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blood advances

Clofarabine-fludarabine-busulfan in HCT for pediatric leukemia: an effective, low toxicity, TBI-free conditioning regimen

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Key Points

- CloFluBu-conditioning results in encouraging EFS for ALL and AML, with low TRM, limited incidence of aGvHD and GF, and no cases of VOD.
- Minimal residual disease status prior to transplantation impacted outcome due to increased relapse risk in both AML and ALL patients.

We prospectively studied clofarabine-fludarabine-busulfan (CloFluBu)-conditioning in allogeneic hematopoietic cell therapy (HCT) for lymphoid and myeloid malignancies and hypothesized that CloFluBu provides a less toxic alternative to conventional conditioning regimens, with adequate antileukemic activity. All patients receiving their first HCT, from 2011-2019, were included and received CloFluBu. The primary endpoint was event-free survival (EFS). Secondary endpoints were overall survival (OS), graft-versus-host disease (GvHD)-relapse-free survival (GRFS), treatment-related mortality (TRM), cumulative incidence of relapse (CIR), acute and chronic GvHD (aGvHD and cGvHD), and veno-occlusive disease (VOD). Cox proportional hazard and Fine and Gray competing-risk models were used for data analysis. One hundred fifty-five children were included: 60 acute lymphoid leukemia (ALL), 69 acute myeloid leukemia (AML), and 26 other malignancies (mostly MDS-EB). The median age was 9.7 (0.5 to 18.6) years. Estimated 2-year EFS was 72.0% ± 6.0 in ALL patients, and 62.4% ± 6.0 in AML patients. TRM in the whole cohort was 11.0% ± 2.6, incidence of aGvHD 3 to 4 at 6 months was 12.3% ± 2.7, extensive cGvHD at 2 years was 6.4% ± 2.1. Minimal residual disease-positivity prior to HCT was associated with higher CIR, both in ALL and AML. CloFluBu showed limited toxicity and encouraging EFS. CloFluBu is a potentially less toxic alternative to conventional conditioning regimens. Randomized prospective studies are needed.

Introduction

Allogeneic hematopoietic cell therapy (HCT) is used as consolidation therapy in roughly 15% of all children with acute lymphoid leukemia (ALL) or acute myeloid leukemia (AML). While HCT is often the only curative treatment option for relapsed or chemo-refractory leukemia, it also comes with high toxicity potency, and relapses still occur. Standard conditioning regimens for HCT in children consist of total body irradiation (TBI) or combined alkylating chemotherapeutics. Administering the optimal dosage is imperative since too little chemotherapy or irradiation can result in graft failure or leukemia relapse, and too high of exposure can cause tissue damage and acute life-threatening complications, as well as notorious late effects after transplantation. The success of HCT thus highly depends on the balance between efficacy and toxicity of the conditioning regimen.

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The full-text version of this article contains a data supplement.

In a recently published multicenter prospective study in pediatric ALL patients, TBI-based conditioning prior to HCT was found to result in higher 2-year event-free survival (EFS) and lower risk of relapse compared with 2 chemotherapy arms.[1] This is an important finding that should, however, be weighed against the well-known late toxic effects of TBI in long-term survivors.[2-5] In AML patients, the effects of conditioning regimens on outcome after HCT are not well studied. Since the 1990s, conditioning with busulfan (Bu), cyclofosfamide (Cy), and melfalan (Mel) is a commonly used preparative regimen[6] with proven efficacy in pediatric AML in terms of EFS (between 61% and 73%).[7,8] Nevertheless, especially in adolescent patients, toxic effects after BuCyMel are of great concern.[8] Therefore, both in ALL and AML, the optimal conditioning regimen resulting in high efficacy and low (long-term) toxicity remains to be identified.

Andersson et al have studied a conditioning regimen containing clofarabine, fludarabine, and busulfan (CloFluBu).[9-11] This combination showed to have synergistic antileukemic activity against ALL and AML blasts in vitro,[9,10] and a remarkable good outcome in adult patients with high-risk leukemias in terms of EFS.[9-11] Containing only a single alkylating drug, CloFluBu-conditioning is a potentially less toxic alternative to TBI in childhood ALL. In addition, this chemo-based conditioning regimen with high antileukemic activity could improve disease control in AML, reducing relapse risk. In a retrospective analysis comparing CloFluBu with other conditioning regimens (BuCy and BuCyMel) in pediatric HCT for AML, CloFluBu showed comparable disease control to BuCyMel, but with less acute graft-versus-host disease (aGvHD).[12] Targeted drug monitoring (TDM) of busulfan was an important factor in preventing toxicity, such as aGvHD. So far, no reports exist on the efficacy and toxicity of CloFluBu conditioning prior to HCT in pediatric ALL.

In the "Dutch COG HCT Working Group" we applied a CloFluBu-conditioning regimen in pediatric patients with high-risk lymphoid and myeloid malignancies undergoing HCT. We here describe our experience on the use of CloFluBu-conditioning in high-risk leukemia patients, aiming for an effective TBI-free regimen with possibly lower late toxicity and at least similar disease control compared with conventional conditioning regimens.

Patients and methods

Patients and procedures

Since August 2011, CloFluBu was the prescribed conditioning regimen for both ALL and AML in the 2 Dutch pediatric BMT centers. The regimen was also used for most patients with other high-risk hematological malignant indications since August 2011 (eg, MDS-EB, JMML, CML, infant ALL, lymphoma). All patients from both Dutch pediatric HCT programs (LUMC, Leiden, The Netherlands; and UMCU/Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands) receiving their first HCT between August 2011 and April 2019 were included. Patient data were collected and registered prospectively. Patients were enrolled, and data were collected only after written informed consent in accordance with the Helsinki Declaration. The study received Institutional Review Board approval for sample and data collection by METC UMCU and CME LUMC.

Leukemia treatment

All patients were treated for leukemia according to (inter)national treatment protocols. For upfront ALL, these were Dutch Childhood Oncology Group (DCOG) ALL-10[13] and DCOG ALL-11, where HCT indication was mainly based on initial disease response and MRD at the end of induction and high risk (HR) genetic characteristics like MLL-AF4. For relapsed ALL, the IntReALL was used, and HCT indications were based on the duration of remission after first therapy, organs involved in relapse, and MRD levels at the end of induction. AML patients received upfront treatment according to NOPHO2004/DB01[14] and NOPHODBHAML 2012, and HCT indication was based on the response of disease after 2 courses and HR genetic characteristics (FLT3ITD, NPM1wildtype). Relapsed AML was considered an HCT indication and was treated according to the international BFM protocol, mainly FLAD/FLA.[15] In poor responders, other chemotherapy was given (clofarabine, Mylotarg).

HCT procedure

CloFluBu-conditioning was administered from day -5 to day -1 prior to HCT. Clofarabine was given at a cumulative dosage of 120 mg/m^2 (4 $\times$ 30 mg/m^2), followed by fludarabine at 40 mg/m^2 (4 $\times$ 10 mg/m^2), and then by IV-administered busulfan, targeted at a cumulative area under the curve (AUC) of 85 to 95 mg*h/L using therapeutic drug monitoring as previously described.[16,17] Antithymocyte globulin (ATG; Thymoglobulin) was added to the conditioning regimen of patients receiving grafts from unrelated donors. From 2013 onwards, patients receiving an unrelated cord blood (CB) transplant for AML generally did not receive ATG, aiming for earlier immune reconstitution to enhance antileukemic, alloimmune effect.

ATG dosing also changed over time, in general, by bringing forward and decreasing the dose in order to promote immune reconstitution post HCT. ATG used to be administered at a dose of 10 mg/kg. Dose adjustments in older children were done from 2011 onwards in UMCU (>40 kg: ATG 7.5 mg/kg, 50% dose reduction was given when preconditioning lymphocyte counts were <300 $\times$ 10^6 T cells/L) and from 2014 onwards in LUMC (20 to 40 kg: ATG 8 mg/kg, >40 kg: ATG 6 mg/kg). Timing of ATG moved from start at day -6 to start at day -9. From 2016 onwards, ATG was dosed using an individualized dosing scheme based on body weight, absolute lymphocyte count, and stem cell source.[18]

Patients received GvHD-prophylaxis and infection prophylaxis according to local protocols. GvHD-prophylaxis consisted of cyclosporin A (CsA; targeted at trough levels of 100 to 250 μg/L depending on indication), combined with either prednisolone 1 mg/kg (CB) or methotrexate 10 mg/m^2 (on day +1, +3, and +6). CB recipients were treated with filgrastim from day +7 after HCT until neutrophils were above 2000 cells/μL. Patients were treated in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms.

Outcomes and definitions

The primary endpoint was EFS. EFS was defined as survival without the events graft failure (GF), therapy-related mortality (TRM), and relapse. Patients without an event were censored at the last follow-up date. Secondary endpoints were OS, TRM, cumulative incidence of relapse (CIR), acute and chronic GvHD (aGvHD grade 2 to 4 and 3 to 4, cGvHD, respectively), GvHD-free relapse-free survival (GRFS), and veno-occlusive disease (VOD). For OS, death from

any cause was considered an event. aGvHD and cGVHD were diagnosed and graded according to Glucksberg and Shulman criteria.[19,20] For GRFS, aGvHD 3 to 4, and extensive cGVHD, relapse and death were considered events. VOD was defined according to the modified Seattle criteria.[21]

Complete remission (CR) was defined as ≤5% bone marrow (BM) blasts and no evidence of extramedullary disease. CR1 was defined as complete remission after first-line treatment, CR2 was defined as complete remission after relapse therapy. Minimal residual disease (MRD) was assessed in BM prior to HCT by polymerase chain reaction (PCR) for immunoglobulin and T-cell receptor gene rearrangements in ALL and by flow cytometry for leukemia-associated immune profile (LAIP) in AML. MRD-negative was defined as $<10^{-4}$ in ALL and <0.1 in AML.

Statistical analysis

We evaluated and described the outcomes of interest in the full cohort, the ALL, AML, and "other" subgroups. ALL patients ≥4 years at transplantation were evaluated as a subcohort next to all ALL patients to evaluate their outcome in the context of literature on TBI-based conditioning regimens given to ALL patients older than 4 years. Apart from the effect of remission state (CR1 vs >2) and MRD state (positive vs negative) prior to HCT, we evaluated the outcomes in patients according to age as a continuous variable, per stem cell source; CB vs BM/peripheral blood, related vs unrelated, use of serotherapy in unrelated, and HLA match/mismatch. We also evaluated outcomes according to age above or below 12 years at transplantation, as this was reported to affect TRM in HCT for pediatric AML.[8]

Kaplan Meier and cumulative incidence models were applied to evaluate the outcomes. Cox proportional hazard and Fine and Gray competing risk models were used for data analysis of the entire distribution of events. Graft failure and TRM were considered as competing events for CIR. Relapse was considered a competing event for TRM. Competing events considered for aGvHD and cGvHD were graft failure, TRM, and relapse. Multivariate analyses were performed with covariates having a *P* value ≤.10 in univariate analysis. We made use of R 4.0.3, with packages *survival*, *survminer*, *ggplot2*, *cmprsk*, and *prodlim* to analyze the data and prepare graphs.

Results

A total of 155 children were included in our study (Table 1). The median age was 9.7 (0.5 to 18.6) years. Of these patients, 60 had ALL (32 in CR1, 24 in CR2, 4 in CR3; 13 MRD-positive, 34 MRD-negative, 13 MRD n/a), 69 AML (28 in CR1, 40 in CR2, 1 in active disease; 6 MRD-positive, 27 MRD-negative, 36 MRD n/a), and 26 had other malignancies (mostly MDS-EB). Most donors were unrelated (119; 77%); and stem cell source was BM in most patients (n = 80, 51%), followed by CB in 66 (43%), and peripheral blood stem cells (PBSC) in 10 (6%). The median follow-up time was 765 (19 to 2994) days. Clinical outcomes are shown in Table 2. Univariate and multivariate analyses for predictors of outcome are depicted in Table 3. Figures 1, 2, 3, and 4 show the impact of MRD, remission status, and age on EFS, GRFS, CIR, and TRM in ALL, ALL ≥4, and AML patients. The effects of all clinical variables on outcomes can be found in supplemental Table 1.

For ALL, the 2-year estimated EFS probability was 72.0% ± 6.0, with significantly lower EFS in patients with MRD-positivity prior to HCT (46.2% ± 13.8 vs 76.7% ± 7.9; hazard ratio [HR] 4.38; 95% confidence interval [CI] 1.55-12.34; *P* = .005). MRD status highly affected 2-year CIR, with 53.8% ± 13.8 in ALL MRD-positive and only 14.4% ± 6.7 in MRD-negative (multivariate HR 7.16; 95% CI 1.96-26.20; *P* = .003). Two-year TRM probability was low with only 5.0% ± 2.8

Table 1. Patient characteristics

		ALL			
	Total, n (%)	ALL total, n (%)	ALL ≥4 y, n (%)	AML, n (%)	Other, n (%)
Patients (n)	155	60	51	69	26
Age (y; range)	9.9	10.2	11.4	10.1	9.5
	(0.5-18.6)	(1.2-18.6)	(4.6-18.6)	(0.8-18.1)	(0.5-17.9)
Gender (female)	68 (44)	24 (40)	22 (43)	31 (45)	13 (50)
CR at HCT					
CR1	66 (43)	32 (53)	26 (51)	28 (41)	6 (23)
CR2	64 (41)	24 (40)	21 (41)	40 (58)	–
CR3	4 (3)	4 (7)	4 (8)	0 (0)	–
no CR	21 (13)	0 (0)	0 (0)	1 (1)	20 (77)
MRD					
Positive	21 (13)	13 (22)	10 (20)	6 (9)	2 (8)
Negative	65 (42)	34 (56)	28 (55)	27 (39)	4 (15)
n.a.	69 (45)	13 (22)	13 (25)	36 (52)	20 (77)
Stem cell source					
uCB	65 (42)	19 (32)	15 (29)	40 (58)	6 (23)
BM	80 (52)	35 (58)	30 (59)	25 (36)	20 (77)
PBSC	10 (6)	6 (10)	6 (12)	4 (6)	0 (0)
Donor unrelated	119 (77)	43 (72)	35 (69)	57 (83)	19 (73)
Serotherapy	87 (56)	40 (67)	33 (65)	28 (40)	19 (73)

in ALL patients (Table 3). TRM was not different in children older or younger than 12 years of age, and age as a continuous variable did also not affect TRM (supplemental Table 1). The probability of developing aGvHD grade 3 to 4 was 8.3% ± 3.6, and for extensive cGvHD this was 5.5% ± 3.1. GRFS was 66.7% ± 6.3. No graft failures and no cases of VOD were noted in patients treated for ALL in our cohort (Table 2).

We also evaluated the outcomes for ALL patients >4 years at transplantation (n = 51). In this subcohort of ALL, the 2-year EFS probability was 72.6% ± 6.6. MRD status prior to HCT influenced the 2-year EFS probability in this subcohort, with 50.0% ± 15.8 in MRD-positive and 74.3% ± 9.3 in MRD-negative ALL (HR 4.13; 95% CI 1.32-12.95; $P = .015$). CIR was highly affected by MRD status, with 50.0% ± 15.8 in MRD-positive and 18.4% ± 8.5 in MRD-negative ALL patients (multivariate HR 4.82; 95% CI 1.14-20.40; $P = .032$). Two-year TRM probability was only 4.0% ± 2.8. Probability of developing aGvHD grade 3 to 4 was 9.8% ± 4.2, and for extensive cGvHD this was 6.6% ± 3.7. GRFS was 66.4 ± 7.0 (Tables 2 and 3).

The 2-year probability of EFS for AML patients in our cohort was 62.4% ± 6.0, which was highly affected by MRD status with EFS of 16.7% ± 15.2 in AML MRD-positive and 67.7% ± 9.6 in AML MRD-negative (multivariate HR 5.66; 95% CI 1.76-18.24; $P = .004$). The overall 2-year CIR was 21.2% ± 5.1. For AML CR1, CIR was 7.5% ± 5.1, and 28.6% ± 7.3 for AML CR2 (multivariate HR 4.65; 95% CI 1.01-21.40; $P = .048$). CIR in MRD-positive patients was 50.0% ± 20.4, significantly higher than in MRD-negative patients 12.8% ± 7.0 (univariate HR 5.64; 95% CI 1.23-25.80; $P = .026$, not enough events for multivariate analysis). Two-year TRM was 15.5% ± 4.5 for all AML patients, with a higher TRM of 26.2% ± 8.5 observed in AML CR1, and only 8.5% ± 4.7 in AML CR2/3 (HR 0.25; 95% CI 0.07-0.94; $P = .040$). Age (younger or older than 12, or as continuous variable) did not affect TRM, nor did stem cell source, HLA match, serotherapy, or MRD status (supplemental Table 1). The probability of developing aGvHD grade 3 to 4 in our cohort of AML patients was 14.6% ± 4.3, and for extensive cGvHD this was 8.1% ± 3.5. Only 1 graft failure and no VOD were noted in patients treated for AML in our cohort. Two-year GRFS was 51.3% ± 6.3.

The patients in our study who were transplanted for other high-risk hematologic malignancies included 11 MDS-EB, 6 infant ALL, 2 CML, 2 M. Hodgkin, 2 JMML, 1 AUL, 1 CNL, and 1 Burkitt lymphoma patient. The 2-year probability of EFS in these patients was

Table 2. Clinical outcomes

		ALL			
	Total cohort (%)	ALL total (%)	ALL ≥4 y (%)	AML (%)	Other (%)
EFS	63.3 ± 4.0	72.0 ± 6.0	72.6 ± 6.6	62.4 ± 6.0	46.2 ± 10.3
CR1	–	81.2 ± 6.9	84.6 ± 1.2	62.4 ± 6.0	–
CR2/3	–	59.9 ± 10.6	58.4 ± 11.6	63.5 ± 7.8	–
MRD+	–	46.2 ± 13.8	50.0 ± 15.8	16.7 ± 15.2	–
MRD−	–	76.7 ± 7.9	74.3 ± 9.3	67.7 ± 9.6	–
age ≤12 y	63.2 ± 4.9	–	–	–	–
age ≥12 y	63.6 ± 7.0	–	–	–	–
OS	70.1 ± 3.9	78.8 ± 5.5	81.2 ± 5.7	66.3 ± 5.9	60.2 ± 10.5
GRFS	56.3 ± 4.2	66.7 ± 6.3	66.4 ± 7.0	51.3 ± 6.3	44.8 ± 10.5
CIR	24.7 ± 3.6	23.0 ± 5.7	23.4 ± 6.3	21.2 ± 5.1	37.6 ± 10.0
CR1	–	12.5 ± 5.9	11.5 ± 6.3	7.5 ± 5.1	–
CR2/3	–	35.9 ± 10.4	36.8 ± 11.4	28.6 ± 7.3	–
MRD+	–	53.8 ± 13.8	50.0 ± 15.8	50.0 ± 20.4	–
MRD−	–	14.4 ± 6.7	18.4 ± 8.5	12.8 ± 7.0	–
TRM	11.0 ± 2.6	5.0 ± 2.8	4.0 ± 2.8	15.5 ± 4.5	12.4 ± 6.7
CR1	–	6.3 ± 4.3	3.8 ± 3.7	26.2 ± 8.5	–
CR2/3	–	4.2 ± 4.1	4.8 ± 4.7	8.5 ± 4.7	–
MRD+	–	0.0 ± 0.0	0.0 ± 0.0	20.8 ± 18.4	–
MRD-	–	8.9 ± 4.9	7.3 ± 5.0	20.1 ± 8.1	–
age ≤12 y	11.2 ± 3.2	5.3 ± 3.7	3.6 ± 3.5	16.1 ± 5.6	–
age ≥12 y	10.5 ± 4.5	4.5 ± 4.4	4.5 ± 4.4	14.3 ± 7.7	–
aGvHD 2-4	25.9 ± 3.5	21.7 ± 5.3	21.6 ± 5.8	29.2 ± 5.5	27.4 ± 8.8
aGvHD 3-4	12.3 ± 2.7	8.3 ± 3.6	9.8 ± 4.2	14.6 ± 4.3	15.8 ± 7.2
cGvHD	12.0 ± 2.7	16.1 ± 4.9	16.8 ± 5.5	11.4 ± 4.1	4.2 ± 4.1
ext. cGvHD	6.4 ± 2.1	5.5 ± 3.1	6.6 ± 3.7	8.1 ± 3.5	4.2 ± 4.1
VOD	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Graft failure	1.3 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 1.2	4.3 ± 4.3
Follow-up (days; range)	765 (19-2994)	951 (58-2994)	913 (58-2994)	643 (19-2607)	488 (45-2628)

Kaplan Meier and Fine and Gray competing risk models were applied to calculate probability of EFS, OS, and GRFS and cumulative incidence of CIR, TRM, aGvHD, cGvHD, GF, and VOD. Percentages and standard errors are provided.

Table 3. Univariate and multivariate analyses for the effect of variables on EFS, GRFS, RR, and TRM.

	Prob./cum. inc. % with SE	Univariate analysis		Multivariate analysis	
		(subd.) HR; 95% CI	*P* value	(subd.) HR; 95% CI	*P* value
ALL					
EFS	72.0 ± 6.0				
MRD−	76.7 ± 7.9				
MRD+	46.2 ± 13.8	4.30; 1.55-11.94	.005*		
GRFS	66.7 ± 6.3				
MRD−	70.5 ± 8.4				
MRD+	38.5 ± 13.5	3.45; 1.31-9.10	.012*		
CIR	23.0 ± 5.7				
CR1	12.5 ± 5.9				
CR2/3	35.9 ± 10.4	3.01; 0.92-9.91	.070	2.97; 0.63-14.10	.170
MRD−	14.4 ± 6.7				
MRD+	53.8 ± 13.8	7.15; 2.21-23.1	.001**	7.16; 1.96-26.20	.003*
TRM	5.0 ± 2.8				
ALL ≥4 y of age					
EFS	72.6 ± 6.6				
MRD−	74.3 ± 9.3				
MRD+	50.0 ± 15.8	4.13; 1.32-12.95	.015*		
GRFS	66.4 ± 7.0				
MRD−	66.6 ± 9.9				
MRD+	40.0 ± 15.5	3.27; 1.11-9.66	.032*		
CIR	23.4 ± 6.3				
CR1	11.5 ± 6.3				
CR2/3	36.8 ± 11.4	3.21; 0.83-12.50	.092	3.19; 0.43; 23.70	.260
MRD−	18.4 ± 8.5				
MRD+	50.0 ± 15.8	5.52; 1.55-19.70	.009*	4.82; 1.14-20.40	.032*
TRM	4.0 ± 2.8			.	
AML					
EFS	62.4 ± 6.0				
MRD−	67.7 ± 9.6				
MRD+	16.7 ± 15.2	5.06; 1.63-15.74	.005*	5.66; 1.76-18.24	.004**
BM/PBSC	79.3 ± 7.5				
CB	48.9 ± 8.4	2.95; 1.18-7.40	.021*	7.23; 0.92-56.76	.060
GRFS	51.3 ± 6.3				
CIR	21.2 ± 5.1				
CR1	7.5 ± 5.1				
CR2/3	28.6 ± 7.3	4.19; 0.95-18.60	.059	4.65; 1.01-21.4	.048*
MRD neg	12.8% ± 7.0				
MRD pos	50.0% ± 20.4	5.64; 1.23-25.80	.026*	(not enough events)	
BM/PBSC	10.3% ± 5.7				
CB	29.8 ± 7.6	3.14; 0.89-11.10	.075	3.26; 0.99-12.00	.076
TRM	15.5 ± 4.5				
CR1	26.2 ± 8.5				
CR2/3	8.5 ± 4.7	0.255; 0.07-0.94	.040*		

Cox proportional hazard and Fine and Gray models were applied to perform univariate analyses of the effect of clinical covariates on outcome. Hazard and subdistribution HR with 95% CI are given for Cox proportional hazard and Fine and Gray models, respectively. Probability of 2-year EFS and 2-year GRFS and cumulative incidence for CIR and TRM are given. *P* values < .05 are deemed statistically significant and are indicated with asterisks based on their significance level: *$P < .05$, **$P < .005$, ***$P < .001$. A full overview of clinical outcomes and univariate analyses of clinical variables is provided in supplemental Table 1.

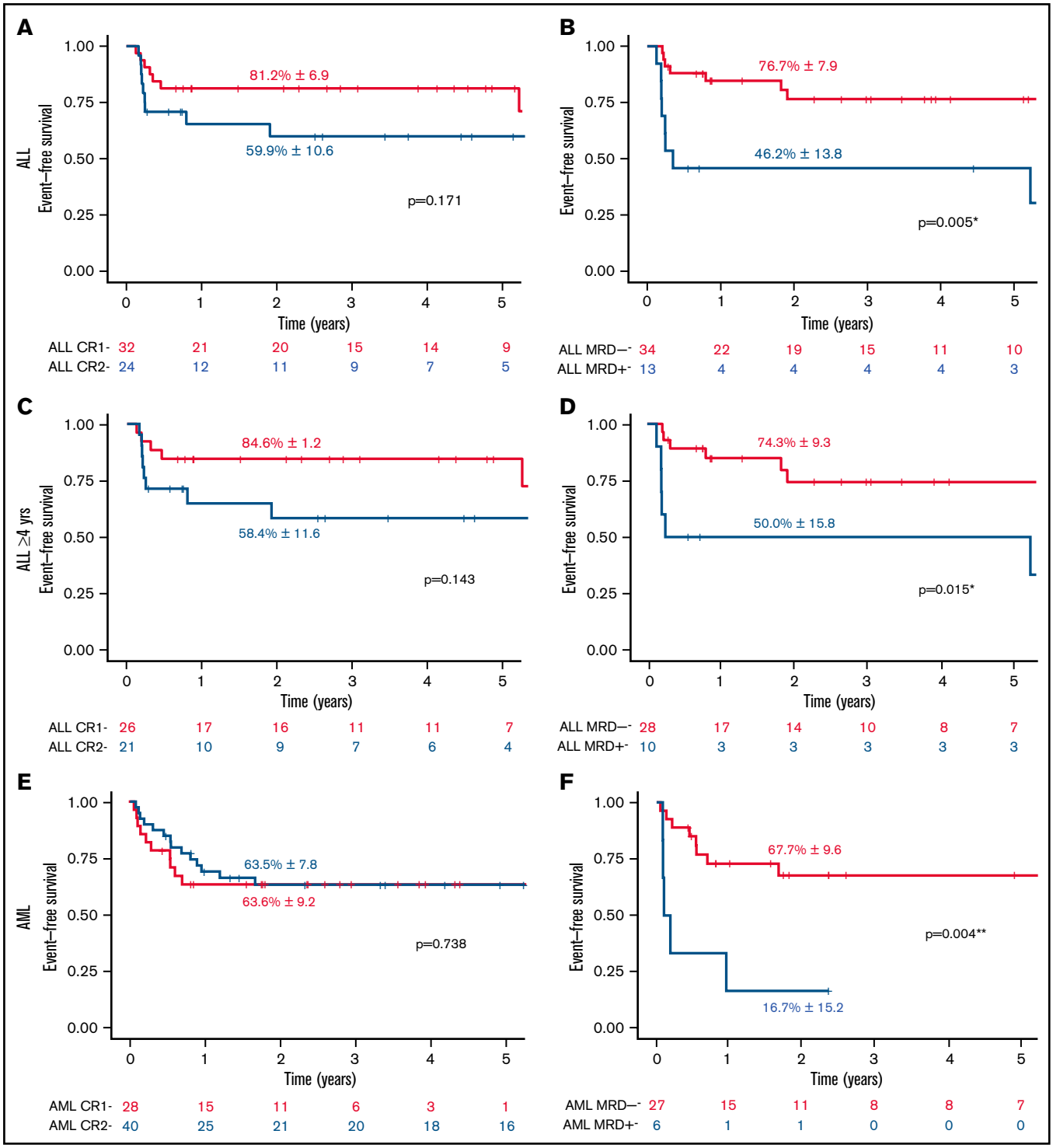

Figure 1. Event-free survival (EFS) probability in all ALL patients. (A-B) EFS probability in ALL patients. ALL patients ≥4 years of age (C-D) and AML patients receiving CloFluBu conditioning (E-F). Two-year EFS with standard errors is provided, with *P* values calculated with the Cox proportional hazard model and correction for covariates where appropriate (Table 2; supplemental Table 1). Patients at risk are depicted below each survival plot.

46.2% ± 10.3, with an OS of 60.2% ± 10.5 and a GRFS of 44.8% ± 10.5. CIR was 37.6% ± 10.0 and TRM 12.4% ± 6.7. Probability of aGvHD grade 3 to 4 was 15.8% ± 7.2, and for extensive cGvHD this was 4.2% ± 4.1. No VOD was noted in these patients, and 1 patient had a graft failure.

Discussion

We report on our experience with CloFluBu-conditioning in a large pediatric HCT cohort, with encouraging EFS for ALL and AML, and with low overall TRM. MRD status prior to

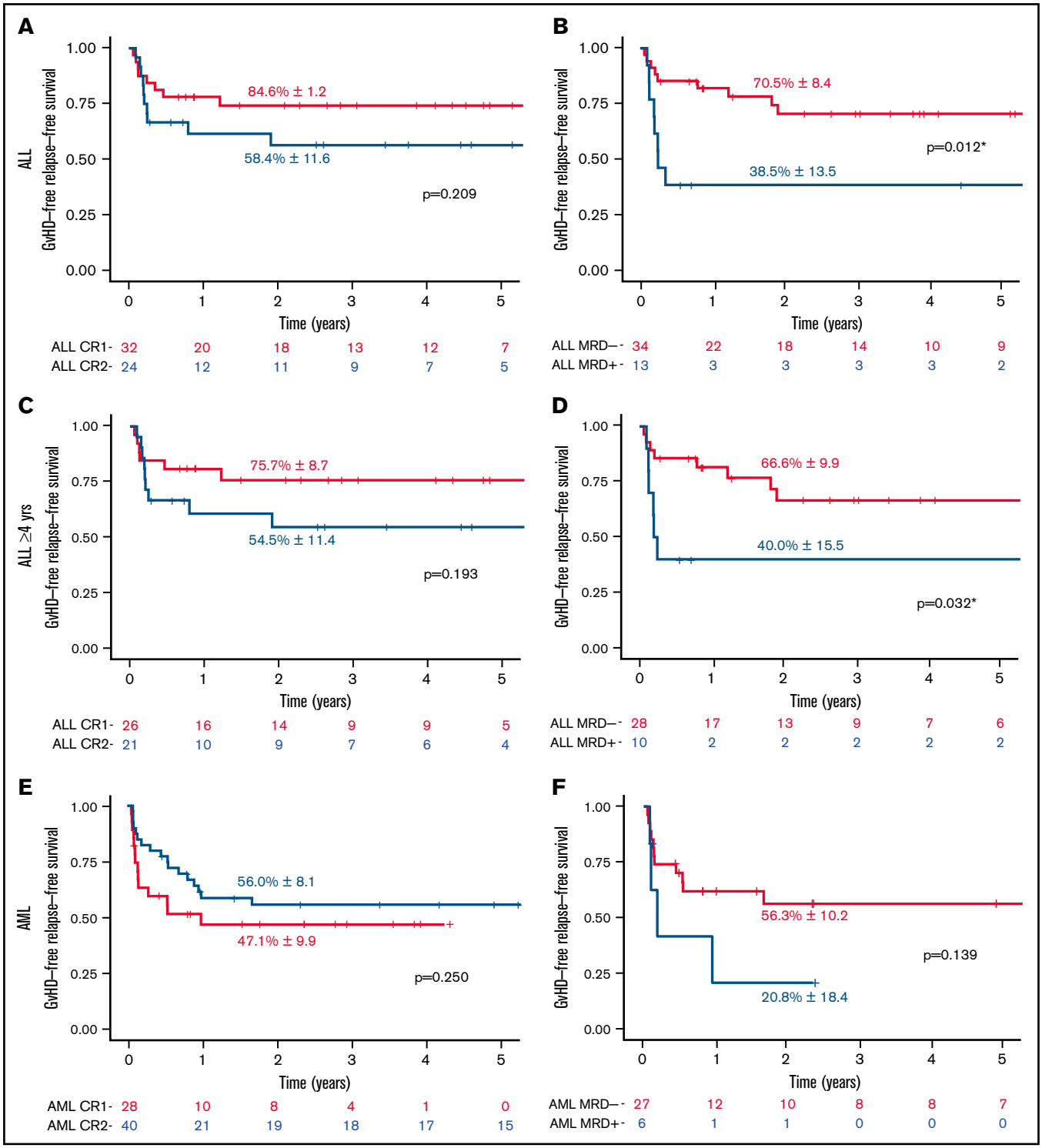

Figure 2. GRFS probability in all ALL patients. GRFS probability in all ALL patients (A-B), ALL patients ≥4 years of age (C-D), and AML patients (E-F) receiving CloFluBu conditioning. Two-year GRFS with standard errors is provided, with *P* values calculated with the Cox proportional hazard model and correction for covariates where appropriate (Table 2; supplemental Table 1). Patients at risk are depicted below each survival plot.

transplantation highly impacted outcome due to increased relapse risk in both MRD-positive AML and ALL patients. Outcomes were comparable for patients younger and older than 12 years of age at HCT. Incidence of aGvHD was low, and only 2 graft failures and no cases of VOD were noted. Our findings indicate that CloFluBu is a good alternative for TBI-based conditioning in ALL and an effective and less toxic strategy in AML patients.

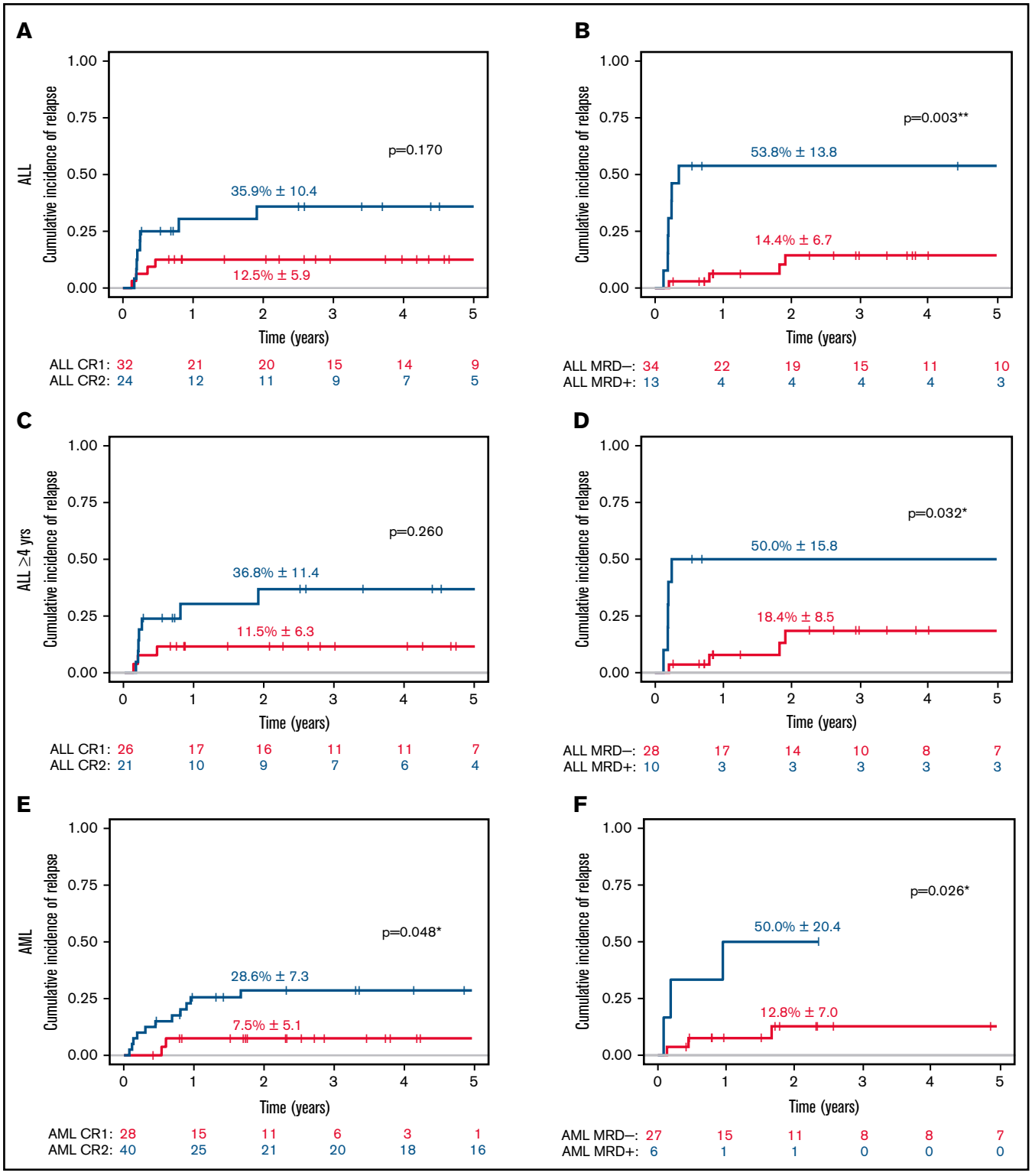

Figure 3. CIR in all ALL patients. CIR in all ALL patients (A-B), ALL patients ≥4 years of age (C-D), and AML patients (E-F) receiving CloFluBu conditioning. Two-year CIR with standard errors is provided, with *P* values calculated with the Fine and Gray model for competing risk analysis and correction for covariates where appropriate (Table 2; supplemental Table 1). Patients at risk are depicted below each survival plot.

Findings from a large prospective randomized study (FORUM study) comparing TBI-based with chemo-based conditioning of fludarabine, thiotepa, and either busulfan or treosulfan, in patients ≥4 years of age with high-risk ALL, were recently published.[1] In this study, TBI showed the most favorable results with significantly better 2-year EFS, OS, and GRFS, with lower CIR, and equal TRM compared with the

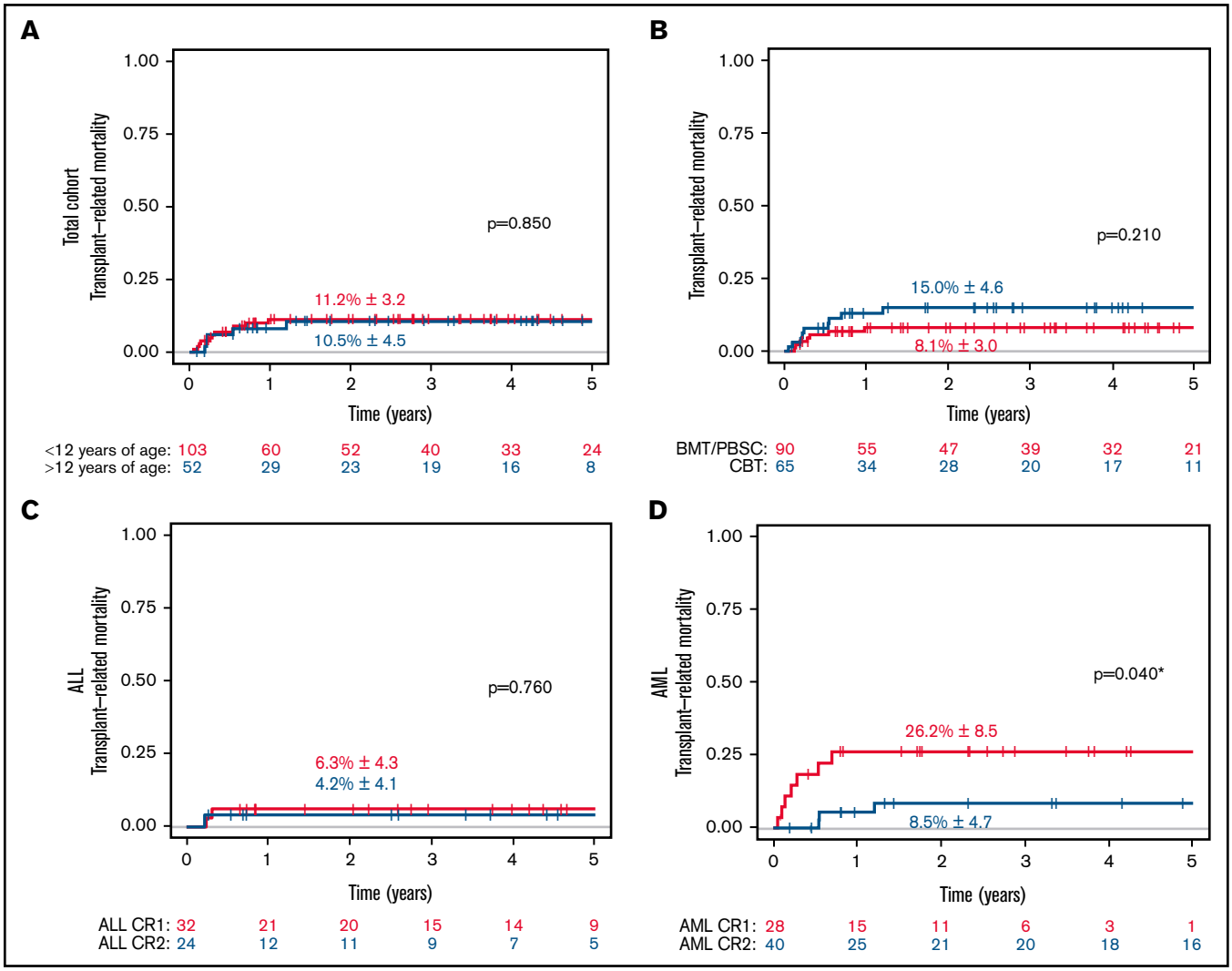

Figure 4. Cumulative incidence of TRM in the total cohort. Cumulative incidence of TRM in the total cohort (A-B), all ALL patients (C), and AML patients (D) receiving CloFluBu conditioning. Two-year TRM with standard errors is provided, with *P* values calculated with the Fine and Gray model for competing risk analysis and correction for covariates where appropriate (Table 2; supplemental Table 1). Patients at risk are depicted below each survival plot.

chemotherapy arms. In our subcohort of ALL patients ≥4 years of age, 51% were transplanted in CR1, and 26% were MRD-positive at time of HCT, compared with 56% in CR1 and 42% MRD-positive in the FORUM cohort. We found an estimated 2-year EFS of 84.6% (95% CI 72-99) in ALL CR1, compared with the 91% (95% CI 83-96) EFS in the "as-treated" TBI arm, and the 67% (95% CI 56-76) EFS in the as-treated chemotherapy arm of the FORUM study (supplemental Figure 1). GRFS was comparable between the TBI arm in FORUM and our cohort, while CIR was lower in the TBI arm of the FORUM study, and TRM was equally low. Notably, the incidence of aGVHD 2 to 4 was lower in our cohort compared with the TBI and chemotherapy arms of the FORUM study. We describe outcome data from a nonrandomized 2-center study and are fully aware of the fact that comparison with a randomized study should be done with great caution. However, because all consecutive patients with ALL received CloFluBu-conditioning (ruling out patient selection that would normally cause bias of the outcomes), and the FORUM trial was done during the same time in a similar group of patients, we believe that a prudent comparison can be made.

In addition, when compared with the 2-year EFS reported in other studies on HCT in pediatric ALL, our 2-year EFS of 72.0% ± 6.0 is relatively high.[1,22,23] This indicates that CloFluBu, with targeted busulfan as the only alkylator, is a promising TBI-free conditioning regimen for high-risk ALL, both in patients ≥4 years of age as well as in younger patients. With these increased survival chances in current high-risk ALL HCT cohorts, late effects from conditioning regimens should be more carefully weighed against treatment success. TBI is implicated in severe late effects as cataracts, growth retardation, infertility, restrictive pulmonary disease, nephrotoxicity, and secondary malignancies,[2-5] which are less frequently seen after chemotherapy-only conditioning regimens, especially with busulfan TDM possibly further limiting toxicity. Therefore, it is of high added value to evaluate and compare the late effects from our CloFluBu-conditioning regimen to the late effects after TBI-based conditioning.

We found a strong impact of MRD status pre-HCT on outcome in ALL in our cohort. MRD data were available in 78% of ALL patients, 28% of them being MRD-positive. MRD positivity was the only

highly significant predictor for CIR and lower GRFS and EFS. This is in line with earlier reports on the prognostic value of pre-HCT MRD in ALL.[24-26] Remarkably, this effect was not observed in the FORUM study, neither in the TBI nor in the chemo arms.[1] This discrepancy might be explained by different definitions of MRD positivity pre-HCT and different approaches to reduce MRD level prior to transplant. In FORUM MRD, positivity was defined as PCR $>10^{-4}$, similar to our study, but in 17% of patients in FORUM, MRD was not based on PCR but on flowcytometry where $>10^{-3}$ was called positive. In our cohort of patients, we aimed for MRD pre-HCT below 10^{-4} and in the FORUM study below 10^{-3}, giving extra chemotherapy in some patients to achieve this situation. The latter possibly resulted in a lower incidence of MRD-positive ALL patients in our study (26% in ALL $\geq$4 years of age) compared with the TBI arm (41%) and the chemo arms (46%) of the FORUM study. Therefore, MRD positivity might be of different relevance in these studies.

In the AML patients in our cohort, we noted encouraging EFS and OS in the mid-60%, which is largely comparable to outcomes reported in other studies on HCT in pediatric AML.[8,27,28] Also, the CIR and TRM were in line with what has been described by others.[24,26,29-31] In these studies as well as our cohort, MRD status highly influenced the risk of relapse in HCT for AML. The 2-year TRM in AML patients in our cohort was comparable to the CIBMTR data[27] and to what was seen in the AML BFM 2007 trial.[8] However, we observed an unexpected high TRM in the 28 patients in AML CR1. This likely has to do with the relatively low number of AML CR1 patients and may in part be explained by a higher incidence of aGvHD grade 3 to 4 in this subgroup (25% in AML CR1 vs 7.5% in AML CR2/3). Variables like stem cell source, use of serotherapy, or age did not explain this high incidence (supplemental Table 1). When looking at the comorbidity index (HCT-CI),[32,33] this subgroup of patients had a low-risk score for TRM. Notably, more recent data in AML patients in our cohort (transplanted after the study period) show a decline in TRM (unpublished data). Unlike previous reports,[8] younger or older age (below or above 12 years at transplantation, respectively) did not affect TRM in our cohort of AML patients. In a retrospective comparison with other busulfan-based conditioning regimens for HCT in pediatric AML, CloFluBu resulted in better leukemia-free survival compared with busulfan-cyclophosphamide (BuCy), and lower aGvHD risk compared with BuCy and BuCyMel conditioning regimens.[12] Our findings indicate that CloFluBu is a successful conditioning regimen for high-risk pediatric AML patients, with respectable survival chances, limited relapse risk, and low risks of aGvHD, VOD, and graft failure.

The limited aGvHD risk in our cohort is largely explained by the targeted busulfan.[12] The impact of optimally targeting chemo-based conditioning regimens is further highlighted by the low incidence of graft failure, TRM, and the high 2-year GRFS in our study. The optimal range for busulfan AUC (78 to 101 mg*h/l) results from a previous study by Bartelink et al[17] where lower AUC resulted in more graft failure and relapse, and higher AUC increased acute toxicity, including aGvHD grade 2 to 4, and TRM. Next to busulfan, studies on fludarabine exposure also show a relation between exposure and outcome, with higher TRM in both over- and under-exposed patients due to graft failure, toxicity, and impaired immune reconstitution.[34] Besides optimizing chemotherapy exposure, serotherapy (rATG) exposure could also be further optimized and individualized since this is related to survival outcomes, graft failure, aGvHD and cGvHD, and relapse after HCT as well.[35-37] Because of the potential impact of targeting busulfan and fludarabine, in addition to individualizing serotherapy, it is of high interest to study HCT outcomes and toxicity after fully targeting CloFluBu-conditioning in relation to other conditioning regimens.

There were no cases of VOD in our entire cohort. With increasing insight in the pathophysiology of transplant-related systemic endothelial diseases, VOD is currently called sinusoidal obstruction syndrome (SOS) and new diagnostic criteria for VOD/SOS in children are proposed.[38] With regard to these criteria, we might have missed some severe SOS cases because we did not include platelet refractoriness, renal function, or impaired coagulation in our clinical definition. However, no patient received defibrotide, and no patient died of a clinical syndrome fitting the new criteria for VOD/SOS.

In general, it is important to emphasize that HCT outcome also depends on upfront therapy, HCT indications, and supportive care protocols. This should be considered when comparing data from different studies. As to HCT procedures, our patient group contains a relatively large number of CBT recipients, many of them not receiving serotherapy (especially in the AML subgroup), and if serotherapy was given, the dosing schedule has been changed during the study, and ultimately was based on a model using lymphocyte count, weight, and stem cell source. This might have influenced the outcome compared with other cohorts. Although in none of the analyses, stem cell source and serotherapy were significant predictors for outcome in our cohort. All our patients received targeted busulfan, aiming for the previously identified optimal exposure of busulfan, which likely influenced overall outcomes as well.

In conclusion, CloFluBu-conditioning provides a promising regimen for pediatric patients with high-risk AML and ALL. This TBI-free, single alkylator regimen comes with a favorable toxicity profile and retains a high antileukemic potency. Particularly for MRD-AML patients, but also ALL CR1 patients and the youngest of patients, it may be considered a safe and effective TBI-free alternative. The toxicity is largely reduced due to the single alkylator configuration and by targeting busulfan exposure. By individualizing the serotherapy and targeting the fludarabine exposure, the regimen can be further refined. More studies, preferably in randomized controlled prospective clinical trials with long-term follow-up, are needed to draw firmer conclusions with regard to the antileukemic effect and late effects of this conditioning regimen. In the near future, a randomized phase 3 trial comparing CloFluBu and BuCyMel in pediatric HCT for AML will open within the NOPHO-DBH consortium. ALL new studies on chemotherapy-based conditioning will most likely be limited to the patients that cannot receive TBI due to young age, organ toxicity, or earlier exposure. A combination based on a single alkylator with clofarabine, with a potential role for etoposide, could be an effective and mildly toxic strategy to study in these patients.

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Authorship

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