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MAJOR ARTICLE

Impact of Baseline and Week 2 and Week 4 Posttransplant CMV Cell-Mediated Immunity on Risk of CMV Infections and Mortality in Recipients of Allogeneic Hematopoietic Cell Transplant

Ella J. Ariza-Heredia,[1] Drew J. Winston,[2] Scott D. Rowley,[3] Kathleen Mullane,[4] Pranatharthi Chandrasekar,[5] Parameswaran Hari,[6] Robin K. Avery,[7] Karl S. Peggs,[8] Deepali Kumar,[9] Rajneesh Nath,[10] Per Ljungman,[11] Sherif B. Mossad,[12] Lynn El Haddad,[13] Dimpy P. Shah,[14] Ying Jiang,[1] Fareed Khawaja,[1] Sanjeet Dadwal,[15] Ted Blanchard,[16] and Roy F. Chemaly[1]

[1]Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA, [2]Ronald Reagan UCLA Medical Center, Los Angeles, California, USA, [3]Hackensack University Medical Center, Hackensack, New Jersey, USA, [4]Department of Medicine, University of Chicago, Chicago, Illinois, USA, [5]Division of Infectious Diseases, Department of Medicine, Karmanos Cancer Center, Wayne State University, Detroit, Michigan, USA, [6]Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, Wisconsin, USA, [7]Division of Infectious Diseases (Transplant Oncology), Johns Hopkins University, Baltimore, Maryland, USA, [8]Department of Haematology, University College London Cancer Institute and University College London Hospitals National Health Service Foundation Trust, London, UK, [9]Transplant Infectious Diseases, University Health Network, Toronto, Canada, [10]Bone Marrow Transplant, Banner MD Anderson Cancer Center, Gilbert, Arizona, USA, [11]Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska Comprehensive Cancer Center, Karolinska University Hospital, Huddinge, and Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, [12]Department of Infectious Diseases, Respiratory Institute, Cleveland Clinic, Cleveland, Ohio, USA, [13]Department of Medicine, University of Florida, Gainesville, Florida, USA, [14]Department of Population Health Sciences, Mays Cancer Center at UT Health San Antonio MD Anderson, San Antonio, Texas, USA, [15]Division of Infectious Diseases, City of Hope, Duarte, California, USA, and [16]Oxford Immunotec USA, Marlborough, Massachusetts, USA

Background. Cytomegalovirus (CMV) infection is a common opportunistic infection after allogeneic hematopoietic cell transplant (alloHCT). We explored whether a change in CMV cell-mediated immunity during the first month after transplant predicts the risk of development of CMV infection and all-cause mortality.

Methods. This follow-up analysis is based on data from the REACT study, a multicenter prospective observational study of recipients of alloHCT who were CMV-seropositive. Production of interferon γ following ex vivo stimulation with CMV antigens IE1 (immediate early 1) and pp65 (phosphoprotein 65) was assessed by CMV ELISPOT assay at baseline and 2 and 4 weeks after transplant. Clinically significant CMV infection (CS-CMVi) was defined as CMV viremia and/or disease necessitating antiviral therapy. We evaluated the impact of CMV CMI changes on the risk of CS-CMVi and post transplant mortality.

Results. The analysis included 226 recipients of alloHCT with CMV cell-mediated immunity data at baseline and 2 and/or 4 weeks after transplant. CS-CMVi occurred in 64 patients (28%). On Cox regression analyses, independent predictors of CS-CMVi included a negative Δ change from baseline to week 2 of pp65 spot counts (hazard ratio, 3.65 [95% CI, 1.65–8.04]; $P = .001$) to week 4 of IE1 spot counts (hazard ratio, 2.79 [95% CI, 1.46–5.35]; $P = .002$), anti-thymocyte globulin conditioning regimen, type of transplant, female sex, and corticosteroid use. Kaplan-Meir analysis showed a significant association of a negative IE1 change from baseline to week 4 and increased all-cause mortality after transplant (log rank test = 0.041).

Conclusions. A decrease in CMV-specific T-cell responses during the first month after transplant may predict CS-CMVi and is associated with all-cause mortality in recipients of alloHCT.

Keywords. cell-mediated immunity; CMV ELISPOT assay; cytomegalovirus; hematopoietic cell transplant.

Cytomegalovirus (CMV) infection is one of the most common infectious complications affecting recipients of allogeneic hematopoietic cell transplant (alloHCT) [1]. Control of CMV replication relies on cell-mediated immunity (CMI), especially CMV-specific CD4+ T-cell response, which precedes the development of CMV-specific antibodies and cytotoxic CD8+ T cells for control of viral replication [2, 3].

CD4+ and CD8+ T cells are implicated in controlling latent CMV infection through a complex interaction involving a CD8+ T-cell response producing interferon γ, interferon α, and a host of other cytokines in response to the presence of CMV. Measuring CMV CMI may be a useful tool to predict the risk of CMV infection or disease [4]. The routine use of CMV CMI

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Correspondence: Roy F. Chemaly, MD, MPH, Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 1460, Houston, TX 77030-4095 (rfchemaly@mdanderson.org). Ella J. Ariza-Heredia, MD, Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 1460, Houston, TX 77030-4095 (eariza@mdanderson.org).

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assays is hindered by (1) limited access to commercially available assays, (2) lack of standardization across platforms (eg, enzyme-linked immunospot [ELISPOT] assays and enzyme-linked immunoassays), and (3) remaining questions related to the utility of information from CMV CMI assays in relation to posttransplant outcomes [5]. CMV ELISPOT assays assess CMV CMI by measuring the release of interferon γ by CD4+ and CD8+ T cells in vitro from isolated peripheral blood mononuclear cells [6]. Evaluation of the clinical utility of CMV ELISPOT assays showed promising results for predicting CMV infections in recipients of alloHCT and solid organ transplant [7–10].

We previously reported that among recipients of alloHCT who were CMV-seropositive and enrolled in a prospective observational study, low CMV CMI within the first 6 months after transplant (as measured by a CMV ELISPOT assay) was associated with an increased risk of clinically significant CMV infection (CS-CMVi) [9]. Furthermore, we noted that among patients with low CMV CMI, the mortality rate within 6 months posttransplant was significantly higher among those who did experience CS-CMVi than among those who did not (37% vs 12%) [9]. In the present analysis, we explored the relationships between the Δ changes in CMV CMI from baseline (before transplant) to specific time points during the first month after transplant and the occurrence of CS-CMVi as well as all-cause mortality.

METHODS

Study Design

This post hoc analysis was based on data from the REACT study [9], a multicenter prospective observational trial conducted at 13 transplant centers in the United States, Canada, and Europe to determine the performance of a CMV ELISPOT assay in predicting immune competence against CMV reactivation after alloHCT. Full details of the trial design and the study population were previously published [9]. Briefly, all enrolled patients underwent an alloHCT from matched or haploidentical related donors or from matched or mismatched unrelated donors. Pediatric patients (<18 years) were excluded. Enrolled patients provided a blood sample within 14 days prior to alloHCT (baseline), at days 14 and 28, and then every 2 weeks for up to 6 months after transplant. At each time point, CMV ELISPOT assay and a central laboratory measurement of CMV viral load by polymerase chain reaction were performed through the Roche Cobas platform (lower limit of detection, 137 IU/mL). CMV reactivation and CMV disease were defined per Ljungman et al [11]. The primary end point was detection of the first CS-CMVi, defined as the first CMV reactivation or CMV disease after hematopoietic cell transplant (HCT) necessitating the start of anti-CMV therapy by each center's institutional protocols [12]. CS-CMVi was assessed subsequent to each time point. All-cause mortality was an exploratory end point. For all the analysis evaluating the impact of CMV CMI after week 2 or 4, CS-CMV infection and mortality that occurred after those time points were included in the analysis. CMV ELISPOT assay was performed in accordance with validated test procedures, which are described in the report of the REACT study [9]. None of the patients received primary prophylaxis for CMV.

In the REACT study, CMV CMI (CMV-specific T-cell activity) was assessed by determining the production of interferon γ following ex vivo stimulation with 2 CMV antigens: immediate early 1 (IE1) and phosphoprotein 65 (pp65). Results were reported in spot counts (SPCs) per 250 000 cells for IE1 and pp65. When the nil control SPCs were <10 per 250 000 cells, they were subtracted from the SPCs obtained for IE1 and pp65. For the current analysis, SPCs were categorized as low if they were ≤100 per 250 000 cells and high if >100 per 250 000 cells, according to our previous results [9]. Changes in SPCs for IE1 and pp65 between baseline and 2 and 4 weeks after transplant were categorized as a positive Δ change if the week 2 or 4 count was greater than or equal to the baseline count, and negative Δ change if the week 2 or 4 count was less than the baseline count. In addition to CMV CMI results, data obtained from medical records included patient demographics, medical history, relevant transplant-related data, corticosteroid use for other than premedication purposes, and laboratory results (including CMV polymerase chain reaction results).

Patient Consent Statement

The protocol was approved by the institutional review board of each site, and all patients signed an informed consent form.

Statistical Analysis

Categorical variables were compared with chi-square or Fisher exact test. Continuous variables were compared through the Wilcoxon rank sum test. Cox proportional hazards models were used to identify factors independently associated with CS-CMVi development and to evaluate the impact of CMV CMI change from baseline to posttransplant on the risk of CS-CMVi development and mortality. For analyses of the impact of CMV CMI on the risk of development of CS-CMVi, separate models were used for week 2 and 4 analyses. Correlations between IE1 and pp65 changes were evaluated via Spearman rank-order correlation analysis. Survival curves of the same patients were estimated by the Kaplan-Meier method and compared with the log-rank test. All tests were 2-sided with a significance level of 0.05. Statistical analyses were performed with SAS version 9.4 (SAS Institute).

RESULTS

This multicenter study followed 241 recipients of alloHCT who were CMV-seropositive. After 2 recipients of blood cord

transplant were excluded, 226 patients had results for CMV CMI at baseline and at 2 and/or 4 weeks after transplant and were included in these analyses. The clinical and demographic characteristics of the 226 patients are summarized in Table 1. Sixty-four patients (28%) had a CS-CMVi during the 6-month follow-up period. Of these 64 patients, 10 (15%) had CMV end organ disease. Most cases of CS-CMVi (57/64) occurred within the first 75 days after HCT (median, 36; IQR, 30–57). Patients with and without CS-CMVi differed in terms of demographic and clinical characteristics, including female sex, receipt of anti-thymocyte globulin (ATG), acute graft-vs-host disease, and corticosteroid use, which were significantly more common in patients with CS-CMVi.

Table 1. Demographic and Clinical Characteristics of the Study Participants (N = 226)

Characteristic	Median (Range) or No. (%)		*P* Value
	CS-CMVi (n = 64)	No CS-CMVi (n = 162)	
Age, y	58 (18–80)	56 (22–78)	.91
Sex			.008
Male	27 (42)	100 (62)	
Female	37 (58)	62 (38)	
Race	61	145	.044
White	42 (69)	123 (85)	
African American	6 (10)	8 (6)	
Asian	6 (10)	9 (6)	
Other	7 (11)	5 (3)	
Unknown	3	17	
Type of transplant	63	160	.021
Matched related donor	15 (24)	69 (43)	
Matched or mismatched unrelated donor	38 (60)	67 (42)	
Haploidentical	10 (16)	24 (15)	
Unknown	1	2	
HCT donor status	63	153	.66
CMV positive	35 (56)	90 (59)	
CMV negative	28 (44)	63 (41)	
Unknown	1	9	
Anti-thymocyte globulin	21 (33)	25 (15)	.004
Posttransplant cyclophosphamide	12 (19)	33 (20)	.78
Total body irradiation	2 (3)	15 (9)	.16
Conditioning regimen	63	154	.91
Myeloablative	30 (48)	72 (47)	
Nonmyeloablative	33 (52)	82 (53)	
Unknown	1	8	
Time from HCT to engraftment d	13 (3–42)	14 (0–42)	.97
At any time during the study period			
Corticosteroid use	62 (97)	113 (70)	<.0001
Acute GVHD	40 (63)	73 (45)	.018
CMV disease	10 (16)		
CMV viral load by PCR, IU/mL[a]	1628 (323–26 364)		
All-cause mortality	16 (25)	20 (12)	.019

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

Abbreviations: CMI, cell-mediated immunity; CMV, cytomegalovirus; CS-CMVi, clinically significant CMV infection; GVHD, graft-vs-host disease; HCT, hematopoietic cell transplant; PCR, polymerase chain reaction.

[a]CMV PCR is provided for all patients who experienced CMV viremia (n = 62).

CMV CMI at Specific Time Points and CS-CMVi Risk

For the Cox regression analyses of the impact of CMV CMI at specific time points on CS-CMVi risk, 2 models were constructed: 1 for baseline and week 2 and 1 for baseline and week 4. In both models, low pp65 SPCs at week 4 were associated with an increased risk for CS-CMVi (adjusted hazard ratio, 2.57 [95% CI, 1.11–5.94]; $P = .028$). IE1 SPCs at any time point were not predictive of CS-CMVi (Supplementary Table 1).

Changes in CMV CMI and CS-CMVi Risk

To determine the effect of Δ changes in CMV CMI on the risk for CS-CMVi, changes were analyzed separately for each antigen (pp65 and IE1) from baseline to week 2 and baseline to week 4 and then correlated with CS-CMVi. Data were available for

Table 2. Changes in CMV CMI and Risk for CS-CMVi

CMV CMI	No. (%)		*P* Value
	CS-CMVi (n = 64)	No CS-CMVi (n = 162)	
SPC/250 000 cells			
IE1 at week 2 (n = 192)	56	136	.76
>100	3 (5)	10 (7)	
≤100	53 (95)	126 (93)	
pp65 at week 2 (n = 192)	56	136	.016
>100	2 (4)	22 (16)	
≤100	54 (96)	114 (84)	
IE1 at week 4 (n = 190)	47	143	.09
>100	3 (6)	23 (16)	
≤100	44 (94)	120 (84)	
pp65 at week 4 (n = 190)	47	143	.11
>100	8 (17)	41 (29)	
≤100	39 (83)	102 (71)	
Δ change[a]			
IE1, baseline to week 2 (n = 152)	43	109	.0005
Positive	13 (30)	67 (61)	
Negative	30 (70)	42 (39)	
pp65, baseline to week 2 (n = 152)	43	109	
Positive	9 (21)	60 (55)	.0001
Negative	34 (79)	49 (45)	
IE1, baseline to week 4 (n = 154)	40	114	
Positive	15 (38)	76 (67)	.001
Negative	25 (63)	38 (33)	
pp65, baseline to week 4 (n = 154)	40	114	
Positive	14 (35)	70 (61)	.004
Negative	26 (65)	44 (39)	

Abbreviations: CMV CMI, cytomegalovirus cell-mediated immunity; CS-CMVi, clinically significant cytomegalovirus infection; IE1, immediate early 1; pp65, phosphoprotein 65; SPC, spot count.

[a]Δ change was considered positive if the week 2 or 4 count was greater than or equal to the baseline count and negative it was less than the baseline count.

152 patients for the week 2 analysis and 154 patients for the week 4 analysis. The median time from sample collection and HCT (baseline) was −3 days (IQR, −5 to −1).

Between baseline and week 2, IE1 had a median SPC change of 0 (IQR, −28 to 0), and pp65 had a median SPC change of −14 (IQR, −175 to 0). Seventy percent (30/43) and 79% (34/43) of patients with a CS-CMVi and 39% (42/109) and 45% (49/109) without a CS-CMVi had negative Δ changes in IE1 SPCs ($P = .0005$) and pp65 SPCs ($P = .0001$), respectively (Table 2). Between baseline and week 4, IE1 had the same median SPC change of 0 (IQR, −17 to 8), and pp65 also had a median SPC change of 0 (IQR, −146 to 41). In total, 63% (25/40) and 65% (26/40) of patients with a CS-CMVi and 33% (38/114) and 39% (44/114) without a CS-CMVi had negative Δ changes in IE1 SPCs ($P = .001$) and pp65 SPCs ($P = .004$), respectively (Table 2).

On Cox regression analyses, independent predictors of CS-CMVi were a negative Δ change for pp65 from baseline to week 2 and a negative Δ change for IE1 from baseline to week 4 (Tables 3 and 4). When CMV CMI was treated as a continuous variable, Cox regression analysis showed that for every decrease of pp65 SPCs by 50 per 250 000 cells from baseline to week 2, the hazard ratio of developing CS-CMVi increased by 12% (1.12; 95% CI, 1.05–1.19). Changes in IE1 from baseline to week 4 were not associated with CS-CMVi when CMV CMI was considered as a continuous variable on univariate or Cox regression analyses ($P = .38$ and $P = .89$, respectively). In addition, ATG use during the conditioning regimen was an independent

Table 3. Cox Regression Analysis: Impact of CMV CMI (IE1 and pp65) Change Between Baseline and Week 2 After Transplant on Risk of Developing CS-CMVi

	Univariate Analysis		Multivariate Analysis	
Variable	Crude HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Age	0.99 (.97–1.02)	.61		
Sex		.07		.004
Male	1 [Reference]		1 [Reference]	
Female	1.74 (.96–3.18)		2.62 (1.35–5.07)	
Race		.18		.011
White	1 [Reference]		1 [Reference]	
African American	1.77 (.62–5.05)		2.19 (.74, 6.50)	
Asian	1.53 (.59–3.95)		1.61 (.60, 4.34)	
Other	2.90 (1.02–8.26)		6.10 (1.98, 18.79)	
Type of transplant		.012		— [a]
Matched related donor	1 [Reference]			
Matched or mismatched unrelated donor	2.65 (1.36–5.19)			
Haploidentical	1.26 (.45–3.54)			
HCT donor status		.87		
CMV negative	1 [Reference]			
CMV positive	1.05 (.56–1.97)			
Anti-thymocyte globulin	2.41 (1.19–4.90)	.015	2.52 (1.18, 5.40)	.018
Posttransplant cyclophosphamide	0.88 (.42–1.84)	.74		
Total body irradiation	0.59 (.14–2.44)	.47		
Conditioning regimen		.43		
Myeloablative	1.27 (.70–2.32)			
Nonmyeloablative	1 [Reference]			
Time from HCT to engraftment, d	1.00 (.95–1.05)	.86		
Corticosteroid use[b] prior to week 2	2.29 (1.25–4.21)	.008	2.16 (1.13–4.13)	.02
CMV CMI change, baseline to week 2				
Δ change in IE1		.001		
Positive	1 [Reference]			
Negative	2.94 (1.53–5.65)			
Δ change in pp65		<.001		.001
Positive	1 [Reference]		1 [Reference]	
Negative	3.73 (1.79–7.79)		3.64 (1.65–8.04)	

Week 2 in the study was chosen as time 0 in the analysis.

Abbreviations: CMI, cell-mediated immunity; CMV, cytomegalovirus; CS-CMVi, clinically significant CMV infection; HCT, hematopoietic cell transplant; HR, hazard ratio; IE1, immediate early 1; pp65, phosphoprotein 65.

[a]Variables were entered into the initial multivariate Cox regression model on the basis of the *P* value on univariate analysis (≤.20) and later removed from the final Cox regression model through the backward elimination procedure.

[b]Corticosteroid use does not include corticosteroids given as part of pre medication.

Table 4. Cox Regression Analysis: Impact of CMV CMI (IE1 and pp65) Change Between Baseline and Week 4 After Transplant on Risk of Developing CS-CMVi

	Univariate Analysis		Multivariate Analysis	
Variable	Crude HR (95% CI)	*P* Value	Adjusted HR (95% CI)	*P* Value
Age	1.00 (.98–1.02)	.95		
Sex		.07		— [a]
Male	1 [Reference]			
Female	1.78 (.95–3.34)			
Race		.31		
White	1 [Reference]			
African American	2.10 (.73–6.01)			
Asian	1.64 (.63–4.27)			
Other	2.15 (.65–7.09)			
Type of transplant		.028		.012
Matched related donor	1 [Reference]		1 [Reference]	
Matched or mismatched unrelated donor	2.74 (1.31–5.74)		2.95 (1.40–6.20)	
Haploidentical	2.15 (.78–5.92)		3.14 (1.11–8.84)	
HCT donor status		.54		
CMV negative	1 [Reference]			
CMV positive	1.23 (.64–2.35)			
Anti-thymocyte globulin	2.64 (1.29–5.41)	.008		
Posttransplant cyclophosphamide	1.22 (.60–2.50)	.58		
Total body irradiation	0.46 (.11–1.91)	.29		
Conditioning regimen		.38		
Myeloablative	1.32 (.71–2.47)			
Nonmyeloablative	1 [Reference]			
Time from HCT to engraftment, d	1.02 (.97–1.07)	.54		
Prior to week 4				
Corticosteroid use [b]	2.93 (1.51–5.68)	.002	2.93 (1.48–5.80)	.002
Acute GVHD	1.08 (.42–2.75)	.88		
CMV CMI change, baseline to week 4				
Δ change in IE1		.001		.002
Positive	1 [Reference]		1 [Reference]	
Negative	2.92 (1.54–5.54)		2.79 (1.46–5.35)	
Δ change in pp65		.003		
Positive	1 [Reference]			
Negative	2.73 (1.42–5.23)			

Week 4 in the study was chosen as time 0 in the analysis.

Abbreviations: CMI, cell-mediated immunity; CMV, cytomegalovirus; CS-CMVi, clinically significant CMV infection; GVHD, graft-vs-host disease; HCT, hematopoietic cell transplant; HR, hazard ratio; IE1, immediate early 1; pp65, phosphoprotein 65.

[a]Variables were entered into the initial multivariate Cox regression model on the basis of the *P* value on univariate analysis (≤.20) and later removed from the final Cox regression model through the backward elimination procedure.

[b]Corticosteroid use does not include corticosteroids given as part of pre medication.

predictor for CS-CMVi after week 4. Spearman correlation coefficient and box plots of the Δ changes in pp65 and IE1 SPCs between baseline and weeks 2 and 4 in patients with and without CS-CMVi are depicted in Figure 1*A* and 1*B* and Figure 2*A*–2*D*. The performance of the negative changes in IE1 and pp65 from baseline to week 2 or 4 and its prediction of CS-CMVi (sensitivity, specificity, positive predictive value, and negative predictive value) are summarized in Supplementary Table 2.

Changes in CMV CMI and Mortality

Overall, all-cause mortality at 6-month follow-up was higher in patients with CS-CMVi ($P = .03019$). Figure 1*C* and 1*D* shows the correlation between IE1 and pp65 changes from baseline to weeks 2 and 4 and mortality. Furthermore, we evaluated the impact of Δ changes in both antigens on patients' mortality at 6 months using Kaplan-Meir curves for survival analysis (Figure 3*A*–3*D*). A negative IE1 change from baseline to week 4 was significantly associated with increased mortality after transplant (log rank test, $P = .041$; Figure 3*D*); however, type of transplant and prior corticosteroid use were the only independent factors associated with mortality by multivariate survival analysis.

DISCUSSION

In this follow-up analysis, we aimed to determine if changes in CMV ELISPOT results during the first month after transplant

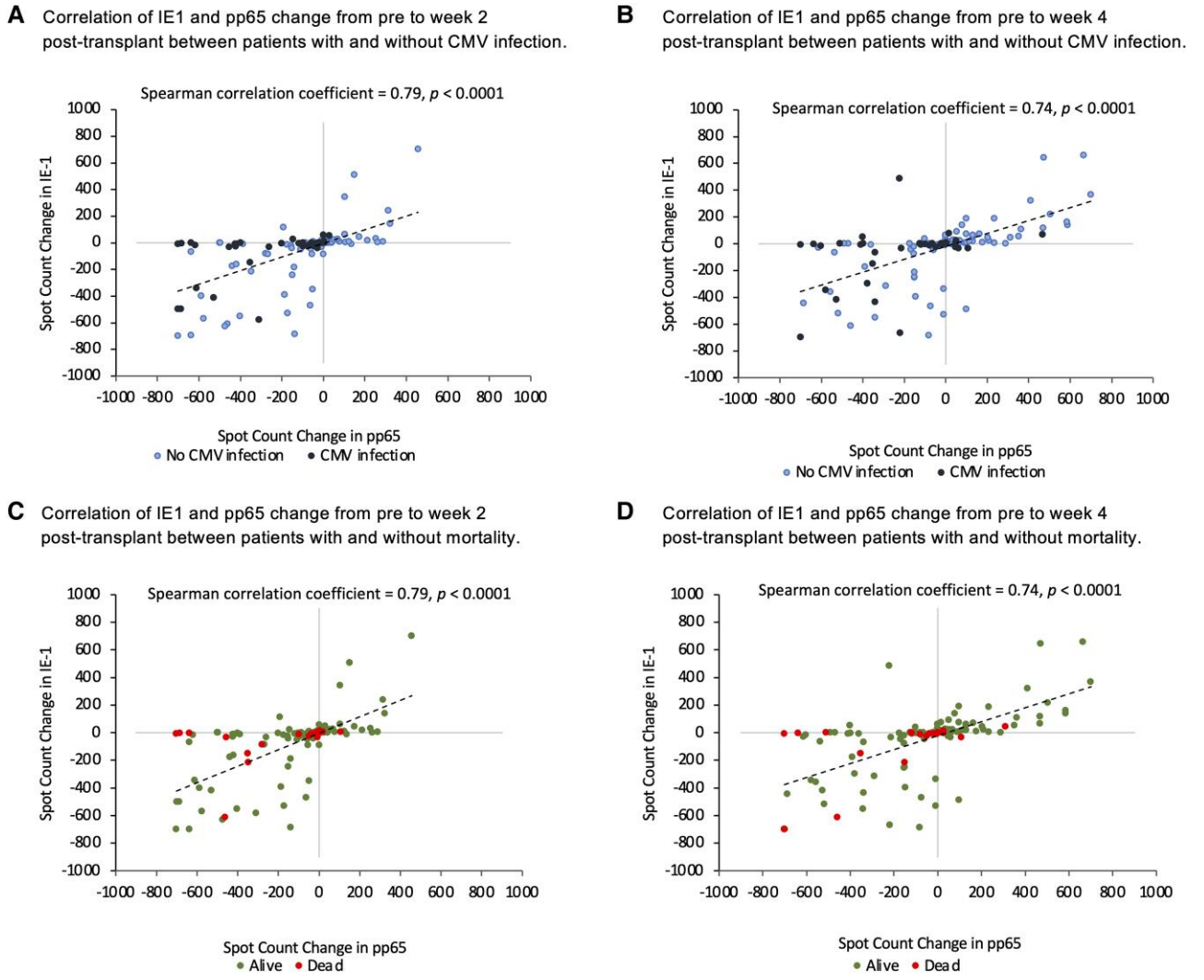

Figure 1. (*A–D*) Correlation of change in response to pp65 and IE1 antigens from baseline to week 2 and from baseline to week 4 in patients with and without CMV and patients alive and dead at the end of the study period. CMV, cytomegalovirus; IE1, immediate early 1; pp65, phosphoprotein 65.

predicts CS-CMVi and all-cause mortality in the first 6 months after transplant. We found that decline (negative Δ change) in CMV CMI, as measured by CMV ELISPOT for pp65 from baseline to week 2 and for IE1 from baseline to week 4, was a predictor of CS-CMVi, along with receiving ATG as part of the conditioning regimen, matched and mismatched unrelated donors, haploidentical transplant, corticosteroid use, and female sex. Furthermore, all-cause mortality at 6-month follow-up was higher in patients with CS-CMVi.

The relationship between negative Δ changes in CMV CMI and CS-CMVi can be explained in part by delayed reconstitution of donor-origin CMV-specific T cells after transplant and the loss of the previously activated recipient CMV-specific T cells as a result of conditioning chemotherapy. The utility of CMV ELISPOT assays in predicting progression of CMV infection has been demonstrated [8, 13–15]; however, few studies have examined the utility of early dynamic changes in CMV CMI in predicting progression of CMV infection. Jung et al [16] reported results similar to ours in a prospective study of 84 recipients of HCT who were CMV-seropositive. The authors found that dynamic changes (day 30 – day 0) in pp65 SPCs (<42 per 200 000 cells) and IE1 SPCs (<4 per 200 000 cells), as measured by ELISPOT assay, were predictors of CMV infection. The authors proposed an algorithm based on their findings denoting that the Δ change in pp65 between days 0 and 30 is sensitive and the Δ change in IE1 between days 0 and 30 is specific for predicting CMV infection, similar to our previous report on diagnostic accuracy [9]. Likewise, our data highlight the utility of pp65 and IE1 Δ changes within a month from transplant to predict the risk for CS-CMVi. Overall, negative changes in IE1 or pp65 values between the time points had good negative predictive value to identify patients at risk for CS-CMVi. Both biomarkers were strong predictors of CS-CMVi, as shown in

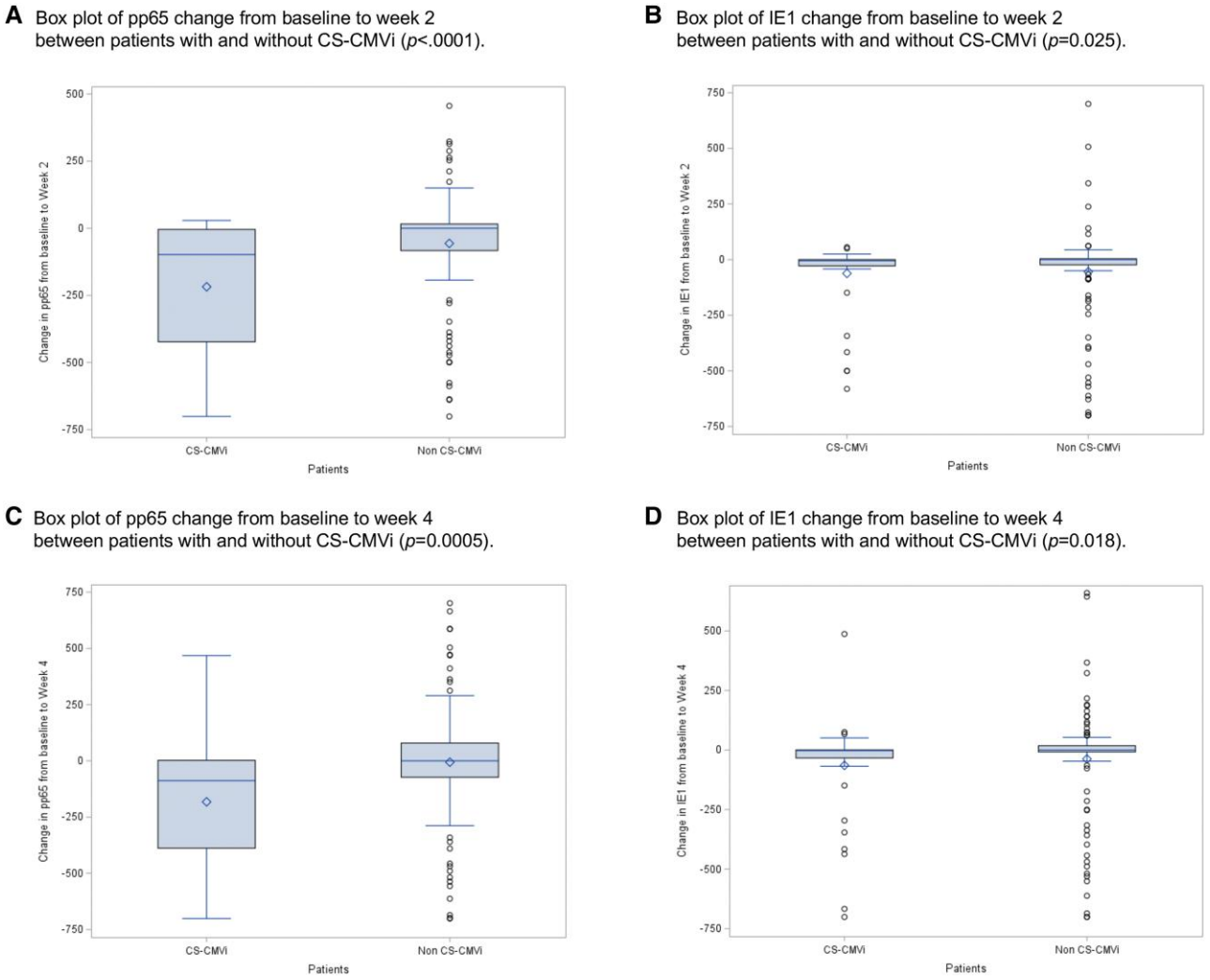

Figure 2. (*A–D*) Change in response to pp65 and IE1 antigens from baseline to week 2 and from baseline to week 4 in patients with and without a CS-CMVi. Box, IQR; vertical lines, minimum and maximum; diamond, mean; horizontal line, median. CS-CMVi, clinically significant CMV infection; IE1, immediate early 1; pp65, phosphoprotein 65.

the univariate analysis and with strong correlation. On multivariate analysis, likely due to small numbers, only one biomarker per time point prevailed as significant: negative changes on pp65 at values at week 2, and negative changes on IE1 antigen at week 4. Our results underscore the dynamic changes of CMV-specific T-cell immunity early after transplant as a predictor, of CS-CMVi rather than at specific time points. Similarly, Chiereghin et al [7] reported on CMV immunomonitoring using CMV CMI during the first month after small bowel/multivisceral transplant to identify patients at risk for CMV infection. In that study, response was considered positive when the number of spot-forming cells was ≥5 in the wells stimulated with peptides. Patients who were CMV-seropositive were classified as "early responders" if they had CMV T-cell reconstitution within the first month after transplant and "late responders" if they had it after the first month. The early responders had asymptomatic or mild CMV infections with lower mean and peak CMV viral loads, and the late responders had moderate or severe CMV infections.

Furthermore, this study also reveals a very interesting result: that a decrease in IE1 SPCs from baseline to week 4 was associated with an increase in all-cause mortality risk at 6 months after transplant. The correlation between lymphocyte recovery and transplant outcomes, including increased risk for infections, is well recognized [17]. The relationship between lymphocyte recovery after transplant and graft-vs-host disease, disease relapse, and mortality is a topic of high interest [18]. We described the relationship between CMV immune recovery and poor outcomes in the REACT study [9]. The data from our current analysis suggest that CMV CMI values from as early as the first month after transplant could serve as predictors of not only CS-CMVi but also mortality after transplant, thus warranting further evaluation [18, 19], especially in the era of CMV chemoprophylaxis with letermovir. Additional studies

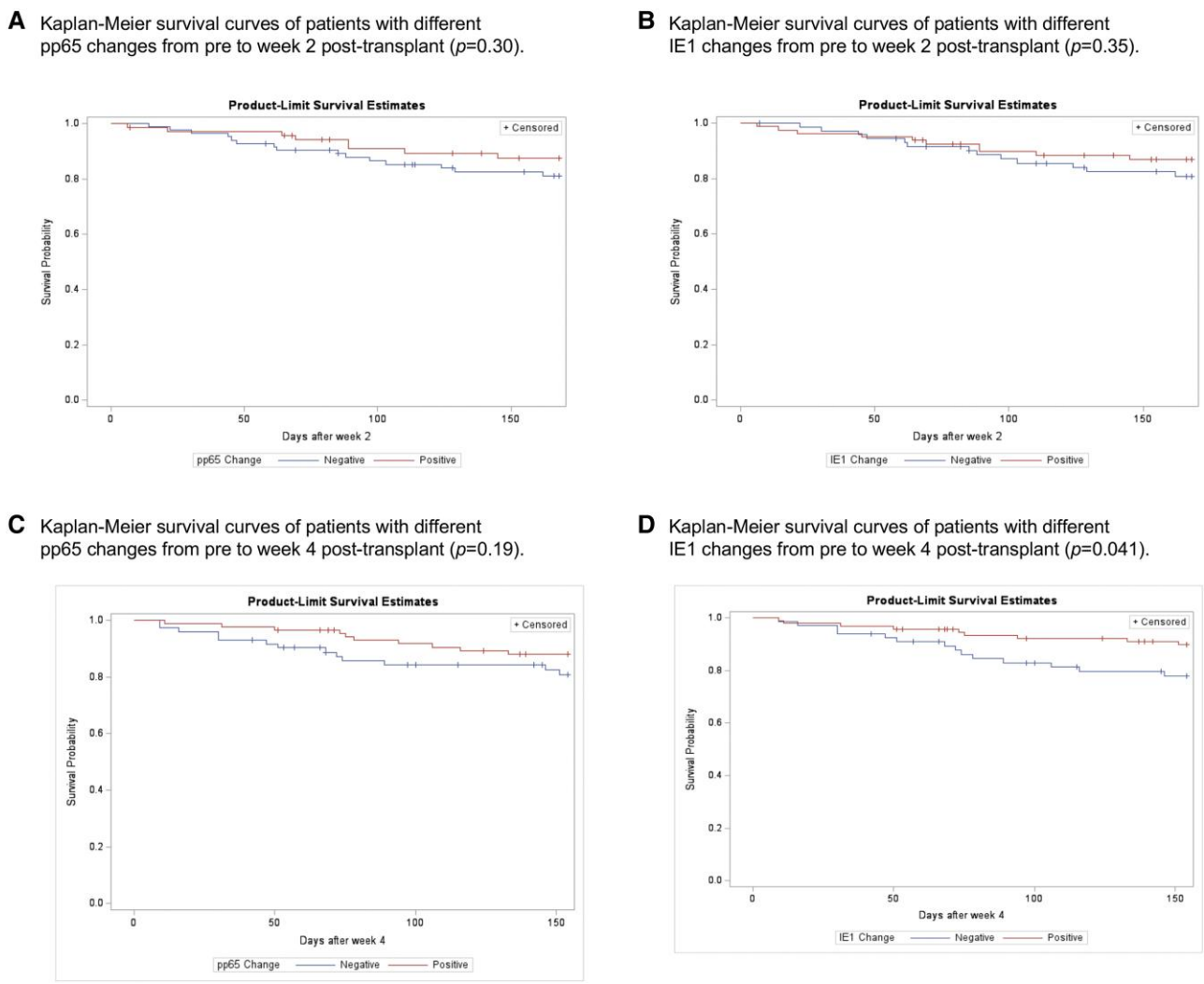

Figure 3. (*A–D*) Kaplan-Meier survival curves in patients with positive and negative changes in pp65 and IE1 SPCs between baseline and weeks 2 and 4 after transplant. IE1, immediate early 1; pp65, phosphoprotein 65; SPC, spot count.

are needed to determine whether the Δ changes or the absolute CMI values are more relevant to predict CS-CMVi or mortality.

Our study is not without limitations. First, as a multicenter trial the study has missing data, such as absolute lymphocyte count, short-term follow-up (up to 6 months from HCT), and cause of death. Also this study was observational; therefore, management of CS-CMVi was not uniform but up to the provider, which may have affected outcomes.

Future directions from this research include evaluating (1) the role of CMV CMI measurement on when to initiate therapy for low CMV viral loads during chemoprophylaxis (eg, letermovir), (2) whether to safely discontinue primary or secondary prophylaxis as well as CMV PCR testing, and (3) the impact of long-term CMV primary or secondary prophylaxis on CMV CMI and transplant outcomes [20, 21].

In summary, changes in CMV-specific T-cell responses from pretransplant to 1 month posttransplant may serve as predictors of CS-CMVi and all-cause mortality in recipients of alloHCT who are CMV-seropositive. Future clinical trials should examine whether such recipients who do not exhibit an increase in CMV CMI would benefit from closer monitoring of CMV viral loads and possibly from prolonged antiviral prophylaxis to improve CMV- and transplant-related outcomes.

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

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and S. D. help with the acquisition of data. F. K. and T. B. helped with editing and reviewing the manuscript. Acquisition of data: D. J. W., K. M., P. C., P. H., R. K. A., K. S. P., D. K., R. N., P. L., S. B. M., D. P. S., E. J. A.-H., and L. E. H. All authors contributed to reviewing and editing the article.

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References

1. Ariza-Heredia EJ, Nesher L, Chemaly RF. Cytomegalovirus diseases after hematopoietic stem cell transplantation: a mini-review. Cancer Lett **2014**; 342:1–8.
2. Lúcia M, Crespo E, Cruzado JM, Grinyó JM, Bestard O. Human CMV-specific T-cell responses in kidney transplantation; toward changing current risk-stratification paradigm. Transpl Int **2014**; 27:643–56.
3. Pourgheysari B, Piper KP, McLarnon A, et al. Early reconstitution of effector memory CD4+ CMV-specific T cells protects against CMV reactivation following allogeneic SCT. Bone Marrow Transplant **2009**; 43:853–61.
4. Giulieri S, Manuel O. QuantiFERON-CMV assay for the assessment of cytomegalovirus cell-mediated immunity. Expert Rev Mol Diagn **2011**; 11:17–25.
5. Kwon JS, Kim T, Kim SM, et al. Comparison of the commercial QuantiFERON-CMV and overlapping peptide-based ELISPOT assays for predicting CMV infection in kidney transplant recipients. Immune Netw **2017**; 17:317–25.
6. Pahl-Seibert MF, Juelch M, Podlech J, et al. Highly protective in vivo function of cytomegalovirus IE1 epitope-specific memory CD8 T cells purified by T-cell receptor-based cell sorting. J Virol **2005**; 79:5400–13.
7. Chiereghin A, Gabrielli L, Zanfi C, et al. Monitoring cytomegalovirus T-cell immunity in small bowel/multivisceral transplant recipients. Transplant Proc **2010**; 42:69–73.
8. El Haddad L, Ariza-Heredia E, Shah DP, et al. The ability of a cytomegalovirus ELISPOT assay to predict outcome of low-level CMV reactivation in hematopoietic cell transplant recipients. J Infect Dis **2018**; 219:898–907.
9. Chemaly RF, El Haddad L, Winston DJ, et al. Cytomegalovirus (CMV) cell-mediated immunity and CMV infection after allogeneic hematopoietic cell transplantation: the REACT study. Clin Infect Dis **2020**; 71:2365–74.
10. Nesher L, Shah DP, Ariza-Heredia EJ, et al. Utility of the enzyme-linked immunospot interferon-γ-release assay to predict the risk of cytomegalovirus infection in hematopoietic cell transplant recipients. J Infect Dis **2016**; 213:1701–7.
11. Ljungman P, Boeckh M, Hirsch HH, et al. Disease definitions working group of the cytomegalovirus drug development, definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis **2017**; 64: 87–91.
12. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. N Engl J Med **2017**; 377:2433–44.
13. Nesher L, Shah DP, Azzi JM, et al. Immune monitoring with the T-SPOT CMV assay of allogeneic hematopoietic cell transplant (allo-HCT) recipients: a proof of concept in the clinical setting. Presented at: ICAAC 2014 54th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 5-9, **2014**; Washington, DC.
14. Gratama JW, Brooimans RA, van der Holt B, et al. Monitoring cytomegalovirus IE-1 and pp65-specific CD4+ and CD8+ T-cell responses after allogeneic stem cell transplantation may identify patients at risk for recurrent CMV reactivations. Cytometry B Clin Cytom **2008**; 74:211–20.
15. Gamadia LE, Remmerswaal EB, Weel JF, Bemelman F, van Lier RA, Ten Berge IJ. Primary immune responses to human CMV: a critical role for IFN-γ-producing CD4+ T cells in protection against CMV disease. Blood **2003**; 101:2686–92.
16. Jung J, Lee HJ, Kim SM, et al. Diagnostic usefulness of dynamic changes of CMV-specific T-cell responses in predicting CMV infections in HCT recipients. J Clin Virol **2017**; 87:5–11.
17. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. Blood **2010**; 115: 3861–8.
18. McIver Z, Melenhorst JJ, Wu C, et al. Donor lymphocyte count and thymic activity predict lymphocyte recovery and outcomes after matched-sibling hematopoietic stem cell transplant. Haematologica **2013**; 98:346–52.
19. Foolad F, Aitken SL, Chemaly RF. Letermovir for the prevention of cytomegalovirus infection in adult cytomegalovirus-seropositive hematopoietic stem cell transplant recipients. Expert Rev Clin Pharmacol **2018**; 11:931–41.
20. Li CR, Greenberg PD, Gilbert MJ, Goodrich JM, Riddell SR. Recovery of HLA-restricted cytomegalovirus (CMV)-specific T-cell responses after allogeneic bone marrow transplant: correlation with CMV disease and effect of ganciclovir prophylaxis. Blood **1994**; 83:1971–9.
21. Cherrier L, Nasar A, Goodlet KJ, Nailor MD, Tokman S, Chou S. Emergence of letermovir resistance in a lung transplant recipient with ganciclovir-resistant cytomegalovirus infection. Am J Transplant **2018**; 18:3060–4.