



# Comparison of reduced-toxicity conditioning protocols using fludarabine, melphalan combined with thiotepa or carmustine in allogeneic hematopoietic cell transplantation

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## Abstract

The age of patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT) has increased during the last decades, mainly due to improved reduced-intensity/toxicity conditioning protocols. A reduced-intensity conditioning based on fludarabine, carmustine/BCNU and melphalan (FBM) has been previously developed at our institution. Since we observed detrimental effects in individual patients with compromised lung function, efforts have been made in order to replace BCNU by thiotepa (FTM) to reduce toxicity. In this study, we retrospectively analyzed the outcome, GvHD incidence, lung function and organ toxicity of patients with a median age of 62 years (range 21–79) transplanted for malignant disease (96.7%, 62.3% in intermediate/advanced disease stage) at our institution after conditioning with FBM ( $n = 136$ ) or FTM ( $n = 105$ ) between 2013 and 2017. Median follow-up was 868 days (range 0–2615). In multivariate analysis for overall survival, no difference was detected between both conditioning protocols in the presence of impaired lung function, age, lower performance, and liver disease previous allo-HCT. In the subgroup analysis, FTM was not inferior to FBM in patients with pulmonary disease prior allo-HCT, lymphoid malignancies, and higher comorbidity index. In conclusion, the reduced-intensity FBM and FTM conditioning protocols show adequate antineoplastic efficacy and are suitable for patients with impaired lung function.

## Introduction

The age of patients undergoing hematopoietic cell transplantation has increased during the last decades, due to reduced-intensity conditioning protocols, which allow hematopoietic cell transplantation in older patients and with reduced performance status [1]. Current research is focused

to develop conditioning protocols with reduced toxicity but with sufficient anti-leukemic effect [2].

BCNU (bis-chloroethylnitrosourea or carmustine) is an alkylating agent that has been successfully used in combination with alkylating agents in conditioning regimens for the treatment of lymphomas and multiple myeloma [3–5] and in the treatment of brain tumors [6]. BCNU is a lipophilic substance, which crosses the blood-brain barrier and produces a prolonged and cumulative myelosuppression [7]. However, high dose of BCNU in conditioning regimen and cumulative dose of BCNU for the treatment of glioblastoma have been associated with severe pulmonary toxicity [8, 9].

Thiotepa (N,N'-triethylenethiophosphoramide) is also an alkylating agent with antineoplastic activity [10], immunosuppressive effects and ability to penetrate the blood-brain barrier [11]. Moreover, thiotepa has a dose-dependent non-hematopoietic toxicity profile, with mucositis the most frequent complication in doses of 10–15 mg/kg followed by sinusoidal obstructive syndrome, transient liver, renal and cardiovascular toxicities in higher doses [12–14]. Due to these properties, thiotepa has been incorporated as chemotherapy agent in conditioning protocols

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**Table 1** Clinical characteristics of the patient cohort at the time of allo-HCT.

Clinical characteristics			
Conditioning protocol	FBM	FTM	<i>p</i> value
Total patients	136	105	
<b>Age at allo-HCT, median (range)</b>	<b>64 (25–79)</b>	<b>59 (21–78)</b>	<b>0.001</b>
<b>Age &lt; 55 years, <i>n</i> (%)</b>	<b>23 (16.9)</b>	<b>40 (38.9)</b>	<b>0.001</b>
Patient sex male, <i>n</i> (%)	79 (58.1)	64 (60.9)	0.65
Donor sex male, <i>n</i> (%)	79 (58.1)	65 (61.9)	0.55
Donor recipient sex pair female to male, <i>n</i> (%)	30 (22.1)	24 (22.8)	0.88
<b>Primary disease, <i>n</i> (%)</b>			<b>0.001</b>
Myeloid	121 (89.0)	68 (64.8)	
Lymphoid	12 (8.8)	32 (30.5)	
Others	3 (2.2)	5 (4.8)	
<b>Disease stage before allo-HCT, <i>n</i> (%)</b>			<b>0.04</b>
Early	61 (45.1)	30 (30.1)	
Intermediate	15 (11.5)	15 (17.8)	
Late	60 (43.4)	60 (52.0)	
<b>Prior therapy lines, <i>n</i> (%)</b>			<b>0.001</b>
Untreated	34 (25.0)	14 (13.3)	
1st line	60 (44.1)	39 (37.1)	
2nd line	20 (14.7)	10 (9.5)	
≥3rd line	22 (16.1)	41 (39.0)	
<b>Prior therapy cycles, mean (range)</b>	<b>3.0 (0–21)</b>	<b>4.5 (0–20)</b>	<b>0.004</b>
<b>Prior therapy ≥ 3 cycles, <i>n</i> (%)</b>	<b>33 (22.1)</b>	<b>42 (40.0)</b>	<b>0.01</b>
<b>Prior auto-HCT</b>	<b>4 (2.9)</b>	<b>21 (20.0)</b>	<b>0.001</b>
<b>Follow-up in days, median (range)</b>	<b>955 (5–2615)</b>	<b>762 (0–1961)</b>	<b>0.001</b>
<b>Donor, <i>n</i> (%)</b>			<b>0.02</b>
Related	37 (24.6)	19 (15.3)	
Unrelated	99 (75.4)	80 (76.7)	
Haplo	0	5 (6.8)	
Twin	0	1 (1.3)	
<b>HLA-A, -B, -DR1, DQ1, <i>n</i> (%)</b>			<b>0.05</b>
Ident	105 (77.0)	76 (71.2)	
Different	31 (22.9)	23 (20.5)	
Haplo-ident	0	5 (6.8)	
Twin	0	1 (1.3)	
<b>HLA-C, <i>n</i> (%)</b>			0.12
Ident	116 (95.1)	68 (93.2)	
Different	6 (4.9)	3 (2.7)	
Haplo-ident	0	3 (4.1)	
<b>EBMT disease risk score, <i>n</i> (%)</b>			0.20
0–2	15 (11.5)	6 (6.8)	
3–4	55 (40.2)	38 (31.5)	
5–7	66 (48.4)	61 (61.6)	
<b>GvHD prophylaxis with in vivo T-cell depletion, <i>n</i> (%)</b>	<b>118 (86.8)</b>	<b>69 (65.7)</b>	<b>0.001</b>
ATG	99 (72.8)	68 (64.7)	
Alemtuzumab (campath)	19 (13.9)	1 (0.9)	
<b>Methotrexate-based, <i>n</i> (%)</b>	<b>30 (22.1)</b>	<b>1 (0.9)</b>	<b>0.001</b>
<b>Karnofsky prior allo-HCT, <i>n</i> (%)</b>			
≤90	113 (83.1)	90 (85.7)	0.58

**Table 1** (continued)

Clinical characteristics			
Conditioning protocol	FBM	FTM	<i>p</i> value
≤80	45 (33.1)	47 (44.7)	0.07
<b>HCT-CI score, median (range)</b>	<b>3 (0–10)</b>	<b>4 (0–10)</b>	<b>0.03</b>
HCT-CI score, <i>n</i> (%)			0.40
0	12 (24.3)	6 (7.6)	
1	12 (5.1)	8 (7.6)	
2	23 (39.0)	13 (18.1)	
3	26 (13.2)	16 (24.8)	
≥4	63 (18.4)	62 (41.9)	

Statistical analysis of categorical variables was performed by Pearson's chi-square and Fisher's exact test; continuous variables by Student's T-test assuming a normal distribution.

*allo-HCT* allogeneic hematopoietic cell transplantation, *GvHD* Graft-versus-Host-Disease, *ATG* anti-thymocyte anti-globulin, *HCT-CI* hematopoietic cell transplantation comorbidity index, *FBM* Fludarabine, BCNU, Melphalan, *FTM* Fludarabine, Thiotepa, Melphalan.

prior autologous hematopoietic cell transplantation (auto-HCT) [15, 16] and allogeneic hematopoietic cell transplantation (allo-HCT) [17, 18].

A reduced-intensity conditioning protocol based on fludarabine, BCNU, and melphalan (FBM) has been developed at our institution [19, 20]. Although this protocol has been shown to have anti-leukemic activity [21] and adequate quality of life [22, 23], patients still developed post-transplant complications as lung function impairment [24, 25]. Recent efforts have been made in order to replace BCNU by thiotepa in conditioning protocols prior auto-HCT [26, 27]. Therefore, to reduce lung toxicity while maintaining anti-leukemic activity, a conditioning protocol for allo-HCT replacing the alkylating agent BCNU by thiotepa (FTM protocol) has been developed [28–30].

In this study, we analyzed and retrospectively compared the outcome of patients, cause of deaths, impact on GvHD incidence, organ function toxicity, hematopoietic and immune system reconstitution after conditioning with the reduced-intensity/toxicity protocols FBM and FTM at our institution during the same time period. Patients treated with the FTM protocol showed similar outcomes compared to FBM protocol, and allowed allo-HCT in patients with impaired lung function and/or low-performance status.

## Material and methods

### Patient selection and characteristics

Patients included in the study were transplanted at the University of Freiburg Medical Center between January 1st

**Table 2** Pulmonary characteristics of the patient cohort at the time of allo-HCT.

Pulmonary clinical characteristics			
Conditioning protocol	FBM	FTM	<i>p</i> value
<b>Pulmonary disease prior allo-HCT, <i>n</i> (%)</b>	<b>44 (33.1)</b>	<b>49 (46.7)</b>	<b>0.04</b>
Bact. pneumonia	16 (11.8)	15 (14.3)	
Fungal pneumonia	8 (5.9)	8 (7.6)	
Atypical pneumonia	5 (3.7)	4 (3.8)	
PjP pneumonia	0	2 (1.9)	
Influenza A pneumonia	0	2 (1.9)	
Asthma/COPD	1 (0.7)	13 (12.3)	
Pulmonary embolism	5 (3.7)	6 (5.7)	
Pleural effusions	4 (2.9)	3 (2.8)	
Previous tuberculosis	4 (2.9)	0	
Bronchitis	1 (0.7)	2 (1.9)	
Sleep apnea	1 (0.7)	2 (1.9)	
Others	4 (2.9)	5 (4.8)	
<b>Current and previous smokers, <i>n</i> (%)</b>	<b>45 (33.1)</b>	<b>49 (46.7)</b>	<b>0.04</b>
Pulmonary lung function test before allo-HCT, <i>n</i> (%) <sup>a</sup>	120 (89.5%)	96 (91.4%)	
<b>FEV1 (% of predicted)</b>	<b>97 (53–145)</b>	<b>95 (23–138)</b>	<b>0.02</b>
<b>FEV1/VCmax ratio</b>	<b>0.75 (0.49–1.03)</b>	<b>0.74 (0.18–0.99)</b>	<b>0.05</b>
<b>FEV1/FVC ratio</b>	<b>0.79 (0.55–0.99)</b>	<b>0.77 (0.35–1.00)</b>	<b>0.02</b>
<b>FVC (% of predicted)</b>	<b>103 (47–159)</b>	<b>99 (43–147)</b>	<b>0.02</b>
<b>MEF50 (% of predicted)</b>	<b>68.3 (19–150)</b>	<b>65.5 (2.5–137)</b>	<b>0.03</b>
MEF25 (% of predicted)	44.8 (9–137)	45.1 (9–103)	0.38
<b>DLCOCsB (% of predicted)</b>	<b>76.9 (36–127)</b>	<b>68.0 (21–106)</b>	<b>0.001</b>
RV (% of predicted)	108.5 (44–349)	110 (59–370)	0.27
RV/TLC ratio	0.39 (0.10–1.40)	0.40 (0.22–1.35)	0.15
TLC (% of predicted)	99.9 (60–159)	100.0 (71.5–159)	0.53
Arterial CO <sub>2</sub> , mm Hg	35 (26–43)	35 (24–44)	0.37
Arterial O <sub>2</sub> , mmHg	77.7 (62–106)	79.0 (59–102)	0.20

Statistical analysis of categorical variables was performed by Pearson's chi-square and Fisher's exact test; continuous variables by Student's T-test assuming a normal distribution.

allo-HCT allogeneic hematopoietic cell transplantation, *RV* residual volume, *TLC* total lung capacity, *VCmax* maximal vital capacity, *FEV1* forced expiratory volume in 1 s, *FEV1/FVC* FEV1/forced vital capacity (FVC), *MEF50* mid-expiratory flow 50%, *MEF25* mid-expiratory flow 25%, *aCO<sub>2</sub>* arterial carbon dioxide, *aO<sub>2</sub>* arterial oxygen, *DLCOCsB* diffusion capacity of carbon monoxide adjusted for hemoglobin level, *FBM* fludarabine, BCNU/carmustine, melphalan, *FTM* fludarabine, thiotepe, melphalan.

<sup>a</sup>Data are presented as median(range).

2013 and December 31st 2017 using reduced-intensity/toxicity protocols FBM or FTM. We included all patients at first allo-HCT. All clinical data were prospectively collected and retrospectively analysed. Patient characteristics are described in Table 1 and Supplementary Table 1 and pulmonary clinical characteristics in Table 2. HCT-CI Score [31] and EBMT disease risk score [32] were evaluated as previously described by standard international guidelines. Causes of death were adapted from previously described criteria [33]. This study was conducted in accordance to the Declaration of Helsinki. Written consent was obtained from

the subjects for data use in clinical research. All patients gave informed consent and the institutional review board of the University of Freiburg Medical Center approved reduced-intensity conditioning protocols. All data were evaluated as of March 31st 2020.

### Conditioning protocol, GvHD prophylaxis and Graft source

Patients were stratified by attending physicians to receive conditioning according to FBM protocol (fludarabine 4 × 30 mg/m<sup>2</sup>, BCNU/carmustine 2 × 150 mg/m<sup>2</sup> and melphalan by patients >55 years:1 × 110 mg/m<sup>2</sup> or <55 years:1 × 140 mg/m<sup>2</sup>) or FTM (fludarabine 4 × 30 mg/m<sup>2</sup>, thiotepe 2 × 5 mg/kg and melphalan for patients >55 years:1 × 110 mg/m<sup>2</sup> or <55 years:1 × 140 mg/m<sup>2</sup>). Caring physicians allocated patients to the two protocols depending mainly on previous therapies, pulmonary toxicity, and comorbidities. G-CSF mobilized peripheral blood stem cell (PBSC) grafts were used in most of the cases (*n* = 239, 99.2%) together with cyclosporine based GvHD prophylaxis (cyclosporine 5 mg/kg b.w. per day, starting day −3, targeted through serum level between 250–350 ng/ml) combined with methotrexate, mycophenolate mofetil, anti T-lymphocyte globulin (previously ATG-F, now ATLG, Grafalon) [34, 35] or alemtuzumab [36]. Two patients received an everolimus-based GvHD prophylaxis and two further patients a tacrolimus-based GvHD prophylaxis.

### Acute and chronic GvHD and organ toxicity

Time to acute GvHD (aGvHD) and chronic GvHD (cGvHD) were defined as the number of days from transplantation to the occurrence of a GvHD episode with characteristic clinical aGvHD or cGvHD symptoms [37, 38]. The grade of GvHD was classified according to Glucksberg's criteria [39]. Conditioning regimen-related toxicity by organ system within the first 30 days after allo-HCT was assessed as previously described [40].

### Hematopoietic reconstitution, bone marrow chimerism, and regeneration of lymphocyte subpopulations

Bone marrow chimerism was measured using standard PCR for microsatellite amplification as previously described [41]. Mixed chimerism was considered when recipient DNA was detected in >1% of PCR reaction. Determination of leucocyte subsets was performed as previously described [42]. Gating of lymphocytes and estimation of the gated lymphocytes were performed according to international guidelines [43].

## Pulmonary function tests (PFTs)

Pulmonary function tests (PFTs) including diffusion capacity for carbon monoxide were routinely performed according to international standards [44, 45] a week before allo-HCT and after 30 days, 3, 6, and 12 months, then repeated on the basis of clinical suspicion of pulmonary disease. PFT parameters were evaluated and expressed as percentage of predicted normal values, calculated using published equations [46], which were based on a population (caucasian, Middle Europe) very similar to the patients included in this study.

## Study Endpoints, Definitions, and Statistical Analysis

Statistical analysis of patient characteristics, PFTs, cumulative incidences, and hazard ratios was conducted using STATA v11.0 (College Station, Texas, USA). Overall survival (OS) was defined as time from allo-HCT until death from any cause, and progression-free survival (PFS) was calculated as time from allo-HCT to death from any cause or relapse, whichever occurred first. For those patients not experiencing the event in question for OS or PFS during follow-up, time to last contact was used as a censored observation. Relapse was defined as detection of disease via cytological and histological assessment after allo-HCT; death without prior relapse was considered as a competing risk and denoted as non-relapse mortality (NRM). For aGvHD and cGvHD cumulative incidences, death without aGvHD/cGvHD was considered as competing risk.

The Cox proportional hazards regression model was used to estimate hazard ratios (HR) and confidence intervals (CI) for OS and PFS. We applied the Fine and Gray model to compare cumulative incidence rates in the presence of competing risks [47, 48] and presented subdistribution hazard ratios (SHR) for relapse incidence, NRM, aGvHD and cGvHD incidence.

Multivariate analyses for OS were conducted using Cox proportional hazards regression model using a backward selection strategy. Covariates including clinical characteristics and pulmonary function tests values with a univariate  $p$  value  $<0.1$  were included. HR and two-sided 95% confidence intervals of prognostic factors for OS were estimated.

Pearson's chi-square and Fisher's exact tests were used to compare categorical variables as appropriate [49] and Student's  $T$ -test to compare continuous variables assuming a normal distribution. Differences in PFTs before allo-HCT and at day +365 were analyzed using mixed-model for longitudinal analysis based on the maximum likelihood method.

## Results

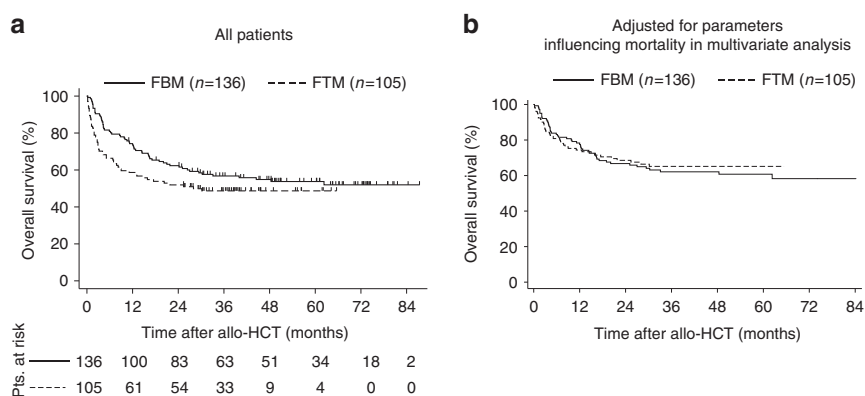
### Clinical features of patients by reduced toxicity conditioning protocols prior allo-HCT

Between January 1st 2013 to December 31st 2017, 241 patients were conditioned with the reduced-intensity/toxicity protocols FBM ( $n = 136$ ) or FTM ( $n = 105$ ) at the Medical Center of the University of Freiburg (Germany). During the observation period, continuously increasing number of patients were treated with FTM. Therefore, patients treated with FBM had a longer follow-up compared to FTM-treated patients (median 955 vs. 762 days,  $p < 0.001$ ). The clinical features of the patients are described in Table 1 and Supplementary Table 1. Compared to patients conditioned with FBM, FTM-treated patients were younger and received less frequently GvHD prophylaxis with in vivo T-cell depletion, including alemtuzumab. Patients treated with FTM suffered more frequently lymphoid malignancies, had a late disease stage defined by EBMT score, received more chemotherapy cycles and had a higher HCT-CI score (Table 1). Patients with pulmonary diseases prior allo-HCT, current/previous smokers, and with impaired lung function were more frequently allocated in the FTM group, due to the patient selection by attending physicians to the presumably less pulmonary toxic protocol (Table 2).

### Impact of reduced toxicity conditioning protocol on outcome after allo-HCT

In the univariate analysis, no differences could be demonstrated for OS between FTM and FBM-treated patients, despite of higher HCT-CI score and impaired lung function (Fig. 1a). To elucidate the impact of reduced-intensity/toxicity conditioning protocols on OS and NRM considering other relevant clinical factors, we performed univariate and multivariate analyses by Cox proportional hazard regression (Supplementary Tables 2–4 and Table 3). Multivariate analysis adjusted for clinical and pulmonary factors impacting the overall survival (FEV1  $<70\%$  of predicted, DLCOcSB  $<60\%$  of predicted, HCT-CI liver, Karnofsky index  $\leq 80\%$  and age) showed no significant difference between both conditioning protocols on overall survival (Table 3, Fig. 1b). Most of the clinical characteristics affecting OS in the survival analysis also increase NRM (Supplementary Tables 3 and 4). Altogether, these data suggest that FTM conditioning is, at least, not inferior regarding outcome to FBM conditioning adjusted for adverse clinical factors.

To define which groups of patients are more suitable for conditioning with FTM compared to FBM, we performed subgroup analyses. Interestingly, FTM conditioning was not inferior in patients with impaired lung function as defined



**Fig. 1 Impact of reduced-intensity/toxicity conditioning on outcome.** Kaplan–Meier curves represent overall survival for (a) unadjusted and (b) adjusted for parameters influencing mortality in multivariate analysis (FEV1 < 70% of predicted, DLCOcSB < 60% of predicted, Karnofsky index < 80%, HCT-CI liver, age < 55 years) by

**Table 3** Multivariate analysis for overall survival of clinical and lung function parameters.

	Hazard ratios for overall survival			
	n	HR	95% CI	p value
FEV1 < 70% of predicted	18	2.47	1.20, 5.06	0.02
DLCOc SB < 60% of predicted	35	1.87	1.15, 3.03	0.02
Karnofsky index ≤ 80%	92	1.70	1.11, 2.58	0.02
HCT-CI score liver	51	1.69	1.04, 2.74	0.03
Age < 55 years	63	0.51	0.30, 0.88	0.02
Conditioning with FTM	105	1.31	0.85, 2.58	0.21

Clinical factors (Supplementary Table 2) with a *p* value < 0.1 in univariate analysis were included in the multivariate analysis with conditioning protocol as hypothesis covariate. Multivariate analysis following backward selection was performed using the Cox proportional hazards regression model.

*HCT-CI* hematopoietic cell transplantation comorbidity index, *DLCOcSB* diffusion capacity of carbon monoxide adjusted for hemoglobin level, *FEV1* forced expiratory volume in 1s, *HR* hazard ratios, *CI* confidence intervals, *FTM* fludarabine, thiotepa, melphalan.

by HCT-CI lung, with pulmonary diseases prior allo-HCT, lymphoid malignances or with HCT-CI score of ≥ 4 (Supplementary Figs. 1–4). These data indicate, that patients with initial impaired lung function and/or several comorbidities might profit by FTM conditioning.

### Cause of death by conditioning regimens

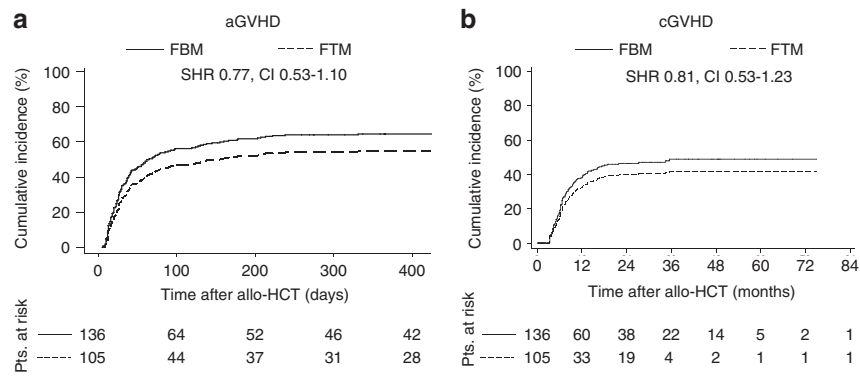
Overall, 116 patients (48.1%) died: 62 patients (45.6%) in the FBM group and 54 patients (51.4%) in the FTM group (Table 4). Mortality before day 100 after allo-HCT were mostly due to infections including pneumonia, sepsis, and fungal infections whereas mortality after day 100 were mostly due to relapses. No significant differences in cause

conditioning in all patients included in this study. Statistical analysis was performed for overall survival by log-rank test. Allo-HCT allogeneic hematopoietic cell transplantation, FBM fludarabine, BCNU, melphalan; FTM: fludarabine, thiotepa, melphalan; Pts., patients.

**Table 4** Cause of mortality by conditioning regimen.

FBM (n = 62/136, 45.6%)	FTM (n = 54/105, 51.4%)
Relapse or progress (n = 28, 20.6%)	Relapse or progress (n = 15, 14.3%)
Non-relapse mortality (n = 34, 25.0%)	Non-relapse mortality (n = 39, 37.1%)
(a) Non-pulmonary (n = 15, 11.0%)	(a) Non pulmonary (n = 20, 19.0%)
GvHD (6×)	CNS Bleeding complications (2×)
Infection/sepsis (3×)	CNS failure/Encephalopathy
CNS failure	GvHD (7×)
Cardiac failure	Sepsis (4×)
Peritonitis	Cardiac failure
Acute renal failure	CMV colitis
Unknown	Fungal infection (2×)
Sudden death	Post-transplant lymphoproliferative disease
	Unknown
(b) Pulmonary (n = 19, 14.0%)	(b) Pulmonary (n = 19, 18.1%)
Pneumonia/pneumonic sepsis (10×)	Pneumonia/pneumogenic sepsis (8×)
Interstitial pneumonitis	Fungal pneumonia
Fungal pneumonia (3×)	PjP-pneumonia
ARDS	ARDS (4×)
Pulmonary embolism	Pulmonary embolism
Bronchial carcinoma	Bronchiolitis obliterans (2×)
Bronchiolitis obliterans	Pulmonary failure
CMV pneumonia	Pneumothorax

Table depicts cause of mortality by conditioning classified in relapse and non-relapse mortality including pulmonary and non-pulmonary causes. *GvHD* Graft-versus-Host-Disease, *ARDS* acute respiratory distress syndrome, *PjP* pneumocystis jirovicii pneumonia, *CMV* cytomegalovirus, *CNS* central nervous system, *FBM* fludarabine, BCNU/carmustine, melphalan, *FTM* fludarabine, thiotepa, melphalan.



**Fig. 2** Incidence of acute and chronic GvHD is not affected by conditioning FTM protocol. Cumulative incidences of (a) acute GvHD (aGvHD) and (b) chronic GvHD (cGvHD) were calculated for FBM and FTM conditioning protocols. Clinical evidence of aGvHD or cGvHD was computed as event, death was considered as competing

of death could be found between FBM and FTM patients, although the patients had different follow-up times.

### Impact of conditioning on GvHD, hematopoietic and immune system reconstitution

Besides its hematopoietic stem cell toxicity, it has been postulated that thiotepa has immunosuppressive properties as conditioning chemotherapy [50, 51]. Therefore, we assessed the impact of reduced toxicity conditioning protocols FBM and FTM on GvHD incidence, hematopoietic, and immune system reconstitution.

No significant differences were found in the cumulative incidence of aGvHD and cGvHD and severity and organs affected by aGvHD and cGvHD (Fig. 2 and data not shown) as well as in blood cells recovery and lymphocyte subpopulations (Supplementary Table 5) between both protocols in our patient cohort. In conclusion, the FTM conditioning protocol does not affect the incidence and organs affected by aGvHD and cGvHD, the hematopoietic and immune system reconstitution compared to the FBM protocol.

### Impact of conditioning on lung function

The main goal of using the FTM protocol at our institution was to reduce the risk of lung toxicity possibly caused by BCNU while maintaining anti-leukemic activity. Therefore, we investigated the impact of FTM- and FBM-conditioning on lung function in patients with available pulmonary function tests prior allo-HCT and 1 year after allo-HCT (Fig. 3, Supplementary Tables 6 and 7). As expected, due to selection of patients with impaired pre-transplant lung function for the FTM protocol, FEV1, FEV1/VCmax ratio, MEF50, and DLCOcSB were lower in the FTM-group. We hypothesize that causes for reduced lung function in FTM-

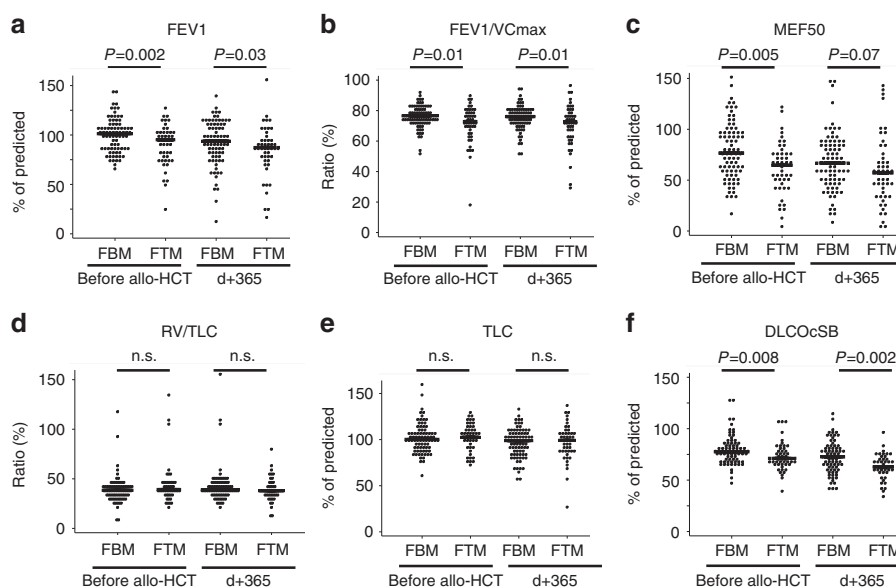
risk and time to last contact as censored event. Statistical differences were calculated by Fine and Gray regression model in the presence of competing-risks. SHRs represent the risk of FTM as compared to FBM. Pts. patients. FBM: fludarabine, BCNU/carmustine, melphalan; FTM fludarabine, thiotepa, melphalan.

treated patients prior allo-HCT were due to pulmonary diseases, smoking, use of lung toxic chemotherapies, irradiation, and previous auto-HCT among others. At 1 year after allo-HCT, statistically differences were still observed in obstructive parameters FEV1, FEV1/VCmax and in DLCOcSB (Fig. 3, Supplementary Table 6). Hence, dynamic changes of VCmax, FEV1, FVC, MEF50 in FBM-treated patients but not in FTM-treated patients were found to be statistically significant (Supplementary Table 7). Loss of 5.5% vital capacity in FBM-treated and 4.5% in FTM treated patients are on the order of the minimum clinically important difference (MCID) in terms of mortality for restrictive lung diseases [52].

In summary, patients treated with FTM protocol had impaired lung function compared to FBM treated patients prior allo-HCT. Significant decreases in several lung function parameters were observed in FBM-treated but not in FTM-treated patients at one year after allo-HCT.

### Regimen-related toxic effects by conditioning protocol

Early toxicity within the first 30 days according to Bearman's regimen-related criteria [40] (Table 5) and frequency of complications after allo-HCT were assessed (Supplementary Table 8). Overall, toxicity related to liver and other organs including bladder, heart, lungs were similar in both groups. Patients conditioned with FTM-protocol developed more frequently mild and moderate mucositis compared to FBM-treated patients (39.7% vs 63.8%,  $p < 0.001$ , Table 5). Regarding complications after allo-HCT, patients conditioned with FTM have less infections by HHV6-virus (13.2% vs 3.8%,  $p < 0.02$ ) and more infections by HSV (4.4% vs 12.4%,  $p < 0.03$ ). As expected due to worse performance status and concomitant comorbidities prior allo-



**Fig. 3 Impact of conditioning protocol on lung function after allo-HCT.** Dot plots represent pulmonary function tests (PFT) values from patients before allo-HCT and at 1 year after allo-HCT. Only patients with lung function available at both time points were included in the analysis. PFT parameters were compared by conditioning protocol from patients with available PFT at both time points (FBM,  $n = 80/99$  alive at d+365, 80.8%; FTM,  $n = 47/61$  alive at d+365, 77.0%). Each dot represent the indicated PFT value from a patient, horizontal bar represents the median. Statistical analysis were performed by Student's

*T*-test assuming a normal distribution. Allo-HCT allogeneic hematopoietic cell transplantation, FEV1 forced expiratory volume in 1 s, FEV1/maximal vital capacity (VC), FEV1/forced vital capacity (FVC); MEF50, mid-expiratory flow 50%; RV/TLC, residual volume (RV)/total lung capacity (TLC); TLC, total lung capacity; DLCOcSB, diffusion capacity of carbon monoxide adjusted for hemoglobin level. FBM: fludarabine, BCNU/carmustine, melphalan; FTM: fludarabine, thiotepa, melphalan.

**Table 5** Conditioning regimen related early toxicity by organ system within the first 30 days after allo-HCT.

Organ	FBM ( $n = 136$ )				FTM ( $n = 105$ )				<i>p</i> value
	Grade 1	Grade 2	Grade 3/4	Total	Grade 1	Grade 2	Grade 3/4	Total	
Mucosa <i>N</i> (%)	33 (24.3)	18 (13.2)	3 (2.2)	54 (39.7)	47 (44.8)	15 (14.3)	5 (4.8)	67 (63.8)	0.001
Kidney <i>N</i> (%)	49 (36.1)	21 (15.4)	11 (8.1)	81 (59.5)	37 (35.2)	16 (15.2)	21 (20.0)	74 (70.5)	0.08
Liver <i>N</i> (%)	39 (28.6)	23 (16.9)	4 (2.9)	67 (49.3)	32 (30.5)	15 (14.3)	7 (6.7)	54 (51.4)	0.74
Others <i>N</i> (%)	12 (8.8)	14 (10.3)	13 (9.5)	39 (28.7)	19 (18.1)	11 (10.5)	11 (10.5)	42 (40.0)	0.07

Data are number of patients (% of total). Toxicity effects and grades was assessed by Bearman's criteria [40]. Other organs include bladder central nervous system, cardiac and lungs toxicity. Statistical analysis performed by Pearson's chi square.

FBM fludarabine, BCNU/carmustine, melphalan, FTM fludarabine, thiotepa, melphalan.

HCT, patients treated with FTM had a higher frequency of respiratory insufficiency, cardiac/hemodynamic, and septic complications, including admission to the intensive care unit (Supplementary Table 8).

## Discussion

Allo-HCT protocols are in continuous evolution and constantly evaluated for higher efficacy, lower toxicity, and better outcomes [53]. The optimal conditioning regimen for patients with several co-morbidities and/or organ toxicity prior allo-HCT is focus of intense research. The

optimization of reduced-intensity/toxicity conditioning protocols might allow allo-HCT to older patients, those with reduced performance status and/or impaired organ function due to prior therapies or disease.

Therefore, we modified our previously described FBM protocol [19, 20], which is very effective in patients with high risk of relapse [21], in order to reduce toxicity and improve outcome by replacing BCNU by thiotepa (FTM). Both protocols are based on the very effective fludarabine/melphalan backbone, for which very low relapse incidence rates in myeloid malignancies are reported [54]. Notably, conditioning with fludarabine, thiotepa, and melphalan have been previously reported by other groups [28–30].

Interestingly, no statistically significant differences were observed on adjusted overall survival rates between FBM- and FTM-treated patients, despite higher HCT-CI score, more previous chemotherapy treatments and impaired lung function in FTM-treated patients. In the multivariate analysis as well as in subgroup analyses, we could not demonstrate significant differences in OS and NRM between FTM and FBM conditioning. These data suggest that the BCNU can be safely substituted by thiotepa, while maintaining anti-neoplastic effect.

In our study, patients with a HCT-CI score  $\geq 4$  have an OS at 24 months of 50% with both protocols and the cumulative incidence of NRM of 32% with FBM and 42% with FTM protocol (Supplementary Fig. 4). Similar data with NRM around 40% were observed in patients with high HCT-CI score in several studies [31, 55]. Other reduced-intensity conditioning protocols using only one alkylating agent as FluBu2 or FluMel show lower NRM rates (about 5%) but higher relapse incidence (about 40%) at 2 years [56, 57]. Hence, patients older than 65 years old showed a NRM of 28% and relapse incidence of 21% at 24 months with the FBM protocol (Supplementary Fig. 5). Of note, a significant difference for NRM was found in this group of age between both protocols without adjusting for HCT-CI score and other covariates associated with mortality. These data suggest there is still a clinical need for improving conditioning protocols for patients with comorbidities and in advanced age in order to reduce NRM.

Changes in lung function were investigated in patients treated with FBM and FTM. Significant differences between 1 year after allo-HCT and before allo-HCT were found in VCmax, FEV1, FVC and MEF50, suggesting increased lung toxicity in FBM-treated patients. Unexpectedly, lung function parameters as TLC, MEF25, FEV1/FVC, and DLCOcSB declined similarly in both FBM- and FTM-treated patients surviving at least one year (Fig. 3, Supplementary Table 7). We hypothesize that thiotepa as well as BCNU may cause comparable lung function deterioration at the doses used in our conditioning protocol in some, but not all, lung function parameters.

Both conditioning protocols showed a similar toxicity profile. However, FTM-treated patients suffered more frequently mild to moderate mucositis. Thiotepa has been associated with mucositis in several studies prior auto-HCT [58] and allo-HCT [12, 28–30]. Eder et al. [12] reported a frequency of 46.8% in 323 ALL patients receiving thiotepa as conditioning. In our cohort, 22.1% of patients conditioned with FBM received methotrexate but 1.4% of FTM-treated patients received methotrexate, suggesting that mucositis is not due to concomitant substances given as GvHD prophylaxis. Patients treated with FTM were younger (see Table 1) and patients  $< 55$  years received 140 mg/m<sup>2</sup> (16.9% in FBM and 38.1% in FTM) while patients

$\geq 55$  years old received 110 mg/m<sup>2</sup> per conditioning protocols. We hypothesize that higher doses of melphalan in the FTM-cohort might have contributed to the higher frequency of mucositis.

Unexpectedly, we found less HHV6 infections in patients treated with FTM than with FBM. Further subgroup analysis showed that 9 out of 20 patients treated with alemtuzumab as GvHD prophylaxis developed HHV6 infections and 19 out of 20 FBM-treated patients received alemtuzumab as GvHD prophylaxis suggesting an association of HHV6 infections with alemtuzumab as confounding variable. Similarly, we found more patients with mixed chimerism at d+30 and d+100 in FBM-treated compared to FTM-treated patients (Supplementary Table 9), caused by deeper immunosuppression due to alemtuzumab and higher frequency of myeloid disease in the FBM cohort.

This study has several limitations. Significant differences found in univariate analysis on outcome variables between conditioning protocols are probably due to imbalanced clinical characteristics of the patients as age, hematologic malignancies, prior therapies before allo-HCT, differences in follow-up and GvHD prophylaxis. In the multivariate analysis and subgroup analysis of patients with clinical features associated with worse prognosis, we did not detect any difference between both conditioning protocols on outcome variables. Second, we performed a retrospective analysis of the patients transplanted at our institution during a specific period of time. There was no randomization of the patients to the conditioning protocols and the selection of the reduced toxicity/intensity protocol was done by choice of the attending physicians depending on disease, prior therapies, and lung function. Therefore, these data should be interpreted with caution. Randomized controlled clinical trials should elucidate which reduced-intensity conditioning protocol is the most effective and the less toxic for specific patient subgroups in the near future.

In conclusion, with FBM and FTM, we have conditioning protocols incorporating two alkylating agents plus fludarabine with an adequate antineoplastic efficacy and suitable for patients with impaired lung function prior allo-HCT, several comorbidities, and reduced performance status prior allo-HCT.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicting financial interest with the submission of this article. JF received research support and speakers honoraria from Medac, Neovii and Riemser. JD-A received speakers honoraria from Roche and travel support from Gilead and Sobi. RZ received speakers honoraria from Incyte, Novartis, Roche, and Mallinckroth.

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