The Journal of Infectious Diseases

MAJOR ARTICLE

Human Herpesvirus 6 DNAemia Is Associated With Worse Survival After Ex Vivo T-Cell–Depleted Hematopoietic Cell Transplant

Yeon Joo Lee,[1,2] Yiqi Su,[1] Christina Cho,[2,3] Roni Tamari,[2,3] Miguel-Angel Perales,[2,3] Ann A. Jakubowski,[2,3,a] and Genovefa A. Papanicolaou[1,2]

[1]Infectious Diseases Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA, [2]Weill Cornell Medical College, New York, New York, USA, and [3]Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA

Background. We examined the correlation between persistent human herpesvirus 6 (HHV-6) DNAemia (p-HHV-6) and absolute lymphocyte count (ALC), platelet count (PLT), and all-cause mortality by 1 year after ex vivo T-cell–depleted (TCD) hematopoietic cell transplant (HCT).

Methods. We analyzed a cohort of adult TCD HCT recipients during 2012–2016 prospectively monitored for plasma HHV-6 by quantitative polymerase chain reaction from day +14 post-HCT through day +100 (D+100). p-HHV-6 was defined as ≥2 consecutive values of ≥500 copies/mL by D+100. PLT and ALC were compared between patients with and without p-HHV-6 using generalized estimating equations (GEE). Multivariable Cox proportional hazard models (PH) were used to identify the impact of p-HHV-6 on 1 year mortality.

Results. Of 312 patients, 83 (27%) had p-HHV-6 by D+100. p-HHV-6 was associated with lower ALC and PLT in the first year post-HCT. In multivariable models, p-HHV-6 was associated with higher mortality by 1 year post-HCT (adjusted hazard ratio, 2.97 [95% confidence interval, 1.62–5.47]; $P = .0005$), after adjusting for age, antiviral treatment, and ALC at D+100.

Conclusions. p-HHV-6 was associated with lower ALC and PLT in the first year post-HCT. p-HHV-6 was an independent predictor of mortality in the first year after TCD HCT.

Keywords. human herpesvirus 6; hematopoietic cell transplant; T-cell depletion; mortality.

Human herpesvirus 6 (HHV-6) viremia occurs in approximately 30%–40% of T-cell–replete hematopoietic cell transplant (HCT) recipients and up to 90% of cord blood allograft recipients with median onset at 3-4 weeks post-HCT and is usually self-limited [1, 2]. HHV-6 encephalitis, a complication of HHV-6 viremia, has a reported incidence between <1% and 21% and is associated with severe neurologic sequelae and mortality [2–8]. Several other posttransplant complications have been associated with HHV-6 viremia, including acute graft-vs-host disease (GVHD), myelosuppression, thrombocytopenia, delayed immune reconstitution, and delirium; however, a direct causal association has yet to be determined [9–17].

HHV-6 infects T cells and is associated with in vivo and in vitro apoptosis of $CD4^+$ T lymphocytes [18, 19]. In a murine herpesvirus model, a murine herpesvirus related to human roseoloviruses infects the thymus and causes T-cell depletion (TCD) [20]. Persistent HHV-6 viremia correlated with decreased lymphocyte proliferation and lymphocyte counts among T-cell–replete HCT [21]. T cells are essential for the control of HHV-6 viremia [17]. A correlation has been shown between HHV-6–specific T cells and protection from HHV-6 viremia [22], and adoptive transfer of HHV-6–specific cells has been used successfully to treat HHV-6 viremia and disease [22, 23].

TCD by $CD34^+$ selection achieves in 4–5 $\log_{10}$ depletion of T-lymphocytes in the allograft TCD by $CD34^+$ selection achieves in 4-5 $\log_{10}$ T cells [24–26]. As a result, recipients of $CD34^+$-selected HCTs are at low risk for acute and chronic GVHD but at increased risk for viral infections, particularly by double-stranded DNA viruses [24]. HHV-6 viremia occurs early post-HCT in up to 60% of TCD HCT recipients; however, the magnitude and duration of HHV-6 viremia vary widely [24]. The long-term sequelae of persistent HHV-6 viremia after TCD HCT have not been studied.

We analyzed a cohort of TCD HCT recipients who were monitored prospectively for HHV-6 by quantitative polymerase chain reaction (qPCR) in the plasma. The objectives of this study were to study (1) the correlation between persistent HHV-6 DNAemia (p-HHV-6) and lymphocyte and platelet counts and (2) the association between p-HHV-6 and overall survival in the first year after TCD HCT.

Received 23 April 2021; editorial decision 10 August 2021; accepted 12 August 2021; published online August 14, 2021.

[a]A. A. J. and G. A. P. contributed equally to this work as co–senior authors.

Presented in part: American Society for Transplantation and Cellular Therapy Meeting, Orlando, Florida, 19–23 February 2020.

Correspondence: Genovefa A. Papanicolaou, MD, Infectious Diseases Service, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA (papanicg@mskcc.org).

The Journal of Infectious Diseases® 2021;XX:1–12

https://doi.org/10.1093/infdis/jiab412

MATERIALS AND METHODS

Study Patients

The study cohort included patients who received their first TCD HCT at Memorial Sloan Kettering Cancer Center (MSKCC) from January 2012 through December 2016. Clinical and laboratory data were extracted from hospital databases and medical record reviews. The study was reviewed and approved by the MSKCC Institutional Review Board.

Conditioning Regimens and Supportive Care

Conditioning regimens included clofarabine/thiotepa/melphalan, busulfan/fludarabine/melphalan, or total body irradiation (TBI)/thiotepa with either cyclophosphamide or fludarabine, as previously described [25, 27]. $CD34^+$ selection was performed by using the CliniMACS CD34 Reagent system (Miltenyi Biotec).

Supportive Care

Antifungal and antibacterial prophylaxis was administered per institutional standard of care [28, 29]. All patients received acyclovir prophylaxis starting 7 days prior to transplant. Cytomegalovirus (CMV)–seropositive recipients (R^+) or recipients with CMV-seropositive donors (D^+) were monitored routinely for CMV by qPCR and treated preemptively per MSKCC standard of care [30, 31].

HHV-6 Monitoring

Monitoring for HHV-6 by qPCR started on day (D) +14 posttransplant and continued weekly through D+60 and at least once every 2 weeks through D+100. HHV-6 PCR in plasma was performed by Viracor-IBT. The linear range of quantitation was $100–1 \times 10^{10}$ copies/mL between January 2012 and March 2013 and $188–1 \times 10^8$ copies/mL after April 2013. Acute GVHD was scored by standard criteria of the Center for International Blood and Marrow Transplant Research [32]. Treatment of HHV-6 DNAemia was at the clinicians' discretion based on viral kinetics and overall clinical picture. There was no routine prophylaxis or preemptive therapy for HHV-6 DNAemia during the study period. Workup for HHV-6 end-organ disease (EOD) was triggered by clinical symptoms, and therapy for EOD was administered per standard of care [33].

CD4+ and CD8+ Monitoring

$CD4^+$ and $CD8^+$ were monitored by flow cytometry at D+100, 6 months, 9 months, and 12 months posttransplant.

Definitions

HHV-6 DNAemia was defined as ≥1 value of quantifiable viral load (VL). p-HHV-6 was defined as ≥2 consecutive VLs ≥500 copies/mL. HHV-6 encephalitis was defined as HHV-6 DNA in cerebrospinal fluid coinciding with acute onset of altered mental status or short-term memory loss or seizures [33]. HHV-6 lower respiratory tract disease was defined as (1) abnormal radiographic findings with lower respiratory tract symptoms and detectable HHV-6 DNA in bronchoalveolar lavage (BAL) in the absence of a causative organism; or (2) HHV-6 PCR positive in lung biopsy with organizing pneumonia and negative immunohistochemical stain (IHC) for CMV and adenovirus; or (3) detectable HHV-6 DNA in BAL and cytopathic changes consistent with the viral infection and negative IHC for CMV and adenovirus. Neutrophil engraftment was defined as the first date of absolute neutrophil count ≥500 cells/mL for 3 consecutive days. Platelet engraftment was defined as the first date of platelet count ≥20 000/mm^3 without transfusion support for 7 days. Comorbidities were assessed using the Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) [34, 35].

Statistical Analysis

Descriptive analyses were used to summarize baseline demographic, clinical, and transplant characteristics. Mann–Whitney rank-sum tests and Student *t* tests were performed to compare continuous variables. χ^2 tests or Fisher exact tests were used to compare categorical variables. Incidences of HHV-6 DNAemia and p-HHV-6 were estimated by cumulative incidence function. Patients were followed for 1 year post-HCT, relapse, second transplant, or death, whichever occurred first. Overall means of platelet counts (PLT), white blood cell counts (WBC), and absolute lymphocyte counts (ALC) between patients with and without p-HHV-6 from D+100 through 1 year posttransplant were compared using mixed-model analysis of variance. Least-square means were compared between patients with and without p-HHV-6 at specified time points in post hoc analyses. $CD4^+$ and $CD8^+$ lymphocyte counts were compared at D+100, 6 months, 9 months, and 12 months posttransplant. A landmark survival analysis from D+100 through 1 year posttransplant was performed by the Kaplan–Meier method, and relevant groups were compared by log-rank test.

Risk factors for repeated measures of blood cell counts from D+100 through 1 year posttransplant were examined by generalized estimating equations (GEEs). Arithmetic mean ratios (AMRs) were reported to estimate the effect of covariates. Risk factors of 1-year mortality were examined by Cox proportional hazard (PH) models with landmark method. Hazard ratios were reported to estimate the effect of covariates on mortality. Potential risk factors included age, sex, underlying diseases, donor's human leukocyte antigen match, TBI, donor and recipient CMV serostatus, HCT-CI, acute GVHD, CMV reactivation, and p-HHV-6. Acute GVHD, CMV reactivation, and p-HHV-6 by D+100 were entered as time-fixed covariates. Cumulative days on antiviral treatments were examined in GEE models. Receipt of antiviral therapy was examined in Cox PH models regardless of significance. Pearson correlation coefficients between these variables were estimated before performing multivariable models, and interactions were tested during

model selection. Variables with P value < .3 in the univariable model were entered in the full multivariable model. These variables were further selected in the final multivariable model using forward and backward selection based on the Akaike information criterion. All tests were 2-sided. P values < .05 were considered statistically significant. All statistical analyses were performed in R, version 4.0.3 software (R Foundation for Statistical Computing).

RESULTS

During the study period, 323 patients received TCD HCT. Eleven patients not monitored for HHV-6 DNAemia were excluded. The remaining 312 patients were included in the analyses. Table 1 shows the baseline characteristics in patients with and without p-HHV-6. One hundred eighty-three (59%) patients were male, 56 (17.9%) received mismatched donor allografts, 184 (59%) were CMV R^+, and 77 (25%) received TBI-containing conditioning. p-HHV-6 was more common among CMV-seronegative recipients (R^-).

Frequency of acute GVHD was similar between the 2 groups (P = .902). The time to neutrophil engraftment (median, 11 days [interquartile range {IQR}, 10–11] vs 10 days [IQR, 10–11]; P = .85) and platelet engraftment (median, 17 days [IQR, 15–20] vs 18 [IQR, 6–23]; P = .18) was similar between patients with and without p-HHV-6, respectively.

Incidence of p-HHV-6 DNAemia and HHV-6 End-Organ Disease

By D+100 post-HCT, 172 (55%) patients developed HHV-6 DNAemia at a median of 28 days (IQR, 24–39) and 83 (27%) developed p-HHV-6 at a median of 33 days (IQR, 25–43.5) (Figure 1). The median maximum VL for p-HHV-6 was 3.7 $\log_{10}$ copies/mL (IQR, 3.4–4.0; range, 2.8–4.7 $\log_{10}$ copies/mL).

Table 1. Baseline Characteristics

Characteristics	Overall (N = 312)	No Persistent HHV-6 DNAemia (n = 229)	Persistent HHV-6 DNAemia (n = 83)	P Value
Age, y, median (IQR)	54.8 (44.5–63.5)	54.7 (44.4–63.5)	55.4 (45.8–64.0)	.823
Sex				.859
Female	129 (41.3)	94 (41.0)	35 (42.2)	
Male	183 (58.7)	135 (59.0)	48 (57.8)	
Underlying disease				.174
Acute leukemia	150 (48.1)	118 (51.5)	32 (38.6)	
Myelodysplastic syndrome	60 (19.2)	38 (16.6)	22 (26.5)	
Chronic leukemia/MPD	24 (7.7)	19 (8.3)	5 (6.0)	
Multiple myeloma	75 (24.0)	52 (22.7)	23 (27.7)	
Nonhematologic malignancies	3 (1.0)	2 (0.9)	1 (1.2)	
Donor				.101
Matched related	96 (30.8)	75 (32.8)	21 (25.3)	
Matched unrelated	160 (51.3)	119 (52.0)	41 (49.4)	
Mismatched unrelated	56 (17.9)	35 (15.3)	21 (25.3)	
Conditioning regimen				.400
Busulfan/fludarabine/melphalan	226 (72.4)	161 (70.3)	65 (78.3)	
Clofarabine/thiotepa/melphalan	9 (2.9)	6 (2.6)	3 (3.6)	
TBI/thiotepa/cyclophosphamide	76 (24.4)	61 (26.6)	15 (18.1)	
TBI/thiotepa/fludarabine	1 (0.3)	1 (0.4)	0 (0.0)	
Total body irradiation				.103
No	235 (75.3)	167 (72.9)	68 (81.9)	
Yes	77 (24.7)	62 (27.1)	15 (18.1)	
Recipient CMV serostatus				.020
Negative	128 (41.0)	85 (37.1)	43 (51.8)	
Positive	184 (59.0)	144 (62.9)	40 (48.2)	
Recipient/donor CMV serostatus				.019
R^-/D^-	91 (29.2)	65 (28.4)	26 (31.3)	
R^-/D^+	37 (11.9)	20 (8.7)	17 (20.5)	
R^+/D^-	69 (22.1)	52 (22.7)	17 (20.5)	
R^+/D^+	115 (36.9)	92 (40.2)	23 (27.7)	
HCT comorbidity index				
0	62 (19.9)	21 (25.3)	41 (17.9)	.077
1–2	109 (34.9)	21 (25.3)	88 (38.4)	
≥3	141 (45.2)	41 (49.3)	100 (43.7)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviation: CMV, cytomegalovirus; D, donor; HCT, hematopoietic cell transplant; HHV-6, human herpesvirus 6; IQR, interquartile range; MPD, myeloproliferative disorder; R, recipient; TBI, total body irradiation; y, year.

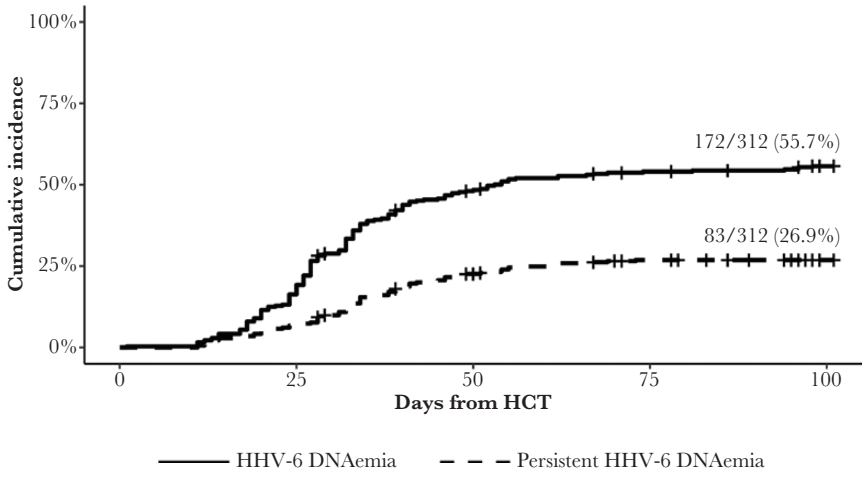


Figure 1. Cumulative incidence of human herpesvirus 6 DNAemia. Tick marks represent censoring events. Abbreviations: HCT, hematopoietic cell transplant; HHV-6, human herpesvirus 6.

HHV-6 EOD was diagnosed in 7 patients (2% of the entire cohort and 8% of patients with p-HHV-6) at a median of 171 days (IQR, 109–180). Maximum VL was higher in patients with HHV-6 EOD (median, 4.9 $\log_{10}$ copies/mL [IQR, 3.9–5.1]) than in patients without HHV-6 EOD (median, 3.2 $\log_{10}$ copies/mL [IQR, 2.7–3.9]) ($P = .003$). Diseases were encephalitis (n = 1), pneumonitis (n = 5), and organizing pneumonia (n = 1). An autopsy was performed on 1 patient with HHV-6 pneumonitis. Prior to death, HHV-6 VL was 125 000 copies/mL (5.1 $\log_{10}$ copies/mL) in the plasma and 30 900 copies/mL (4.5 $\log_{10}$ copies/mL) in the tracheal aspirate. Lung pathology was consistent with viral pneumonitis; IHC for CMV, adenovirus, and human herpes simplex virus (HSV) types 1 and 2 was negative.

Antiviral Treatment

One hundred thirteen (49%) patients without p-HHV-6 received antiviral treatment. One hundred ten patients received ganciclovir or valganciclovir [(Val)GCV] (94 patients) or foscarnet (16 patients) as first preemptive therapy for CMV. The remaining 3 patients received foscarnet for acyclovir-resistant HSV (2 patients) and nonspecified virus (1 patient).

Forty-five (54%) patients with p-HHV-6 received antiviral treatment; the indications were CMV preemptive therapy (n = 24) and HHV-6 infection (n = 21). Nineteen patients received (Val)GCV for CMV (18 patients) and HHV-6 (1 patient). Twenty-six patients received foscarnet for CMV (6 patients) and HHV-6 (20 patients). The choice and duration of antivirals were at physicians' discretion.

Correlation of Persistent HHV-6 DNAemia With Platelet and Lymphocyte Counts

PLT, ALC, and WBC through 1 year post-HCT were compared between patients with and without p-HHV-6 (Figure 2, Supplementary Figures 1 and 2). PLT and ALC were lower in patients with p-HHV-6 over time ($P = .014$ and $P < .0001$, respectively). WBC counts were similar between the 2 groups ($P = .467$).

When T-lymphocyte subsets were compared between the 2 groups, patients with p-HHV-6 had lower $CD4^+$ counts at D+100 and 6-months post-HCT (Figure 3A) and lower $CD8^+$ counts at D+100 and 12 months post-HCT (Figure 3B).

The association between blood counts and p-HHV-6 was examined using GEEs (Figure 4, Supplementary Table 1). p-HHV-6 was associated with lower PLT (adjusted AMR [aAMR], 0.84 [95% confidence interval {CI}, .72–.99]; $P = .034$); ALC (aAMR, 0.72 [95% CI, .57–.92]; $P = .003$), $CD4^+$ counts (aAMR, 0.49 [95% CI, .32–.75]; $P = 0.001$), and $CD8^+$ counts (aAMR, 0.63 [95% CI, .40–.99]; $P = .048$).

Additional predictors for lower PLT were acute GVHD grade 2–4 ($P = .027$), HCT-CI ≥3 ($P = .013$), and longer cumulative days on (Val)GCV ($P = .001$). Additional predictors for lower ALC were myelodysplastic syndrome ($P = .001$), mismatched donor ($P = .002$), TBI-containing regimen ($P = .034$), and more cumulative days on (Val)GCV ($P = .002$). In contrast, CMV reactivation ($P < .0001$) and time of ALC measurement ($P < .0001$) were associated with higher ALC.

For $CD4^+$ counts, in addition to p-HHV-6, mismatched donor ($P = .007$), cumulative days on (Val)GCV ($P = .001$), and cumulative days on foscarnet ($P = .003$) were associated with lower $CD4^+$. In contrast, CMV reactivation ($P = .0005$) and time of $CD4^+$ measurement post-HCT ($P < .0001$) were associated with increased $CD4^+$. The interaction between CMV reactivation and days on foscarnet was examined. Incremental increase on days on foscarnet was associated with 121% increase in $CD4^+$ among patients with CMV reactivation and 20% decrease in $CD4^+$ among patients without CMV reactivation.

For $CD8^+$ counts, in addition to p-HHV-6, mismatched donor ($P = .008$) and older age ($P = .002$) were associated with

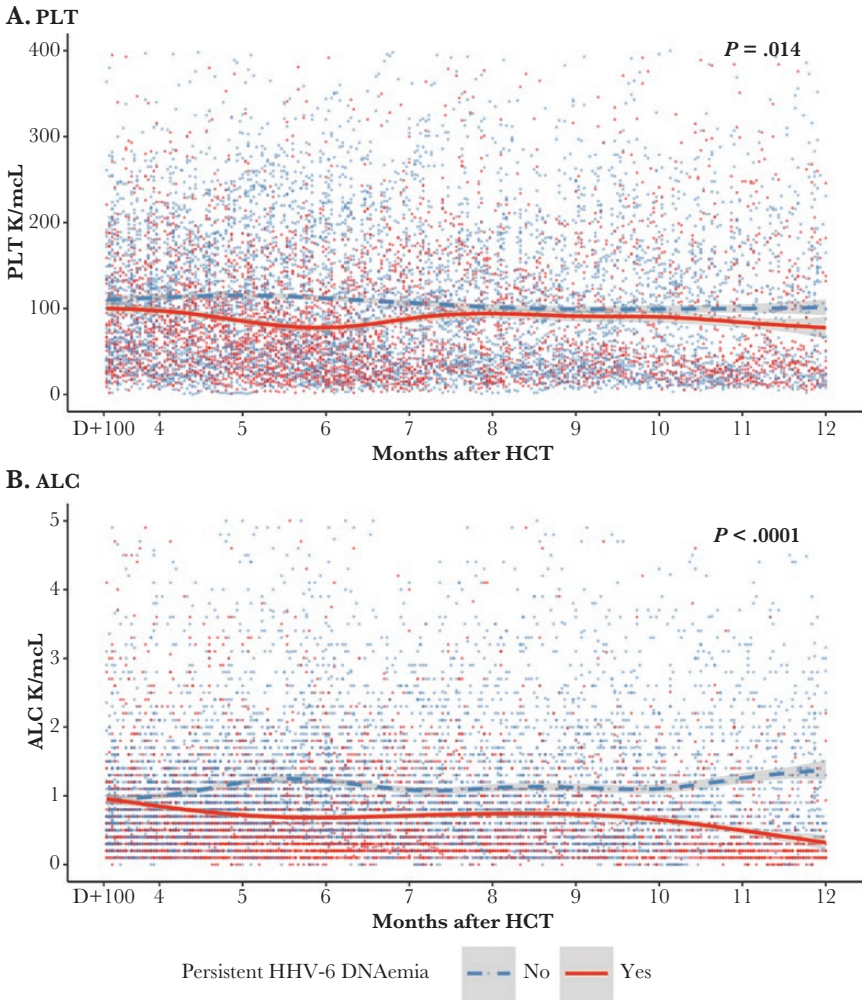


Figure 2. Curved scatter plot of platelet count (PLT) and absolute lymphocyte count (ALC) through 1 year after hematopoietic cell transplant (HCT). PLT and ALC over time in patients with persistent human herpesvirus 6 (HHV-6) DNAemia (line) and without persistent HHV-6 DNAemia (dotted line). In mixed-model analysis of variance, all values from day 100 through 1 year post-HCT are included. *A*, Patients with persistent HHV-6 DNAemia had lower PLT ($P = .014$). Least-square means of PLT were different at 4 months ($P = .035$), 5 months ($P = .044$), and 6 months ($P = .036$) post-HCT. *B*, Patients with persistent HHV-6 DNAemia had lower ALC ($P < .0001$). Least-square means of ALC were different at 5 months ($P = .025$), 6 months ($P = .004$), 7 months ($P = .002$), 8 months ($P = .016$), 11 months ($P = .022$), and 12 months ($P = .046$) post-HCT.

lower $CD8^+$. In contrast, CMV reactivation ($P < .0001$) and time of $CD8^+$ measurement post-HCT ($P < .0001$) were associated with higher $CD8^+$.

Correlation of Persistent HHV-6 DNAemia With Survival

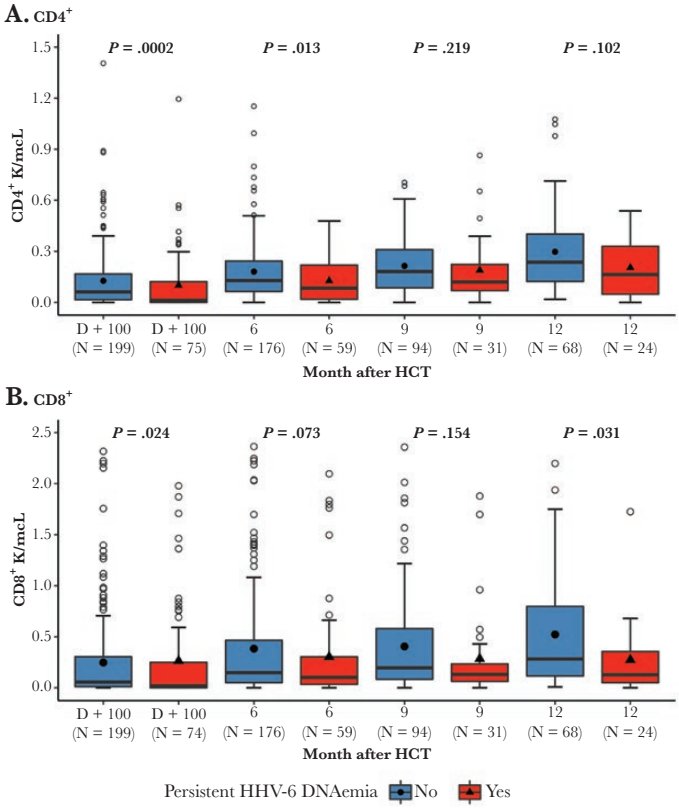
A landmark survival analysis was performed for patients alive on D+100. At D+100, 80 (96%) and 206 (90%) patients with and without p-HHV-6 were alive, respectively ($P = .102$). Overall survival at 1 year was lower for patients with p-HHV-6 than for patients without p-HHV-6 (58.8% vs 79.4%, respectively) ($P = .0001$; Figure 5).

Persistent HHV-6 by D+100 was entered as a categorical variable in multivariable models for mortality, and was associated with increased mortality (adjusted hazard ratio, 2.97 [95% CI, 1.62–5.47]; $P = .0005$) after adjusting for age, antiviral treatment, and ALC at D+100 (Table 2).

Cause of Death

By 1 year posttransplant, 27 of 83 (32.5%) patients and 49 of 229 (21.4%) patients with and without p-HHV-6 died (Supplementary Table 2). Infection was the most common cause of death in both groups, accounting for 37.0% and 36.7% of deaths among patients with and without p-HHV-6, respectively ($P = .979$). Transplant-related mortality accounted for 22.2% and 24.5% of deaths in patients with and without p-HHV-6, respectively ($P = 1.000$). Among patients alive at D+100, the proportion of patients with grade 2–4 acute GVHD by D+100 was similar between the 2 groups ($P = .881$). The proportion of patients with grade 2–4 GVHD by D+100 who were alive by 1 year post-HCT was similar between the 2 groups ($P = .140$).

Next, we compared GVHD-attributable deaths by 1 year post-HCT between the 2 groups. Overall, 11 deaths were attributed to GVHD, accounting for 29.6% and 6.1% of all



C. Median (IQR) of $CD4^+$ and $CD8^+$ lymphocyte counts.

Time from HCT	$CD4^+$		$CD8^+$	
	No persistent HHV-6 DNAemia	Persistent HHV-6 DNAemia	No persistent HHV-6 DNAemia	Persistent HHV-6 DNAemia
D + 100	0.06 (0.02–0.2)	0.01 (0–0.1)	0.06 (0–0.4)	0.02 (0–0.2)
6 months	0.1 (0.06–0.2)	0.08 (0.02–0.2)	0.2 (0.1–0.5)	0.1 (0–0.3)
9 months	0.2 (0.09–0.3)	0.1 (0.07–0.2)	0.2 (0.1–0.6)	0.1 (0.1–0.2)
12 months	0.2 (0.1–0.4)	0.2 (0.05–0.3)	0.3 (0.1–0.8)	0.1 (0.1–0.4)

CD4 and CD8: K/mcL

Figure 3. Comparison of $CD4^+$ (*A*) and $CD8^+$ (*B*) lymphocyte counts between patients without and with persistent human herpesvirus 6 (HHV-6) DNAemia at prespecified time points. Boxes represent the 25th to 75th percentiles. Horizontal lines within boxes represent the median, triangles represent the mean, whiskers represent the range, and circles represent outliers. *C*, Median (interquartile range [IQR]) of $CD4^+$ and $CD8^+$ lymphocyte counts at prespecified time points. $CD4^+$ and $CD8^+$ lymphocyte counts were compared between patients without and with persistent HHV-6 DNAemia at day 100 (D+100) and 6, 9, and 12 months post–hematopoietic cell transplant (HCT).

deaths in patients with p-HHV-6 and without p-HHV-6, respectively (P = .042). Among 8 patients with p-HHV-6 who died of GVHD, only 2 patients had acute GVHD grade 2–4 by D+100.

DISCUSSION

We analyzed a cohort of ex vivo TCD/$CD34^+$-selected HCT recipients to assess the impact of p-HHV-6 by D+100 on HCT outcomes. HHV-6 encephalitis was rare (1 of 312 patients) in the first year post-HCT and comparable with rates reported in T-cell–replete HCT allografts [6]. In multivariable analyses, p-HHV-6 was an independent predictor for lower PLT, ALC, $CD4^+$, and $CD8^+$ counts through 1 year post-HCT. Importantly, p-HHV-6 was an independent predictor of all-cause mortality at 1 year post-HCT.

$CD34^+$-selected allografts contain a low number of donor T cells, resulting in low GVHD rates and delayed immune reconstitution with reported median $CD4^+$ T-cell count 135 cells/μL and 252 cells/μL at 6 months and 12 months, respectively (lower limit of normal, 359 cells/μL) [36, 37]. Given the critical role of T cells in controlling HHV-6 viremia [18–20], we expected rates of HHV-6 encephalitis in TCD to be higher than reported for T-cell–replete HCT. Surprisingly, the incidence of HHV-6 encephalitis was 0.03% for the entire cohort and 1.2% among patients with p-HHV-6. Similar rates are reported in T-cell–replete recipients, from matched donor allografts or cord blood transplant recipients who did not receive antithymocyte globulin [2]. In contrast, rates up to 11.5% have been reported after in vivo T-cell depletion with alemtuzumab or 28% among cord blood allograft recipients who received

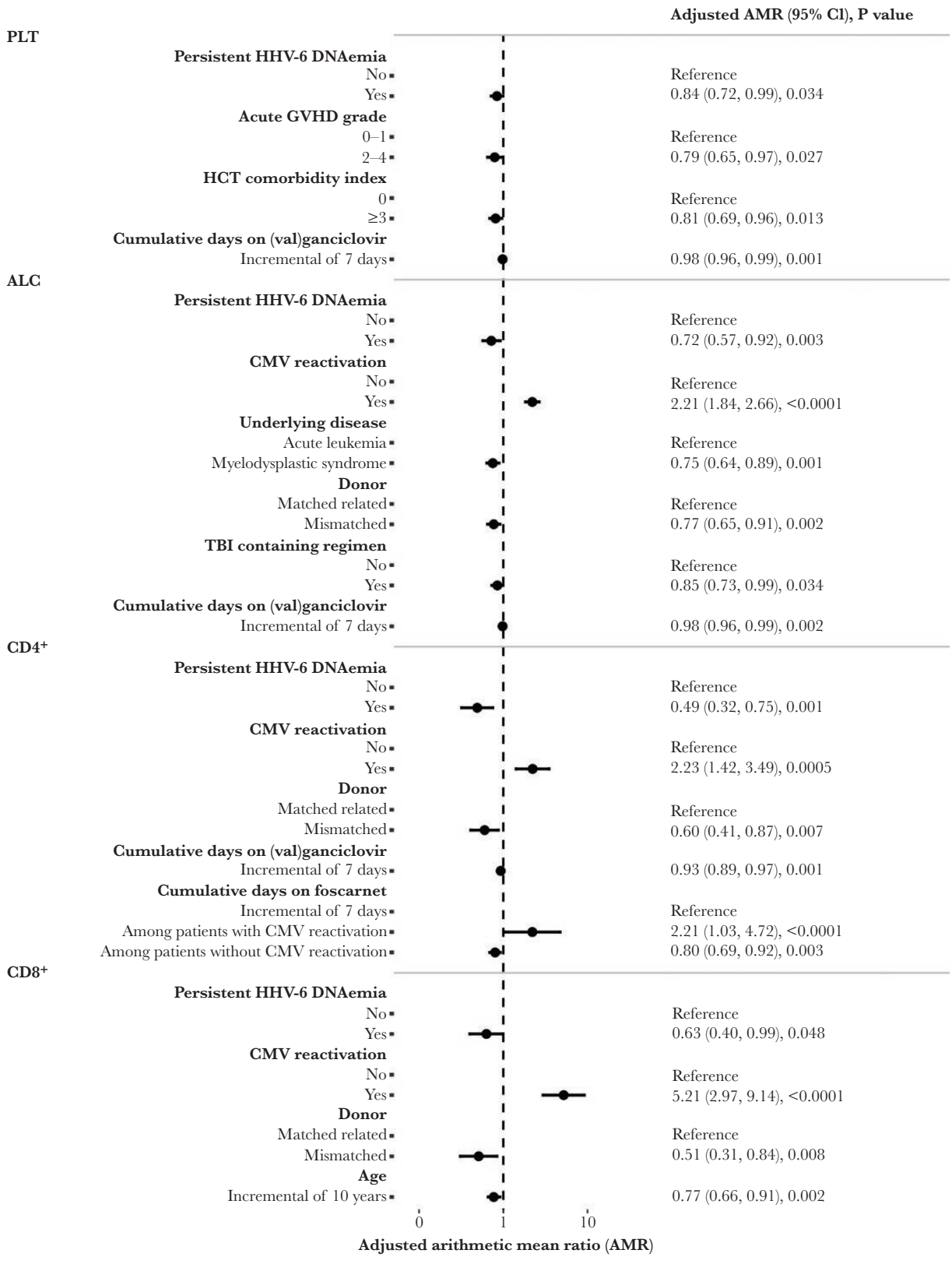


Figure 4. Multivariable predictors for blood counts from day 100 through 1 year post– hematopoietic cell transplant. Abbreviations: ALC, absolute lymphocyte count; AMR, arithmetic mean ratio; CI, confidence interval; CMV, cytomegalovirus; GVHD, graft-vs-host disease; HCT, hematopoietic cell transplant; HHV-6, human herpesvirus 6; PLT, platelet count; TBI, total body irradiation.

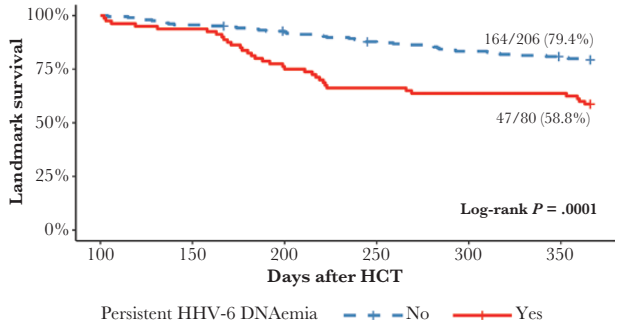


Figure 5. Landmark survival analysis from day 100 (D+100) through 1 year post–hematopoietic cell transplant. Patients alive at D+100 were included in the landmark analysis (n = 286). Tick marks represent censored patients. Abbreviations: HCT, hematopoietic cell transplant; HHV-6, human herpesvirus 6.

antithymocyte globulin [8, 38]. In our cohort, CMV and HHV-6 viremias occurred at a similar time post-HCT [24]. Foscarnet was used for treatment of HHV-6 in 20 (~25%) patients with p-HHV-6 viremia. Among patients with p-HHV-6, 54% received antiviral treatment by D+100. Foscarnet use may have decreased the incidence of HHV-6 encephalitis, although the role of preemptive treatment of HHV-6 viremia for prevention of HHV-6 encephalitis remains controversial [39]. Interestingly, 6 patients (7% p-HHV-6) had pulmonary complications associated with p-HHV-6. An effect of HHV-6 replication in the lung has been reported in experimental models and clinical studies. Reactivation of a murine homolog of HHV-6 caused acute lung injury [40]. Occult infections, including HHV-6, have been associated with idiopathic pulmonary syndrome [41]; yet a causal relationship and the underlying pathophysiology are poorly understood.

Next, we examined the impact of p-HHV-6 on WBC, ALC, and PLT. In vitro, HHV-6 infects bone marrow cells and inhibits granulocyte, macrophage, and megakaryocyte growth [42, 43]. HHV-6 reactivation has been associated with delayed platelet engraftment, frequent platelet transfusion post-HCT [14], and delayed immune reconstitution [16, 17, 21]. In our cohort, we did not observe any effect of p-HHV-6 on platelet engraftment, yet patients with p-HHV-6 had lower PLT but similar WBC in the first 1-year post-HCT.

Importantly, ALC in the first year post-HCT was lower in patients with p-HHV-6. When we examined $CD4^+$ and $CD8^+$ counts at prespecified time points, $CD4^+$ was lower at D+100 and 6 months posttransplant and $CD8^+$ was lower at D+100 and 12 months posttransplant. In GEE analyses, HHV-6 was an independent predictor for lower ALC, $CD4^+$, and $CD8^+$ counts after adjustment for covariates including cumulative antiviral therapy. In contrast, CMV reactivation was independently associated with higher ALC, $CD4^+$, and $CD8^+$.

In pediatric HCT recipients [37], HHV-6 viremia was associated with lymphopenia and treatment of HHV-6 viremia was associated with improved $CD4^+$ T-cell reconstitution [17]. Currently, there are no antivirals approved for HHV-6. A post hoc analysis of the brincidofovir trial for CMV prevention showed a reduced incidence of HHV-6 DNAemia in patients randomized to brincidofovir [44]. Further studies are needed to assess if the prevention or preemption of HHV-6 impacts immune recovery.

Persistent HHV-6 was an independent predictor of mortality after adjusting for age, antiviral treatment, and ALC at D+100. Because increased ALC at D+100 was associated with lower mortality, the interaction between ALC at D+100, and p-HHV-6 was examined and was dropped during the selection process. An association between HHV-6 viremia and increased mortality has been previously reported in T-cell–replete HCT [14, 45]. HCT recipients with HHV-6–positive results in BAL had increased overall mortality and death from respiratory failure compared with patients without HHV-6 in BAL [46].

In ex vivo TCD HCT, T-cell reconstitution and $CD4^+$ T-cell function correlate with improved HCT outcomes, including survival [36, 47, 48]. We postulate that delayed immune reconstitution and late onset or worsening of acute GVHD after D+100 may have contributed to worse survival of patients with p-HHV-6. Deaths due to GVHD were more frequent among patients with p-HHV-6 than patients without p-HHV-6. Importantly, 6 of the 8 (75%) patients with p-HHV-6 who died of GVHD had only mild (grade 0–1) acute GVHD by D+100. We hypothesize that delayed immune reconstitution [36] and immune dysregulation related to p-HHV-6 may have favored late onset or worsening of acute GVHD and may explain the disproportionate number of deaths attributed to GVHD among patients with p-HHV-6.

There are several limitations to our study. First, CMV R^+ comprised almost 60% of our cohort. During the study, the majority of CMV R^+ developed CMV viremia and received preemptive therapy, which may have impacted the incidence of HHV-6 DNAemia and immune reconstitution. Poor immune reconstitution (including low ALC and $CD4^+$ counts) are associated with viral infections and an increased risk of death after TCD HCT. While all efforts were made to adjust for covariates in our retrospective analyses, future randomized studies with an antiviral active against HHV-6 may be required to assess the impact of HHV-6 on mortality. Since December 2017, CMV prevention with letermovir has been implemented in our institution [31]. It is plausible that effective prevention of CMV viremia and reducing toxicities related to CMV antivirals may enhance immune recovery and indirectly mitigate adverse outcomes related to other viruses including HHV-6. Because of the low numbers of HHV-6 disease, we could not assess differences in rates of HHV-6 disease among CMV R^+ and CMV R^-. Second, acute GVHD rates by D+100 were not different among patients with and without p-HHV-6; however, our study was not powered to detect difference given the low incidence of GVHD in TCD HCT and late-onset acute GVHD after D+100

Table 2. Univariable and Multivariable Cox Proportional Hazard Models for All-Cause Mortality by 1 Year Posttransplant

	Univariable Analysis		Multivariable Analysis	
Characteristic	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
Age				
Incremental of 10 y	1.44 (1.09–1.90)	.004	1.49 (1.13–1.96)	.005
Sex				
Female	Reference		…	
Male	1.05 (.57–1.94)	.877	…	
Underlying disease				
Acute leukemia	Reference		…	
Myelodysplastic syndrome	1.36 (.57–3.25)	.483	…	
Multiple myeloma	2.74 (1.35–5.56)	.005	…	
Chronic leukemia/MPD	0.86 (.20–3.79)	.843	…	
Nonhematologic malignancies	4.38 (.57–33.42)	.155	…	
Donor				
Matched related	Reference		…	
Mismatched	2.17 (1.13–4.17)	.021	…	
TBI-containing regimen				
No	Reference		…	
Yes	0.39 (.15–1.00)	.050	…	
Donor CMV serostatus				
Negative	Reference		…	
Positive	1.55 (.84–2.85)	.163	…	
Recipient CMV serostatus				
Negative	Reference		…	
Positive	1.39 (.74–2.62)	.305	…	
HCT comorbidity index				
0	Reference		…	
1–2	2.14 (.78–5.84)	.138	…	
≥3	2.23 (.84–5.91)	.108	…	
CMV reactivation[a]				
No	Reference		…	
Yes	1.24 (.68–2.28)	.480	…	
Persistent HHV-6 DNAemia[a]				
No	Reference		Reference	
Yes	3.30 (1.80–6.05)	.0001	2.97 (1.62–5.47)	.0005
Acute GVHD grade (by D+100)[a]				
0–1	Reference		…	
2–4	1.90 (.95–3.77)	.068	…	
Antiviral therapy				
No			Reference	
Yes	3.34 (1.64–6.80)	.0009	4.47 (2.19–9.13)	<.0001
ALC at D+100				
Incremental of 1 K/mcL	0.27 (.13–.57)	.0007	0.24 (.12–.49)	.0001

Patients alive at D+100 are included in the analysis.

Abbreviation: ALC, absolute lymphocyte count; CI, confidence interval; CMV, cytomegalovirus; D+100, day 100 after transplant; GVHD, graft-vs-host disease; HCT, hematopoietic cell transplant; HHV-6, human herpesvirus 6; MPD, myeloproliferative disorder; TBI, total body irradiation.

[a]CMV reactivation, persistent HHV-6 DNAemia, and acute GVHD were included as time-fixed covariates.

was not examined. The relationship between early p-HHV-6 and late onset or worsening of acute GVHD merits further investigation in larger cohorts of TCD HCT. Third, missing $CD4^+$ and $CD8^+$ count measurements at 9 and 12 months may have introduced a bias. Acknowledging these limitations, our study shows a negative correlation between p-HHV-6 DNAemia and immune recovery and overall survival at 1 year after ex vivo TCD HCT. Novel therapeutic interventions are needed to mitigate the impact of HHV-6 on immune reconstitution and survival after ex vivo TCD HCT.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of

all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health (NIH).

Financial support. This work was supported by the NIH (award number P01 CA23766) and the NIH/National Cancer Institute (Cancer Center Support Grant number P30 CA008748).

Potential conflicts of interest. G. A. P. has served as an investigator for Chimerix, Astellas, Merck & Co, and Shire/Takeda; has received research grant support from Merck, Astellas, and Chimerix; has received consulting and other fees from Chimerix, Astellas, Merck, Cidara, Amplyx, AlloVir, Shionogi, Partners Therapeutics, ADMA Biologics, and Siemens Healthineers; and serves in a volunteer capacity as Chair of the Infectious Disease Special Interest Group of the American Society for Transplantation and Cellular Therapy (ASTCT). M. A. P. reports honoraria from AbbVie, Astellas, Bristol-Myers Squibb, Celgene, Equilium, Incyte, Karyopharm, Kite/Gilead, Merck, Miltenyi Biotec, MorphoSys, Novartis, Nektar Therapeutics, Omeros, Takeda, and VectivBio AG; serves on data and safety monitoring boards for Cidara Therapeutics, Medigene, Sellas Life Sciences, and Servier; serves on the scientific advisory board of NexImmune; has ownership interests in NexImmune and Omeros; has received research support for clinical trials from Incyte, Kite/Gilead, Miltenyi Biotec, and Novartis; and serves in a volunteer capacity as a member of the Board of Directors of the ASTCT and Be The Match (National Marrow Donor Program), as well as on the Executive Committee of the Center for International Blood and Marrow Transplant Research Cellular Immunotherapy Data Resource. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Miura H, Kawamura Y, Hattori F, et al. Late-phase human herpesvirus 6B reactivation in hematopoietic stem cell transplant recipients. Transpl Infect Dis **2018**; 20:e12916.
2. Olson AL, Dahi PB, Zheng J, et al. Frequent human herpesvirus-6 viremia but low incidence of encephalitis in double-unit cord blood recipients transplanted without antithymocyte globulin. Biol Blood Marrow Transplant **2014**; 20:787–93.
3. Zerr DM. Human herpesvirus 6 and central nervous system disease in hematopoietic cell transplantation. J Clin Virol **2006**; 37(Suppl 1):S52–6.
4. Ogata M, Oshima K, Ikebe T, et al. Clinical characteristics and outcome of human herpesvirus-6 encephalitis after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant **2017**; 52:1563–70.
5. Mori Y, Miyamoto T, Nagafuji K, et al. High incidence of human herpes virus 6-associated encephalitis/myelitis following a second unrelated cord blood transplantation. Biol Blood Marrow Transplant **2010**; 16:1596–602.
6. Fujimaki K, Mori T, Kida A, et al. Human herpesvirus 6 meningoencephalitis in allogeneic hematopoietic stem cell transplant recipients. Int J Hematol **2006**; 84:432–7.
7. Zerr DM, Gupta D, Huang ML, Carter R, Corey L. Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients. Clin Infect Dis **2002**; 34:309–17.
8. Hill JA, Koo S, Guzman Suarez BB, et al. Cord-blood hematopoietic stem cell transplant confers an increased risk for human herpesvirus-6–associated acute limbic encephalitis: a cohort analysis. Biol Blood Marrow Transplant **2012**; 18:1638–48.
9. Aoki J, Numata A, Yamamoto E, Fujii E, Tanaka M, Kanamori H. Impact of human herpesvirus-6 reactivation on outcomes of allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant **2015**; 21:2017–22.
10. Phan TL, Carlin K, Ljungman P, et al. Human herpesvirus-6B reactivation is a risk factor for grades II to IV acute graft-versus-host disease after hematopoietic stem cell transplantation: a systematic review and meta-analysis. Biol Blood Marrow Transplant **2018**; 24:2324–36.
11. Zerr DM, Boeckh M, Delaney C, et al. HHV-6 reactivation and associated sequelae after hematopoietic cell transplantation. Biol Blood Marrow Transplant **2012**; 18:1700–8.
12. Zerr DM, Fann JR, Breiger D, et al. HHV-6 reactivation and its effect on delirium and cognitive functioning in hematopoietic cell transplantation recipients. Blood **2011**; 117:5243–9.
13. Dulery R, Salleron J, Dewilde A, et al. Early human herpesvirus type 6 reactivation after allogeneic stem cell transplantation: a large-scale clinical study. Biol Blood Marrow Transplant **2012**; 18:1080–9.
14. Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. Clin Infect Dis **2005**; 40:932–40.
15. Ljungman P, Wang FZ, Clark DA, et al. High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. Br J Haematol **2000**; 111:774–81.

16. Admiraal R, de Koning CCH, Lindemans CA, et al. Viral reactivations and associated outcomes in the context of immune reconstitution after pediatric hematopoietic cell transplantation. J Allergy Clin Immunol **2017**; 140:1643–50.e9.
17. de Koning C, Admiraal R, Nierkens S, Boelens JJ. Human herpesvirus 6 viremia affects T-cell reconstitution after allogeneic hematopoietic stem cell transplantation. Blood Adv **2018**; 2:428–32.
18. Yasukawa M, Inoue Y, Ohminami H, Terada K, Fujita S. Apoptosis of CD4+ T lymphocytes in human herpesvirus-6 infection. J Gen Virol **1998**; 79(Pt 1):143–7.
19. Inoue Y, Yasukawa M, Fujita S. Induction of T-cell apoptosis by human herpesvirus 6. J Virol **1997**; 71:3751–9.
20. Patel SJ, Zhao G, Penna VR, et al. A murine herpesvirus closely related to ubiquitous human herpesviruses causes T-cell depletion. J Virol **2017**; 91:e02463.
21. Wang FZ, Linde A, Dahl H, Ljungman P. Human herpesvirus 6 infection inhibits specific lymphocyte proliferation responses and is related to lymphocytopenia after allogeneic stem cell transplantation. Bone Marrow Transplant **1999**; 24:1201–6.
22. Tzannou I, Papadopoulou A, Naik S, et al. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, Epstein-Barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. J Clin Oncol **2017**; 35:3547–57.
23. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV-6 infections after HSCT. Sci Transl Med **2014**; 6:242ra83.
24. Huang YT, Kim SJ, Lee YJ, et al. Co-infections by double-stranded DNA viruses after ex vivo T cell-depleted, CD34+ selected hematopoietic cell transplantation. Biol Blood Marrow Transplant **2017**; 23:1759–66.
25. Tamari R, Oran B, Hilden P, et al. Allogeneic stem cell transplantation for advanced myelodysplastic syndrome: comparison of outcomes between CD34+ selected and unmodified hematopoietic stem cell transplantation. Biol Blood Marrow Transplant **2018**; 24:1079–87.
26. Cho C, Perales MA. Expanding therapeutic opportunities for hematopoietic stem cell transplantation: T cell depletion as a model for the targeted allograft. Annu Rev Med **2019**; 70:381–93.
27. Jakubowski AA, Small TN, Kernan NA, et al. T cell-depleted unrelated donor stem cell transplantation provides favorable disease-free survival for adults with hematologic malignancies. Biol Blood Marrow Transplant **2011**; 17:1335–42.
28. Rosillo C, Avila AM, Huang YT, et al. Sequential systematic anti-mold prophylaxis with micafungin and voriconazole results in very low incidence of invasive mold infections in patients undergoing allogeneic hematopoietic stem cell transplantation. Transpl Infect Dis **2018**; 20:e12897.
29. Seo SK, Xiao K, Huang YT, et al. Impact of peri-transplant vancomycin and fluoroquinolone administration on rates of bacteremia in allogeneic hematopoietic stem cell transplant (HSCT) recipients: a 12-year single institution study. J Infect **2014**; 69:341–51.
30. Zavras P, Su Y, Fang J, et al. Impact of preemptive therapy for cytomegalovirus on toxicities after allogeneic hematopoietic cell transplantation in clinical practice: a retrospective single-center cohort study. Biol Blood Marrow Transplant **2020**; 26:1482–91.
31. Lin A, Maloy M, Su Y, et al. Letermovir for primary and secondary cytomegalovirus prevention in allogeneic hematopoietic cell transplant recipients: real-world experience. Transpl Infect Dis **2019**; 21:e13187.
32. Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR severity index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol **1997**; 97:855–64.
33. Ward KN, Hill JA, Hubacek P, et al. Guidelines from the 2017 European Conference on Infections in Leukaemia for management of HHV-6 infection in patients with hematologic malignancies and after hematopoietic stem cell transplantation. Haematologica **2019**; 104:2155–63.
34. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the disease risk index for allogeneic stem cell transplantation. Blood **2014**; 123:3664–71.
35. Sorror ML, Logan BR, Zhu X, et al. Prospective validation of the predictive power of the hematopoietic cell transplantation comorbidity index: a Center for International Blood and Marrow Transplant Research Study. Biol Blood Marrow Transplant **2015**; 21:1479–87.
36. Goldberg JD, Zheng J, Ratan R, et al. Early recovery of T-cell function predicts improved survival after T-cell depleted allogeneic transplant. Leuk Lymphoma **2017**; 58:1859–71.
37. Small TN, Papadopoulos EB, Boulad F, et al. Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions. Blood **1999**; 93:467–80.
38. Vu T, Carrum G, Hutton G, Heslop HE, Brenner MK, Kamble R. Human herpesvirus-6 encephalitis following allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant **2007**; 39:705–9.
39. Ishiyama K, Katagiri T, Hoshino T, Yoshida T, Yamaguchi M, Nakao S. Preemptive therapy of human herpesvirus-6 encephalitis with foscarnet sodium for high-risk patients after hematopoietic SCT. Bone Marrow Transplant **2011**; 46:863–9.
40. Zhou X, O'Dwyer DN, Xia M, et al. First-onset herpesviral infection and lung injury in allogeneic hematopoietic

cell transplantation. Am J Respir Crit Care Med **2019**; 200:63–74.
41. Seo S, Renaud C, Kuypers JM, et al. Idiopathic pneumonia syndrome after hematopoietic cell transplantation: evidence of occult infectious etiologies. Blood **2015**; 125:3789–97.
42. Knox KK, Carrigan DR. In vitro suppression of bone marrow progenitor cell differentiation by human herpesvirus 6 infection. J Infect Dis **1992**; 165:925–9.
43. Isomura H, Yoshida M, Namba H, et al. Suppressive effects of human herpesvirus-6 on thrombopoietin-inducible megakaryocytic colony formation in vitro. J Gen Virol **2000**; 81:663–73.
44. Hill JA, Nichols WG, Marty FM, et al. Oral brincidofovir decreases the incidence of HHV-6B viremia after allogeneic HCT. Blood **2020**; 135:1447–51.
45. de Pagter PJ, Schuurman R, Visscher H, et al. Human herpes virus 6 plasma DNA positivity after hematopoietic stem cell transplantation in children: an important risk factor for clinical outcome. Biol Blood Marrow Transplant **2008**; 14:831–9.
46. Hill JA, Vande Vusse LK, Xie H, et al. Human herpesvirus 6B and lower respiratory tract disease after hematopoietic cell transplantation. J Clin Oncol **2019**; 37:2670–81.
47. de Koning C, Prockop S, van Roessel I, et al. $CD4^+$ T-cell reconstitution predicts survival outcomes after acute graft-versus-host-disease: a dual-center validation. Blood **2021**; 137:848–55.
48. van Roessel I, Prockop S, Klein E, et al. Early $CD4^+$ T cell reconstitution as predictor of outcomes after allogeneic hematopoietic cell transplantation. Cytotherapy **2020**; 22:503–10.