Fludarabine/TBI 8 Gy versus fludarabine/treosulfan conditioning in patients with AML in first complete remission: A Study from the Acute Leukemia Working Party of the EBMT

Gesine Bug (G.Bug@em.uni-frankfurt.de)
Goethe University Frankfurt, University Hospital https://orcid.org/0000-0003-2359-131X

Myriam Labopin
Hôpital Saint-Antoine https://orcid.org/0000-0003-4514-4748

Riitta Niittyvuopio
Helsinki University Hospital

Matthias Stelljes
University of Muenster https://orcid.org/0000-0002-9331-5145

Christian Reinhardt
University Hospital, Dept. of Bone Marrow Transplantation, Essen

Inken Hilgendorf
Universitätsklinikum Jena https://orcid.org/0000-0003-2038-9730

Nicolaus Kroeger
University Medical Center Hamburg-Eppendorf https://orcid.org/0000-0001-5103-9966

Ain Kaare
Tartu University Hospital https://orcid.org/0000-0002-3170-1016

Wolfgang Bethge
Medical Center, University of Tuebingen https://orcid.org/0000-0001-6052-2618

Kerstin Schaefer-Eckart
Department of Bone Marrow Transplantation, Internal Medicine V, North Hospital, Nuernberg

Mareike Verbeek
Technical University Munich

Stephan Mielke
Karolinska Institute and University Hospital

Kristina Carlson
Uppsala University Hospital

Ali Bazarbachi
American University of Beirut Medical Center https://orcid.org/0000-0002-7171-4997

Alexandros Spyridonidis
University Hospital of Patras https://orcid.org/0000-0003-3097-2532

Bipin Savani
Vanderbilt University Medical Center
Arnon Nagler
Chaim Sheba Medical Center https://orcid.org/0000-0002-0763-1265
Mohamad Mohty
Hôpital St Antoine, Sorbonne University, INSERM UMRs 938 https://orcid.org/0000-0002-7264-808X

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Abstract

The optimal reduced intensity conditioning (RIC) regimen is a matter of debate. We retrospectively compared conditioning with fludarabine plus fractionated total body irradiation of 8 Gy (FluTBI) and fludarabine plus treosulfan 30, 36 or 42 g/m^2 (FluTreo) in 754 patients with AML above the age of 40 years undergoing an allogeneic hematopoietic stem cell transplant (HSCT) in first complete remission (CR). In a multivariate analysis, FluTBI was associated with a significantly lower probability of relapse than FluTreo (hazard ratio (HR) 0.59 [95% CI, 0.38–0.93], p = 0.023). There was no significant difference in leukemia-free survival (LFS), overall survival (OS), graft-versus-host disease-free and relapse-free survival (GRFS), or acute and chronic graft-versus-host disease (GVHD). After balancing patient characteristics by propensity score matching of 115 patients in each group, FluTBI retained its significantly lower probability of relapse compared to FluTreo (18.3% vs. 34.7%, p = 0.018) which was counteracted by a higher non-relapse mortality (16.8 vs. 5.3%, p = 0.02). Thus, OS and GRFS at 2 years were similar between groups (OS 66.9% vs. 67.8%, GRFS 50.3% vs. 45.6%). We conclude that both conditioning regimens are effective and safe, but FluTBI may better be reserved for younger patients below the age of 55 years.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission (CR) is the treatment of choice for the majority of patients with acute myeloid leukemia (AML) (1), (2), (3), but its antileukemic efficacy needs to be balanced against the risk of non-relapse morbidity and mortality (4). To address the higher non-relapse mortality (NRM) associated with increasing age, especially with myeloablative conditioning (MAC) regimens, reduced intensity conditioning (RIC) has been widely adopted. (5) These regimens may result in higher relapse rates especially in patients with measurable residual disease (MRD) as demonstrated in the large randomized BMT CTN 0901 trial (6), (7). While fludarabine is universally used as a component of RIC regimens to ensure stable engraftment of donor hematopoiesis, the optimal combination partner remains contentious. Alkylating agents such as IV busulfan 6.4 mg/kg (FB2) or melphalan ≤ 150 mg/m2 (FluMel) as well as reduced doses of fractionated total body irradiation (TBI) of 8 Gy or less are frequently employed, but these RIC regimens differ in their ability to retain strong antileukemic activity while conferring only modest non-hematologic toxicity as assessed by the risk of NRM (8), (9), (10), (11).

Treosulfan is a prodrug of a bifunctional alkylating agent with stem cell depleting and broad antileukemic activity. It gained approval in the EU and Canada as part of conditioning based on a pivotal randomized trial including patients with AML in CR or MDS at increased risk of mortality with MAC. The RIC regimen of fludarabine and treosulfan (30 g/m^2 total dose) resulted in improved relapse-free survival (RFS) and OS compared to FB2 (64% and 71% vs. 50% and 56% at 2 years, respectively), mainly due to reduction of late NRM from 22–11%. (12) In a randomized comparison of TBI-based MAC (i.e., fractionated TBI of 12 Gy and cyclophosphamide, TBI 12 Gy/Cy) with a RIC regimen of fludarabine and fractionated TBI of 8 Gy

in AML in first CR (CR1), patients with AML aged 41 to 60 years treated in the RIC arm achieved favorable RFS of 76% at 1 year and a sustained low NRM of 13% at 10 years (13), (14).

To date, no direct comparison of fludarabine and treosulfan (FluTreo) with fludarabine and TBI 8 Gy (FluTBI) has been performed. We hypothesize that FluTreo may be a lower toxicity alternative to the FluTBI 8 Gy regimen in AML patients aged above 40 years in CR1, who may not be prime candidates for MAC regimens.

Subjects And Methods

Data collection

Data for this retrospective multicenter study were retrieved from the registry of the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT), a nonprofit, scientific society representing > 600 transplant centers, mainly located in Europe. Centers commit to reporting all consecutive HSCT and follow-ups once a year. Data are entered, managed, and maintained in a central database and validated by verification of the computer printout of the entered data, cross-checking with the national registries, and on-site visits to selected teams. All patients gave informed consent authorizing the use of their personal information for research purposes. This study was approved by the ALWP of the EBMT institutional review board and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Criteria for patient selection

Patients were included if they had (1) a diagnosis of AML in CR1 (MRD positive or negative); (2) an age > 40 years; (3) received their first allogeneic HSCT between 2009 and 2019; (4) a matched sibling donor (MSD) or 10/10 HLA-matched unrelated donor (MUD), and if (5) peripheral blood stem cells or bone marrow was used as the stem cell graft; (6) the conditioning regimen consisted of either fludarabine and treosulfan (30, 36 or 42 g/m^2, FluTreo) or fludarabine and fractionated TBI 8 Gy (4 x 2 Gy or 2 x 4 Gy, FluTBI). *In vivo* T- cell depletion (TCD) with anti-thymocyte globulin (ATG) was allowed, but transplantations from haploidentical donors, umbilical cord blood stem cells or using post-transplant cyclophosphamide or *ex-vivo* T-cell depletion were excluded.

Statistical analysis

The primary endpoint of this study was OS; secondary endpoints included LFS, cumulative incidence of relapse (CIR), NRM, incidence of acute and chronic graft-versus-host disease (GVHD) as well as survival free of grade III-IV acute GVHD, chronic GVHD, and relapse (GRFS) (15). Acute and chronic GVHD were diagnosed according to the modified Glucksberg criteria and modified Seattle criteria, respectively (16), (17).

Patient, disease, and transplant characteristics were compared by using the χ^2 or Fisher's exact test for categorial variables and the Mann-Whitney or Kruskal Wallis test for continuous variables. Probabilities for OS, LFS and GRFS were calculated using Kaplan-Meier estimates, and cumulative incidence (CI) curves for relapse, NRM, acute and chronic GVHD using a competing risk model: for relapse, death served as competing risk, whereas relapse and death were competing risks for NRM and GVHD (18). Univariate analyses were performed using the log-rank test for LFS, OS, and GRFS, and Gray's test for CI estimates (19). A Cox' s proportional hazards model was used for multivariate analyses by including all variables differing significantly between the groups, and factors known to influence outcomes. In order to take into account the heterogeneity in the effect of a characteristic or a treatment across centers, we introduce a random effect in Cox multivariate models (20).

For propensity score matching, exact matching was performed in a 1:1 ratio for donor type, secondary AML and adverse risk cytogenetics and nearest neighbor matching for age at HSCT, time from diagnosis to HSCT, female to male transplant, Karnofsky performance score (KPS) and *in vivo* TCD. We compared 115 patients in each conditioning group. All tests were two-sided with the type 1 error rate fixed at 0.05. SPSS 27.0 (IBM Corp., Armonk, NY, USA) and R 4.0.2 (R Core Team 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.Rproject.org/)), were used for all statistical analyses.

Results

Patients and transplant procedures

A total of 754 patients with AML are included in this analysis, of whom 617 received FluTreo and 137 FluTBI conditioning. Patients in the FluTBI group were significantly younger with a median age of 53.7 (range, 40.1–70.7) vs. 60.7 (range 40.1–77.5) years. They had been transplanted a median of two years earlier (2014 vs. 2016) and had longer follow-up (median 45.9 vs. 26.9 months), Table 1. Additional statistically significant differences between the groups included a higher proportion of MSD with FluTBI (64.2% vs. 36.3%, $p < 0.0001$) as opposed to 10/10 MUD (35.8% vs. 63.7%, $p < 0.0001$), a shorter time from diagnosis to HSCT (3.8 (range, 1.8–16.2) vs. 4.7 (range, 1.7–22.9 months)) and a higher proportion of patients with *de novo* AML (84.7% vs. 76%). The groups did not differ significantly in terms of adverse risk cytogenetics according to European LeukemiaNet (ELN) 2017, patient or donor sex, KPS, or pre-transplant MRD status, although information on the latter parameter was available for only 287 patients.

Table 1
Patient, donor, and transplant characteristics according to conditioning regimen for all patients

	FluTBI (n = 137)	FluTreo (n = 617)	P
Median patient age, years (range)	53.7 (40.1–70.7)	60.7 (40.1–77.5)	< 0.0001
Karnofsky performance score			
<90%	27 (20.1%)	133 (22.2%)	0.60
≥90%	107 (79.9%)	465 (77.8%)	
Missing	3	19	
Diagnosis			
De novo AML	116 (84.7%)	469 (76%)	0.028
Secondary AML	21 (15.3%)	148 (24%)	
Cytogenetic risk group			
Good	4 (3.7%)	15 (3.4%)	0.074
Intermediate	75 (70.1%)	256 (58.9%)	
Poor	28 (26.2%)	164 (37.7%)	
NA/failed	30	182	
Not adverse	109 (79.6%)	453 (73.4%)	0.14
Adverse	28 (20.4%)	164 (26.6%)	
Median interval from diagnosis to HSCT, months (range)	3.8 (1.8–16.2)	4.7 (1.7–22.9)	< 0.0001
Median year of HSCT (range)	2014 (2009–2019)	2016 (2009–2019)	< 0.0001
MRD status pre-transplant			
MRD negative	31 (64.6%)	143 (59.8%)	0.54
MRD positive	17 (35.4%)	96 (40.2%)	
Missing	89	378	
Donor			

HLA: Human leukocyte antigen, CMV: Cytomegalovirus, TBI: Total body irradiation; ATG: Anti-thymocyte globulin, MTX: Methotrexate, MMF: Mycophenolate mofetil, CI: Confidence interval, NA: not applicable

	FluTBI (n = 137)	FluTreo (n = 617)	P
Matched sibling	88 (64.2%)	224 (36.3%)	< 0.0001
10/10 HLA matched unrelated	49 (35.8%)	393 (63.7%)	
Patient sex			
Male	84 (61.3%)	329 (53.3%)	0.089
Female	53 (38.7%)	288 (46.7%)	
Donor/patient sex			
Female/male	26 (19%)	107 (17.4%)	0.66
Other combinations	111 (81%)	508 (82.6%)	
Missing	0	2	
Donor/patient CMV status			
Donor negative/patient negative	34 (26.0%)	109 (17.9%)	0.11
Donor positive/patient negative	11 (8.4%)	37 (6.1%)	
Donor negative/patient positive	30 (22.9%)	155 (25.6%)	
Donor positive/patient positive	56 (42.7%)	307 (50.5%)	
Missing	6	9	
TBI fractions		NA	
4 x 2 Gy	40 (29.2%)		
2 x 4 Gy	16 (11.7%)		
unknown	81 (59.1%)		
Treosulfan dose	NA		
3 x 10 g/m^2		167 (27.1%)	
3 x 12 g/m^2		122 (19.8%)	
3 x 14 g/m^2		328 (53.2%)	
In vivo T-cell depletion			

HLA: Human leukocyte antigen, CMV: Cytomegalovirus, TBI: Total body irradiation; ATG: Anti-thymocyte globulin, MTX: Methotrexate, MMF: Mycophenolate mofetil, CI: Confidence interval, NA: not applicable

	FluTBI (n = 137)	FluTreo (n = 617)	P
No	74 (54%)	202 (32.7%)	< 0.0001
ATG	63 (46%)	415 (67.3%)	
GvHD prevention			
Cyclosporin A + MTX	117 (85.4%)	425 (68.9%)	0.0003
Cyclosporin A + MMF	10 (7.3%)	124 (20.1%)	
Other	10 (7.3%)	68 (11.0%)	
Median follow-up, months [95% CI]	45.88 [35.83–56.96]	26.92 [24.19–31.03]	0.033

HLA: Human leukocyte antigen, CMV: Cytomegalovirus, TBI: Total body irradiation; ATG: Anti-thymocyte globulin, MTX: Methotrexate, MMF: Mycophenolate mofetil, CI: Confidence interval, NA: not applicable

The major difference in relation to transplant procedures was the significantly more frequent use of *in vivo* TCD with ATG in the FluTreo group (67.3% vs. 46%, $p < 0.0001$), Table 1.

Multivariate analysis on the entire patient population

After adjusting for confounding factors in a multivariate Cox analysis (Table 2), FluTBI conditioning was associated with a significantly lower probability of relapse than FluTreo (hazard ratio (HR) 0.59 [95% CI, 0.38–0.93], $p = 0.023$). There was no significant difference in LFS, OS, GRFS, or acute and chronic GVHD. Adverse risk cytogenetics and year of HSCT had a significant negative impact on relapse incidence, LFS and OS. Adverse risk cytogenetics also had a significant negative impact on GRFS.

Table 2
Multivariate analysis of factors associated with outcomes on the entire patient population

Endpoint	Factor	Hazard ratio (95% CI)	P value
Relapse	FluTBI vs. FluTreo	0.59 (0.38–0.93)	0.023
	Year of HSCT	0.95 (0.9-1)	0.034
	Adverse cytogenetics	2.55 (1.86–3.48)	< 0.0001
NRM	Age (HR per 10 years)	1.8 (1.35–2.42)	< 0.0001
	KPS ≥90%	0.43 (0.28–0.66)	0.0004
LFS	Age (HR per 10 years)	1.2 (1.02–1.41)	0.026
	Year of HSCT	0.95 (0.91–0.99	0.009
	Adverse cytogenetics	2 (1.53–2.61)	< 0.0001
	KPS ≥90%	0.66 (0.5–0.87)	0.003
OS	Age (HR per 10 years)	1.38 (1.16–1.65)	0.0004
	Year of HSCT	0.95 (0.91-1)	0.037
	Adverse cytogenetics	1.83 (1.36–2.45)	< 0.0001
	KPS ≥90%	0.68 (0.51–0.92)	0.013
GRFS	Adverse cytogenetics	1.72 (1.34–2.2)	< 0.0001
	In vivo TCD	0.63 (0.44–0.89)	0.009
Acute GVHD II-IV	none		
Chronic GVHD	Female donor to male recipient	1.63 (1.19–2.23	0.002
	In vivo TCD	0.43 (0.28–0.66)	< 0.0001

HR: Hazard ratio, CI: Confidence interval, NRM: non-relapse mortality, LFS: leukemia-free survival, OS: overall survival, GVHD: graft-versus-host disease, GRFS: Survival free of grade III-IV acute GVHD, chronic GVHD, and relapse. HSCT: hematopoietic stem cell transplantation, KPS: Karnofsky performance score, TCD: T cell depletion.

All models were adjusted for the following factors: age (per 10 years); year of transplantation; diagnosis: secondary vs. de novo AML; cytogenetics: adverse vs. not adverse risk; interval from diagnosis to transplantation; donor/recipient sex: female to male versus other combinations; Karnofsky performance status; donor type: 10/10 HLA matched unrelated vs. matched sibling; in vivo T-cell depletion. Only factors with p value < 0.05 are shown.

Pair-match analysis on propensity score

Because of the substantial difference in patient numbers between the two conditioning groups and significant differences in demographic and transplant-related parameters, we used propensity score matching to reduce the treatment assignment bias and create two patient groups of 115 each that were comparable for all observed covariates. Patient characteristics in the FluTBI and FluTreo group were well balanced in terms of age (median 55.2 vs. 54.9 years, KPS < 90% 22.6% and 23.5%, respectively), secondary AML (13% each), adverse cytogenetics (15.7% each), female donor to male recipient (19.1% vs. 17.4%) and time from diagnosis to HSCT (median 3.8 (range, 1.8–16.2) and 4.5 (range, 1.7–16.2) months, respectively), Table 3. An identical proportion of patients in both groups received grafts from MSD (61.7%) or 10/10 HLA-MUD (38.3%). In both groups, GVHD prophylaxis consisted predominantly of cyclosporin A (CSA) plus methotrexate (85.2% for FluTBI vs. 73.9% for FluTreo, p = ns), CSA and mycophenolate mofetil were given to 8.7% and 18.3% of patients in the FluTBI and FluTreo groups, respectively (p = ns). A similar proportion of patients in both groups (52.2% and 53.9%) received additional *in vivo* T-cell depletion with ATG.

Table 3
Patient, donor, and transplant characteristics according to conditioning regimen for patients included in the propensity score analysis

	FluTBI (n = 115)	FluTreo (n = 115)	P
Median patient age, years (range)	55.2 (40.1–70.7)	54.9 (40.4–74.9)	0.96
Karnofsky performance score			
<90	26 (22.6%)	27 (23.5%)	0.88
>=90	89 (77.4%)	88 (76.5%)	
Diagnosis			
De novo AML	100 (87%)	100 (87%)	1
Secondary AML	15 (13%)	15 (13%)	
Cytogenetic risk group			
Good	4 (4.5%)	5 (6.5%)	
Intermediate	66 (75.0%)	54 (70.1%)	
Poor	18 (20.5%)	18 (23.4%)	
NA/failed	27	38	
Not adverse	97 (84.3%)	97 (84.3%)	1
Adverse	18 (15.7%)	18 (15.7%)	
FLT3 ITD			
negative	65 (77.4%)	50 (67.6%)	0.17
positive	19 (22.6%)	24 (32.4%)	
missing	31	41	
NPM1			
wildtype	64 (76.2%)	50 (67.6%)	0.39
mutated	20 (23.8%)	21 (30%)	
missing	31	45	
Median interval from diagnosis to HSCT, months (range)	3.8 (1.8–16.2)	4.5 (1.7–16.2)	0.15

HLA: Human leukocyte antigen; CMV: Cytomegalovirus; ATG: Anti-thymocyte globulin; TBI: Total body irradiation; MTX: Methotrexate; MMF: Mycophenolate mofetil, CI: Confidence interval; NA: Not applicable

	FluTBI (n = 115)	FluTreo (n = 115)	P
Median year of HSCT (range)	2014 (2009–2019)	2016 (2009–2019)	0.005
MRD status pre-transplant			
MRD negative	37 (61.7%)	30 (58.8%)	0.76
MRD positive	23 (38.3%)	21 (41.2%)	
Missing	55	64	
Donor			
Matched sibling	71 (61.7%)	71 (61.7%)	1
10/10 HLA matched unrelated	44 (38.3%)	44 (38.3%)	
Patient sex			
Male	68 (59.1%)	64 (55.7%)	0.59
Female	47 (40.9%)	51 (44.3%)	
Donor/patient sex	70 (60.9%)	71 (61.7%)	0.89
Female/male	22 (19.1%)	20 (17.4%)	0.73
Other combinations	93 (80.9%)	95 (82.6%)	
Donor/patient CMV status			
Donor negative/patient positive	27 (24.3%)	28 (24.6%)	0.97
Other combinations	84 (75.7%)	86 (75.4%)	
Missing	4	1	
TBI fractions		NA	
4 x 2 Gy	33 (28.7%)		
2 x 4 Gy	15 (13.0%)		
unknown	67 (58.3%)		
Treosulfan dose	NA		
3 x 10 g/m^2		27 (23.5%)	
3 x 12 g/m^2		23 (20.0%)	

HLA: Human leukocyte antigen; CMV: Cytomegalovirus; ATG: Anti-thymocyte globulin; TBI: Total body irradiation; MTX: Methotrexate; MMF: Mycophenolate mofetil, CI: Confidence interval; NA: Not applicable

	FluTBI (n = 115)	FluTreo (n = 115)	P
3 x 14 g/m^2		65 (56.5%)	
In vivo T-cell depletion			
No	60 (52.2%)	62 (53.9%)	0.79
ATG	55 (47.8%)	53 (46.1%)	
GVHD prevention			
Cyclosporin A + MTX	98 (85.2%)	85 (73.9%)	0.08
Cyclosporin A + MMF	10 (8.7%)	21 (18.3%)	
Other	7 (6.1%)	9 (7.8%)	
Median follow-up, months [95% CI]	42.37 [31.52–53.77]	23.2 [20.44–32.74]	0.14

HLA: Human leukocyte antigen; CMV: Cytomegalovirus; ATG: Anti-thymocyte globulin; TBI: Total body irradiation; MTX: Methotrexate; MMF: Mycophenolate mofetil, CI: Confidence interval; NA: Not applicable

All but one patient in each group engrafted. Median follow-up of living patients was 42.4 months (range, 31.5–53.8) in the FluTBI and 23.2 months (range, 20.4–32.7) in the FluTreo group (p = 0.14). FluTBI was associated with a significantly lower CIR of 18.3% vs. 34.7% in FluTreo (p = 0.018, HR 0.51 (95% CI, 0.29–0.89)), but a higher NRM of 16.8% vs. 5.3%, p = 0.02, HR 3.0 (95% CI, 1.19–7.59), Fig. 1A-B. This difference in NRM was due exclusively to the higher NRM in patients ≥ 55 years of age (Table 4). LFS and OS were similar in the FluTBI and FluTreo groups (64.9% vs. 60.0%, HR 0.84 (95% CI, 0.54–1.21) and 66.9% vs. 67.8%, HR 1.08 (95% CI, 0.67–1.75)), respectively, Fig. 1C-D. Infection was the leading cause of death following FluTBI (n = 12, 34.3% vs. n = 3, 9.4% with FluTreo), whereas AML recurrence was the predominant cause of death in the FluTreo group (n = 15, 46.9% vs. n = 10, 28.6%). The frequency of death due to GVHD, multiorgan failure or interstitial pneumonitis did not differ between the two groups. Two patients developed a secondary malignancy after TBI conditioning, results not shown.

Table 4
Univariate analysis by age group for the pair-match analysis

		FluTreo (n = 58)	FluTBI (n = 56)	FluTBI vs FluTreo HR (95% CI)	p (cluster = pair)
Age < 55 years	Relapse	33.6%[20.9–46.9]	20.5%[10.4–33]	0.51 (0.23–1.13)	0.10
	NRM	4.7%[0.8–14.6]	6%[1.5–15]	1.68 (0.29–9.76)	0.57
	LFS	61.7%[46.3–73.8]	73.5%[58.7–83.8]	0.62 (0.3–1.28)	0.19
	OS	67%[50.1–79.3]	77.2%[62.4–86.7]	0.65 (0.3–1.42)	0.28
	GRFS	46.6%[31.4–60.4]	59.7%[44.6–72]	0.69 (0.41–1.18)	0.18
	Acute GVHD II-IV	21.4%[11.8–33]	20.1%[10.7–31.7]	0.89 (0.37–2.14)	0.79
	Acute GVHD III-IV	5.4%[1.4–13.5]	7.3%[2.3–16.3]	1.28 (0.28–5.72)	0.75
	Chronic GVHD	54.7%[38.3–68.4]	35.7%[22.1–49.5]	0.51 (0.29–0.93)	0.027
	Extensive chronic GVHD	19.6%[8.8–33.6]	10.7%[3.8–21.6]	0.64 (0.24–1.74)	0.39
		FluTreo (n = 57)	**FluTBI (n = 59)**	**FluTBI vs FluTreo HR (95% CI)**	**p (cluster = pair)**
Age > 55 years	Relapse	35.7%[21.9–49.8]	16.3%[7.3–28.5]	0.52 (0.24–1.13)	0.10
	NRM	5.8%[1.5–14.7]	27.6%[15.9–40.7]	3.74 (1.23–11.43)	**0.02**
	LFS	58.4%[42.7–71.3]	56%[40.6–68.9]	1.09 (0.62–1.91)	0.78
	OS	68.7%[51.6–80.9]	56.2%[40.5–69.3]	1.67 (0.89–3.15)	0.11
	GRFS	45%[30.1–58.8]	40.4%[26.2–54.2]	0.98 (0.62–1.55)	0.92

NRM: Non-relapse mortality; LFS: Leukemia-free survival; OS: Overall survival; GVHD: Graft-versus-host disease; GRFS: Survival free of grade III-IV acute GVHD, chronic GVHD, and relapse. All results are at 2 years except acute GVHD at 180 days post HSCT.

		FluTreo (n = 58)	FluTBI (n = 56)	FluTBI vs FluTreo HR (95% CI)	p (cluster = pair)
	Acute GVHD II-IV	20%[10.6–31.5]	25.4%[15.1–37.1]	1.21 (0.57–2.59)	0.62
	Acute GVHD III-IV	12.7%[5.5–23]	5.1%[1.3–12.9]	0.34 (0.09–1.31)	0.12
	Chronic GVHD	39%[24.3–53.4]	49.5%[34.4–63]	1.31 (0.78–2.21)	0.3
	Extensive chronic GVHD	19.7%[8.9–33.5]	22.7%[12-35.5]	0.91 (0.45–1.82)	0.78

NRM: Non-relapse mortality; LFS: Leukemia-free survival; OS: Overall survival; GVHD: Graft-versus-host disease; GRFS: Survival free of grade III-IV acute GVHD, chronic GVHD, and relapse. All results are at 2 years except acute GVHD at 180 days post HSCT.

There was no statistically significant difference between FluTBI and FluTreo in the CI of acute GVHD II-IV (22.8% vs. 20.7%, HR 1.05), GVHD III-IV (6.2% vs. 9.0%, HR 0.59), chronic GVHD (42.6% vs. 47.5%, HR 0.81) or extensive chronic GVHD (16.8% vs. 19.6%, HR 0.76), results not shown, resulting in similar GRFS of 50.3% and 45.6%, HR 0.83, respectively (Fig. 1E).

Discussion

Because of its manageable extramedullary toxicity profile and satisfactory anti-leukemic activity in a randomized registration study, the combination of fludarabine and treosulfan 30 g/m^2 has been increasingly adopted as the RIC of choice in patients with AML and MDS who are ineligible for MAC (12). In a large retrospective study, this conditioning regimen using higher treosulfan doses was shown to be tolerable and effective in patients with more advanced AML and a median age of 57 years (21). We hypothesized that FluTreo might be an alternative to RIC with FluTBI 8 Gy in patients above 40 years with AML in CR1, for whom favorable long-term outcomes have recently been reported in a randomized comparison with TBI 12 Gy/Cy MAC (13), (14). Our study demonstrates that FluTBI conditioning prior to 10/10 HLA matched allogeneic HSCT achieves good leukemic control in this patient population with a low relapse rate of 19.2% and modest NRM of 16.2%. The probabilities of LFS and OS at two years (64.6% and 68%, respectively) match the outcome data reported for the subgroup of patients aged 41–60 years in the FluTBI 8 Gy RIC arm of the German MAC vs. RIC trial.

Our hypothesis that the antileukemic efficacy of FluTreo would be equivalent to that of FluTBI was not borne out by the results of our multivariate analysis, which demonstrated a significantly higher CIR with FluTreo compared with FluTBI conditioning. Moreover, the relapse rate of 32.7% in our FluTreo cohort was higher than in the FluTreo arm (24.6%) of the randomized registration trial (12). This may be attributable

to differences in the patient population, as the latter study included not only AML, but also 30% of MDS patients, with MDS patients experiencing fewer relapses (Supplementary Appendix, (12)). In addition, a larger proportion of patients in the randomized German study developed chronic, and in particular mild chronic GvHD, which may have contributed to the lower relapse rate. As chronic GVHD did not significantly contribute to mortality in either study, the lower incidence of chronic GVHD seen in our analysis is consistent with the higher observed CIR.

An additional possibility is that differences in the treosulfan dose may have contributed to the unexpectedly high relapse rate although there is no conclusive evidence of greater antileukemic efficacy of higher treosulfan doses. NRM however proved to be higher with 42 g/m^2 compared to the 30 g/m^2 dose in the pivotal randomized study which was stopped after an interim analysis had shown prolonged neutropenia and subsequent serious infectious complications with fludarabine and treosulfan 42 g/m^2 total dose compared to the RIC regimen FB2. The protocol was amended to a reduced treosulfan dose of 30 g/m2 and demonstrated superior overall and relapse-free survival of patients in the FluTreo arm. (12), (22) In our study, NRM was low despite most patients receiving treosulfan doses higher than 30 g/m^2.

Considering the high relapse rate with FluTreo in our study, the lack of difference in survival, NRM, and incidence and severity of GVHD between the two conditioning regimens in our multivariate analysis was initially unexplained. This raised the possibility that age, which was not well balanced between the FluTreo and FluTBI groups, would be a relevant confounding factor. To address this, we performed a propensity score analysis which allowed us to compare two age-matched cohorts of 115 patients with each conditioning regimen. The propensity score analysis indeed demonstrated a profound age-dependency of NRM with FluTBI, in agreement with the randomized study by Bornhäuser *et al.* evaluating FluTBI 8 Gy versus TBI 12 Gy/Cy. Moreover, we demonstrate that a 55 year age threshold discriminates between patients with low and high NRM, even though this does not translate into inferior OS and RFS in the older age cohort. This discrepancy may be due to the fact that the lower relapse rate with FluTBI did not reach statistical significance given the relatively small number of patients. Nevertheless, it appears advisable to employ FluTBI with considerable caution in patients 55 years and above.

Comparing the whole patient cohort in our analysis and the randomized study evaluating FluTBI 8 Gy, NRM in the FluTBI group in our study seemed to be somewhat higher, with the caveat of a different duration of follow-up (16.2% at 2 years vs. 13.0% at 10 years). Another possible explanation for this difference in NRM is the TBI schedule used in these two studies: whereas the randomized study consistently administered TBI in four fractions of 2 Gy, our analysis also included patients in whom 8 Gy TBI was delivered in 2 fractions of 4 Gy. A retrospective study comparing delivery of 12 Gy TBI in one or two fractions over 3 days suggested a higher risk of organ toxicity, but not NRM with the 1-day fractionation (23). Additional confounding variables might have been introduced by the heterogeneity of TBI techniques used in different centers (24) and/or by center preferences in the application of only FluTBI (8 centers), only FluTreo (52 centers) or both (15 centers). However, we found no such center effects (data not shown) (20).

In addition to age, the level of MRD at the time of HSCT is a well-known determinant of relapse rate and outcome. There was no significant difference in the proportion of MRD positive and negative patients in the two conditioning groups in our study. As MRD levels at transplant were available for only a minority of patients, a separate analysis of this parameter would not have yielded statistically meaningful results, which is a limitation of the present analysis.

Taken together, our large retrospective analysis including propensity score matching demonstrates that the reduced-intensity FluTBI and FluTreo conditioning regimens result in comparable survival in patients with AML undergoing HSCT in CR1. A more detailed analysis of relapse rate and NRM in relation to patient age strongly suggests that the choice of these two conditioning regimens should be made after consideration of an individual patient´s risk of toxicity-related mortality. Although we did not identify patient subgroups who derived significant benefit from the enhanced antileukemic activity of FluTBI, patients < 55 years of age with high-risk leukemias and a low HCT-specific comorbidity index (HCT-CI) may do better with FluTBI. Robust long-term outcome data consistent with this concept have been reported (14).

NRM with FluTreo was remarkably low even in patients at higher risk of toxic death and could likely be the preferred type of conditioning for patients with such risk features. (5) It will also be of interest to determine whether the more user friendly chemotherapy based FluTreo regimen may have fewer long-term side effects than TBI based conditioning, provided outcome is the same. Our results strongly suggest that strategies that build on the excellent tolerability of FluTreo and focus on reducing the higher relapse rate associated with this regimen conceptually have promise. Recently, O´Hagan Henderson *et al.* demonstrated the feasibility of fludarabine and treosulfan 42 g/m^2 in combination with high-dose cytarabine in 77 patients with poor-risk myeloid neoplasms, 54% of whom were not in CR. In the subgroup of 58 AML patients, OS and CIR at 3 years were 44% and 43%, respectively. (25) The combination of FluTreo with TBI 2 Gy has been pioneered by Gyurkocza *et al*. in AML and MDS patients (26), analogous to the successful sequential conditioning regimen of fludarabine and melphalan followed by TBI 8 Gy in relapsed and refractory AML (27). Addition of low-dose TBI to FluTreo was associated with considerable gastrointestinal toxicity but nevertheless a low NRM of 8% and a CIR of 27% at 2 years. (26) Conspicuously, this approach did not appear to mitigate the high post-transplant relapse rate in patients with MRD pre-HSCT as opposed to patients who were MRD negative (CIR 70% and 18%, respectively). This highlights the need to also explore additional approaches such as post-transplant maintenance strategies and more effective pre-transplant therapies.

Declarations

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Author Contributions

GB designed the study and wrote the manuscript, ML performed the statistical analysis. AB, AS, BNS, AN and MM revised the manuscript and all the authors reviewed and accepted its final version. RN, MS, HCR, IH, NK, AK, WB, KSE, MV, SM and KC were the principal investigators at the centers recruiting the highest number of patients for the study. Members of the contributing institutions are listed as Supplementary Information.

Competing Interests

GB received research support from Novartis and honoraria and travel expenses from Jazz, Celgene, Novartis, and Gilead. HCR received consulting and lecture fees from Abbvie, AstraZeneca, Vertex, Novartis and Merck and research funding from Gilead Pharmaceuticals and AstraZeneca. HCR is a co-founder of CDL Therapeutics GmbH. IH received honoraria from Novartis and Abbvie and has obtained travel, accommodation or expenses from Medac GmbH, Janssen-Cilag, Jazz Pharmaceuticals and Celgene. WB received honoraria from Miltenyi, Celgene, Gilead und Novartis and travel expenses from Medac. All other authors declare no competing financial interests.

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Data availability statement

Upon request to the ALWP of the EBMT (Dr Myriam Labopin).

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Figures

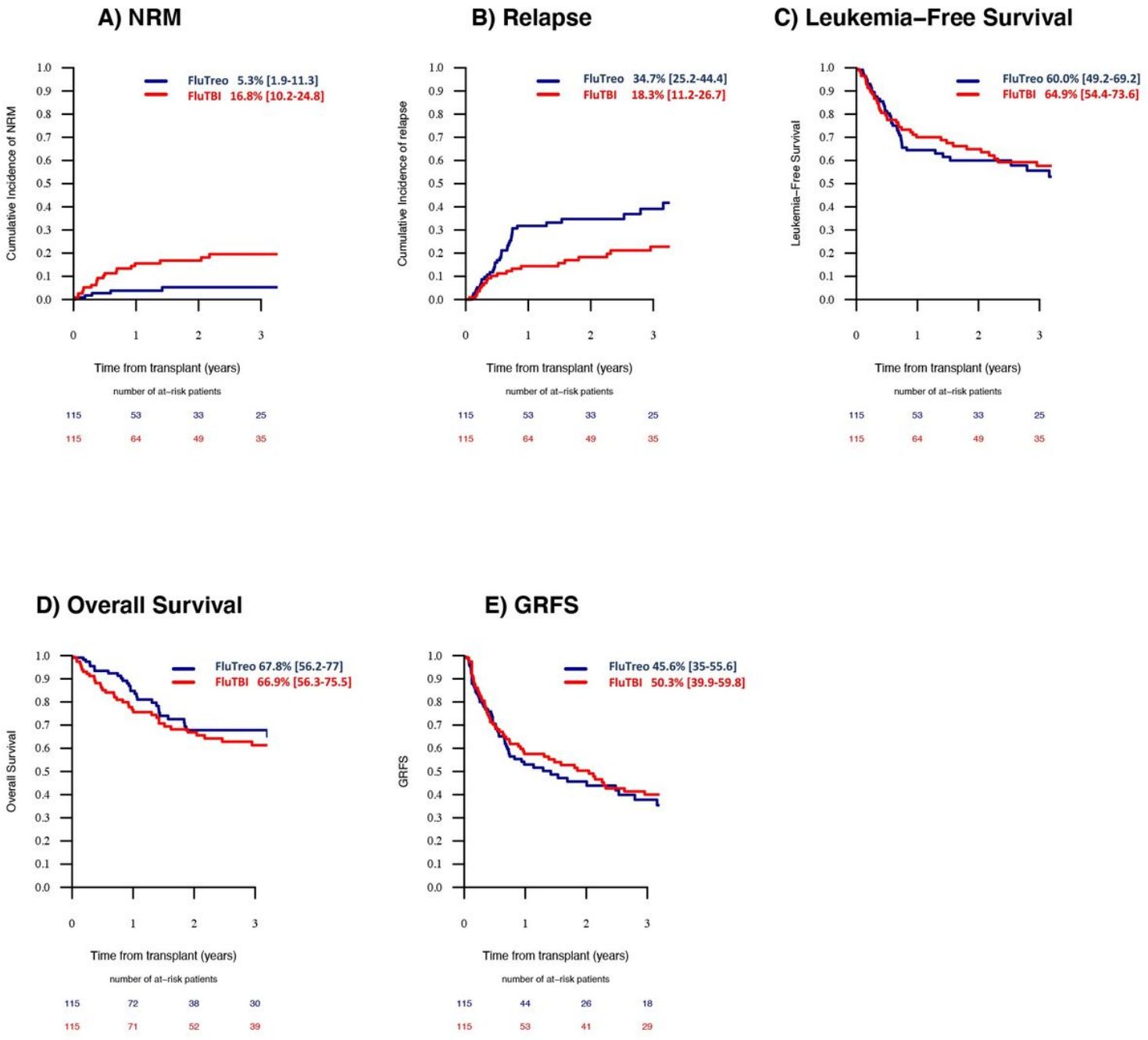

Figure 1

Comparison of FluTreo and FluTBI conditioning regimens. A: Non-Relapse Mortality. B: Relapse Incidence. C: Leukemia-free Survival. D: Overall Survival. E: Graft-versus-Host Disease (GVHD) and Relapse-free

Survival. All results are at 2 years except acute GVHD at 180 days post-hematopoietic stem cell transplantation.

Supplementary Files

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