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Treosulfan/fludarabine versus thiotepa/busulfan/fludarabine for allogeneic haematopoietic cell transplantation in lymphoma in the post-transplant cyclophosphamide era: A GETH-TC study

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Summary

Allogeneic haematopoietic cell transplantation (alloHCT) remains a potentially curative strategy for relapsed or refractory lymphoid malignancies, even in the post-chimeric antigen receptor T-cell and bispecific antibody era. While reduced-intensity conditioning regimens offer lower non-relapse mortality (NRM), relapse rates remain high, and optimal conditioning strategies in the setting of post-transplant cyclophosphamide (PTCy) prophylaxis remain undefined. In this retrospective, international multicentre study, the primary end-point was NRM. We compared treosulfan/fludarabine (Treo/Flu) versus thiotepa/busulfan/fludarabine (TBF) in 178 adults with lymphoid malignancies undergoing first alloHCT with PTCy and peripheral blood grafts. Three-year NRM was 14.0% with Treo/Flu versus 33.0% with TBF. On multivariate analysis, Treo/Flu was associated with significantly lower 3-year NRM (hazard ratio [HR] 0.44; 95% confidence interval [CI], 0.22–0.87; $p = 0.018$). Conditioning regimen was not independently associated with overall survival (OS) or progression-free survival (PFS), and relapse incidence was similar between regimens. Moderate to severe chronic graft-versus-host disease (GVHD) was higher

Marta Peña and Lorenzo Lazzari—Co-first author.

with Treo/Flu (26.0% vs. 9.9%; HR 2.43; 95% CI, 1.09–5.43; $p = 0.03$), while GVHD-free/relapse-free survival (GFRS) was comparable. Findings were consistent in a prespecified propensity score-matched sensitivity analysis. These findings support Treo/Flu as a potentially safer reduced-toxicity conditioning option than TBF in the context of PTCy-based GVHD prophylaxis for lymphoid malignancies and warrant prospective validation.

KEYWORDS
allogeneic haematopoietic cell transplantation, lymphoid malignancies, post-transplant cyclophosphamide, reduced-intensity conditioning, treosulfan/fludarabine

INTRODUCTION

Allogeneic haematopoietic cell transplantation (alloHCT) represents a potentially curative approach for patients with relapsed or refractory lymphomas. This is valid also in the era of chimeric antigen receptor (CAR) T-cell therapies and bispecific antibodies for B-cell lymphomas.[1] Over the last decades, reduced-intensity conditioning (RIC) regimens have been shown to be non-inferior to myeloablative (MAC) regimens in this setting.[2,3] In fact, the higher relapse or progression incidence associated with RIC regimens is offset by a lower non-relapse mortality (NRM), a finding that has been observed in both B-cell and T-cell lymphomas.[4,5]

Studies specifically addressing the most effective RIC regimens for non-Hodgkin lymphomas (NHL) have shown that fludarabine/melphalan is associated with poorer outcomes, mainly driven by higher NRM.[6] Similarly, a study conducted exclusively in patients with Hodgkin lymphoma (HL) identified fludarabine/cyclophosphamide as the regimen associated with the worst outcomes, primarily due to late-onset NRM.[7] Despite the differences in toxicity profiles, the incidence of relapse or progression after RIC alloHCT remains approximately 40%–50% for both HL and NHL, representing the leading cause of death in this setting. Notably, only a minority of patients included in these studies received post-transplant cyclophosphamide (PTCy) as graft-versus-host disease (GVHD) prophylaxis.

In response to this limitation, the so-called reduced toxicity conditioning (RTC) regimens have been developed in recent years. The aim of such regimens is to preserve the lower NRM, typical of RIC, while achieving higher disease control rates, typical of MAC regimens. Although the introduction of RTC regimens—such as thiotepa/busulfan/fludarabine (TBF) or treosulfan/fludarabine (Treo/Flu)—has been extensively studied in the myeloid setting, their use in lymphomas has been reported only in a few studies. In a recent study by Peña et al., the use of TBF in lymphoma patients was associated with a low 3-year relapse/progression incidence of 19%, albeit at the cost of a substantial NRM of 32%.[8] Treo/Flu, at reduced intensity dosages, has shown improved survival in myeloid malignancies due to reduced NRM compared with busulfan/fludarabine-based RIC regimen[9] However, its application in lymphoma has been rarely reported and only in small, monocentric cohorts.[10]

In our study, we hypothesized that a newer RTC regimen such as Treo/Flu would be associated with improved survival outcomes compared to a classical regimen such as TBF in the setting of lymphomas while using PTCy as GVHD prophylaxis.

METHODS

Patient selection

We conducted a retrospective, international, multicentre study including adult patients (≥18 years old) diagnosed with lymphoid malignancies who received a first alloHCT across 11 institutions included in the Spanish registry and one Italian institution between March 2014 and November 2023. Inclusion criteria included receiving conditionings based on Treo/Flu or TBF, having a low/intermediate-transplant intensity score, receiving PTCy-based GVHD prophylaxis and peripheral blood stem cell grafts. Data were collected retrospectively. The study was approved at the ethics committee of the *Hospital de Bellvitge* (EOM008/24) and conducted in accordance with the Declaration of Helsinki. The study was coordinated by the *Grupo Español de Trasplante Hematopoyético y Terapia Celular* (GETH-TC).

Definitions

Response to last line of therapy before alloHCT was defined as per Lugano criteria.[11] Conditioning regimen intensity was defined by the transplant conditioning intensity (TCI) score according to previous studies.[12] Survival outcomes definitions followed standard criteria.[13]

Statistical methods

Continuous variables were compared using the two-sample *t*-test, and categorical variables were compared using the Pearson chi-squared test. Time-to-event outcomes were measured from the date of alloHCT, with patients censored at last follow-up in the absence

of the relevant event. The primary end-point was NRM. Secondary end-points included overall survival (OS), progression-free survival (PFS), GVHD-free/relapse-free survival (GFRS), relapse incidence/progression of disease (RI/POD), cumulative incidence of grade II–IV and III–IV acute GVHD, moderate-to-severe chronic GVHD and haematological recovery. OS, PFS and GFRS rates were estimated using the Kaplan–Meier method. Median follow-up was calculated using the reverse Kaplan–Meier estimator. Univariable analyses were performed using the log-rank test for Kaplan–Meier outcomes and Gray's test for cumulative-incidence outcomes.[14] Multivariable analyses were conducted using Cox proportional hazards models for OS, PFS and GRFS, and Fine–Gray subdistribution hazard models for NRM, RI/POD, acute GVHD, chronic GVHD and haematological recovery.[15] Results were reported as hazard ratios (HRs) with corresponding 95% confidence intervals (95% CIs). All clinically relevant covariates were entered into the multivariable models irrespective of their statistical significance in univariable analyses. For clarity and conciseness, only variables that remained statistically significant in the final multivariable models are presented in the tables. All statistical tests were two-sided, with $p < 0.05$ indicating statistical significance. A shared frailty model with transplant centre as a random effect was applied in the multicentre TBF cohort but not in the Treo/Flu cohort, which was predominantly derived from a single centre (approximately 90% of patients), precluding reliable estimation of between-centre variability. The centre-level random effect was considered non-significant if the corresponding p-value exceeded 0.05.

Propensity score matching (PSM) was performed to reduce baseline imbalances between Treo/Flu and TBF recipients. Propensity scores were estimated using a logistic regression model including age (≥median vs. <median), haematopoietic cell transplantation-specific comorbidity index (HCT-CI) (≥3 vs. <3), Eastern Cooperative Oncology Group (ECOG) performance status (≥1 vs. 0), donor type (matched related donor [MRD] vs. matched unrelated donor [MUD]/Haploidentical), donor age (≥30 vs. <30 years), Disease Risk Index (DRI) (high vs. low/intermediate) and number of previous treatment lines (1–3 vs. ≥4). Matching was performed 1:1 using nearest-neighbour matching without replacement. Covariate balance before and after matching was assessed using standardized mean differences (SMDs) and visualized with a Love plot. Matching improved balance across multiple clinical variables; however, some covariates—most notably disease type and DRI—remained partially imbalanced because of limited overlap in their distribution between treatment groups. Therefore, PSM was used as a supplementary sensitivity approach, while the primary inference relies on multivariable regression models adjusting for these factors. SMDs were used instead of p-values because they provide a sample size-independent measure of imbalance. Baseline tables report SMDs accordingly, and p-values were intentionally omitted. Primary analyses were conducted on the whole cohort, and findings were supported by univariable analyses in the matched cohort. All analyses were performed on complete-case data using R (version 4.4.1).[16] Missing data were handled using a complete case approach. For multivariable models, only patients with complete covariate information were included, and variables with substantial missingness were not entered. For propensity score estimation, cases with missing data in any of the variables used to compute the score were excluded prior to matching. No imputation was performed.

RESULTS

Patient characteristics

A total of 178 patients with lymphoid malignancies were included, of whom 65 (37%) received Treo/Flu regimen and 113 (63%) received TBF. Baseline patient-, disease- and transplant-related characteristics for the entire cohort are presented in Table 1. Among survivors, the median follow-up for the overall cohort was 3.63 years (interquartile range 2.44–4.72).

Engraftment

Primary graft failure occurred in five patients (4%) in the TBF group, whereas no cases were observed in the Treo/Flu cohort. By day +30, the cumulative incidence of neutrophil engraftment was 90.8% (95% CI, 79.9%–95.8%) for Treo/Flu and 90.7% (95% CI, 83.0%–95.0%) for TBF ($p = 0.255$). Platelet engraftment at day +30 was achieved in 47.9% (95% CI, 35.2%–59.6%) of patients receiving Treo/Flu and 41.4% (95% CI, 32.1%–40.4%) of those receiving TBF ($p = 0.290$) (Table 2). In the multivariable analysis, the likelihood of achieving neutrophil engraftment by day +30 was significantly lower among recipients of haploidentical grafts compared with MRD (HR 0.48; 95% CI, 0.31–0.75, $p = 0.001$). None of the evaluated covariates had a significant impact on platelet recovery time (Table 3).

Non-relapse mortality and relapse incidence/progression of disease incidence

Among TBF recipients, 52 patients died during follow-up, including 15 (28.8%) deaths due to relapse and 37 (71.2%) attributable to transplant-related toxicities. The latter comprised infections ($n = 19$), sinusoidal obstruction syndrome ($n = 7$), GVHD ($n = 8$), primary graft failure ($n = 1$), alveolar haemorrhage (n = 1) and transplant-associated thrombotic microangiopathy ($n = 1$). In the Treo/Flu cohort, 21 patients died, of whom 10 (47.6%) succumbed to relapse and 11 (52.4%) to transplant-related causes. Transplant-related deaths were due to sinusoidal obstruction syndrome ($n = 2$), GVHD ($n = 3$) and infections ($n = 4$).

TABLE 1 Patient characteristics of the whole population.

	TBF[a] (*n* = 113)	Treo/Flu[b] (*n* = 65)	SMD
Age, years			0.251
Median (range)	52.7 (25.3–70.1)	50 (21.2–68.5)	
Recipient sex, *n* (%)			0.005
Female	42 (37)	24 (37)	
Male	71 (63)	41 (63)	
Disease type, *n* (%)			0.838
Aggressive B-cell lymphoma	46 (41)	10 (15)	
Indolent B-cell lymphoma	40 (35)	18 (28)	
Hodgkin lymphoma	12 (11)	26 (40)	
T-cell lymphoma	15 (13)	11 (17)	
DRI, *n* (%)			2.016
Very low	0	21 (32)	
Low	24 (21)	37 (56)	
Intermediate	67 (59)	5 (8)	
High	16 (14)	2 (3)	
Very high	3 (3)	0	
Missing	3 (3)	0	
Ann-Arbor stage at alloHCT, *n* (%)			0.827
I–II	13 (11)	22 (34)	
III–IV	89 (79)	34 (52)	
Missing	11 (10)	9 (14)	
Disease status at alloHCT, *n* (%)			0.214
CR	73 (65)	45 (69)	
PR	30 (26)	17 (26)	
SD	4 (3)	2 (3)	
PD	6 (5)	1 (1)	
ASCT before alloHCT, *n* (%)	52 (46)	39 (60)	0.283
ECOG performance status at alloHCT, *n* (%)			0.468
0	71 (63)	54 (83)	
≥1	42 (37)	11 (17)	
Previous lines of treatment, *n* (%)			0.019
1–3	65 (57.6)	38 (58.5)	
≥4	48 (42.4)	27 (41.5)	
Prior CAR-T cell exposure[c], *n* (%)	5 (4.4)	1 (1.5)	0.170
Prior BsAb exposure[d], *n* (%)	8 (7.1)	1 (1.5)	0.275
Prior CPI exposure[e], *n* (%)	10 (8.8)	16 (24.6)	0.432
Donor type, *n* (%)			0.541
MRD	45 (40)	6 (10)	
MUD	37 (33)	35 (54)	
Haplo	31 (27)	24 (37)	
HCT-CI score, *n* (%)			0.282
<3	63 (56)	39 (60%)	
≥3	50 (44)	24 (37%)	
CMV status (donor/recipient)			0.541
Negative/negative	10 (9)	13 (20)	
Negative/positive	20 (18)	19 (29)	
Positive/negative	10 (9)	7 (11)	
Positive/positive	72 (64)	26 (40)	
Transplant conditioning intensity			0.314
Low	34 (30)	11 (17)	
Intermediate	79 (70)	54 (83)	
Female donor to male recipient	23 (20%)	11 (17%)	0.020
Donor age			0.541
<30 years	33 (29)	32 (49)	
≥30 years	72 (64)	33 (51)	
Missing	8 (7)	0	
Year of alloHCT			0.152
<2019	57 (50)	28 (43)	
≥2019	56 (50)	37 (57)	
Median follow-up among survivors, days (range)	673 (39–2548)	1054 (138–3326)	—

Abbreviations: alloHCT, allogeneic haematopoietic cell transplantation; ASCT, autologous stem cell transplant; BsAb, Bispecific antibodies; CAR-T, Chimeric antigen receptor T-cell; CMV, cytomegalovirus; CPI, immune checkpoint inhibitors; CR, Complete response; DRI, disease risk index; ECOG, eastern cooperative oncology group performance status; Haplo, Haploidentical donor; HCT-CI, Haematopoietic Cell Transplantation-specific Comorbidity Index; MRD, Matched related donor; MUD, Matched unrelated donor; PD, Progressive disease; PR, partial response; SD, stable disease; SMD, Standardized Mean Difference; TBF, thiotepa/busulfan/fludarabine; Treo/Flu, treosulfan/fludarabine.

[a]10/113 thiotepa 5 mg/sm, busulfan 3.2 mg/sm, fludarabine 150 mg/sm; 24/113 thiotepa 5 mg/sm, busulfan 6.4 mg/sm, fludarabine 150 mg/sm; 8/113 thiotepa 10 mg/sm, busulfan 3.2 mg/sm, fludarabine 150 mg/sm; 63/113 thiotepa 10 mg/sm, busulfan 6.4 mg/sm, fludarabine 150 mg/sm; 8/113 thiotepa 10 mg/sm, busulfan 9.6 mg/sm, fludarabine 150 mg/sm.

[b]11/65 = treosulfan 30 mg/sm, fludarabine 150 mg/sm; 2/65 = treosulfan 36 mg/sm, fludarabine 150 mg/sm; 1/65 = treosulfan 36 mg/sm, fludarabine 150 mg/sm, melphalan 100 mg/sm; 49/65 = treosulfan 42 mg/sm, fludarabine 150 mg/sm; 2/65 = treosulfan 42 mg/sm, fludarabine 150 mg/sm, melphalan 100 mg/sm.

[c]Prior CAR T-cell therapy is reported for descriptive purposes only, as the very low numbers did not allow for comparative analyses. One patient in the Treo/Flu cohort (brexucabtagene autoleucel) and five patients in the TBF cohort (3 treated with tisagenlecleucel and 2 with axicabtagene ciloleucel) had previously received CAR T-cell therapy.

[d]Prior bispecific antibody exposure is reported for descriptive purposes only; no statistical comparison was performed due to the very low numbers. In total, one patient in the Treo/Flu cohort (1.5%) had received prior glofitamab, and eight patients in the TBF cohort (7.1%) had previously received bispecific antibodies (4 glofitamab, 3 epcoritamab and 1 odronextamab).

[e]Prior immune checkpoint inhibitors is reported for descriptive purposes only; no statistical comparison was performed due to the very low numbers. In total, nine patients in the Treo/Flu cohort (13.8%) had received prior nivolumab and seven patients (10.8%) had received pembrolizumab. In the TBF cohort, eight patients (7%) had received prior nivolumab and two patients (1.8%) had received pembrolizumab.

TABLE 2 Univariable analysis of the whole population.

	TBF ($n=113$)	Treo/Flu ($n=65$)	HR		Frailty (for TBF centres)*
	% (95% CI)	% (95% CI)	% (95% CI)	*p*-value	*p*-value
+30-day neutrophil engraftment	90.7 (83.0–95.0)	90.77 (79.9–95.8)	0.83 (0.60–1.14)	0.255	0.80
+30-day platelet engraftment	41.4 (32.1–50.4)	47.9 (35.2–59.6)	1.20 (0.85–1.68)	0.290	0.84
3-year OS	54.5 (45.9–64.5)	70.8 (60.1–83.4)	0.61 (0.36–1.01)	0.056	0.95
3-year PFS	45.2 (36.8–55.5)	66.0 (55.7–79.7)	0.57 (0.35–0.93)	**0.023**	0.90
3-year NRM	33.0 (24.0–42.0)	14.0 (6.8–24.0)	0.44 (0.22–0.87)	**0.017**	0.20
3-year RI/POD	22.0 (15.0–30.0)	22.0 (12.0–34.0)	1.08 (0.56–2.09)	0.800	0.06
3-year GRFS	39.8 (31.7–49.9)	43.8 (33.1–57.8)	0.86 (0.57–1.29)	0.470	0.83
3-year grade II–IV acute GVHD	23.0 (16.0–31.0)	26.0 (16.0–37.0)	1.14 (0.62–2.09)	0.700	0.94
3-year grade III–IV acute GVHD	9.8 (5.2–16.0)	14.0 (6.8–23.0)	1.35 (0.57–3.19)	0.500	0.92
3-year moderate–severe chronic GVHD	9.9 (3.3–13.0)	26.0 (11.0–31.0)	2.73 (1.26–5.90)	**0.007**	0.95

Note: Bold values indicate statistically significant *p* values.

Abbreviations: CI, confidence interval; GFRS, graft-versus-host disease-free/relapse-free survival; GVHD, graft-versus-host disease; NRM, non-relapse mortality; OS, overall survival; PFS, progression-free survival; RI/POD, relapse incidence/progression of disease; TBF, thiotepa, busulfan, fludarabine; Treo/Flu, treosulfan, fludarabine.

*All patients treated with Treo/Flu originated from a single centre; centre-level variability could only be evaluated within the TBF cohort.

The 3-year cumulative incidence of NRM was 14.0% (95% CI, 6.8–24.0%) in the Treo/Flu group compared with 33.0% (95% CI, 24.0–42.0%) in the TBF group ($p=0.017$) (Table 2, Figure 1A). In the multivariable analysis, Treo/Flu remained independently associated with a significantly lower 3-year NRM (HR 0.44; 95% CI, 0.22–0.87; $p=0.018$). Haploidentical donor and female donor/male recipient sex mismatch were also independently associated with increased NRM (haploidentical donor: HR 3.22; 95% CI, 1.62–6.84; $p=0.020$; female donor/male recipient: HR 2.12; 95% CI, 1.17–3.84; $p=0.012$). Age was additionally associated with higher NRM (HR 2.06; 95% CI, 1.14–3.70; $p=0.016$).

The 3-year cumulative incidence of RI/POD did not differ between regimens: 22.0% (95% CI, 12.0–34.0%) for Treo/Flu and 22.0% (95% CI, 15.0–30.0%) for TBF ($p=0.800$) (Figure 1B). Progressive disease at the time of transplant, compared with complete response, was independently associated with a higher risk of RI/POD (HR 7.98; 95% CI, 2.61–24.4; $p=0.001$) (Table 3).

Survival outcomes

The 3-year OS was 70.8% (95% CI, 60.1%–83.4%) in the Treo/Flu group versus 54.5% (95% CI, 45.9%–64.5%) in the TBF group ($p=0.056$). Corresponding 3-year PFS rates were 66.0% (95% CI, 55.7%–79.7%) and 45.2% (95% CI, 36.8%–55.5%) respectively ($p=0.023$) (Table 2, Figure 1C,D). In the multivariable analysis, conditioning regimen was not independently associated with OS or PFS, and the effect estimate for Treo/Flu did not reach statistical significance for either end-point (OS: HR 0.72; 95% CI, 0.42–1.22; $p=0.22$; PFS: HR 0.59, 95% CI, 0.34–1.04; $p=0.07$). OS was negatively affected by ECOG performance status ≥1 (HR 1.86; 95% CI, 1.07–2.85, $p=0.024$) and by the use of haploidentical versus MRD grafts (HR 1.92; 95% CI, 1.00–3.61, $p=0.048$). Older age was associated with inferior PFS (HR 1.91; 95% CI, 1.17–3.11, $p=0.008$) (Table 3).

Graft-versus-host disease

At 3 years, the cumulative incidence of grade II–IV acute GVHD was 26.0% (95% CI, 16.0%–37.0%) in the Treo/Flu group and 23.0% (95% CI, 16.0%–31.0%) in the TBF group ($p=0.700$). Grade III–IV acute GVHD occurred in 14.0% (95% CI, 6.8%–23.0%) for Treo/Flu and 9.8% (95% CI, 5.2%–16.0%) for TBF ($p=0.500$) (Table 2, Figure 2A,B). These differences were not statistically significant in multivariable analyses. Hodgkin lymphoma, compared with aggressive B-cell lymphoma, was independently associated with a higher incidence of both grade II–IV and grade III–IV acute GVHD (Table 3). The 3-year cumulative incidence of moderate to severe chronic GVHD was 26.0% (95% CI, 11.0%–31.0%) in the Treo/Flu compared with 9.9% (95% CI, 3.3%–13.0%) in the TBF cohort ($p=0.007$) (Table 2). In multivariable analysis, Treo/Flu was associated with a significantly higher incidence of moderate to severe chronic GVHD (HR 2.43, 95% CI, 1.09–5.43, $p=0.030$) (Table 3, Figure 2C).

GVHD-free, relapse-free survival (GRFS)

The 3-year GRFS was 43.8% (95% CI, 33.1–57.8%) in the Treo/Flu group and 39.8% (95% CI, 31.7–49.9%) in the TBF

TABLE 3 Multivariable analysis of the whole population.

Outcome	HR (95% CI)	*p*-value
Platelet recovery	—	—
Neutrophil recovery		
Haplo vs. MRD	0.48 (0.31–0.75)	0.001
NRM		
Treo/Flu vs. TBF	0.44 (0.22–0.87)	0.018
Haplo vs. MRD	3.22 (1.52–6.84)	0.020
Donor female/receptor male vs. other	2.12 (1.17–3.84)	0.012
Age (> vs. ≤ Median)	2.06 (1.14–3.70)	0.016
Relapse		
Disease status (PD vs. CR)	7.98 (2.61–24.4)	0.001
OS		
ECOG performance status ≥1 (vs. 0)	1.86 (1.07–2.85)	0.024
Haplo (vs. MRD)	1.92 (1.00–3.61)	0.048
PFS		
Age (> vs. ≤ Median)	1.91 (1.17–3.11)	0.008
Grade II–IV acute GVHD		
Hodgkin Lymphoma vs. Aggressive B-cell lymphoma	3.15 (1.32–7.56)	0.01
Grade III–IV acute GVHD		
Hodgkin Lymphoma vs. Aggressive B-cell lymphoma	2.97 (0.96–9.20)	0.056
Moderate–severe chronic GVHD		
Treo/Flu vs. TBF	2.43 (1.09–5.43)	0.030
GRFS		
ECOG performance status ≥1 vs. 0	1.71 (1.15–2.56)	0.008
Haplo (vs. MRD)	1.95 (1.13–3.35)	0.010

Abbreviations: CI, Confidence interval; CR, Complete response; ECOG, Eastern cooperative oncology Group; GRFS, GVHD-free/relapse-free survival; GVHD, graft-versus-host disease; Haplo, Haploidentical donor; MRD, Matched related donor; NRM, non-relapse mortality; OS, overall survival; PD, Progressive disease; PFS, progression-free survival; TBF, Thiotepa, busulfan, fludarabine conditioning; Treo/Flu, Treosulfan/fludarabine conditioning.

group ($p = 0.470$) (Tables 2 and 3, Figure 2D). In the multivariable analysis, ECOG performance status ≥1 and the use of haploidentical donor (vs. MRD donor) were both independently associated with reduced GRFS.

Propensity score matching analysis

After applying PSM, 63 matched pairs were obtained. Table S1 summarizes the baseline characteristics of the matched population, and the corresponding Love plot demonstrates a substantial improvement in covariate balance between groups (Figure S1). In the univariable analysis, Treo/Flu was associated with a significantly lower 3-year NRM (HR 0.48; 95% CI, 0.23–0.98, $p = 0.045$) and a significantly higher incidence of moderate-to-severe chronic GVHD (HR 2.93; 95% CI, 1.13–7.57, $p = 0.027$) compared with TBF. No other significant differences were observed for the remaining outcomes. Table 4 summarizes the results of the univariable analysis in the matched population.

DISCUSSION

Studies specifically addressing the most effective conditioning regimens for alloHCT in HL and NHL remain limited. Available data suggest that MAC and certain RIC regimens, such as fludarabine/melphalan and fludarabine/cyclophosphamide, are associated with inferior outcomes, largely due to unacceptably high rates of NRM.[6,7] Although RIC regimens have more favourable toxicity profiles, the incidence of relapse or progression after alloHCT continues to occur in approximately 40%–50% of patients, making it the leading cause of death in this setting. In response to these limitations, RTC regimens have emerged as a promising alternative, aiming to balance antitumour efficacy with lower toxicity. Regimens such as Treo/Flu and TBF exemplify this approach, offering a more favourable toxicity profile than conventional MAC, while potentially achieving better disease control than traditional RIC.[8] In particular, Treo/Flu has shown improved outcomes in myeloid malignancies in comparison with busulfan-based regimens.[9,17,18] However, data on its use in lymphoid malignancies remain limited.[10,19,20]

In this study, we explored two different RTC regimens classified as TCI low or intermediate, Treo/Flu and TBF, in patients with HL and NHL undergoing alloHCT with a PTCy-based GVHD prophylaxis. After adjusting for baseline imbalances using PSM, Treo/Flu was associated with significantly lower NRM. Relapse incidence, GRFS, PFS, OS and engraftment rates were comparable between the two groups. These results suggest that the potential benefit of Treo/Flu conditioning may also expand to alloHCT for patients with lymphoid malignancies. Differences in NRM between the two conditioning regimens were further reflected in the pattern of cause-specific mortality. Among TBF recipients, more than half of all transplant-related deaths were infection-related, accompanied by additional regimen-associated toxicities such as sinusoidal obstruction syndrome and GVHD. This concentration of early infectious and toxicity-related fatalities suggests that TBF conditioning may confer a higher vulnerability to severe complications in the peri-transplant period. In contrast, Treo/Flu recipients experienced fewer transplant-related deaths overall, consistent with the more favourable toxicity profile previously described for this regimen. The consistency in the direction of the findings in the matched cohort reinforces that the lower NRM observed with Treo/Flu likely reflects true differences in regimen-related toxicity rather than residual baseline imbalances.

In our cohort, univariable analyses showed that Treo/Flu was associated with a significant improvement in PFS,

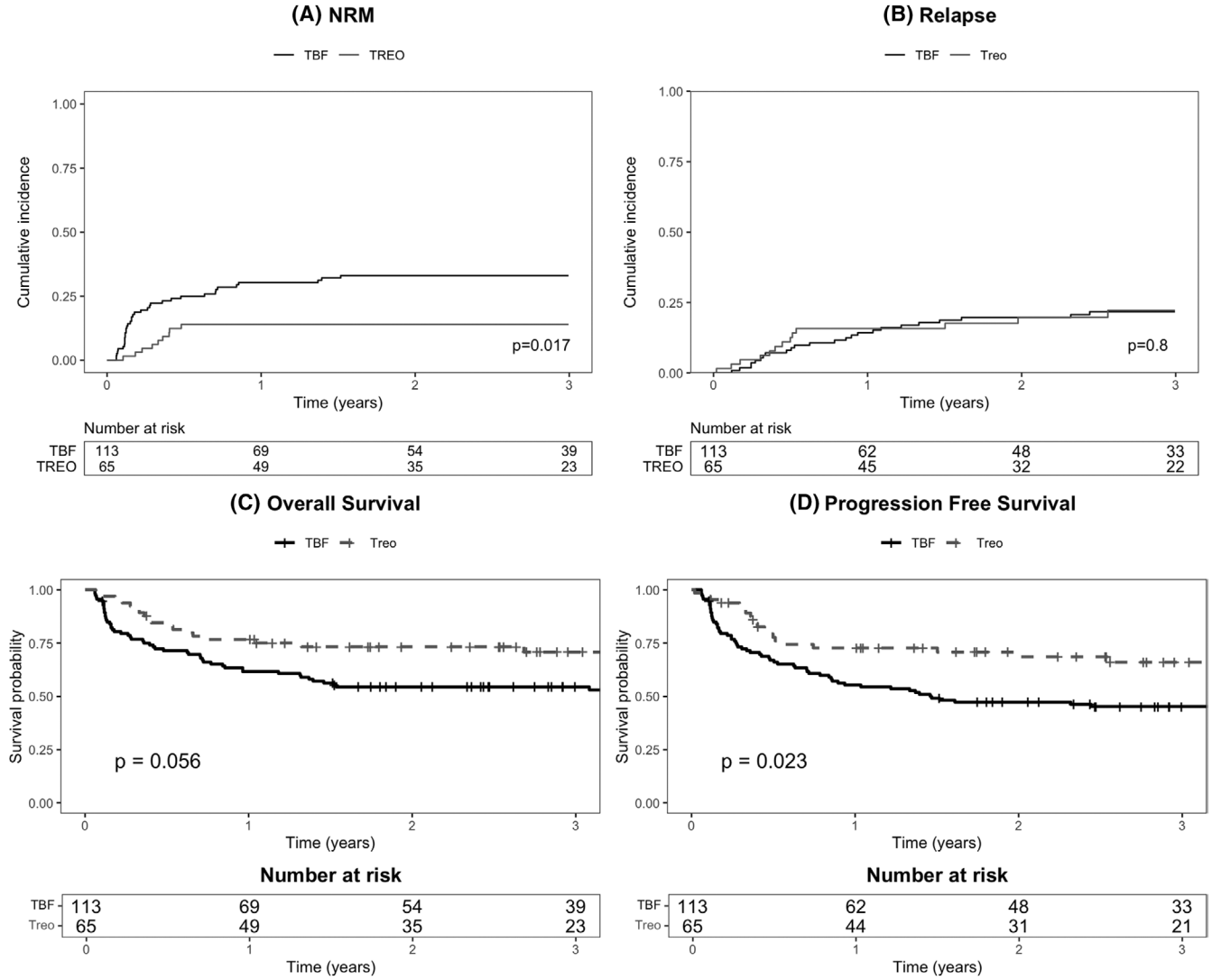

FIGURE 1 Visualization of univariable outcome analysis. (A) non-relapse mortality (NRM), (B) Relapse/progression of disease (POD), (C) overall survival (OS) and (D) progression-free survival (PFS).

whereas the benefit in OS did not reach conventional statistical significance despite a directionally consistent and clinically meaningful effect size. However, after adjustment for relevant patient- and transplant-related covariates, conditioning regimen was not independently associated with either PFS or OS. This attenuation of effect after adjusted analyses, including both multivariable modelling and PSM, is not unexpected in contemporary lymphoma transplantation, where multiple competing prognostic factors may substantially influence long-term outcomes. Indeed, in our multivariable models, ECOG performance status ≥1 and the use of haploidentical donors emerged as strong independent predictors of inferior OS, while age significantly impacted PFS. Taken together, these findings indicate that, while Treo/Flu may be associated with improved disease control in unadjusted analyses, the most consistent and independent benefit observed in this study is the reduction in NRM, rather than a direct effect on long-term survival. Experiences with PTCy have demonstrated acceptable long-term survival and remarkably low rates of chronic GVHD when used in combination with RIC and bone marrow grafts in haploidentical alloHCT.[21,22] In line with more recent evidence, PTCy has also emerged as a preferred GVHD prophylaxis for patients with lymphoid malignancies undergoing matched donor alloHCT.[23,24] In our cohort, the universal application of a PTCy-based prophylaxis, together with the use of peripheral blood stem cell grafts across all donor sources, was associated with favourable survival outcomes and acceptable GVHD rates. However, patients receiving Treo/Flu experienced a higher incidence of moderate-to-severe chronic GVHD in comparison with those in the TBF cohort. Several hypotheses may explain this observation. One possibility is that Treo/Flu, while less toxic, may allow for more robust immune reconstitution, which could enhance alloreactive responses in the absence of early immunologic suppression from the addition of other agents such as thiotepa. Furthermore, variations in the extent of tissue injury induced by different conditioning agents could modulate the inflammatory environment and

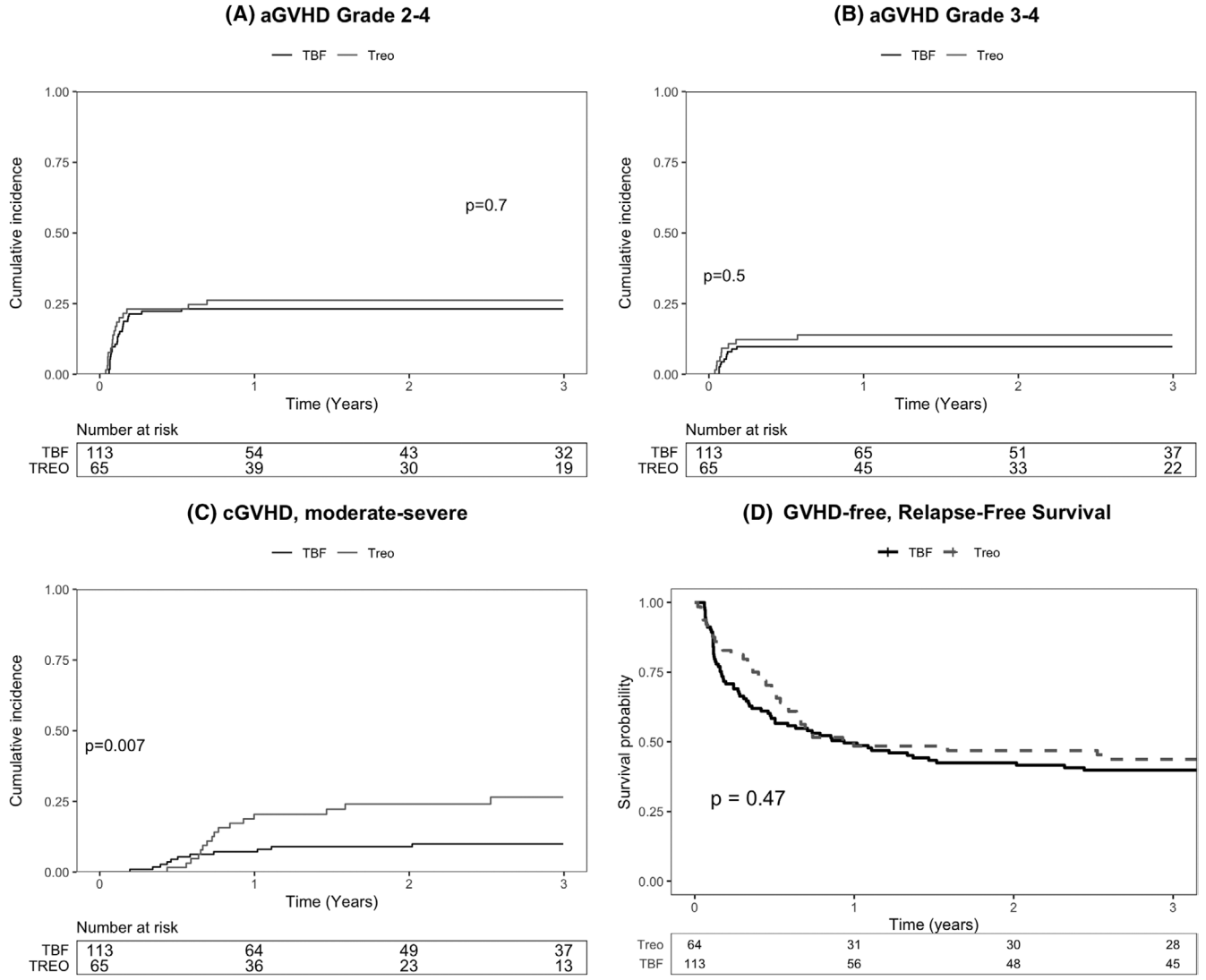

FIGURE 2 Visualization of univariable outcome analysis. (A) Acute graft-versus-host disease Grade 2–4, (B) acute graft-versus-host disease Grade 3–4, (C) Chronic graft-versus-host disease grade moderate–severe and (D) graft-versus-host disease -progression-free survival.

TABLE 4 Univariable analysis of the matched population.

	TBF ($n=63$)	Treo/flu ($n=63$)	HR	
	% (95% CI)	% (95% CI)	% (95% CI)	*p*-value
+30-day neutrophil engraftment	85.8 (72.8–92.9)	91.9 (80.9–96.7)	1.03 (0.72–1.48)	0.873
+30-day platelet engraftment	50.3 (37.2–62.1)	34.9 (23.1–47.1)	1.47 (0.99–2.17)	0.055
3-year OS	58.7 (47.7–72.1)	71.5 (60.79–84.2)	0.66 (0.37–1.19)	0.170
3-year PFS	52.1 (80.41–0.66)	66.7 (55.3–80.5)	0.67 (0.38–1.16)	0.154
3-year NRM	32 (21–43)	14 (7.0–24)	0.48 (0.23–0.98)	**0.045**
3-year RI/POD	16 (8.2–26)	21 (11–33)	1.45 (0.65–3.29)	0.370
3-year GRFS	46.3 (0.35–0.60)	43.5 (32.8–57.8)	0.98 (0.61–1.58)	0.960
3-year grade II–IV acute GVHD	22 (13–33)	25 (15–37)	1.11 (0.55–2.22)	0.780
3-year grade III–IV acute GVHD	7.9 (2.9–16)	14 (7.0–24)	1.58 (0.57–4.34)	0.380
3-year moderate-to-severe chronic GVHD	9.6 (3.8–18.0)	27 (16–39)	2.93 (1.13–7.57)	**0.027**

Note: Bold values indicate statistically significant *p* values.

Abbreviations: CI, confidence interval; GFRS, graft-versus-host disease-free/relapse-free survival; GVHD, Graft-versus-host disease; NRM, non-relapse mortality; OS, overall survival; PFS, progression-free survival; RI/POD, relapse incidence/progression of disease; TBF, thiotepa, busulfan, fludarabine; Treo/Flu, treosulfan, fludarabine.

influence GVHD pathogenesis. Additionally, differences in diagnostic sensitivity and surveillance practices between centres, particularly regarding TBF conditioning, may have contributed to the observed disparity and should be taken into consideration when interpreting these findings. However, the frailty random-effects model did not demonstrate a significant centre-level effect.

This study provides valuable insights but has inherent limitations, including the heterogeneity of the study population and the retrospective design. Several baseline covariates differed between the two conditioning groups. Notably, substantial imbalances were present in disease type, DRI and Ann Arbor stage, each of which may influence RI/POD, NRM and OS. Additional differences in ECOG performance status, donor type and donor age were also observed and represent clinically relevant prognostic factors. To mitigate the potential impact of these imbalances, these variables were incorporated into the multivariable Cox and Fine–Gray models, which did not demonstrate attenuation of the association between conditioning regimen and the primary or secondary endpoints. The PSM analysis, used as a complementary sensitivity approach, further supported these findings, although some residual imbalance persisted due to limited overlap in certain clinical covariates. While unmeasured confounding cannot be entirely excluded, the concordance between adjusted analyses and the matched cohort, together with the use of real-world data reflecting routine clinical practice, supports the robustness and external validity of our results.

In conclusion, our findings support Treo/Flu as a potentially less toxic and more effective conditioning regimen for lymphoma patients undergoing alloHCT, particularly in individuals at higher risk for NRM. The recent inclusion of PD-1 blockades, CAR T-cell therapies and bispecific antibodies before or after alloHCT calls for a manageable safety profile in terms of conditioning regimen. The reported increase in chronic GVHD in treosulfan-exposed patients warrants attention and should be weighed against the observed reduction in early mortality, especially in patients with pre-existing comorbidities or higher transplant risk scores. Prospective randomized studies are warranted to confirm these observations and to refine conditioning strategies for patients with lymphoid malignancies.

AUTHOR CONTRIBUTIONS

Marta Peña and Alberto Mussetti designed the study. Juan A. Carbonell Asins, Carlos Peña and Diego F. Martínez performed the statistical analysis. Marta Peña, Lorenzo Lazzari, Diego F. Martínez, Raffaella Greco and Alberto Mussetti created the figures and wrote the manuscript. All the co-authors contributed to patient care and data collection, provided valuable input during the study, and reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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