Open Forum Infectious Diseases

MAJOR ARTICLE

Cytomegalovirus Viral Load Continues to Predict Poor Outcomes in Adults and Children Despite Improved Hematopoietic Cell Transplantation Success

Alicja Sadowska-Klasa,[1,2] Hu Xie,[3] Danniel Zamora,[1] Alpana Waghmare,[1,4,5] Joshua A. Hill,[1,3,4] Elizabeth R. Duke,[6] Margaret L. Green,[4] Masumi Ueda Oshima,[7] Brenda M. Sandmaier,[7,8] Keith R. Jerome,[1,4] Wendy M. Leisenring,[3] and Michael Boeckh[1,3,4]

[1]Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA, [2]Department of Hematology and Transplantology, Medical University of Gdańsk, Gdańsk, Poland, [3]Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, Washington, USA, [4]Division of Allergy and Infectious Diseases, University of Washington, Seattle, Washington, USA, [5]Center for Clinical and Translational Research, Seattle Children's Hospital, Seattle, Washington, USA, [6]Veterans Affairs Palo Alto Health Care System, Palo Alto, California, USA, [7]Division of Hematology and Oncology, University of Washington, Seattle, WA,USA, and [8]Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, WA, USA

Background. Recent advances of graft-versus-host disease prevention strategies have led to improved overall survival after hematopoietic cell transplantation (HCT). Whether cytomegalovirus (CMV) viral load continues to predict CMV disease, overall mortality, and nonrelapse mortality is poorly defined.

Methods. CMV-seropositive patients undergoing allogeneic HCT between 2007 and 2017 with weekly CMV DNA polymerase chain reaction surveillance and preemptive therapy were analyzed. Multivariate Cox proportional hazards models were used to estimate the association between CMV viral load by day 100 at different thresholds with CMV disease and overall and nonrelapse mortality up to 1 year post-HCT.

Results. Of 1539 patients who received a transplant in the study period, 1349 survived >100 days after HCT and were included in the analyses. By day 100 post-HCT, CMV reactivation at any level was observed in 76%, with the lowest incidence at all levels in young children. Pediatric patients had significantly less CMV disease compared to the adult population. CMV reactivation was associated with a higher risk of CMV disease (adjusted hazard ratio, 6.8 [95% confidence interval, 3.54–13]); it was also associated with overall and nonrelapse mortality among day 100 survivors, although the association was diminished recently. The strongest correlation was still apparent between viral load and mortality in patients with a viral load >3 $\log_{10}$ and lymphocyte count <300 cells/μL.

Conclusions. CMV viral load continued to be a strong predictor for CMV disease. With improved transplantation techniques, the association of viral load with overall and nonrelapse mortality is diminished, but the effect was still present in patients with severe immunosuppression.

Keywords. CMV viral load; association with mortality; CMV disease.

Cytomegalovirus (CMV) viral load has been accepted as a surrogate endpoint for phase 3 clinical trials in hematopoietic cell transplant (HCT) recipients due to its association with CMV disease and overall mortality (OM) and nonrelapse mortality (NRM) after HCT, and recent interventional trials have used it as a primary endpoint. However, the data supporting the surrogacy claim are derived from older cohorts and trials when transplantation techniques differed significantly and OM and NRM rates were higher [1–3]. Furthermore, current US Food and Drug Administration approvals for pediatric populations rely on clinical trials conducted in adult patients, but this population lacks detailed data on viral load used as an endpoint. With the significant decline in mortality in transplant recipients, the role of CMV viremia as a contributing factor to NRM has not been consistently identified [4–7]. However, viral load is now used as a key part of the clinically significant CMV infection (csCMV) endpoint in recently completed [8–10], ongoing, and planned advanced-stage clinical trials of novel agents in HCT recipients for both treatment and prophylaxis.

A critical part of the determination of viral load as a surrogate is the accurate and comprehensive accounting for confounders and effect modifiers while considering causal pathways of the clinical endpoints [11, 12]. Previous studies

Received 07 July 2025; accepted 19 September 2025; published online 26 September 2025
Correspondence: Michael Boeckh, MD, PhD, Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Center, 1100 Fairview Ave N, Seattle, WA 98109, USA (mboeckh@fredhutch.org); Alicja Sadowska-Klasa, MD, PhD, Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Center, 1100 Fairview Ave N, Seattle, WA 98109, USA (asadowsk@fredhutch.org).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofaf612

used only graft-versus-host disease (GVHD) as a marker of the underlying immunosuppression. Thus, in the case of the association of CMV viral load and mortality endpoints, the models need to control for the underlying immunosuppression and the toxicity associated with the antiviral agents used for treatment.

This study sought to define the impact of CMV viral load on CMV disease and OM and NRM in the era characterized by modern GVHD prevention strategies and improved survival [1] in different age groups, including younger children and adolescents. We examined this question in a large cohort of patients receiving transplant before the adoption of universal prophylaxis, as large enough datasets in the letermovir era are slowly emerging [13]. We specifically analyzed how immunosuppression, as measured by lymphopenia and GVHD severity, and CMV treatment–related toxicities affect the correlation between CMV viral load and CMV disease, NRM, and OM.

METHODS

In this observational, retrospective cohort study, we analyzed CMV viremia and clinical outcomes in pediatric and adult patients receiving their first HCT at Fred Hutchinson Cancer Center (Fred Hutch; Seattle, Washington, USA) in 2 time periods: 1 January 2007 through 28 February 2013, which established the association of CMV viral load with NRM and OM [14]; and a later period. 1 March 2013 through 31 December 2017, which constitutes a period reflecting changing transplant practices and improved OM and NRM [1]. None of the included patients received letermovir prophylaxis. Further cohort details are available in the Supplementary Methods [15–17].

Eligible patients were CMV seropositive and provided written consent to have their clinical data used for retrospective research. The study protocol was approved by the Fred Hutch Institutional Review Board.

Preemptive antiviral therapy was initiated per institutional protocol, when viral load exceeded 50 IU/mL in high-risk groups (cord blood or human leukocyte antigen [HLA]–mismatched transplants, patients receiving at least 1 mg/kg body weight of prednisone or equivalent, or T-cell depletion) and when the viral load reached 150 IU/mL in the rest of the study population. After 100 days posttransplantation, patients who were thought to be at risk of late CMV disease were recommended to continue weekly CMV polymerase chain reaction (PCR) monitoring and started preemptive therapy if the viral load was >250 IU/mL [13]. For cord blood HCT recipients, we have used 3 different antiviral prevention strategies since 2006 involving high-dose valacyclovir and pretransplant valganciclovir prophylaxis as well as preemptive therapy at any CMV detection, as previously described [18].

Foscarnet-related renal dysfunction was defined as a kidney injury (doubling creatinine concentration from baseline) that occurred after start of foscarnet treatment or within 14 days after the stop date of treatment [19]. Ganciclovir (GCV)– or valganciclovir (VGCV)–related neutropenia was defined as nonrelapse-related neutropenia (absolute neutrophil count [ANC] <500 cells/μL) after the start of preemptive therapy or neutropenia that occurred within 14 days after the end of an antiviral treatment for PCR positivity in patients with an ANC >1000 cells/μL at the time of CMV infection [20].

Statistical Analysis

Kaplan-Meier and cumulative incidence estimation methods were used to initially estimate the incidence of OM and NRM, and CMV reactivation and CMV disease after transplantation. Cox proportional hazards regression models were used to estimate the association between CMV plasma viral load after transplantation with OM and NRM by 1 year using landmark analyses in patients who survived >100 days post-HCT to estimate correlation between maximum viral load and lymphopenia before day 100, and NRM and OM by 1 year post-HCT. For the association of viral load with CMV disease by day 100 or 1 year, post-HCT viral load and lymphopenia were considered time-dependent variables (lymphopenia was required to precede viral load). To show the relative contributions of viral load and lymphopenia graphically, as has previously been done in the human immunodeficiency virus setting [21], restricted cubic spline regression with 3 knots placed at the 25th, 50th, and 75th percentiles was used to explore the nonlinear association between CMV viral load in $\log_{10}$ scale and absolute lymphocyte counts (ALCs) with CMV disease rate, OM rate, and NRM rate. The spline curves fitted over the average CMV viral load in $\log_{10}$ scale stratified by ALC and transplant year were presented for data visualization [22].

Demographic and clinical factors assessed as potential covariates in each of the models were age at HCT, donor age, race, donor race, sex, donor sex, donor CMV serostatus, HLA matching, underlying disease risk index [23], HCT-specific comorbidity index [24], conditioning regimen intensity, cell source, year of transplantation, GVHD prophylaxis regimen, peak acute GVHD (time dependent), chronic GVHD requiring immunosuppressive treatment (time dependent), foscarnet-related renal dysfunction, lymphopenia, and GCV/VGCV-related neutropenia (time dependent). All covariates with $P < .05$ in the univariable analyses were selected into the multivariable models. Statistical significance was defined as 2-sided $P < .05$. SAS version 9.4 TS1M6 software (SAS Institute, Cary, North Carolina, USA) was used for all statistical analyses.

RESULTS

Of the 2137 patients initially selected for the study, 596 were excluded because of their negative CMV serostatus, and 5 did not sign informed consent. The remaining 1536 CMV-seropositive

Table 1. Basic Demographics of the Study Group

Variable	Category	Before 28 Feb 2013 (n = 789)	After 1 Mar 2013 (n = 747)	*P* Value
Age at transplant, y	0–11	71 (9)	88 (12)	.167
	12–17	43 (5)	38 (5)	
	18–40	161 (20)	145 (19)	
	41–60	316 (40)	266 (36)	
	>60	198 (25)	210 (28)	
Sex	Female	371 (47)	347 (46)	.823
	Male	418 (53)	400 (54)	
Female to male transplanth	Female to male	170 (22)	166 (22)	.935
Race	White	622 (79)	556 (74)	<.001
	American Indian or Alaska Native	15 (2)	17 (2)	
	Asian	73 (9)	78 (10)	
	Black or African American	17 (2)	25 (3)	
	Multiple	33 (4)	12 (2)	
	Native Hawaiian or other Pacific Islander	11 (1)	17 (2)	
	Unknown	18 (2)	42 (6)	
HCT-CI score	0–1	282 (36)	271 (36)	<.001
	2	118 (15)	111 (15)	
	≥3	266 (34)	351 (47)	
	Unknown	123 (16)	14 (2)	
Donor race	White	153 (19)	206 (28)	<.001
	Non-White	86 (11)	89 (12)	
	Unknown	550 (70)	452 (61)	
Donor sex	Female	355 (45)	326 (44)	.82
	Male	393 (50)	384 (51)	
	Missing	41 (5)	37 (5)	
Donor age, y	Median (range)	35.2 (1.0–77.8)	32.4 (0.2–74.5)	.211
CMV serostatus	Donor positive/Recipient positive	333 (42)	323 (43)	.682
	Donor negative/Recipient positive	456 (58)	424 (57)	
Conditioning regimen	Myeloablative	453 (57)	434 (58)	<.001
	Nonmyeloablative	235 (30)	127 (17)	
	Reduced intensity	101 (13)	186 (25)	
T-cell depletion	No	773 (98)	693 (93)	<.001
	Yes	16 (2)	54 (7)	
Cell source	Bone marrow	155 (20)	118 (16)	.143
	Cord blood	101 (13)	101 (14)	
	Peripheral stem cells	533 (68)	528 (71)	
Donor type	HLA-matched related	240 (30)	200 (27)	.023
	HLA-matched unrelated	358 (45)	323 (43)	
	HLA-haploidentical	54 (7)	62 (8)	
	Cord blood	101 (13)	101 (14)	
	HLA-mismatched unrelated	36 (5)	61 (8)	
GVHD prophylaxis	MMF + CNI	350 (44)	292 (39)	<.001
	MTX + CNI	313 (40)	272 (36)	
	PTCy-based	71 (9)	67 (9)	
	Other ± CNI	16 (2)	21 (3)	
	Sirolimus-based	39 (5)	95 (13)	
Underlying disease	Aplastic anemia	24 (3)	38 (5)	<.001
	Acute lymphoblastic leukemia	103 (13)	106 (14)	
	Acute myeloid leukemia	263 (33)	275 (37)	
	Chronic lymphocytic leukemia	58 (7)	11 (1)	
	Hodgkin lymphoma	16 (2)	11 (1)	
	Myelodysplastic syndrome	97 (12)	127 (17)	
	Chronic myeloproliferative neoplasms	67 (8)	54 (7)	
	Non-Hodgkin lymphoma	81 (10)	48 (6)	
	Plasma cell neoplasms	46 (6)	27 (4)	
	Primary immunodeficiency	16 (2)	22 (3)	

Table 1. Continued

Variable	Category	Before 28 Feb 2013 (n = 789)	After 1 Mar 2013 (n = 747)	*P* Value
	Other nonmalignant disorders	18 (2)	28 (4)	
Disease risk index	High	292 (37)	158 (21)	<.001
	Intermediate	64 (8)	64 (9)	
	Low	433 (55)	525 (70)	
No. of transplants	1	668 (85)	660 (88)	.035
	>1	121 (15)	87 (12)	
Acute GVHD	Grade 0–2	676 (86)	653 (87)	.319
	Grade 3–4	113 (14)	94 (13)	
Chronic GVHD	No	215 (27)	308 (41)	<.001
	Yes	574 (73)	439 (59)	
Early CMV disease before day 100	No	740 (94)	709 (95)	.341
	Yes	49 (6)	38 (5)	
Late CMV disease after day 100	No	737 (93)	711 (95)	.135
	Yes	52 (7)	36 (5)	
csCMV infection by 1 y	No	273 (35)	265 (35)	.72
	Yes	516 (65)	482 (65)	
Maximum viral load, IU/mL	Negative	160 (20)	183 (24)	<.001
	Positive to 150	231 (29)	139 (19)	
	>150–1000	329 (42)	363 (49)	
	>1000	69 (9)	62 (8)	
ALC day 100 cells/μL (last value prior to day 100)	<100	54 (7)	45 (6)	.72
	100–300	81 (10)	83 (11)	
	>300	654 (83)	619 (83)	
GCV/VGCV treatment by day 100	No	337 (43)	326 (44)	.713
	Yes	452 (57)	421 (56)	
GCV/VGCV-related neutropenia by day 100	No	701 (89)	677 (91)	.25
	Yes	88 (11)	70 (9)	
FCN treatment by day 100	No	593 (75)	573 (77)	.478
	Yes	196 (25)	174 (23)	
FCN-related renal dysfunction by day 100	No	726 (92)	691 (93)	.721
	Yes	63 (8)	56 (7)	

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

Abbreviations: ALC, absolute lymphocyte count; CMV, cytomegalovirus; CNI, calcineurin inhibitor; csCMV, clinically significant cytomegalovirus infection; FCN, foscarnet; GCV/VGCV, ganciclovir/valganciclovir; GVHD, graft-versus-host disease; HCT-CI, hematopoietic cell transplantation–specific comorbidity index; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; MTX, methotrexate; PTCy, posttransplantation cyclophosphamide.

HCT recipients were included in the final analysis. Numbers of pediatric and adult patients were similar between the 2 time periods. The basic demographic and clinical characteristics of the study group are presented in Table 1.

By day 100 after HCT, CMV reactivation at any level was observed in 76% of patients (Figure 1*A*), with no significant differences between the 2 time periods, and the numbers of high-level viremia did not change over the years (Supplementary Figure 1*A*). The incidence of CMV reactivation at different viral load thresholds varied between age groups (Figure 1*B*–*D*), with the lowest incidence at all levels in the youngest children (aged 0–11 years). Viral loads were similar between adolescents (aged 12–18 years) and adults overall; however, higher viral loads appeared to be more common in adult patients (Figure 1*C* and 1*D*). Peak viral loads during the first 100 days were higher in patients at high risk for CMV reactivation [9] (Supplementary Figure 1*B*). Antiviral drug utilization in the different age groups is shown in Supplementary Table 1.

Clinically significant CMV infection was observed in 61% of the study population by day 100, with no differences between adults and adolescents, but with a lower incidence in patients <12 years old (Figure 2*A*). No difference in csCMV infection incidence was observed between the 2 time periods (before 28 February 2013 and after 28 February 2013), including the entire study population (Supplementary Figure 1*C*).

In the first year after transplantation, 156 patients developed CMV disease (10.3% [95% confidence interval {CI}, 8.8%–11.2%]) with gastrointestinal disease being most common, followed by pneumonia. The incidence of CMV disease did not differ between younger children and adolescents; however, pediatric patients combined had significantly less CMV disease compared to the adult population. The numbers of proven gastrointestinal disease were significantly higher in the adult group (Figure 2*B*–*D*).

GCV- or VGCV-based preemptive therapy was used in 873 (57%) patients. Foscarnet treatment was applied in 370 (24%)

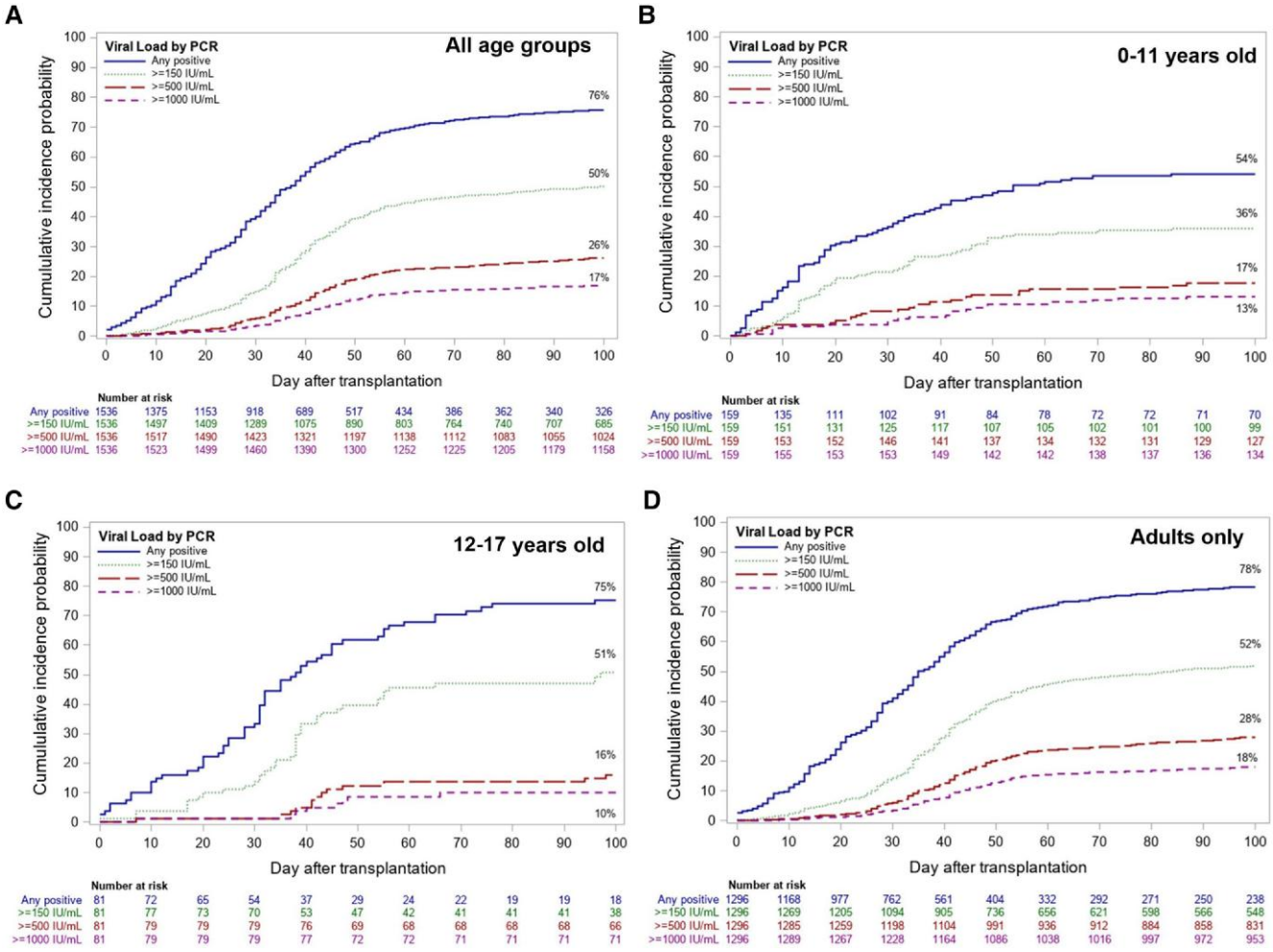

Figure 1. Cumulative incidence of cytomegalovirus (CMV) reactivation by day 100, stratified by viral load and by age group: all age groups (*A*), younger children 0–11 years old (*B*), adolescents 12–17 years old (*C*), and adults ≥18 years old (*D*).

cases; however, foscarnet as a first-line therapy was used in only 67 (4%) patients.

Among patients receiving antiviral treatment, GCV/VGCV-related neutropenia and foscarnet-associated kidney injury occurred in 158 (18%) and 119 (32%) patients, respectively. The median time to the onset of treatment-related toxicities after initiation of the therapy was 23 days (range, 2–54 days) for GCV/VGCV and 15 days (range, 1–67 days) for foscarnet.

Risk Factors for csCMV Infection

In multivariate models, including all patients and both time periods, the risk of developing csCMV infection was the highest in HLA-mismatched transplant recipients and patients suffering from acute grade 3–4 or chronic GVHD. It was decreased in younger children and in patients with sirolimus-based GVHD prophylaxis protocols (Supplementary Figure 3).

Lymphopenia as Confounder for the Viral Load CMV Disease Association

The role of viral load on the risk of CMV disease was assessed in multivariate models including both time periods and all ages. Models were adjusted for the year of transplantation, age, GVHD prophylaxis regimens, race, chronic GVHD (time dependent), and lymphopenia <100 cells/μL (time dependent) (Supplementary Figure 2). CMV reactivation was associated with a higher risk of CMV disease (adjusted hazard ratio [aHR], 6.8 [95% CI, 3.54–13]), and the risk was the highest when viral load exceeded 1000 IU/mL (aHR, 15 [95% CI, 7.29–30.6]). This effect was diminished by antiviral treatment but remained significant (aHR, 4.86 [95% CI, 2.25–10.5]). Patients <18 years of age had significantly decreased risk for developing CMV disease (aHR, 0.15 [95% CI, .05–.40]) (Figure 2*B* and Supplementary Figure 2).

Among day 100 survivors throughout the entire study period, we assessed the relative contribution of lymphopenia and CMV

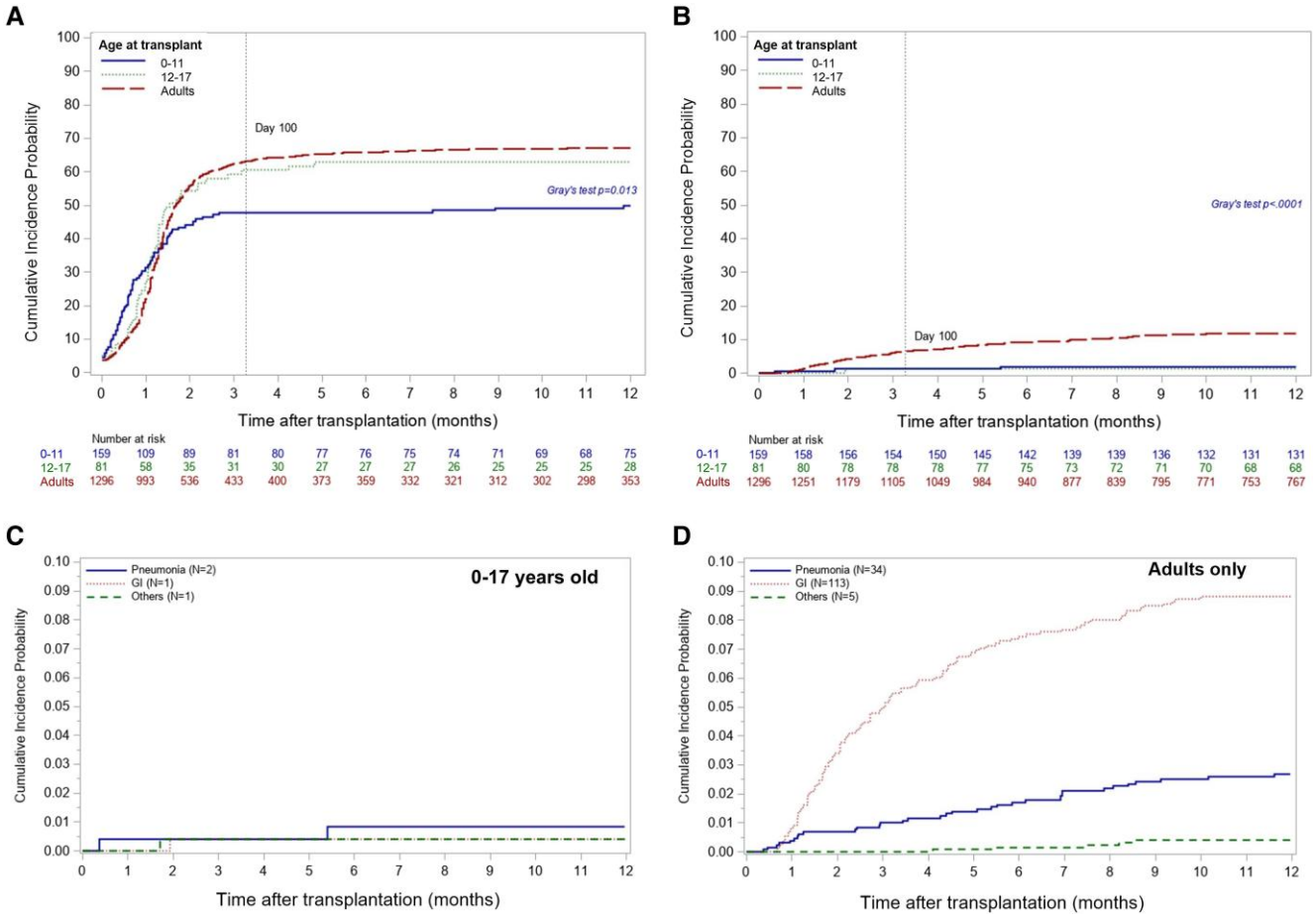

Figure 2. Cumulative incidence by 12 months posttransplant of clinically significant cytomegalovirus (CMV) infection (*A*), CMV disease (*B*), organ-specific manifestation in children aged 0–17 years (*C*), and organ-specific manifestation in adults aged ≥18 years (*D*), stratified by age of hematopoietic cell transplant recipient (*A* and *B*) or organ affected (*C* and *D*). Abbreviation: GI, gastrointestinal.

viral load to the risk of CMV disease using cubic spline analysis. Each declining lymphocyte counts and increasing CMV viral load increased the risk of CMV disease (Figure 3*A*). Next, we examined whether the association of CMV viral load with or without lymphopenia changed over time (Figure 3*B*). Viral load was associated with increased risk of CMV disease among patients with lymphopenia in both periods, with a plateau between 1.5 and 3.0 $\log_{10}$ when most patients received preemptive antiviral therapy. Among nonlymphopenia patients, the association was less pronounced, especially in the earlier cohort.

Overall and Nonrelapse Mortality

By 1 year post-HCT, 424 (28%) deaths were observed, 142 (9.2%) within the first 100 days after HCT. The probability of overall survival was 91% (95% CI, 88%–93%) by day 100, and 72% (95% CI, 69%–75%) by 1 year post-HCT. Cumulative NRM was 7% (95% CI, 6%–8%) by day 100% and 17% (95% CI, 15%–19%) by 1 year post-HCT.

There was a differential impact of CMV viral load on OM and NRM among day 100 survivors in the 2 time periods (Figure 4), with a diminished effect of viral load on both OM and NRM in the more recent time period (Supplementary Tables 2 and 3).

In multivariate Cox analyses, lymphopenia was adjusted to account for the level of immunosuppression. These results were confirmed for different viral thresholds (with the highest impact seen when the viral load exceeded >1000 IU/mL) and csCMV infection in the earlier period (Supplementary Tables 2 and 3, Supplementary Figure 4). Neither foscarnet-related renal toxicity nor GCV-related neutropenia affected NRM in multivariable models.

When we analyzed the impact of viral load on outcomes in different age groups in both time periods combined, NRM was significantly decreased in the pediatric population (aHR, 0.23 [95% CI, .10–.51]) irrespective of viral load; however, higher NRM was observed in children with the highest viremia

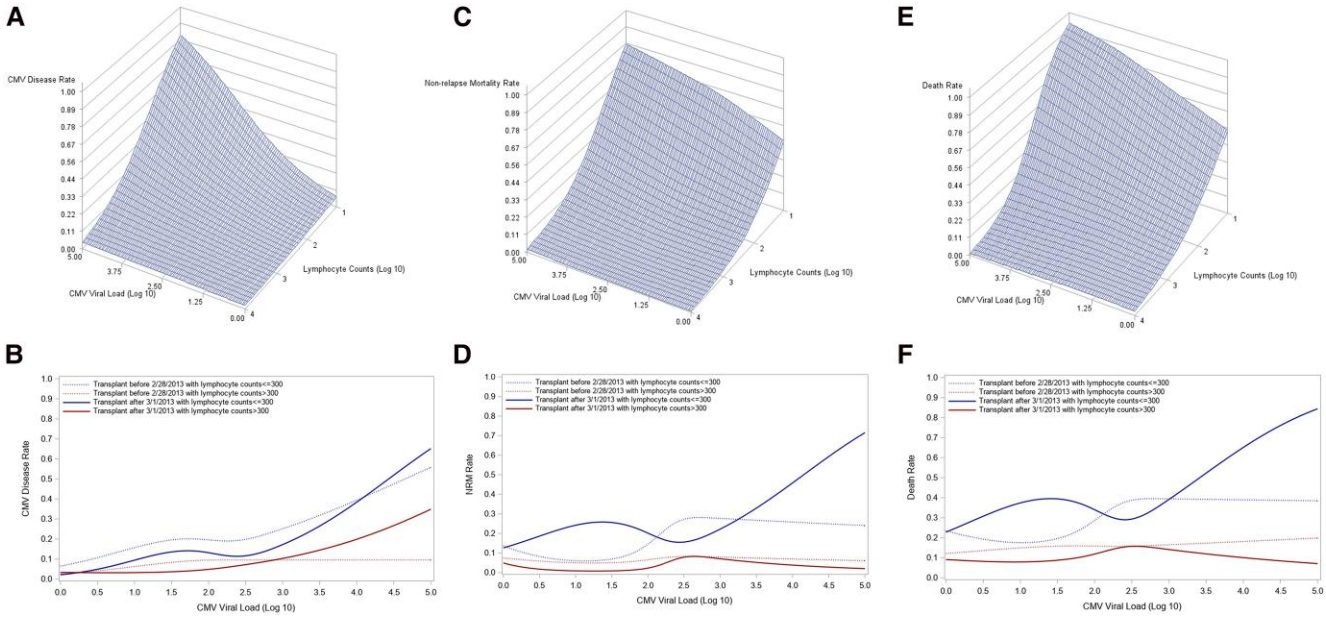

Figure 3. *A*, Restricted cubic splines for cytomegalovirus (CMV) disease risk including all patients from both time periods (before and after 28 Feb 2013), showing the effect of increasing CMV viral load relative to decreasing absolute lymphocyte count (ALC). *B*, Restricted cubic splines with binary presentation of lymphocyte counts and viral load presented in $\log_{10}$ scale, stratified by time period. Blue lines represent lymphocyte counts ≤300 cells/μL observed at day 100 post–hematopoietic cell transplant, red lines >300 cells/μL; the earlier period is indicated by the dotted lines while the later period is shown as solid lines. The panels show CMV disease rates (y-axis) associated with increasing CMV viral load values (x-axis) with (blue line) and without (red line) lymphopenia. Restricted cubic splines for nonrelapse mortality (NRM) (*C*) and overall mortality (OM) (*E*) for all patients showing the effect of increasing CMV viral load relative to decreasing lymphocyte counts. The effects of the early (before 28 Feb 2013) and later (28 Feb 2013) time period on NRM and OM are shown in panels *D* and *F*, respectively. The panels show NRM and OM rates (y-axis) associated with increasing CMV viral load values (x-axis) with (blue line) and without (red line) lymphopenia. Viral load threshold and lymphocyte counts are presented in $\log_{10}$ scale.

(7.41%) (Figure 4*E*). In adult patients, the effect of viral load was more apparent (Figure 4*F*).

The synergistic interaction between levels of lymphopenia and CMV viral load on OM and NRM in the entire study period is shown in Figure 3*C* and 3E, respectively. The impact of the study period in a model stratified by lymphopenia is depicted in Figure 3*D* and 3*F* and shows that the strongest correlation between viral load and OM and NRM was observed in patients with a viral load >3 $\log_{10}$ and lymphocyte count ≤300 cells/μL in the more recent study period.

DISCUSSION

In this study, we defined the impact of CMV viral load on CMV disease and OM and NRM in the era characterized by novel GVHD prevention strategies and improved survival (2013–2017). We also characterized the impact of age on viral kinetics and clinical outcomes. The study showed that CMV viral load continues to be a strong predictor of CMV disease and for NRM in patients with lymphopenia. In younger children, the incidence of csCMV infection is approximately 20% lower than in adolescents and adults, and CMV disease was virtually absent.

The recognition of CMV viral load as a surrogate for CMV disease and all-cause and NRM has been a major advance that has led to the approval of 2 antiviral drugs [8, 9] and to the incorporation of viral load as primary endpoints in numerous ongoing trials. However, the data used to establish surrogacy are >10 years old [3, 14, 25–27] and improved transplantation practices, including GVHD prophylaxis regimens and corticosteroid treatment regimens, have led to improved survival and other clinical outcomes [1, 2]. This raises the question whether the original assumptions of surrogacy are still valid in the era with improved transplant outcomes. Importantly, all currently and planned protocols of antivirals and vaccines use primary endpoints that include viral load either by itself or in combination with CMV disease. We chose a large dataset that extended to the time right before CMV prophylaxis with letermovir was adopted at our center, thereby providing the most current unbiased cohort representative of current practices and unaffected by the introduction of prophylaxis. Although the effect of antiviral prophylaxis should be captured by the viral load surrogate according to the Prentice criteria [28] such an analysis would likely require an even larger cohort with similar follow-up, which is presently not available. Also, recent real-world data indicate that clinical utilization and access to

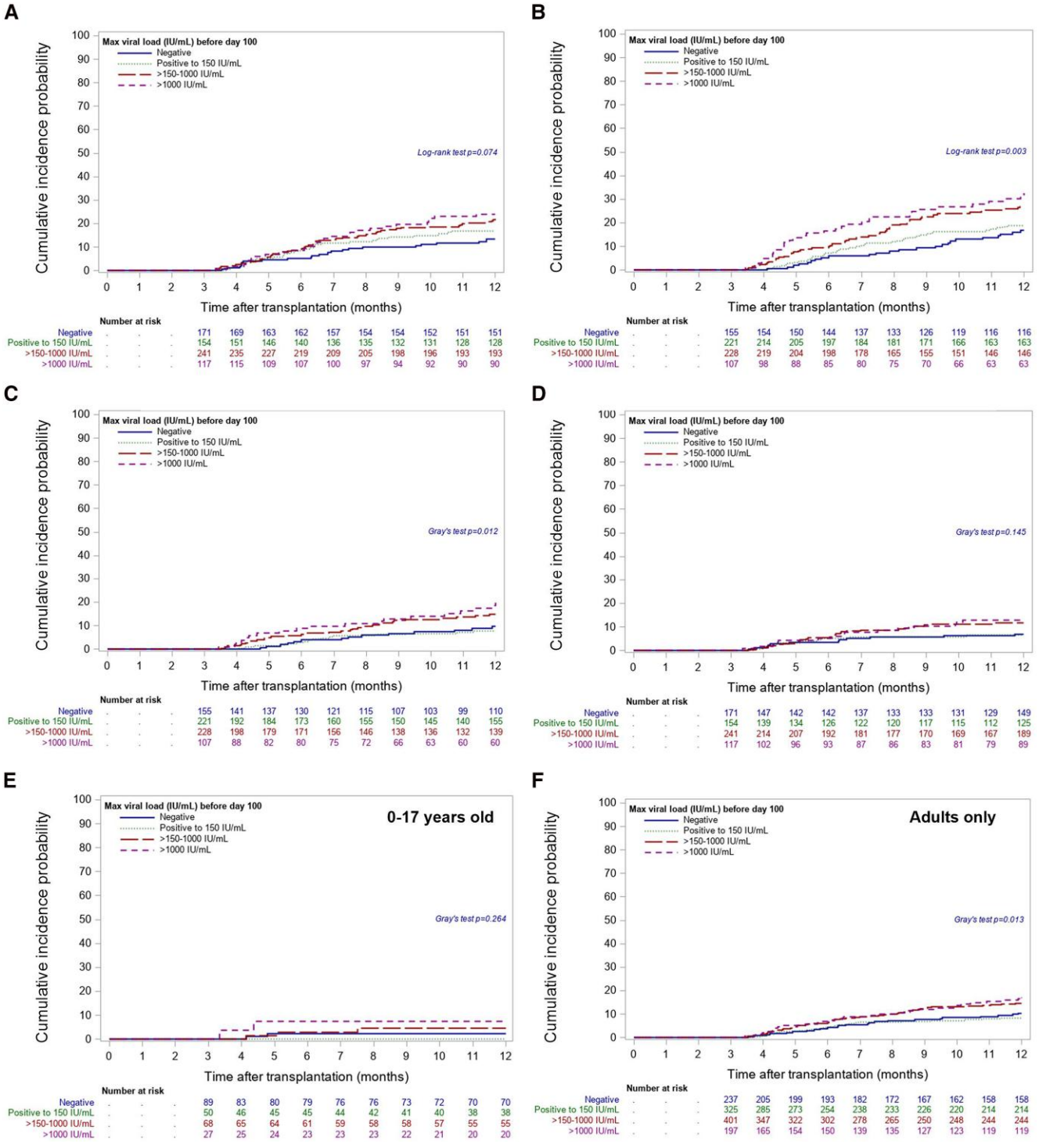

Figure 4. Cumulative incidence of overall mortality (*A* and *B*) and nonrelapse mortality (*C* and *D*) by 1 year after hematopoietic cell transplant among day 100 survivors transplanted before 28 Feb 2013 (*A* and *C*) and after 28 Feb 2013 (*B* and *D*), stratified by different viral load thresholds. *E* and *F*, Cumulative incidence of nonrelapse mortality during both time periods (before and after 28 Feb 2013) in children 0–17 years old (*E*) and adults (*F*), stratified by cytomegalovirus viral load.

letermovir vary across the world. In the United States and selected countries in Europe and Asia, letermovir utilization is high; however, the situation differs significantly in other parts of world [29]. Thus, preemptive therapy continues to be used.

Limitations of prior studies of CMV viral load associations with clinical outcomes include the insufficient consideration of the confounding immunosuppression and toxicities of the antiviral treatment (ganciclovir or foscarnet toxicities), which

have been independently associated with poor outcomes in earlier studies [20, 30]. Multivariate models that accounted for these factors showed that viral load remained strongly associated with CMV disease in both the study periods. However, with the development of new transplantation techniques, supportive care, and improved overall transplant success rates, the associations of CMV viral load with OM and NRM diminished and did not reach statistical significance in the more recent period (Figure 4 and Supplementary Table 2). A detailed look at impact of lymphopenia using cubic spline analyses showed that the associations with OM and NRM remained significant in highly immunocompromised hosts, as measured by lymphopenia (Figure 3*C*–*F*). This is plausible because lymphopenia was the strongest predictor of death overall and the impact of viral load became more apparent in this setting.

We also examined whether the viral load kinetics and disease differed between children, adolescents, and adults. csCMV infection occurred less frequently in children <11 years but was not different between adolescents and adults. The very low incidence of CMV disease in pediatric patients throughout the observation period (Figure 2*C*) was somewhat unanticipated. This difference may have been partially related to the less frequent use of gastrointestinal endoscopies. Bronchoscopies were not different between age groups (Supplementary Table 1). A higher proportion of children and adolescents received bone marrow and cord blood transplantation and T-cell–depleting agents, which could be a plausible explanation for the difference. At our center, recipients of cord blood transplants received high-dose acyclovir and were preemptively treated at any viral load level [18], resulting in a high level of protection from breakthrough CMV disease. Another reason may be a higher utilization of preemptive therapy in pediatric patients, at lower viral load levels compared to adult patients, likely due to a higher use of T-cell–depleting agents.

While the analyses of viral load association with clinical endpoints included children and adolescents, we were unable to perform a formal stratified analyses that included only children due to the smaller number of patients and outcomes. Overall, the data suggest that preemptive therapy with the thresholds used in this study is highly effective.

This study has important implications for clinical care, research, and drug and vaccine development. First, that CMV viral load continued to be associated with CMV disease, and with OM and NRM rates in more immunosuppressed HCT patients, is reassuring and supports the continued use of viral load as part of a clinical trial's primary endpoints. It also is consistent with recent studies that did not find associations with mortality endpoints in the overall population [4]. The fact that mortality rates are declining [1, 2] and that the association of CMV viral load with OM and NRM was only seen in more immunosuppressed patients, will make it more difficult to demonstrate an impact of any antiviral interventions on mortality. Such demonstration will require randomized trials with a large proportion of patients with high levels of immunosuppression or analyses of large, pooled populations from different centers. Clinically, the study supports the continued use of prophylaxis to suppress CMV viremia and disease. The data on the association of CMV disease and mortality are particularly strong in adults while larger studies are needed in pediatric populations to demonstrate associations with OM and NRM separately.

Strengths of this study include the large sample size, the more accurate assessment of immunosuppression compared to previous studies, a more comprehensive inclusion of possible confounders, a detailed review of clinical outcomes in different age groups, the use of uniform diagnostic and therapeutic management throughout the study period, and a rigorous statistical analysis. However, the study has limitations that should be noted, including the retrospective nature and the relatively small proportion of patients receiving posttransplant cyclophosphamide, sirolimus, and T-cell depletion–based GVHD prophylaxis regimens. Younger age, which was associated with a lower incidence of CMV disease, could also be a protective factor due to other unknown risk factors (eg, comorbidities) that we were unable to explore in the context of this retrospective study.

In conclusion, CMV viral load continues to be a strong predictor for CMV disease. The apparent differences by age of viral load kinetics and CMV disease prevention effectiveness with preemptive therapy require confirmation in larger cohorts. With improved transplant techniques, the association of viral load with OM and NRM is diminished but the effect is still present in patients with severe immunosuppression.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. A. S.-K., and M. B. contributed to conception and design. A. S.-K., M. L. G., A. W., M. U. O., K. R. J., J. A. H., D. Z., and B. M. S. were responsible for provision of study materials and data collection. A. S.-K., M. B., H. X., and W. M. L. conducted data analysis and interpretation. A. S.-K. and M. B. prepared the initial draft of the manuscript. All authors provided a critical review and final approval of the manuscript and are accountable for all aspects of the work.

Acknowledgments. We thank Chris Davis and Ryan Basom for database services; Louise Kimball, Rachel Blazevic, Nina Ozbek, Ashley Patajo, Jocelyn Meyer, Clarissa Santiano, Cindy Thai, and the Clinical Research team for medical records review; and Alex Downing and Jessica Lawler for text and graphs corrections.

Data availability. The data that support the findings of this study are available from the corresponding author (M. B.) upon reasonable request.

Financial support. This study was an investigator-initiated study by supported by grants from the Polish National Agency for Academic Exchange, the Medical University of Gdańsk, Poland, and the Joel D. Meyers Scholarship Endowment at the Fred Hutchinson Cancer Center, Seattle, Washington (to A. S.-K.). Additional support was provided by program funds and the National Institutes of Health (grant numbers CA15704 to

H. X. and W. M. L., CA078902 to B. M. S., and K23AI163343 to D. Z.). Funding for the earlier part of the cohort has been previously reported (Green et al [14]).

Potential conflicts of interest. M. B. has received research support and fees from Merck & Co. and Moderna Therapeutics, and personal fees from GlaxoSmithKline, Allovir, Takeda, SymBio, and EvrysBio. J. A. H. has received grants from Allovir, Deverra, GeoVax, Merck, Oxford Immunotec, and Takeda, and consulting fees from Century Therapeutics, CSL Behring, Eversana, ExeVir, GeoVax, Gilead, Grifols, Karius, Medscape, Modulus, Pfizer (previously Amplyx/Medpace), Sanofi, SentiBio, Symbio, and Takeda. A. W. has received research support from Merck, Pfizer, Ansun Biopharma, Shionogi, and GSK, and consulting fees from Merck, AstraZeneca, GSK, and Vir Biotechnology. All other authors report no potential conflicts.

References

1. McDonald GB, Sandmaier BM, Mielcarek M, et al. Survival, nonrelapse mortality, and relapse-related mortality after allogeneic hematopoietic cell transplantation: comparing 2003–2007 versus 2013–2017 cohorts. Ann Intern Med **2020**; 172: 229–39.
2. Penack O, Peczynski C, Mohty M, et al. How much has allogeneic stem cell transplant-related mortality improved since the 1980s? A retrospective analysis from the EBMT. Blood Adv **2020**; 4:6283–90.
3. US Food and Drug Administration, Center for Drug Evaluation and Research. Cytomegalovirus in transplantation: developing drugs to treat or prevent disease. Guidance for industry. **2020**; Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/cytomegalovirus-transplantation-developing-drugs-treat-or-prevent-disease. Accessed 25 June 2025.
4. Solano C, Vazquez L, Gimenez E, et al. Cytomegalovirus DNAemia and risk of mortality in allogeneic hematopoietic stem cell transplantation: analysis from the Spanish Hematopoietic Transplantation and Cell Therapy Group. Am J Transplant **2021**; 21:258–71.
5. Stern A, Su Y, Dumke H, et al. Cytomegalovirus viral load kinetics predict cytomegalovirus end-organ disease and mortality after hematopoietic cell transplant. J Infect Dis **2021**; 224:620–31.
6. Gimenez E, Torres I, Albert E, et al. Cytomegalovirus (CMV) infection and risk of mortality in allogeneic hematopoietic stem cell transplantation (allo-HSCT): a systematic review, meta-analysis, and meta-regression analysis. Am J Transplant **2019**; 19:2479–94.
7. Sadowska-Klasa A, Leisenring WM, Limaye AP, Boeckh M. Cytomegalovirus viral load threshold to guide preemptive therapy in hematopoietic cell transplant recipients: correlation with cytomegalovirus disease. J Infect Dis **2024**; 229: 1435–9.
8. Papanicolaou GA, Silveira FP, Langston AA, et al. Maribavir for refractory or resistant cytomegalovirus infections in hematopoietic-cell or solid-organ transplant recipients: a randomized, dose-ranging, double-blind, phase 2 study. Clin Infect Dis **2019**; 68:1255–64.
9. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med **2017**; 377:2433–44.
10. Marty FM, Winston DJ, Chemaly RF, et al. A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for cytomegalovirus prophylaxis in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant **2019**; 25:369–81.
11. Fleming TR, DeMets DL. Surrogate end points in clinical trials: are we being misled? Ann Intern Med **1996**; 125:605–13.
12. Groenwold RHH, Palmer TM, Tilling K. To adjust or not to adjust? When a "confounder" is only measured after exposure. Epidemiology **2021**; 32:194–201.
13. Sadowska-Klasa A, Ozkok S, Xie H, et al. Late cytomegalovirus disease after hematopoietic cell transplantation: significance of novel transplantation techniques. Blood Adv **2024**; 8:3639–51.
14. Green ML, Leisenring W, Xie H, et al. Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study. Lancet Haematol **2016**; 3:e119–27.
15. Zamora D, Duke ER, Xie H, et al. Cytomegalovirus-specific T-cell reconstitution following letermovir prophylaxis after hematopoietic cell transplantation. Blood **2021**; 138:34–43.
16. Ljungman P, Chemaly RF, Khawaya F, et al. Consensus definitions of cytomegalovirus (CMV) infection and disease in transplant patients including resistant and refractory CMV for use in clinical trials: 2024 update from the Transplant Associated Virus Infections Forum. Clin Infect Dis **2024**; 79:787–94.
17. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant **2015**; 21:389–401.e1.
18. Hill JA, Pergam SA, Cox E, et al. A modified intensive strategy to prevent cytomegalovirus disease in seropositive umbilical cord blood transplantation recipients. Biol Blood Marrow Transplant **2018**; 24:2094–100.
19. Makris K, Spanou L. Acute kidney injury: definition, pathophysiology and clinical phenotypes. Clin Biochem Rev **2016**; 37:85–98.
20. Nakamae H, Storer B, Sandmaier BM, et al. Cytopenias after day 28 in allogeneic hematopoietic cell transplantation: impact of recipient/donor factors, transplant conditions and myelotoxic drugs. Haematologica **2011**; 96:1838–45.
21. Spector SA, Hsia K, Crager M, Pilcher M, Cabral S, Stempien MJ. Cytomegalovirus (CMV) DNA load is an independent predictor of CMV disease and survival in advanced AIDS. J Virol **1999**; 73:7027–30.
22. Gauthier J, Wu QV, Gooley TA. Cubic splines to model relationships between continuous variables and outcomes: a guide for clinicians. Bone Marrow Transplant **2020**; 55:675–80.
23. Armand P, Gibson CJ, Cutler C, et al. A disease risk index for patients undergoing allogeneic stem cell transplantation. Blood **2012**; 120:905–13.
24. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)–specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood **2005**; 106:2912–9.
25. Duke ER, Williamson BD, Borate B, et al. CMV viral load kinetics as surrogate endpoints after allogeneic transplantation. J Clin Invest **2021**; 131:e133960.
26. Gor D, Sabin C, Prentice HG, et al. Longitudinal fluctuations in cytomegalovirus load in bone marrow transplant patients: relationship between peak virus load, donor/recipient serostatus, acute GVHD and CMV disease. Bone Marrow Transplant **1998**; 21:597–605.
27. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet **2000**; 355:2032–6.
28. Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. Stat Med **1989**; 8:431–40.
29. GYR Research. Global letermovir drugs market insights, forecast to 2030. **2024**. Available at: https://www.qyresearch.com/reports/3316311/letermovir-drugs. Accessed 25 June 2025.
30. Kizilbash SJ, Kashtan CE, Chavers BM, Cao Q, Smith AR. Acute kidney injury and the risk of mortality in children undergoing hematopoietic stem cell transplantation. Biol Blood Marrow Transplant **2016**; 22:1264–70.