Letermovir prophylaxis for cytomegalovirus is associated with risk of post-transplant lymphoproliferative disorders after haploidentical stem cell transplantation

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) emerge as the most frequently reactivated opportunistic viruses after haploidentical stem cell transplantation (haplo-SCT).[1-3] As members of the family of herpesviruses, CMV and EBV are reported to be linked to causing clinical manifestations, resulting in co-infection rates ranging from 10% to 32.7%.[4,5] Additionally, CMV treatment coincides with EBV reactivation, potentially affecting the progression of EBV reactivation and EBV-associated diseases.[6,7] Letermovir prophylaxis effectively reduces CMV infection after haplo-SCT.[8,9] However, its impact on EBV remains unclear.

This study compared letermovir recipients (letermovir group) to a control group receiving standard polymerase chain reaction (PCR)-guided pre-emptive therapy (non-letermovir group) in two independent cohorts. In both cohorts, letermovir significantly reduced clinically significant CMV infection and refractory CMV infection within 180 days after the transplant. While the incidence of EBV viremia was similar in the two groups, letermovir recipients exhibited higher peak EBV titers and had an increased risk of post-transplant lymphoproliferative disorder (PTLD) within the first 180 days. In both univariate and multivariate analyses, the use of letermovir was significantly linked to a higher risk of PTLD. These findings indicate that while letermovir prophylaxis is highly effective in preventing CMV complications after haplo-SCT, it may also be associated with an increased risk of PTLD, highlighting the need for caution and further research to explore this potential link in clinical practice.

Patients were divided into two cohorts based on transplant date. Cohort 1 (June 2022 to December 2022) included letermovir-treated haplo-SCT patients in whom letermovir use depended on a patient's choice and drug availability. Cohort 2 (January 2023 to June 2023) (NCT05656599) included haplo-SCT patients who regularly received letermovir and met our inclusion criteria as part of our registered clinical trial (clinicaltrials.gov NCT05656599). Non-letermovir controls received PCR-guided therapy, matched 1:1 to letermovir patients by gender, age, and disease via propensity score matching. Inclusion criteria were first haplo-SCT, age over 14 years, and letermovir started within 28 days and continued to 100 days after the transplant. Patients with genetic metabolic disorders, bone marrow fibrosis or who died early within 28 days were excluded. Patients in whom CMV or EBV reactivated before letermovir or within 7 days of letermovir administration were also excluded. The study received approval from the ethics committee of Peking University People's Hospital. Transplant protocols, including conditioning, stem cell mobilization, and acute graft-*versus*-host disease (GvHD) prophylaxis were consistent with those of our previous studies. CMV and EBV were monitored by real-time quantitative PCR on plasma DNA twice weekly during hospitalization and at least once weekly after discharge up to 180 days, or as needed. CMV >1,000 copies/mL and EBV >500 copies/mL were the thresholds for positive results.[10-12] Clinically significant CMV infection was defined as CMV viremia requiring pre-emptive treatment or CMV disease.[8] Refractory CMV infection was defined as CMV viremia that persisted or increased after at least 2 weeks of antiviral therapy.[10,13] EBV-associated PTLD was diagnosed as proven or probable based on a published definition.[12] EBV-associated hemophagocytic syndrome (HLH) was defined by meeting HLH-2004 diagnostic criteria, having active EBV infection, and excluding other causes.[14] Other herpesviruses were not routinely monitored, but testing was performed on patients with clinically suspicious manifestations.

In the letermovir group, prophylaxis began after stable neutrophil engraftment. Patients with clinically significant CMV stopped letermovir and were given ganciclovir, foscarnet, and immunoglobulin if refractory; letermovir was resumed once CMV was controlled within 100 days. In patients with EBV DNA >500 copies/mL in two consecutive tests with a rapid increase or when a patient presented with clinical symptoms of EBV-related complications, rituximab therapy was typically initiated within 24 hours, along with a reduction of immunosuppressive therapy if conditions permitted. EBV-specific T-cell therapy was also considered for high-risk or rituximab-refractory patients.[12] All haplo-SCT recipients received acyclovir prophylaxis for herpesviruses for 1 year, regardless of whether they were receiving letermovir. Statistical analyses were performed using R software.

The characteristics of the patients are summarized in Table 1. In cohort 1, 178 haplo-SCT patients (89 per group) were analyzed. Letermovir prophylaxis began at a median of 19 days after haplo-SCT and lasted 84 days (range, 62-112 days) in the letermovir group. In cohort 2, 464 haplo-SCT patients (232 per group) had similar baseline characteristics, with letermovir started at a median of 16 days after SCT and continued for 84 days.

In cohort 1 (Figure 1A), the 180-day cumulative incidence of clinically significant CMV infection was lower in the letermovir group (39.7%, 95% confidence interval [95% CI]: 29.4-49.7%) than in the non-letermovir group (80.9%, 95% CI: 70.9-87.8%, $P<0.001$). Similar results were observed in cohort 2 (Figure 1B), with the cumulative incidence being 21.4% (95% CI: 16.2-27.2%) in the letermovir group *versus* 78.4% (95% CI: 72.5-83.2%) in

the non-letermovir group (*P*<0.001). The incidence of refractory CMV was also lower in the letermovir groups in both cohorts (Figure 1C, D). Delayed CMV reactivation and decreased CMV peak titer and duration are also notable benefits that were observed (*Online Supplementary Figure S1*).

Despite a similar incidence of EBV viremia in the letermovir and non-letermovir groups (Figure 1E, F), the letermovir group exhibited a higher peak EBV titer (5,110 copies/mL) compared to that in the non-letermovir group (1,950 copies/mL, *P*=0.010) (*Online Supplementary Figure S1*). Importantly, letermovir recipients showed an increased risk of PTLD (Figure 1G, H; *Online Supplementary Table S1*). In cohort 1, eight cases of PTLD (5 proven, 3 probable) in the letermovir group and two cases (both probable) in the non-letermovir group,resulting in cumulative incidences of 9.0% (95% CI: 4.2-16.1%) and 2.25% (95% CI: 0.4-7.1%), respectively (Figure 1G), showing a statistically significant difference (*P*=0.049). Cohort 2 provided further support for these findings, with 23 cases of PTLD (9.9%, 12 proven, 11 probable) in the letermovir group and six (2.6%, 2 proven, 4 probable) in the non-letermovir group, leading to cumulative incidences of 9.9% (95% CI: 6.5-14.2%) and 2.6% (95% CI: 1.1-5.3%), respectively (*P*=0.001) (Figure 1H), highlighting the association between letermovir prophylaxis and increased PTLD risk. Of note, four patients in the letermovir group developed EBV-associated HLH, whereas none in the non-letermovir group did so, resulting in a higher cumulative incidence of HLH in the letermovir group (1.7% *vs*. 0%, *P*=0.044). Risk factors for PTLD after haplo-SCT are summarized in Table 2. In the univariate analysis of the entire group of 642 patients, letermovir administration, EBV peak titer ≥2,420 copies/mL (median), and duration of EBV DNAemia ≥10 days (median) emerged as significant risk factors for developing

Table 1. Patients' characteristics.

Characteristics	Cohort 1				Cohort 2			
	Total N=178	LMV N=89	No LMV N=89	*P*	Total N=464	LMV N=232	No LMV N=232	*P*
Age at SCT in years, median (range)	42(14-66)	45 (16-66)	41 (14-61)	0.052	38 (14-70)	40 (14-70)	37 (14-64)	0.133
Age ≥40 years, N (%)	102 (57.3)	54 (60.7)	48 (53.9)	0.363	226 (48.7)	123 (49.8)	103 (41.7)	0.071
Male/female, N/N	94/84	49/40	45/44	0.548	290/204	144/103	146/101	0.855
Follow-up in days, median (range)	465 (33-573)	458 (33-568)	481 (66-573)	0.263	327 (28-728)	273 (28-352)	625 (28-728)	<0.001
Disease type, N (%)				0.965				0.103
Acute myeloid leukemia	74 (41.6)	37 (41.6)	37 (41.6)		194 (41.8)	97 (41.8)	97 (41.8)	
Acute lymphoblastic leukemia	64 (36.0)	33 (37.1)	31 (34.8)		127 (27.4)	54 (23.2)	73 (31.5)	
Myelodysplastic syndrome	26 (14.6)	13 (14.6)	13 (14.6)		82 (17.7)	42 (18.1)	40 (17.2)	
Aplastic anemia	6 (3.4)	2 (2.2)	4 (4.5)		32 (6.9)	21 (9.1)	11 (4.7)	
Others	8 (4.5)	4 (4.5)	4 (4.5)		29 (6.3)	18 (7.8)	11 (4.7)	
Transplant regimen, N (%)				0.496				0.186
Bu/Cy/ATG	139 (78.1)	68 (76.5)	71 (79.8)		369 (79.5)	180 (77.6)	189 (81.5)	
Bu/Flu/Cy/ATG	6 (3.4)	2 (2.2)	4 (4.5)		32 (6.9)	21 (9.1)	11 (4.7)	
RIC-Bu/Flu/Cy/ATG	33 (18.5)	19 (21.3)	14 (15.7)		63 (13.6)	31 (13.4)	32 (13.8)	
Donor CMV serological status, N (%)				<0.001				0.999
Donor-positive	155 (87.1)	71 (79.8)	84 (94.4)		443 (95.5)	221 (95.3)	222 (95.7)	
Donor-negative	20 (11.2)	18 (20.2)	2 (2.2)		17 (3.7)	9 (3.9)	8 (3.4)	
Unknown	3 (1.7)	0 (0.0)	3 (3.4)		4 (0.9)	2 (0.9)	2 (0.9)	
Patient CMV serological status, N (%)				0.621				0.175
Patient-positive	174 (97.8)	88 (98.9)	86 (96.6)		455 (98.1)	225 (97.0)	230 (99.1)	
Patient-negative	4 (2.2)	1 (1.1)	2 (2.2)		9 (1.9)	7 (3.0)	2 (0.9)	
Donor EBV serological status, N (%)				0.225				0.345
Donor-positive	167 (93.8)	84 (94.4)	83 (93.3)		440 (94.8)	217 (93.5)	223 (96.1)	
Donor-negative	8 (4.5)	5 (5.6)	3 (3.4)		20 (4.3)	13 (5.6)	7 (3.0)	
Unknown	3 (1.7)	0 (0.0)	3 (3.4)		4 (0.9)	2 (0.9)	2 (0.9)	
Patient EBV serological status, N (%)				0.118				0.094
Patient-positive	171 (96.1)	88 (98.9)	83 (93.3)		440 (94.8)	224 (96.6)	216 (93.1)	
Patient-negative	7 (3.9)	1 (1.1)	6 (6.7)		24 (5.2)	8 (3.4)	16 (6.9)	

LMV: letermovir; SCT: stem cell transplantation; Bu: busulfan; Cy: cyclophosphamide; ATG: antithymocyte globulin; Flu: fludarabine; RIC: reduced intensity conditioning; CMV: cytomegalovirus; EBV: Epstein-Barr virus.

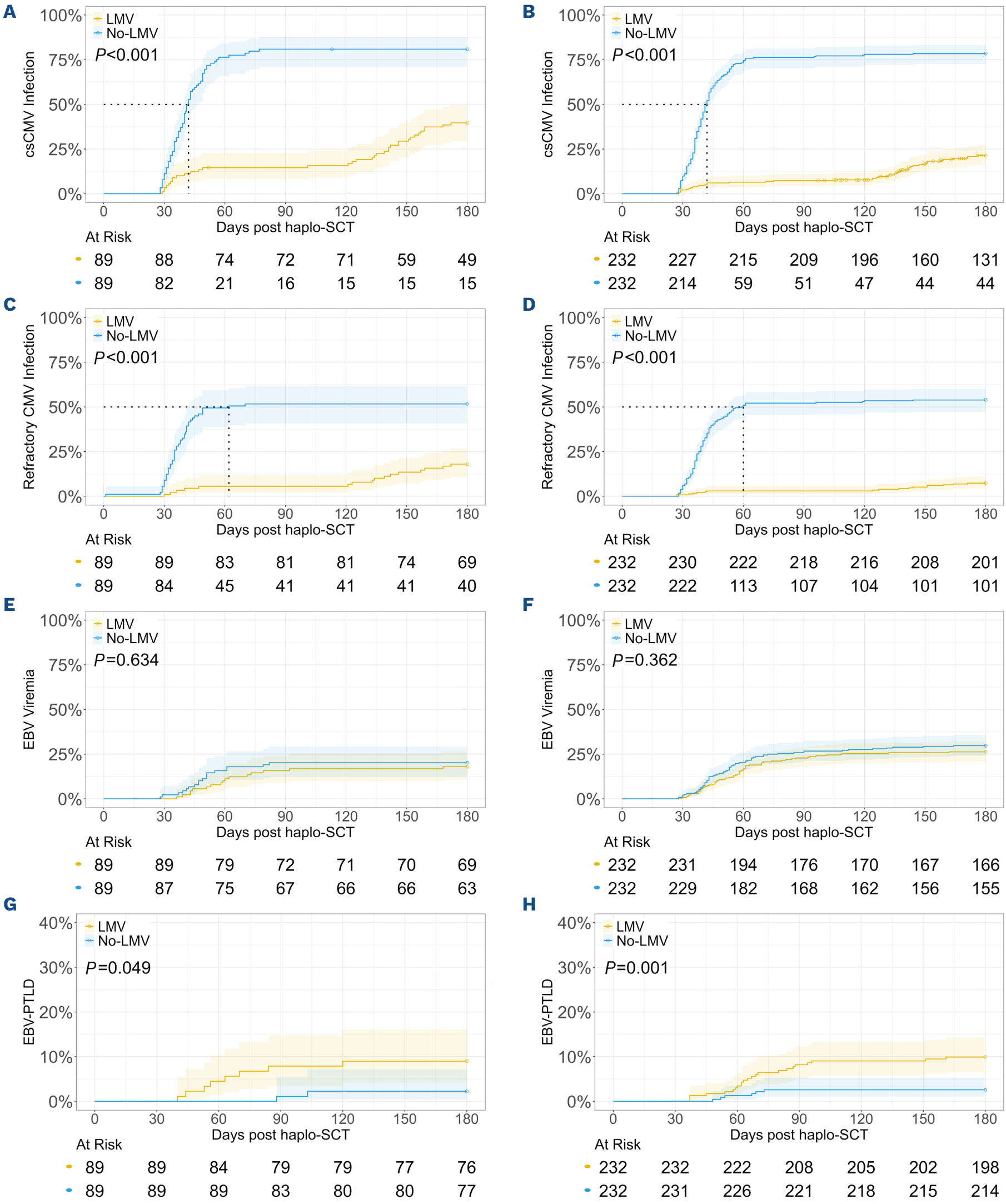

Figure 1. Cytomegalovirus and Epstein-Barr virus infection. Cumulative incidence of clinically significant cytomegalovirus (CMV) infection in cohort 1 (A) and cohort 2 (B), and refractory CMV infection in cohort 1 (C) and cohort 2 (D), Epstein-Barr virus (EBV) viremia in cohort 1 (E) and cohort 2 (F), EBV-post-transplant lymphoproliferative disease in cohort 1 (G) and cohort 2 (H). csCMV: clinically significant cytomegalovirus; haplo-SCT: haploidentical stem cell transplantation; LMV: letermovir; PTLD: post-transplantation lymphoproliferative disorder.

Table 2. Univariate analysis and multivariate analysis of risk factors for post-transplant lymphoproliferative disorder after haploidentical stem cell transplantation.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Patients' sex (male *vs.* female)	0.899 (0.471-1.719)	0.748	-	-
Patients' age at SCT (≥40 y *vs.* <40 y)	0.938 (0.419-2.104)	0.877	-	-
Underlying disease (acute leukemia *vs.* not)	1.004 (0.500-2.017)	0.945	-	-
Patient EBV serological status P+ *vs.* P-)	1.590 (0.317-7.986)	0.573	-	-
Donor EBV serological status (D+ *vs.* others)	0.931 (0.258-3.362)	0.913	-	-
Acute GvHD after SCT (grade 2-4 *vs.* grade 0-1)	1.819 (0.711-4.650)	0.212	-	-
Steroid treatment (≥1 mg/kg/day prednisone or its equivalent *vs.* not)	1.290 (0.452-3.682)	0.634	-	-
CMV DNAemia after SCT (yes *vs.* no)	1.349 (0.615-2.961)	0.455	-	-
EBV peak titer after SCT (≥2,180 *vs.* <2,180)	3.384 (1.009-11.350)	0.048	3.598 (1.126-11.496)	0.031
EBV DNAemia duration in days after SCT (≥10 *vs.* <10)	136.791 (14.746-1,268.936)	<0.001	123.124 (13.617-1,113.285)	<0.001
Letermovir administration (yes *vs.* no)	4.693 (1.905-11.558)	<0.001	3.679 (1.669-8.111)	0.001

HR: hazard ratio; 95% CI: 95% confidence interval; SCT: stem cell transplantation; EBV: Epstein-Barr virus; P+: patient EBV-seropositive; P-: patient EBV-seronegative; D+: donor EBV-seropositive; GvHD: graft-*versus*-host disease; CMV: cytomegalovirus.

PTLD. Multivariate analysis identified letermovir administration (hazard ratio [HR]=3.68, 95% CI: 1.70-8.11, *P*=0.001), median EBV peak titer ≥2,420 copies/mL (HR=3.60, 95% CI: 1.13-11.50, *P*=0.031), duration of EBV DNAemia ≥10 days (HR=123.12, 95% CI: 13.62-1,113.29, *P*<0.001) remained the independent predictors for the development of PTLD. These associations were also confirmed in cohort 2 of 464 patients (*data not shown*). Our data strongly indicate that letermovir prophylaxis may contribute to an elevated risk of PTLD after haplo-SCT.

The cumulative incidences of various transplant outcomes were comparable in the letermovir and non-letermovir groups (*Online Supplementary Figure S2*). With regard to grade 2-4 acute GvHD at 100 days, there was no difference between the letermovir and non-letermovir groups in cohort 1 (24.7% in both, *P*=0.953), and the results in cohort 2 were similar (18.1% *vs*. 22.0%, *P*=0.312). Relapse incidence at 180 days was also comparable (cohort 1: 4.5% for both, *P*=0.997; cohort 2: 5.2% *vs*. 9.1%, *P*=0.111). Turning attention to treatment-related mortality, the studies in both cohort 1 and cohort 2 demonstrated comparable cumulative incidences in the letermovir and non-letermovir groups (cohort 1: 6.7% vs. 10.1%, *P*=0.414; cohort 2: 6.3% *vs*. 3.9%, *P*=0.251). Infection-related mortality was not significantly different in either cohort (cohort 1: 5.6% *vs*. 9.0%, *P*=0.380; cohort 2: 6.0% *vs*. 2.6%, *P*=0.070). At 180 days, overall survival rates were comparable in the two cohorts (cohort 1: 93.9% *vs*. 89.8%, *P*=0.292; cohort 2: 93.5% *vs*. 94.4%, *P*=0.694).

This study of two independent cohorts shows that letermovir prophylaxis after haplo-SCT effectively reduces CMV complications but is unexpectedly linked to a higher risk of EBV-related disease, especially PTLD. This is the first study to suggest a potential association between letermovir and increased EBV-related diseases. Although the observed association between letermovir and increased PTLD may be multifactorial, our data underscore the need for caution regarding this link. Despite increased PTLD cases, transplant-related mortality, infection-related mortality, and overall survival remained unaffected. Given the clear benefit of letermovir in reducing clinically significant CMV, letermovir prophylaxis remains advisable for high-risk CMV patients. However, the increased PTLD risk needs careful monitoring, and further research is needed to explore the potential mechanisms.

Limitations of our study include its single-center design, lack of contemporaneous controls, missing data of some histological confirmation of PTLD and the use of other anti-DNA viral agents. Prospective, multicenter studies are needed to assess clinical factors affecting outcomes and to confirm our findings. Additionally, our study focused on haplo-SCT with high-dose antithymocyte globulin, so the findings require validation in other transplant contexts. Data on immune reconstitution were not collected; future studies should examine antiviral immune recovery.

In conclusion, while letermovir prophylaxis is highly effective in preventing CMV after haplo-SCT, it may also be associated with an increased risk of PTLD. Our results highlight the need for caution and further research to explore this potential link in clinical practice. Additional studies are essential to optimize post-transplant management and carefully balance the benefits and risks of letermovir use.

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https://doi.org/10.3324/haematol.2024.286265

Received: July 15, 2024.
Accepted: November 22, 2024
Early view: November 28, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

X-JH designed the study. X-YP, QH, L-JL and H-LS collected and analyzed data. X-JH, X-YP, QH, and LC wrote the manuscript. All authors provided patients' data and gave final approval of the manuscript.

Funding

This work was funded by Being Nova Program (grant number 20220484076), Major Program of the National Natural Science Foundation of China (grant number 82293630), Peking University Medicine Fund for world's leading discipline or discipline cluster development (grant number 71003Y3035), and Peking University Clinical Scientist Training Program (grant number BMU2024PYJH012).

Data-sharing statement

The data that support the findings of this study are available upon reasonable request from the corresponding author.

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