its toxicity [5,7,11–15]. However, to our knowledge, no study has assessed the impact of the duration of the rest period between the two phases of the sequential regimen or questioned the standard 3-day rest period of the FLAMSA-RIC protocol. Our study aims to focus on the timing of the sequential regimens, rather than the content of these regimens. In this setting, we decided to compare a long sequential regimen to a short sequential regimen, stratified by the duration of the rest period between the cytoreductive chemotherapy and the conditioning regimen. The idea behind a long-bridge-to-transplant regimen (LBTT) is to separate toxicities associated with each phase allowing more time to recover, while a short-bridge-to-transplant regimen (SBTT) aims to reduce the duration of aplasia and hospital stay.

Materials and methods

This real-world retrospective cohort study used data from the hematology departments of two Belgian university hospitals, CHU UCL Namur – Godinne (CHUNG) and Cliniques universitaires Saint-Luc (CUSL), collected between January 2013 and December 2021. The study compared the efficacy and toxicity of two sequential conditioning regimens before allo-HSCT. The timeframe between the cytoreductive chemotherapy and the RIC defined the study arms: the LBTT arm included regimens with a rest period longer than seven days, and the SBTT arm with a rest period of seven days or less. This 7-day cut-off, corresponding to the median of the cohort, was selected to optimally distinguish standard “short” sequential protocols (typically ~3 days as described in FLAMSA-like protocols) from “long” bridge strategies (typically ~10 days), while accounting for real-world logistical variations around these time points.

We reviewed all sequential conditioning regimens’ records of both hospitals during the aforementioned period. Patients were eligible if they were diagnosed with relapsed/refractory acute myeloid leukemia (r/rAML), high-risk myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN) or an overlap syndrome (MDS/MPN), aged 18 to 80 years, with active disease at the time of conditioning regimen and with an indication of allo-HSCT according to current guidelines and scientific knowledge. All patients meeting those criteria were included, regardless of disease type or status, donor characteristics and previous therapies (including previous allo-HSCT). All sequential conditioning regimens were considered, irrespective of their frequency in the literature. Supportive treatments followed local policies.

The primary outcome was progression-free survival (PFS), defined as the time from allo-HSCT to disease progression or death, using criteria from the European Society for Blood and Marrow Transplantation (EBMT) [16]. Secondary outcomes included overall survival (OS), GVHD-free and relapse-free survival (GRFS), measurable residual disease-free survival (MRD-FS), non-relapse mortality (NRM), acute and chronic GVHD incidence, relapse incidence (RI), neutrophil and platelet engraftment, remission at 100 days, overall and post-allo-HSCT hospitalization duration, proven infections, ICU admissions, transfusions, CMV/EBV reactivation, macrophage activation syndrome, and early rehospitalization. Due to the retrospective and real-world setting of this study and the evolution of technical and clinical thresholds between 2013 and 2021, MRD recurrence was defined pragmatically as the re-emergence of disease markers (molecular, cytogenetic, immunophenotypic, or loss of donor chimerism) that were recorded as clinically significant by the treating physician according to Yinstitutional standards at the time. Detailed definitions are provided in the Supplementary data (Table S1, Table S2).

Statistical analyses used the *t*-test for continuous variables and the χ^2 test for categorical variables or Fisher’s exact test when expected cell frequencies were < 5. Survival outcomes, as well as time to neutrophil and platelet engraftment, NRM, and RI were analyzed using the Kaplan-Meier method and compared using the log-rank test in univariate analysis. In this retrospective cohort of limited size (n=82), Kaplan-Meier estimates were primarily used for descriptive and comparative purposes; for NRM and RI, competing events were censored and may lead to an overestimation of absolute cumulative incidences. Multivariate analysis adjusted for EBMT risk score (Table S3), HCT-CI comorbidity score (Table S4), and disease type. Due to the expected small sample size, variables were dichotomized for multivariate analysis: therefore, we analyzed low- and intermediate-risk scores (0 to 4 for EBMT; 0 to 2 for HCT-CI) versus high-risk scores (> 4 for EBMT, > 2 for HCT-CI) and r/rAML versus the other malignancies. Multivariate analysis used Cox regression for survival outcomes, logistic regression for binary secondary outcomes, and linear regression for continuous variables. Results are reported with 95% confidence intervals (*95%CI*) and expressed as adjusted Hazard Ratios (*aHR*), Odds Ratios (*aOR*) and mean Differences (*aD*). Statistical significance was set at a *P*-value < 0.05. All analyses were performed using R software version 4.1.1.

Results

Patient population and characteristics

A total of 82 allo-HSCT were selected, 40 at CHUNG and 42 at CUSL, with a median patient age of 58 years. SBTT was more common at CUSL (75%), while LBTT was predominant at CHUNG (76%) according to their local procedures. The median time from diagnosis to allo-HSCT was 8 months for LBTT and 12 months for SBTT. About half of the patients had r/rAML while the others suffered from MDS, MPN, or MDS/MPN, with similar proportions across both arms. At the initiation of sequential conditioning regimen, 40% were in relapse after a complete remission, while 23% were refractory to prior chemotherapy, including those with primary induction failure and those who became refractory after multiple treatments. For some patients with MPN or MDS, bridge procedure was performed as primary induction therapy (n=5). Median EBMT scores were similar (5) in both groups. However, HCT-CI scores tended to be higher in the SBTT group compared to the LBTT group, with medians of 3 and 2, respectively. Blast percentages for AML and MDS were similar between the groups, with medians of 15% and 16%, respectively. Detailed patient and disease characteristics are presented in Table 1.

Donor population and characteristics

Donors were equally male and female. In both groups, 20% of the transplants involved a female donor and a male recipient. The median donor age was 30 years in the SBTT group and 36 years in the LBTT group. There was a higher proportion of matched unrelated donors in the SBTT group (57% vs. 37% in LBTT), while LBTT group involved more matched related donors (34% vs. 14% in SBTT). Most transplants consisted of peripheral blood stem cells (98%).

Table 1
Baseline characteristics of patients and donors.

	SBTT (n = 44)	LBTT (n = 38)	*P*
Center, n (%)			< 0.001
CHUNG	11 (25)	29 (76)	
CUSL	33 (75)	9 (24)	
Time from diagnosis to allo-HSCT (days), median (IQR)	359 (151-708)	234 (156-388)	0.30
Patient age (years), median (IQR)	58 (47-63)	57 (46-64)	0.98
Patient sex, n (%)			0.30
Female	20 (45)	13 (34)	
Male	24 (55)	25 (66)	
Pathology, n (%)			0.18
r/rAML	28 (64)	16 (42)	
MDS	7 (16)	8 (21)	
MPN	5 (11)	5 (13)	
MDS/MPN	4 (9)	9 (24)	
Blastosis (%), median (IQR)	15 (9-42)	16 (12-25)	0.80
Status, n (%)			0.46
Refractory	9 (20)	10 (26)	
Relapse after CR	21 (48)	12 (32)	
Acutisation	10 (23)	13 (34)	
Other	4 (9)	3 (8)	
Previous HSCT, n (%)			0.087
Allo-HSCT	6 (14)	5 (13)	
Auto-HSCT	0 (0)	4 (11)	
None	38 (86)	29 (76)	
EBMT score, median (IQR)	5 (4-6)	5 (4-5)	0.14
HCT-CI score, median (IQR)	3 (1-4)	2 (0-3)	0.091
Donor age (years), median (IQR)	30 (24-40)	36 (27-54)	0.10
Donor type, n (%)			0.10
MSD	6 (14)	13 (34)	
MUD	25 (57)	14 (37)	
MMUD	6 (14)	7 (18)	
Haplo-identical	7 (16)	4 (11)	
Donor sex, n (%)			1.00
Female	22 (50)	19 (50)	
Male	22 (50)	19 (50)	
Graft origin, n (%)			1.00
PBSC	43 (98)	37 (97)	
BM	1 (2)	1 (3)	
ABO compatibilty, n (%)			0.50
Compatible	22 (50)	17 (45)	
Minor incompatibility	13 (30)	9 (24)	
Major incompatibilty	9 (20)	12 (32)	

SBTT short bridge-to-transplantation, *LBTT* long bridge-to-transplantation, *P* p-value, *n* number of patients, *IQR* interquartile range, *CHUNG* CHU UCL Namur – Godinne, *CUSL* Cliniques universitaires Saint-Luc, *allo-HSCT* allogeneic stem cell transplantation, *r/rAML* refractory or relapsed acute myeloid leukemia, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *MDS/MPN* overlap myelodysplastic/myeloproliferative syndrome, *CR* complete remission, *auto-HSCT* autologous stem cell transplantation, *EBMT* European Society for Blood and Marrow Transplantation, *HCT-CI* Hematopoietic Cell Transplantation-specific Comorbidity Index, *MSD* matched sibling donor, *MUD* matched unrelated donor, *MMUD* mismatched unrelated donor, *PBSC* peripheral blood stem cells, *BM* bone marrow.

Cytoreductive and conditioning regimens

The sequential conditioning included a median rest period of 10 days for LBTT compared to 4 days for SBTT. Overall, the most commonly used sequential conditioning regimen was high-dose Cytarabine (AraC) followed by Fludarabine and Melphalan (AraC-FluMel), representing about a quarter of both LBTT and SBTT procedures. Higher AraC doses were used in the LBTT arm, which frequently included 6 days of AraC at 2000 mg/m^2, while SBTT favored 3 days regimen at 1500 mg/m^2. The combination of Mitoxantrone and AraC (MITARA) was frequently administered as a LBTT, but rarely as SBTT, while FLAMSA-RIC was the second most frequently used SBTT regimen (20%). The combination of Clofarabine and AraC, forming the CLARA-RIC regimen, was also commonly used, particularly as LBTT (21%), though predominantly before 2017 in both groups. Over time, cytoreductive regimens evolved with increasing use of AraC monotherapy in SBTT and of MITARA in LBTT. RIC regimens also evolved, particularly in SBTT where Fludarabine and Busulfan (FluBu) were progressively replaced by FluMel from 2018 onward.

For immunosuppression, cyclosporine was predominantly used in LBTT (73%), while tacrolimus was preferred in SBTT (59%) according to local policies. Over time, mycophenolate mofetil was favored over methotrexate in both groups. In vivo T-cell depletion with anti-thymocyte globulin (ATG) was performed in most cases (80% SBTT vs. 68% LBTT). Detailed regimens are presented in Table 2 and in the Supplementary data (Table S5).

Engraftment and Remission

Out of the 82 allo-HSCT, 77 resulted in successful engraftment, with a median time to neutrophil engraftment of 17 days and platelet engraftment of 20 days in both groups. Five patients died within the first month post-allo-HSCT, four after SBTT (9.1%) and one after LBTT (2.6%). No deaths occurred between the start of sequential conditioning and day 0 (the day of allo-HSCT). The mean hospital stay post-allo-HSCT was about four weeks, with no significant difference between the groups. Among patients with available chimerism data at one month, 97% of LBTT and 82% of SBTT patients achieved full donor chimerism. At day 100, 76% of LBTT patients and 65% of SBTT patients who were alive were in complete remission (CR).

Table 2
Cytoreductive, conditioning and immunosuppressive regimens.

	SBTT (n = 44)	LBTT (n = 38)	*P*
Cytoreductive regimens, n (%)			/
AraC	20 (45)	16 (42)	
CLARA	7 (16)	8 (21)	
MITARA	2 (5)	11 (29)	
FLAMSA	9 (20)	1 (3)	
TEC	1 (2)	0 (0)	
Other	5 (11)	2 (5)	
RIC regimens, n (%)			/
FluMel	20 (45)	27 (71)	
FluBu	13 (30)	7 (18)	
FluTBICy	11 (25)	4 (11)	
TBI use, n (%)			0.062
No TBI	33 (75)	35 (92)	
2 Gy	11 (25)	3 (8)	
Ciclosporin and Tacrolimus use, n (%)			0.03
Ciclosporin	17 (39)	24 (73)	
Tacrolimus	26 (59)	14 (27)	
Other	1 (2)	0 (0)	
Mycophenolate Mofetil and Methotrexate use, n (%)			0.08
Mycophenolate Mofetil	32 (72)	34 (89)	
Methotrexate	11 (25)	4 (11)	
Other	1 (2)	0 (0)	
ATG use, n (%)	35 (80)	26 (68)	0.25
Letermovir use (total n = 81), n (%)	11 (26)	5 (13)	0.16
DLI use (total n = 81), n (%)			0.24
Prophylactic	1 (2)	1 (3)	
Therapeutic	9 (20)	4 (11)	

SBTT short bridge-to-transplantation, *LBTT* long bridge-to-transplantation, *P* p-value, *n* number of patients, *AraC* cytarabine, *CLARA* Clofarabine – Cytarabine, *MITARA* Mitoxantrone – Cytarabine, *FLAMSA* Fludarabine – Cytarabine – Amsacrine, *TEC* Thiotepa – Etoposide – Cyclophosphamide, *RIC* reduced-intensity conditioning, *FluMel* Fludarabine – Melphalan, *FluBu* Fludarabine – Busulfan, *FluTBICy* Fludarabine – Total body irradiation – Cyclophosphamide, *TBI* total body irradiation, *ATG* anti-thymocyte globulin, *DLI* donor lymphocyte infusion. For Letermovir and DLI, information about 1 patient was missing in SBTT.

Overall treatment results and survival

After a median follow-up of 33.3 months, the estimated 2-year PFS was 57.2% (*95%CI* [43–76%]) in the LBTT group, compared to 35.1% (*95%CI* [23–53%]) for SBTT patients (Figs. 1, 2, Table S6). Median PFS was reached at just over four years for LBTT and six months for SBTT. OS and GRFS were similar between both arms, with 2-year OS rates of 62.1% (*95%CI* [48–80%]) and 40.9% (*95%CI* [28–60%]) for LBTT and SBTT, respectively, and GRFS rates of 29.4% and 6.3%, respectively (Figs. 1, 2). Only the MRD-FS was significantly better in the LBTT group (54.7%; *95%CI* [41–73%] vs. SBTT 23.2%; *95%CI* [13.1–40.9%]) at 2 years (Figs. 1, 2). In most cases, MRD recurrence was based on loss of chimerism, either alone or in combination with other MRD markers (Table S7). In multivariate analysis, the SBTT group retained a poorer MRD-FS compared to the LBTT group (*aHR* 2.15; *95%CI* [1.16–3.99]; *P*=0.02).

Toxicity and relapse

Non-relapse mortality (NRM) was 11% and 18% at 6 months and 2 years after LBTT, respectively, compared to 26% and 40% after SBTT, with no significant difference in the rate of non-relapse mortality after adjusting for covariates (*aHR* 1.75; *95%CI* [0.68–4.53]; *P*=0.25; Fig. 3). Both LBTT and SBTT were associated with similar relapse rates, with 18% vs. 33.1% at 6 months and 33% vs. 43.5% at 2 years, with no significant difference in relapse velocity (*aHR* 1.68; *95%CI* [0.77–3.64]; *P*=0.19; Figs. 2, 3).

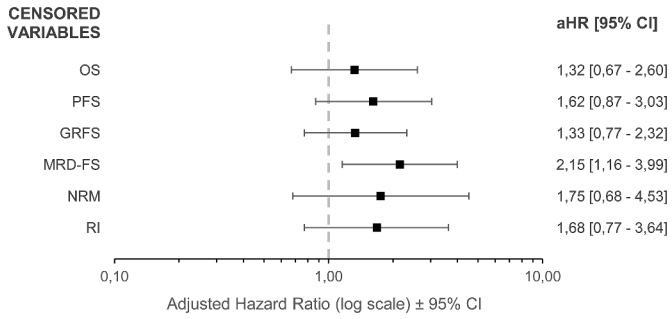

Fig. 2. Adjusted Hazard Ratios with *95%CI* for censored variables. Patient numbers were SBTT (n=44) and LBTT (n=38). Abbreviations: *OS* Overall survival, *PFS* Progression-free survival, *GRFS* Graft-versus-host-disease-free survival, *MRD-FS* Measurable residual disease-free survival, *NRM* non-relapse mortality, *RI* relapse incidence, *CI* Confidence interval. The adjusted Hazard Ratios (*aHR*) are calculated using LBTT as the reference and adjusted through multivariate analysis. A survival variable with an *aHR* > 1.00 favors LBTT.

Acute and Chronic GVHD

Acute GVHD occurred in about half of the patients in both groups,

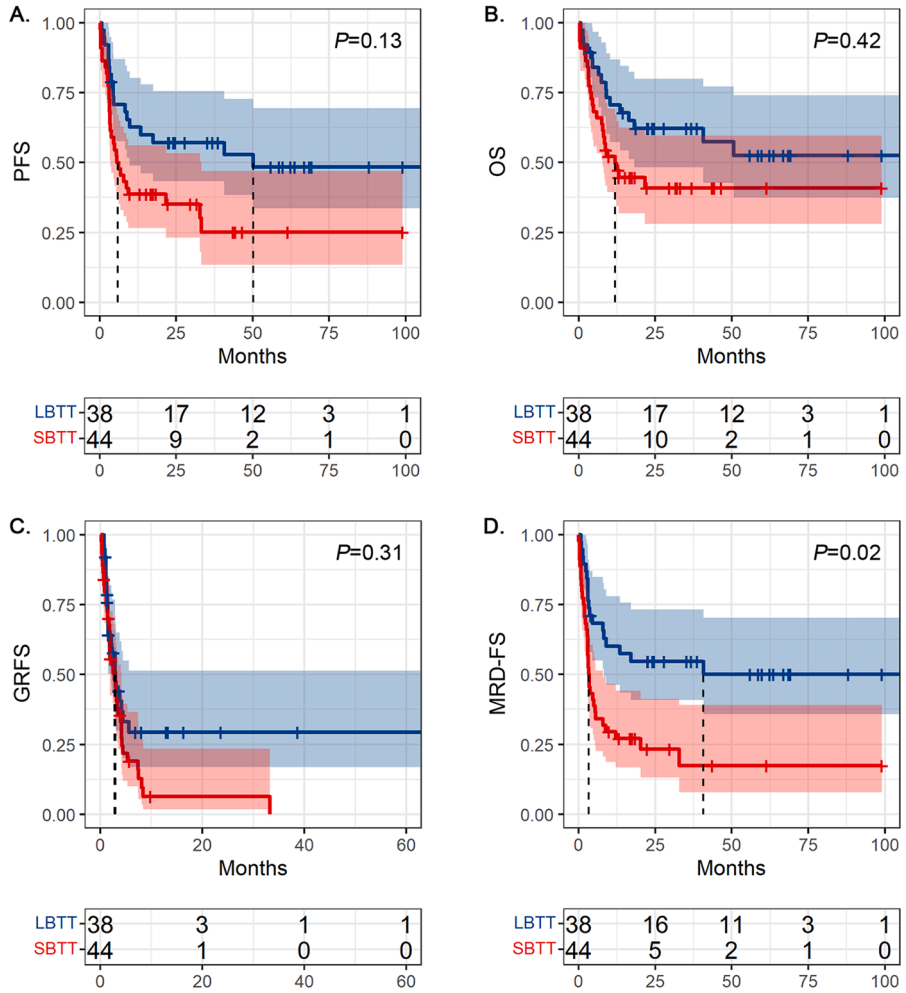

Fig. 1. Comparison of transplant outcomes between short (SBTT) and long (LBTT) bridge-to-transplantation. (A) Progression-free survival (PFS). (B) Overall survival (OS. (C) Graft-versus-host-disease-free survival (GRFS). (D) Measurable residual disease-free survival (MRD-FS). Long-bridge-to-transplantation (LBTT) outcomes are in blue, Short-bridge-to-transplantation (SBTT) outcomes are in red.

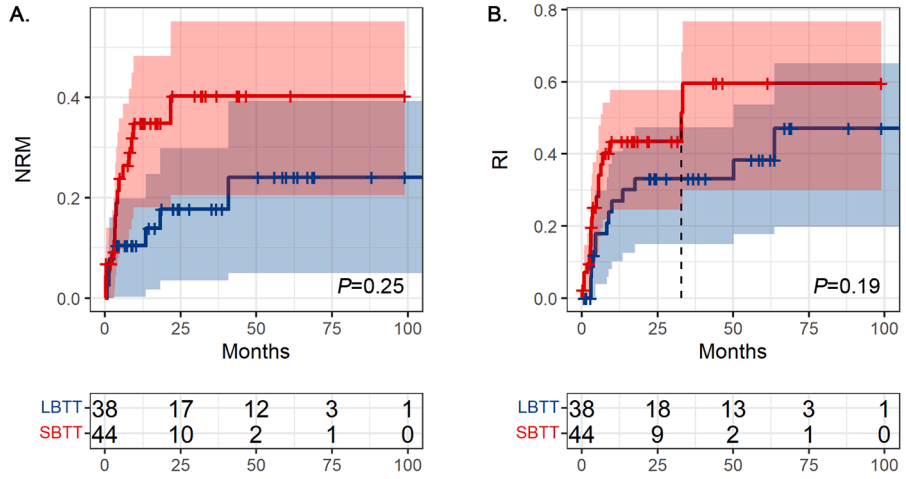

Fig. 3. Comparison of relapse and toxicity rates between short (SBTT) and long (LBTT) bridge-to-transplantation. (A) Cumulative incidence of non-relapse mortality (NRM). (B) Cumulative incidence of relapse (RI). Long-bridge-to-transplantation (LBTT) outcomes are in blue, Short-bridge-to-transplantation (SBTT) outcomes are in red.

with similar grade III-IV rates of 20.4% for SBTT and 15.4% for LBTT (*P*=0.80). Chronic GVHD occurred in 49% of SBTT patients and 63% of LBTT patients, without a significant difference (*aOR* 0.69; *95%CI* [0.26–1.83]; *P*=0.46; Table S8). Regarding cGVHD severity, moderate-to-severe forms were observed in 31.8% of patients in the SBTT arm versus 21.1% in the LBTT arm (*P*=0.40, Fig. S1, supplementaru material).

Other Complications

The extended procedure with LBTT approach resulted in a longer aplasia duration, with a median of 31 days versus 26 days fors SBTT. Both groups experienced similar rates of febrile neutropenia, proven infectious complications and ICU admissions. Hematological toxicity required an average of 14 units of packed red blood cells and 19 platelet concentrates, consistent across both bridging types (Fig. S2, supplementaru material). CMV and EBV reactivation rates were comparable, though EBV reactivation was slightly more frequent after SBTT (*aOR* 2.22; *95%CI* [0.84-6.08]; *P*=0.11; Fig. 4, Table S8).

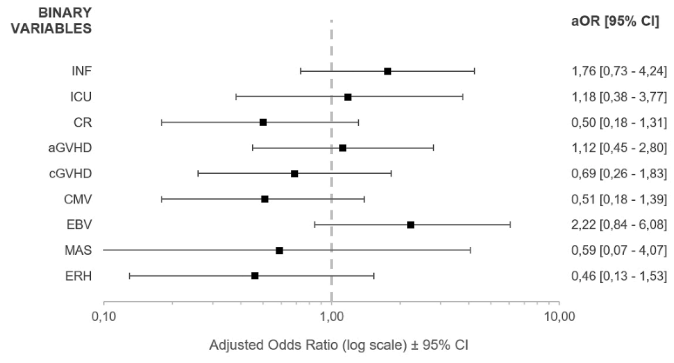

Fig. 4. Adjusted Odds Ratios with *95%CI* for binary variables. Patient numbers were SBTT (n=44) and LBTT (n=38) unless otherwise specified. Abbreviations: *INF* proven infectious complications; *ICU* intensive care unit admission; *CR* complete remission at day+100, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease (SBTT n=43), *CMV* cytomegalovirus reactivation (SBTT n=41); *EBV* Epstein-Barr virus reactivation (SBTT n=42; LBTT n=35), *MAS* macrophage activation syndrome (SBTT n=42), *ERH* early rehospitalization rate, *CI* Confidence interval. Adjusted Odds Ratios (*aOR*) are calculated using LBTT as the reference and adjusted through multivariate analysis. An *aOR* > 1.00 implies a higher frequency in the SBTT arm, favoring LBTT for adverse events (all but CR) but SBTT for efficacy endpoints (CR).

Post-transplant maintenance therapy

Donor lymphocyte infusions (DLI) were administered to 10 patients in the SBTT group (23%) and 5 patients in the LBTT group (13%). Among these, one patient in each group received prophylactic DLI according to institutional protocol at the time, while all other DLI were administered therapeutically for documented disease relapse (n=10) or MRD recurrence (n=3). Other targeted therapies administered post-transplant (hypomethylating agents, FLT3 inhibitors, or BCL-2 inhibitors) were given in the context of documented disease relapse or MRD recurrence rather than as prophylactic maintenance strategies.

Discussion

This bicentric retrospective study represents the first clinical investigation specifically examining the impact of rest period duration in sequential conditioning regimens for allo-HSCT. Our findings suggest that the duration of the rest period between cytoreductive chemotherapy and conditioning does not fundamentally affect the prognosis of patients undergoing allo-HSCT following a sequential conditioning strategy. After a median follow-up of 33 months, no significant difference was observed in PFS or OS between LBTT and SBTT strategies. However, a statistically significant improvement in MRD-FS was detected in the LBTT group. Moreover, effect estimates for other clinical outcomes consistently favored LBTT, although without reaching statistical significance, likely reflecting limited statistical power. The signal of improved MRD-FS after LBTT should be interpreted cautiously due to the limits discussed below, but it warrants careful consideration given that MRD status has emerged as a critical prognostic factor in allo-HSCT, with MRD-positive patients experiencing significantly higher relapse rates and inferior survival [17,18].

The observed difference in MRD relapse might be partially explained by in vitro findings reported by Popat *et al.* [19], who noted that sequential administration of conditioning can synchronize leukemic cells in a proliferative state, increasing their sensitivity to subsequent chemotherapy [20]. Karp *et al.* previously suggested that initial chemotherapy may trigger the release of soluble factors or humoral substances – yet unidentified – that could synergistically enhance the cytotoxic effect of a second chemotherapy administered 8 to 10 days later [21,22]. Besides, several clinical studies have shown the benefit of a sequential administration rather than a simultaneous administration of induction or salvage chemotherapies [23,24], although none specifically

addressed the optimal duration of the rest period between treatment phases. Together, these findings support the biological plausibility of an effect driven by treatment timing rather than regimen composition alone.

Beyond potential effects on disease control, the LBTT strategy may also facilitate improved toxicity management, allowing for the use of higher cumulative chemotherapy doses and/or more intensive agents (e.g. Mitoxantrone, Melphalan) without increasing NRM. Notably, whereas the use of FluMel – more frequently used in the LBTT arm – as conditioning usually leads to higher NRM in the literature, no significant difference in terms of toxicity was observed between the two arms in this study (2-year NRM 0.24; *95%CI* [0.05-0.39] after LBTT vs. 0.40; *95%CI* [0.21-0.55] after SBTT). Besides, the longer aplasia duration associated with LBTT (median of 31 days vs. 26 days with SBTT regimens) did not translate into increased infection rates, ICU transfers or prolonged hospitalization after allo-HSCT. Altogether, these findings suggest that the temporal separation of cytoreductive chemotherapy and conditioning may allow better recovery of healthy tissues, particularly the gastrointestinal mucosa, thereby mitigating toxicity despite prolonged cytopenias.

Our study's survival, relapse, and non-relapse mortality rates align with existing literature on sequential conditioning for active myeloid pathologies. Literature reports 2-year PFS of 26% to 63%, and OS of 30% to 70%, depending on disease type, stage, and used conditioning regimens [7,12–14,25–30]. In our study, LBTT demonstrated particularly favorable outcomes with 2-year PFS and OS rates of 57% and 62%, respectively. These favorable survival rates after LBTT are consistent with a low relapse incidence of 33%, compared to literature rates of 35% to 69% at 2 years. Additionally, toxicity was controlled with a 2-year NRM of 18% in the LBTT group versus 12% to 35% in the literature. In our study, the numerically higher 2-year NRM rate of 40% seen after SBTT, before multivariate adjustment, may partly reflect the higher HCT-CI, greater proportion of unrelated donors, and higher proportion of r/rAML compared to the LBTT group. Finally, the mitoxantrone-containing MITARA regimen, often used in LBTT regimens, might also contribute to its favorable figures in comparison with the literature, and further research could explore this topoisomerase II inhibitor, known for its efficacy and better cardiac tolerance than other anthracyclines [31].

As mentioned before, our study's findings are subject to some limitations, primarily due to its retrospective nature, patient and disease heterogeneity, and the limited power associated with small sample size, which are common challenges in this field. In order to alleviate those potential biases, the multivariate analysis has taken into account donor type heterogeneity, along with variables like pathology type, comorbidity level, and transplant risk, but the small sample size required binary covariable dichotomization, limiting the identification of independent risk factors. In addition, MRD assessment lacked uniform quantitative thresholds across centers and over time, reflecting evolving standards and methodologies; however, all MRD analyses were performed in the same reference laboratory, for both centers. While this pragmatic approach limits precision, it reflects real-world practice and differential ascertainment bias appears minimal given similar monitoring rates between groups.

A potential center effect must also be considered, as SBTT was predominantly performed at CUSL (75%) while LBTT was favored at CHUNG (76%). Beyond the rest period duration, centers differed in some transplant-related practices. For example, CUSL preferentially used tacrolimus while CHUNG favored cyclosporine for GVHD prophylaxis. ATG administration was less common in the LBTT group, reflecting institutional practice: at CHUNG, ATG was not routinely used for matched related donor transplants until the later years of the study period. Regarding donor selection, until 2018, CHUNG referred unrelated donor transplants to CUSL, resulting in a higher proportion of matched related donors in the LBTT arm (34% vs. 14%). The influence of donor type in sequential conditioning is debated [26,30,32] with studies showing no survival differences between related and unrelated donors. This is explained by a balance between increased toxicity but fewer relapse with unrelated donors due to the graft-versus-leukemia (GVL) effect [13,25]. We also mitigated this bias by adjusting for the EBMT risk score (which accounts for donor type) in our multivariate analysis. While these center-specific differences represent a limitation inherent to bicentric retrospective studies, it is important to note that both centers are Belgian university hospitals within the same transplant network (UCL Transplant Team) with similar healthcare infrastructure, drug availability and adherence to European (EBMT) transplant guidelines. This shared context likely ensured comparable standards of care and supportive care capabilities, despite their evolution over the study period.

Finally, differences in cytoreductive and conditioning regimens between both arms and centers may limit the interpretation of the results. These variations may confound the observed effects of rest period duration, making it difficult to disentangle the impact of timing from the intensity or composition of regimens that longer rest periods allow. While the two most common cytoreductive regimens, AraC and CLARA, were used similarly between SBTT and LBTT, differences might arise from FLAMSA, mainly used as SBTT, and MITARA, as LBTT, which have not been compared to our knowledge. Mitoxantrone, often used as induction, consolidation, or salvage chemotherapy, is rarely included in sequential conditioning protocols, making it difficult to assess against FLAMSA. Moreover, recent data challenge the concept of pre-transplant cytoreduction as a bridge strategy in MDS, suggesting that cytoreduction does not consistently improve post-transplant outcomes [33]. Regarding the conditioning, FluMel was more common in LBTT, and FluBu in SBTT. Some studies have shown a relative advantage of FluMel in OS and PFS, mainly in MDS, due to lower RI in comparison with FluBu [34,35]. The literature also shows higher NRM after FluMel [35,36] but, as mentioned before, this does not seem to have negatively affected NRM in the LBTT arm. The effect of FluMel alone seems then insufficient to explain our study results. Additionally, the SBTT arm included more matched unrelated donors, whose enhanced GVL effect might have counterbalanced the potentially inferior disease control associated with FluBu.

Overall, our results should be interpreted as hypothesis-generating, rather than establishing definitive superiority of either approach or demonstrating a clear advantage in MRD control. Nevertheless, the consistent trends observed across outcomes and the interesting signal of improved MRD-FS support a shift in focus from the sole composition of conditioning regimens toward their temporal organization. Moreover, extending the resting period may allow the administration of more intensive or higher-dose chemotherapy with improved toxicity management, which could be particularly relevant for high-risk patients, while MRD control remains a key determinant of allo-HSCT efficacy. Future research should also explore the integration of targeted therapies, such as BCL-2 and FLT3 inhibitors, as well as anti-CD117 monoclonal antibodies [37], into conditioning regimens, including sequential ones.

Conclusion

This real-world bicentric retrospective study suggests that the duration of the rest period between sequential cytoreductive chemotherapy and conditioning before allo-HSCT does not significantly influence patient outcomes. Within study limitations, LBTT appears feasible and at least not inferior to standard SBTT approaches. The statistically significant improvement in MRD-FS observed with LBTT represents an interesting signal that warrants further investigation, particularly as effect estimates for other outcomes consistently favored LBTT despite not reaching statistical significance. The potential benefit of LBTT is biologically plausible and may relate to improved recovery of healthy tissues, the ability to administer more intensive or higher-dose chemotherapy with better toxicity management, and potential cell-

cycle synchronization effects that may enhance leukemic cell sensitivity to subsequent conditioning, as suggested by preclinical data. Although these findings should be interpreted cautiously given limitations related to center-specific practices, patient heterogeneity, and regimen variations, they provide valuable real-world evidence supporting the safety and feasibility of LBTT strategies and highlight the importance of investigating the timing of administration of conditioning regimens in larger, prospective, randomized studies.

Authorship contributions

V.A. collected data from patients' records, reviewed the literature, analyzed the results and wrote the manuscript. A.S., E.C. and F.D. were involved in patients' treatment in CHUNG and completed patients' records. C.B. managed data of the patients treated in CHUNG. B.B. conducted the statistical analysis. X.P. conducted the research in CUSL, was involved in patients' treatment, completed patients' records and corrected the manuscript. C.G. designed the study, conducted the research in CHUNG, was involved in patients' treatment, completed patients' records and corrected the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.retram.2026.103586.

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