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Overcoming minimal residual disease using intensified conditioning with medium-dose etoposide, cyclophosphamide and total body irradiation in allogeneic stem cell transplantation for Philadelphia chromosome-positive acute lymphoblastic leukemia in adults

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ABSTRACT

Background aims: An intensified conditioning regimen incorporating medium-dose etoposide (VP16) is an option for patients with acute lymphoblastic leukemia (ALL). However, the prognostic impacts of the addition of VP16 to cyclophosphamide (CY) and total body irradiation (TBI) in patients with Philadelphia chromosome-positive (Ph+) ALL with regard to minimal residual disease (MRD) status have not been elucidated.
Methods: The authors retrospectively compared the outcomes of patients with Ph+ ALL who underwent allogeneic transplantation following VP16/CY/TBI (n = 101) and CY/TBI (n = 563).
Results: At 4 years, the VP16/CY/TBI group exhibited significantly better disease-free survival (DFS) (72.6% versus 61.7%, $P = 0.027$) and relapse rate (11.5% versus 21.1%, $P = 0.020$) and similar non-relapse mortality (16.0% versus 17.2%, $P = 0.70$). In subgroup analyses, the beneficial effects of the addition of VP16 on DFS were more evident in patients with positive MRD status (71.2% versus 48.4% at 4 years, $P = 0.022$) than those with negative MRD status (72.8% versus 66.7% at 4 years, $P = 0.24$). Although MRD positivity was significantly associated with worse DFS in patients who received CY/TBI (48.4% versus 66.7%, $P < 0.001$), this was not the case in those who received VP16/CY/TBI (71.2% versus 72.8%, $P = 0.86$).
Conclusions: This study demonstrated the benefits of the addition of VP16 in Ph+ ALL patients, especially those with positive MRD status. VP16/CY/TBI could be a potential strategy to overcome the survival risk of MRD positivity.

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Introduction

Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) is a hematologic malignancy of lymphoid precursor cells characterized by breakpoint cluster region–Abelson murine leukemia viral oncogene homolog 1 (*BCR-ABL1*) fusion gene transcripts [1,2]. Outcomes associated with Ph+ ALL have been dramatically improved by the clinical application of tyrosine kinase inhibitors (TKIs), but survival outcomes after treatment with a combination of TKIs and chemotherapy are still suboptimal [3–7], and consolidation therapy with allogeneic stem cell transplantation (SCT) remains the standard strategy for long-term survival [1,2]. However, residual disease burden affects patient survival outcomes even after allogeneic SCT [8], and minimal residual disease (MRD) can be a risk factor for post-SCT relapse in these patients [9,10], just as it is for patient cohorts without SCT [11,12].

To conquer MRD at allogeneic SCT and obtain optimal relapse-free survival, the authors have focused on the intensification of conditioning regimens. The authors' previous studies indicated that the addition of medium-dose (30–40 mg/kg) etoposide (VP16) to a conventional conditioning regimen with cyclophosphamide (CY) and 12 Gy of total body irradiation (TBI) resulted in better transplant outcomes for patients with ALL [13–15]. The beneficial effects of this intensified VP16/CY/TBI regimen were more evident in higher-risk ALL, including Ph+ ALL, second complete remission or later, and higher initial white blood cell count at diagnosis [15]. Considering the prognostic effects of MRD status on the outcomes of patients with Ph+ ALL [9,10], it is conceivable that impacts associated with the addition of VP16 to CY/TBI can vary according to MRD status. Therefore, the selection of conditioning regimen could be optimized by MRD status at SCT [16]. However, the prognostic effects of the addition of VP16 to CY/TBI in patients with Ph+ ALL with regard to MRD status at SCT have not been fully elucidated, and there are no data available on the potential of VP16/CY/TBI to overcome the survival risk of positive MRD status at SCT.

Therefore, the authors retrospectively compared the transplant outcomes of Ph+ ALL adult patients with or without MRD who underwent allogeneic SCT following conditioning with VP16/CY/TBI and standard CY/TBI using nationwide registration data on behalf of the ALL working group of the Japan Society for Hematopoietic Cell Transplantation.

Methods

Patients

The clinical data were provided by the Transplant Registry Unified Management Program 2 of the Japanese Data Center for Hematopoietic Cell Transplantation [17,18]. Patients with Ph+ ALL aged ≥16 years who underwent bone marrow transplantation (BMT) or peripheral blood SCT (PBSCT) at first complete remission between 2002 and 2019 following a total 120 mg/kg of CY (divided into 2 days) and a total 12 Gy of TBI (divided into four to six fractions) with or without medium-dose VP16 (total 30–40 mg/kg or approximately 1000 mg/m^2) were included. Because the number of patients transplanted from mismatched related donors (n = 24) and the number of patients who received anti-thymocyte globulin for T-cell depletion (n = 30) were small, these patients were excluded from the study. The authors also excluded data for patients whose MRD data with molecular level at SCT were not available (n = 45). Finally, 664 patients were included in the study. The selection of conditioning regimen was at the discretion of each attending physician or institutional policy. The authors' study protocol adhered to the principles of the Declaration of Helsinki, and approval for this retrospective study was obtained from the data management committees of the Transplant Registry Unified Management Program and the institutional review board of Tokai University School of Medicine (19R-141).

Definitions of covariates

MRD status was assessed using qualitative or quantitative polymerase chain reaction of the *BCR-ABL1* translocation gene in bone marrow just before conditioning regimens for transplantation. Additional cytogenetic abnormalities were defined when one or more cytogenetic abnormalities were detected in addition to *t*(9;22)(q34;q11). Disease-free survival (DFS) was defined as the time between transplantation and the first relapse or death. Relapse was defined as hematologic or extramedullary evidence of disease. Neutrophil engraftment was defined as an absolute neutrophil count of at least 0.5×10^9 cells/L three consecutive times, with the first day being considered the recovery day. Platelet engraftment was defined as a platelet count of at least 20×10^9 cells/L without transfusion three consecutive times [19]. Acute and chronic graft-versus-host disease (GVHD) was diagnosed and graded at each center according to previously reported criteria [20,21]. HLA disparity was defined as an HLA mismatch in which the recipient and donor had a mismatch of at least one serological level in related BMT or PBSCT and in which the allele level in unrelated BMT or PBSCT was detected. Prophylactic TKI use was defined when any TKI was administered in a leukemia-free state after SCT.

Statistical analyses

The authors compared patient and transplant characteristics using Fisher exact test for categorical variables and Mann–Whitney U-test for continuous variables. The probabilities for DFS were estimated using the Kaplan–Meier method. Univariate and multivariate analyses for survival were performed using the log-rank test and Cox proportional hazard regression model, respectively. The cumulative incidence of relapse and non-relapse mortality (NRM) was evaluated using Gray test by considering relapse and NRM as competing risks. For the multivariate analysis, Fine–Gray models were constructed. The factors associated with significance ($P < 0.05$) in the univariate analyses as well as the basic clinical characteristics were subjected to multivariate analyses, and both hazard ratio (HR) and 95% confidence interval (CI) were calculated. A two-tailed $P< 0.05$ was considered statistically significant. All statistical analyses were performed using EZR (Jichi Medical University Saitama Medical Center, Saitama, Japan), which is a graphical user interface for R (The R Project for Statistical Computing, Vienna, Austria) [22].

Results

Patient characteristics

Of the 664 patients, 101 received conditioning with VP16/CY/TBI, whereas 563 received conditioning with CY/TBI. Table 1 summarizes the patient and transplant characteristics according to conditioning regimen. The median total dose of VP16 in the VP16/CY/TBI group was 30 mg/kg (range, 30–40). Patients in the VP16/CY/TBI group were significantly younger than those in the CY/TBI group. Approximately 70% of patients in both the CY/TBI and VP16/CY/TBI groups had achieved MRD negativity before SCT. Positive MRD status was diagnosed by a quantitative method in approximately three quarters of the patients. No significant differences were observed with respect to the other parameters. Prophylactic TKIs were administered in 36 (6.4%) patients in the CY/TBI group and nine (8.9%) patients in the

Table 1
Patient and transplant characteristics according to conditioning regimen.

Variable		CY/TBI (n = 563)	VP16/CY/TBI (n = 101)	*P* value
Age, years, median (range)		41 (16–60)	37 (17–55)	0.002*
	<38, n (%)	213 (37.8)	52 (51.5)	0.011*
	≥38, n (%)	350 (62.2)	49 (48.5)	–
Sex, n (%)	Male	318 (56.5)	58 (57.4)	0.91
	Female	245 (43.5)	43 (42.6)	–
ECOG PS, n (%)	0	381 (67.7)	70 (69.3)	0.82
	≥1	182 (32.3)	31 (30.7)	–
BCR-ABL1 breakpoint, n (%)	Minor	413 (78.8)	73 (73.7)	0.11
	Major	99 (18.9)	20 (20.2)	–
	Major/minor	12 (2.3)	6 (6.1)	–
ACA, n (%)		107 (19.0)	14 (13.9)	0.26
WBCs ≥30 000/μL at diagnosis, n (%)		316 (56.1)	47 (46.5)	0.083
MRD status at SCT, n (%)	Negative	405 (71.9)	70 (69.3)	0.63
	Positive	158 (28.1)	31 (30.7)	–
	Quantitative	105 (77.2)	21 (75.0)	0.84
	Qualitative	30 (22.1)	7 (25.0)	–
Stem cell source, n (%)	BM	421 (74.8)	78 (77.2)	0.71
	PB	142 (25.2)	23 (22.8)	–
Donor relationship, n (%)	Matched related	222 (39.4)	43 (42.6)	0.85
	Matched unrelated	200 (35.5)	34 (33.7)	–
	Mismatched unrelated	141 (25.0)	24 (23.8)	–
HCT-CI, n (%)	0	343 (70.6)	76 (75.2)	0.39
	≥1	143 (29.4)	25 (24.8)	–
Female donor to male recipient, n (%)		195 (34.8)	34 (33.7)	0.91
TKI use before SCT, n (%)	DA	208 (36.9)	47 (46.5)	0.29
	IMA	288 (51.2)	48 (47.5)	–
	PON	16 (2.8)	3 (3.0)	–
	None	23 (4.1)	2 (2.0)	–
Prophylactic TKI use after SCT, n (%)		36 (6.4)	9 (8.9)	0.39
TBI fraction, n (%)	Six	373 (70.4)	68 (73.1)	0.62
	Four	157 (29.6)	25 (26.9)	–
GVHD prophylaxis, n (%)	CyA-based	246 (44.0)	40 (39.6)	0.45
	Tac-based	313 (56.0)	61 (60.4)	–
Year at transplantation, n (%)	2002–2012	304 (54.0)	51 (50.5)	0.52
	2013–2019	259 (46.0)	50 (49.5)	–
Days from diagnosis to SCT, days, median (range)		171 (45–532)	171 (90–348)	0.72
Follow-up period for survivors, days, median (range)		1897 (59–6385)	1960 (30–4181)	0.73

ACA, additional cytogenetic abnormality; BM, bone marrow; CyA, cyclosporine A; DA, dasatinib; ECOG, Eastern Cooperative Oncology Group; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; IMA, imatinib; PB, peripheral blood; PON, ponatinib; PS, performance status; Tac, tacrolimus; WBCs, white blood cells.
* Statistically significant.

VP16/CY/TBI group ($P = 0.39$) after SCT. The median follow-up period for survivors was 5.2 years (range, 0.1–17.5).

Effects of the addition of VP16 to CY/TBI in the whole cohort

At 4 years, DFS in the VP16/CY/TBI group was significantly higher than that in the CY/TBI group (72.6% versus 61.7%, $P = 0.027$) (Figure 1A). After adjustment for age and MRD status at SCT (see supplementary Table S1), VP16/CY/TBI was associated with significantly better DFS (HR, 0.63, 95% CI, 0.43–0.94, $P = 0.023$) (Table 2). This prognostic effect of VP16/CY/TBI was also significant when prophylactic TKI administration after SCT was included as a time-dependent covariate in the multivariate analysis (see supplementary Table 2). The other significant prognosticator for DFS was MRD status at SCT (Table 2). With regard to relapse rate and NRM, a lower relapse rate was observed in the VP16/CY/TBI group compared with the CY/TBI group (11.5% versus 21.1% at 4 years, $P = 0.020$) (Figure 1B), whereas no difference in NRM was seen between the two groups (16.0% versus 17.2% at 4 years, $P = 0.70$) (Figure 1C). In the multivariate analyses, VP16/CY/TBI was associated with significant relapse reduction (HR, 0.49, 95% CI, 0.26–0.91, $P = 0.025$), whereas VP16/CY/TBI did not increase NRM (HR, 0.94, 95% CI, 0.56–1.56, $P = 0.80$) (Table 2). The other significant factors for relapse and NRM were MRD status at SCT (HR, 2.19, $P < 0.001$) and patient age (HR, 1.55, $P = 0.033$), respectively.

Effects of the addition of VP16 stratified by MRD status at SCT

The authors next compared transplant outcomes of the two groups stratified by MRD status at SCT (Figure 2). With regard to patients with positive MRD status at SCT (MRD [+]), DFS was significantly higher in the VP16/CY/TBI group compared with the CY/TBI group (71.2% versus 48.4% at 4 years, $P = 0.022$) (Figure 2A). After adjusting for other covariates (see supplementary Table 3), VP16/CY/TBI was a significant favorable factor for both DFS itself (HR, 0.50, 95% CI, 0.25–0.99, $P = 0.048$) (Table 2) and DFS when prophylactic TKI administration after SCT was included as a time-dependent covariate in the multivariate analysis (see supplementary Table 2). Regarding MRD levels, DFS in the VP16/CY/TBI group was numerically higher than that observed in the CY/TBI group for both quantitative (68.6% versus 55.1% at 4 years, $P = 0.16$) (see supplementary Figure 1A) and qualitative (68.6% versus 39.2% at 4 years, $P = 0.27$) (see supplementary Figure 1B) MRD (+) groups, although the difference did not reach significance because of the small number of patients in each subgroup. The cumulative incidence of relapse was lower in the VP16/CY/TBI group than in the CY/TBI group, but the difference did not reach significance (17.8% versus 33.1% at 4 years, $P = 0.068$)

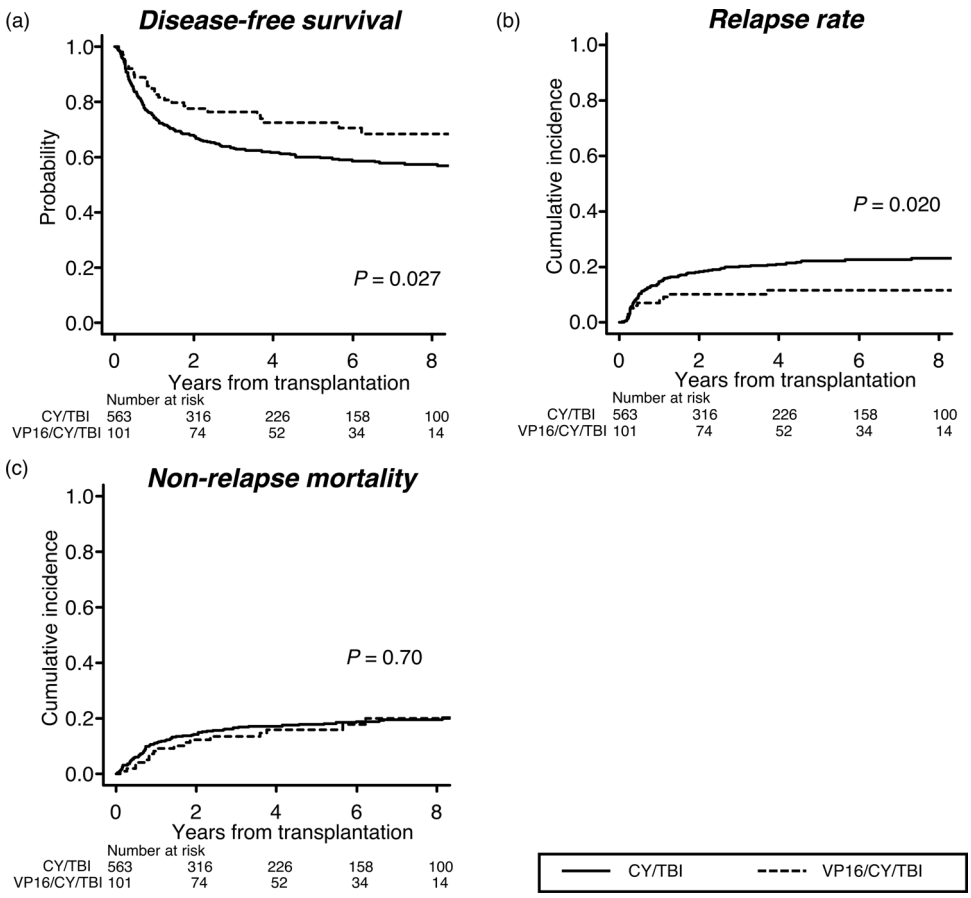

Fig. 1. Transplant outcomes according to conditioning regimen. (A) DFS. (B) Relapse rate. (C) NRM. Relapse rate and NRM are the competing risks in each analysis.

(Figure 2B). In the multivariate analysis, VP16/CY/TBI tended to be associated with relapse reduction (HR, 0.54, 95% CI, 0.21–1.41, $P = 0.20$) (Table 2), although the difference was not significant. The VP16/CY/TBI and CY/TBI groups exhibited similar NRM (11.0% versus 18.5% at 4 years, $P = 0.39$) (Table 2, Figure 2C).

By contrast, with regard to patients with negative MRD status at SCT (MRD [−]), VP16/CY/TBI did not significantly affect DFS (72.8% versus 66.7% at 4 years, $P = 0.24$) (Figure 2D), even in the multivariate analysis (HR, 0.77, 95% CI, 0.47–1.24, $P = 0.28$) (Table 2; also see supplementary Table 2). The relapse rate was lower in the VP16/CY/TBI group than in the CY/TBI group, but the difference did not reach significance (8.9% versus 16.5% at 4 years, $P = 0.094$) (Figure 2E). NRM in the VP16/CY/TBI group was comparable to that observed in the CY/TBI group (18.3% versus 16.8% at 4 years, $P = 0.92$) (Figure 2F). After adjusting for other covariates (see supplementary Table 4), compared with CY/TBI, VP16/CY/TBI tended to be associated with relapse reduction (HR, 0.50, 95% CI, 0.21–1.18, $P = 0.11$) (Table 2), although the difference was not significant.

Prognostic risk of MRD status stratified by conditioning regimen

Given the beneficial effects of VP16/CY/TBI, especially in patients with MRD positivity, the authors analyzed the prognostic impact of MRD status according to conditioning regimen (Figure 3). As expected, MRD positivity at SCT was significantly associated with worse DFS in patients who received conditioning with CY/TBI (66.7% in the MRD [−] group versus 48.4% in the MRD [+] group at 4 years, $P < 0.001$) (Figure 3A). By contrast, the MRD (−) and MRD (+) groups exhibited comparable DFS with regard to patients who received conditioning with VP16/CY/TBI (72.8% versus 71.2% at 4 years, $P = 0.86$) (Figure 3B). With respect to relapse, MRD positivity at SCT was significantly associated with higher relapse rate in patients who received conditioning with CY/TBI (16.5% in the MRD [−] group versus 33.1% in the MRD [+] group at 4 years, $P < 0.001$) (Figure 3C). However, in patients who received conditioning with VP16/CY/TBI, MRD status at SCT did not affect relapse rate (8.9% in the MRD [−] group versus 17.8% in the MRD [+] group at 4 years, $P = 0.27$) (Figure 3D).

Post-transplant complications and cause of death

Supplementary Figure 2 shows the engraftment of neutrophils and platelets. The median time to neutrophil engraftment was 16 days in both the VP16/CY/TBI (range, 10–31) and CY/TBI (range, 8–42) groups, whereas the median time to platelet engraftment was 26 days and 25 days in the VP16/CY/TBI (range, 7–114) and CY/TBI (range, 6–135) groups, respectively. With regard to post-transplant complications, the cumulative incidence of grade II–IV (35.7% in the VP16/CY/TBI group versus 36.9% in the CY/TBI group, $P = 0.86$) (see supplementary Figure 3A) and grade III–IV (8.9% in the VP16/CY/TBI group versus 8.2% in the CY/TBI group, $P = 0.88$) (see supplementary Figure 3B) acute GVHD at 100 days was comparable between the two groups. Similarly, the cumulative incidence of all-grade (38.8% versus 37.6% at 2 years, $P = 0.97$) (see supplementary Figure 3C) and extensive (26.5% versus 22.5% at 2 years, $P = 0.56$) (see supplementary Figure 3D) chronic GVHD in the VP16/CY/TBI group did not differ from that observed in the CY/TBI group. There were no significant differences between groups with regard to the incidence of other infectious or organ complications (see supplementary Table 5). During the analysis period, a total of 197 deaths (23 in the VP16/CY/TBI group and 174 in the CY/TBI group) occurred. The leading cause of death was disease progression followed by infection and GVHD in the CY/TBI group and infection followed by disease progression and GVHD in the VP16/CY/TBI group (Table 3).

Table 2
Multivariate analysis for DFS, relapse and NRM.

Variable		Whole		MRD (+)		MRD (−)	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
DFS							
Conditioning regimen	CY/TBI	Reference	–	Reference	–	Reference	–
	VP16/CY/TBI	0.63 (0.43–0.94)	0.023*	0.50 (0.25–0.99)	0.048*	0.77 (0.47–1.24)	0.28
Age, years	<38	Reference	–	Reference	–	Reference	–
	≥38	1.33 (0.94–1.59)	0.14	1.37 (0.90–2.09)	0.15	1.13 (0.81–1.58)	0.47
MRD status at SCT	Negative	Reference	–	–	–	–	–
	Positive	1.74 (1.33–2.26)	<0.001*	–	–	–	–
Stem cell source	BM	–	–	Reference	–	–	–
	PB	–	–	1.83 (1.18–2.86)	0.007*	–	–
Relapse							
Conditioning regimen	CY/TBI	Reference	–	Reference	–	Reference	–
	VP16/CY/TBI	0.49 (0.26–0.91)	0.025*	0.54 (0.21–1.41)	0.20	0.50 (0.21–1.18)	0.11
MRD status at SCT	Negative	Reference	–	–	–	–	–
	Positive	2.19 (1.53–3.12)	<0.001*	–	–	–	–
Stem cell source	BM	Reference	–	Reference	–	Reference	–
	PB	1.27 (0.77–2.08)	0.35	2.41 (1.16–4.98)	0.018*	0.75 (0.39–1.45)	0.40
Donor relationship	Matched related	Reference	–	–	–	Reference	–
	Matched unrelated	0.89 (0.54–1.46)	0.64	1.81 (0.88–3.72)	0.11	0.47 (0.24–0.91)	0.025*
	Mismatched unrelated	0.62 (0.35–1.11)	0.11	0.57 (0.20–1.64)	0.31	0.56 (0.28–1.11)	0.094
Female donor to male recipient	No	–	–	–	–	Reference	–
	Yes	–	–	–	–	1.77 (1.11–2.82)	0.017*
NRM							
Conditioning regimen	CY/TBI	Reference	–	Reference	–	Reference	–
	VP16/CY/TBI	0.94 (0.56–1.56)	0.80	0.59 (0.22–1.58)	0.30	1.09 (0.60–1.97)	0.78
Age, years	<38	Reference	–	Reference	–	Reference	–
	≥38	1.55 (1.04–2.32)	0.033*	2.22 (1.07–4.60)	0.032*	1.36 (0.84–2.21)	0.21
Donor relationship	Matched related	Reference	–	Reference	–	Reference	–
	Matched unrelated	0.95 (0.58–1.57)	0.85	0.70 (0.26–1.91)	0.48	1.10 (0.61–1.99)	0.75
	Mismatched unrelated	1.44 (0.86–2.42)	0.17	1.40 (0.46–4.25)	0.55	1.56 (0.86–2.85)	0.14
GVHD prophylaxis	CyA-based	Reference	–	Reference	–	Reference	–
	Tac-based	1.30 (0.83–2.05)	0.25	0.97 (0.37–2.52)	0.95	1.47 (0.86–2.51)	0.16

BM, bone marrow; CyA, cyclosporine A; PB, peripheral blood; Tac, tacrolimus.
* Statistically significant.

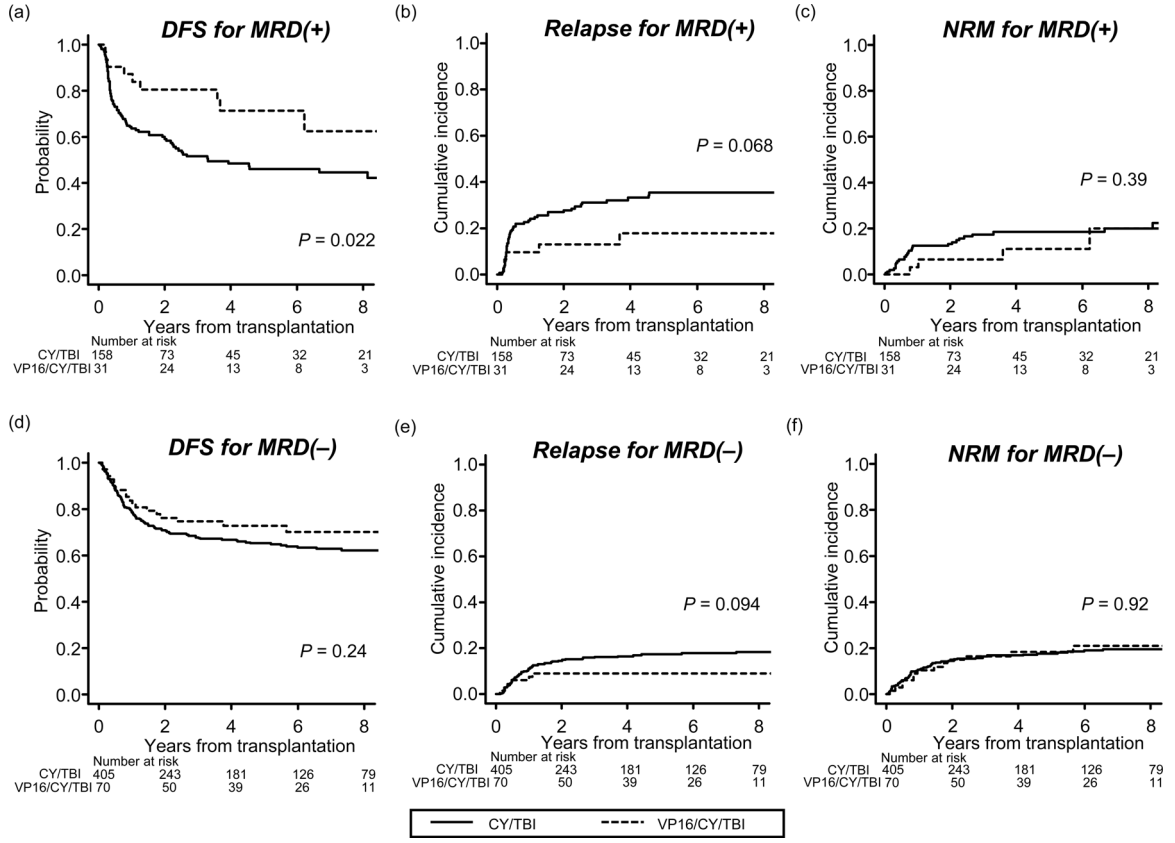

Fig. 2. Transplant outcomes according to conditioning regimen stratified by MRD status at SCT. DFS, relapse rate and NRM in patients with (A–C) positive and (D–F) negative MRD status at SCT. Relapse rate and NRM are the competing risks in each analysis.

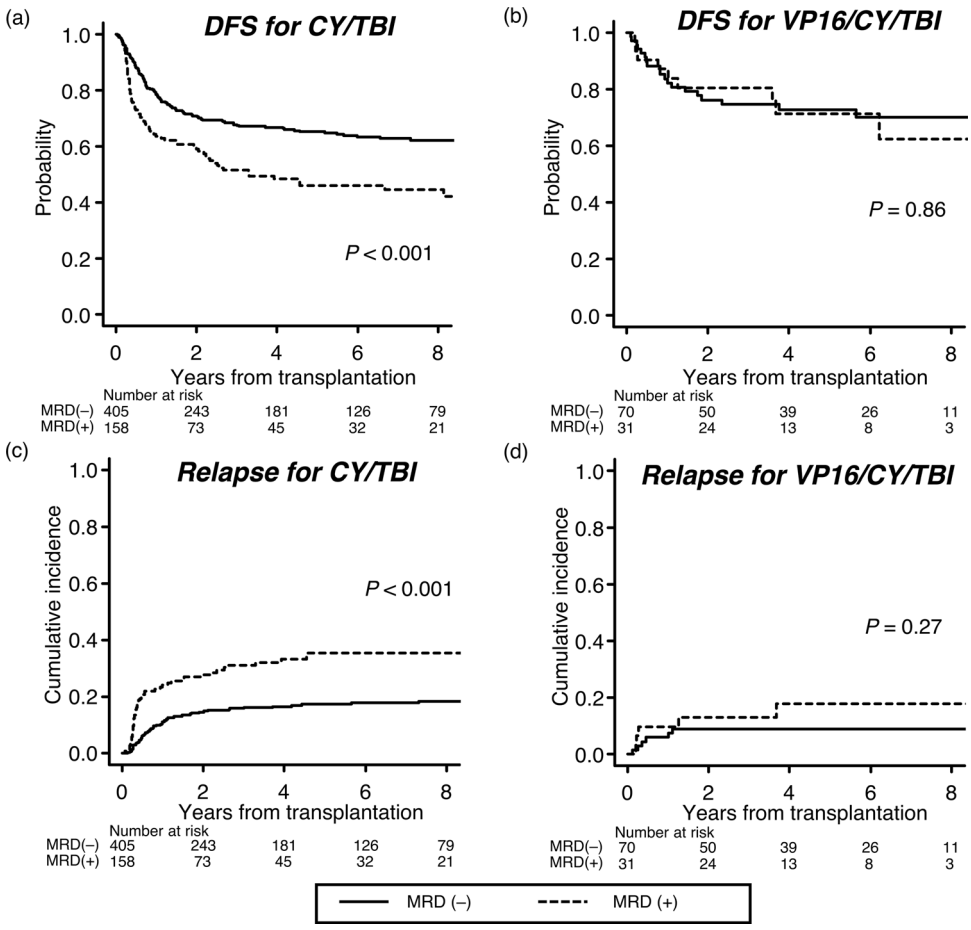

Fig. 3. Transplant outcomes according to MRD status at SCT stratified by conditioning regimen. DFS and relapse rate in patients who received conditioning with (A,C) CY/TBI and (B, D) VP16/CY/TBI. NRM is the competing risk in the analysis for relapse rate.

Discussion

The current study focused on patients with first complete remission of Ph+ ALL, which is a biologically distinct entity characterized by the fusion gene *BCR-ABL1*, and demonstrated that conditioning with VP16/CY/TBI in patients with Ph+ ALL resulted in better DFS and relapse reduction and similar NRM compared with CY/TBI. This additional effect of VP16 was more evident in patients with MRD positivity, for whom VP16 could offset the negative prognostic impact of MRD.

The synergistic effects of VP16 and CY have long been cited as one of the rationales for intensified VP16/CY/TBI regimens [23], and intensified conditioning regimens incorporating VP16 have been used in patients with Ph+ ALL since the non-TKI era. In addition, administration of VP16 can provoke certain chemokine and immunomodulatory effects [24], which might cause stronger graft-versus-leukemia effects. The lower incidence of relapse after SCT in the VP16/CY/TBI group can be attributed to these additional effects as well as the original pharmaceutical function of VP16 (inhibiting DNA synthesis) as a topoisomerase II inhibitor.

In spite of the significantly stronger anti-leukemia effects of the intensified VP16/CY/TBI regimen, NRM and incidence of post-transplant complications were similar between the CY/TBI and VP16/CY/TBI regimens. To date, studies have demonstrated a wide variety of impacts associated with VP16 on post-transplant complications, with some finding the related toxicities to be significant [25,26] and others finding them to be negligible or at least comparable to those observed with CY/TBI [13,15,27–29]. This discrepancy can be partially attributed to the dose of VP16. In general, large doses of VP16 (60 mg/kg or more) appear to be associated with severe adverse effects such as mucositis [25,26,30]. By contrast, medium-dose VP16 (40 mg/kg or less) can be safely administered without exacerbation of mucositis, leading to similar post-transplant complications and NRM between the VP16/CY/TBI and CY/TBI groups [13,15,25,28]. A pharmacokinetics study of VP16, in which medium-dose VP16 efficaciously maintained the optimal concentration [31], supports these observations. Nevertheless, the authors' results should be carefully interpreted because the VP16/CY/TBI group comprised significantly younger patients than the CY/TBI group, which might work as a selection bias.

In addition to the finding that VP16/CY/TBI can reduce relapse without increasing NRM in patients with Ph+ ALL in first complete remission as a whole, the authors' subgroup analysis demonstrated

Table 3
Cause of death according to conditioning regimen.

Cause	CY/TBI (n = 174)	VP16/CY/TBI (n = 23)	*P* value
Disease progression, n (%)	49 (28.2)	6 (26.1)	0.92
Infection, n (%)	47 (27.0)	7 (30.4)	
GVHD, n (%)	19 (10.9)	3 (13.0)	
Organ failure, n (%)	17 (9.8)	2 (8.7)	
Interstitial pneumonitis, n (%)	14 (8.0)	1 (4.3)	
VOD/TMA, n (%)	14 (8.0)	1 (4.3)	
Hemorrhage, n (%)	7 (4.0)	2 (8.7)	
Second malignancy, n (%)	3 (1.7)	1 (4.3)	
Graft failure, n (%)	2 (1.1)	0 (0.0)	
Other, n (%)	2 (1.1)	0 (0.0)	

TMA, thrombotic microangiopathy; VOD, veno-occlusive disease.

that the prognostic effects of the addition of VP16 to CY/TBI were more evident in patients with MRD positivity. As a result, the previously established negative impacts of MRD at SCT on survival in patients with Ph+ ALL [9–12] were not observed to be a significant risk factor for DFS in the authors' study, and the survival curve and cumulative incidence of relapse in patients with MRD positivity receiving VP16/CY/TBI were virtually identical to those observed in patients with MRD negativity receiving CY/TBI (see supplementary Figure 4). Based on the similar incidence of GVHD and the prevalence of prophylactic TKI use between the two groups, negation of the survival risk associated with positive MRD status can be at least partly attributed to the relapse reduction provided by the anti-leukemia effects of VP16. Therefore, the addition of VP16 to the standard conditioning regimen with CY/TBI could be a promising strategy for patients with MRD positivity. The results demonstrating that the addition of VP16 is more effective in MRD (+) patients than MRD (−) patients suggest the potential anti-leukemia effects of VP16/CY/TBI in chemotherapy-resistant residual disease compared with CY/TBI. Given that the widely accepted first-line multi-agent chemotherapies in Japan do not include VP16 [32,33], VP16-naive residual disease may respond to an intensified regimen incorporating this agent.

The current study has several limitations. First, the authors' database did not contain data on genetic abnormalities, although recent evidence has shown that certain somatic mutations, such as *IKZF1*[34] and *CDKN2A/2B*[35] deletions, are associated with poor outcomes in patients with Ph+ ALL. The authors' findings with regard to the superiority of the VP16/CY/TBI regimen should be validated in each patient subgroup with these mutations. Second, the reason for the selection of the conditioning regimen in each patient was not available because this was a registry data-based retrospective study. Selection of the conditioning regimen could be affected by each attending physician's or institution's policy. Multivariate and subgroup analyses based on pre-transplant patient characteristics could adjust these confounding factors to some extent, but selection or institutional biases cannot be completely excluded. Third, the number of patients with MRD positivity who received VP16/CY/TBI was small, and the statistical power to detect this difference can be relatively weak. Subgroup analyses according to the different copy numbers in MRD (+) patients were insufficient. Furthermore, although prophylactic TKI administration after SCT has recently become a standard strategy, only a small proportion of patients in the authors' study received prophylactic TKIs. This could be due to the inclusion criteria of this study, which included patients who underwent allogeneic SCT as far back as the early 2000s. Given these limitations, the authors' results should be carefully validated in other cohorts. However, the authors' results can provide a promising strategy for Ph+ ALL patients, especially those with positive MRD status, which may be useful from the perspective of influence on SCT and cost-effectiveness in comparison with new therapeutic agents—namely, inotuzumab [36], the causative drug for the development of veno-occlusive disease [37], ponatinib [38], and blinatumomab [39]. Moreover, VP16/CY/TBI might be a valid option for patients who do not achieve MRD negativity even after receiving the new therapeutic agents [37–39].

Conclusions

The addition of VP16 to the standard conditioning regimen with CY/TBI offered better outcomes after allogeneic SCT in patients with Ph+ ALL, which could overcome the adverse prognostic factor of positive MRD status at SCT. The authors' study, if validated in a larger number of patients, may provide a strategy for better outcomes and modify the therapeutic algorithm in patients with Ph+ ALL.

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Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions

Conception and design of the study: KH, MM-F, SK and Y.Arai. Acquisition of data: TF, YO, ND, MT, YM, YK, TA, TE, ST, NU, TI, JK, MO and YA. Analysis and interpretation of data: KH, MM-F, SK and Y.Arai. Drafting or revising the manuscript: TF, YO, ND, MT, YM, YK, TA, TE, ST, NU, TI, JK, MO and Y.Atsuta. All authors have approved the final article.

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Supplementary materials

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