

Human cytomegalovirus control in allogeneic stem cell transplant recipients in the letermovir era – emerging humoral and cellular players

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Abstract

Allogeneic hematopoietic stem cell transplant (alloSCT) recipients frequently experience late-onset human cytomegalovirus (HCMV) reactivations following termination of letermovir prophylaxis. Letermovir prophylaxis extends the window for protective B- and T-cell reconstitution; however, our understanding of humoral responses and their contribution to HCMV immune control remains limited. Combining serological and flow cytometric analyses in 42 HCMV-seropositive alloSCT recipients, we herein provide the first comprehensive longitudinal (days 90-270 after transplant) characterization of HCMV-specific humoral responses, natural killer (NK)-cell phenotypes, and $\gamma\delta$ T cells in the letermovir era. HCMV controllers showed predominantly HCMV-specific IgG-driven responses, higher pre-reactivation $V\delta 1^+$ $\gamma\delta$ T-cell frequencies, and stronger expansion of “memory-like” NK cells than patients with clinically significant CMV infection. In contrast, patients with clinically significant CMV infection showed delayed HCMV-specific IgG production, IgM-skewed responses, and stronger post-reactivation expansion of memory B cells and $V\delta 1^+$ $\gamma\delta$ T cells. Early (day 90) $\gamma\delta$ T-cell reconstitution was associated with subsequent HCMV control. HCMV-specific IgG levels correlated only weakly with $\gamma\delta$ T cells but showed distinct associations with “memory-like” NK-cell reconstitution in HCMV controllers, suggesting synergisms between humoral and cellular immunity. Collectively, these findings highlight a need to study anti-HCMV immune protection beyond type 1 T cells and refine risk stratification models in alloSCT patients by inclusion of novel immune markers such as $\gamma\delta$ T-cell frequencies and phenotypes. Leveraging the extended B-cell reconstitution window created by letermovir, novel immunotherapies (e.g., therapeutic antibodies) and future vaccines might boost humoral anti-HCMV immunity and benefit from synergisms with $\gamma\delta$ T cells and “memory-like” NK cells in improving HCMV control.

Introduction

Reactivation of human cytomegalovirus (HCMV) remains a major complication following allogeneic hematopoietic stem cell transplantation (alloSCT).¹⁻³ With late-onset HCMV reactivations after letermovir cessation leading to clinically significant CMV infections (csCMVi) in 25 to 57% of cases,⁴⁻⁷ further investigation into the immune mechanisms controlling HCMV is critically needed.

While T cells are well-established as critical players in HCMV immunity,⁸⁻¹⁰ the role of humoral immunity has been more scarcely studied. Historically, this was due

to the slow reconstitution of B cells and the predominance of early-onset HCMV reactivations before day 100 after transplant.¹¹ However, in the letermovir era, late-onset HCMV reactivations often occur when B-cell reconstitution is more advanced,¹² and antibody responses may play a crucial role in HCMV control through viral neutralization, CD16-mediated recognition of virions, antibody-dependent cellular cytotoxicity (ADCC), and cytokine secretion.¹³⁻¹⁵

Furthermore, other immune cell populations that are poorly explored in the letermovir era, such as “memory-like” NK cells and $\gamma\delta$ T cells, have known anti-HCMV properties and

interact with HCMV-specific antibodies via the immunoglobulin (Ig) G-Fc receptor CD16,¹³⁻¹⁵ suggesting a potential interplay between humoral and cellular immunity that warrants further investigation.

To address these knowledge gaps, we conducted a longitudinal analysis of peripheral blood mononuclear cells and serum samples from 42 HCMV-seropositive alloSCT recipients at four post-transplant timepoints: day 90 (prior to letermovir cessation), day 150 (around the median time

of first HCMV reactivation), and days 210 and 270 (following HCMV reactivation). We compared patients who controlled HCMV reactivation with those who developed csCMVi to investigate the roles of humoral anti-HCMV responses, “memory-like” NK cells, and $\gamma\delta$ T cells in HCMV defense (Figure 1A). Thereby, we provide novel insights into HCMV immune control in the letermovir era beyond antigen-specific T cells, informing improved strategies for risk stratification, vaccination, and immunotherapy.

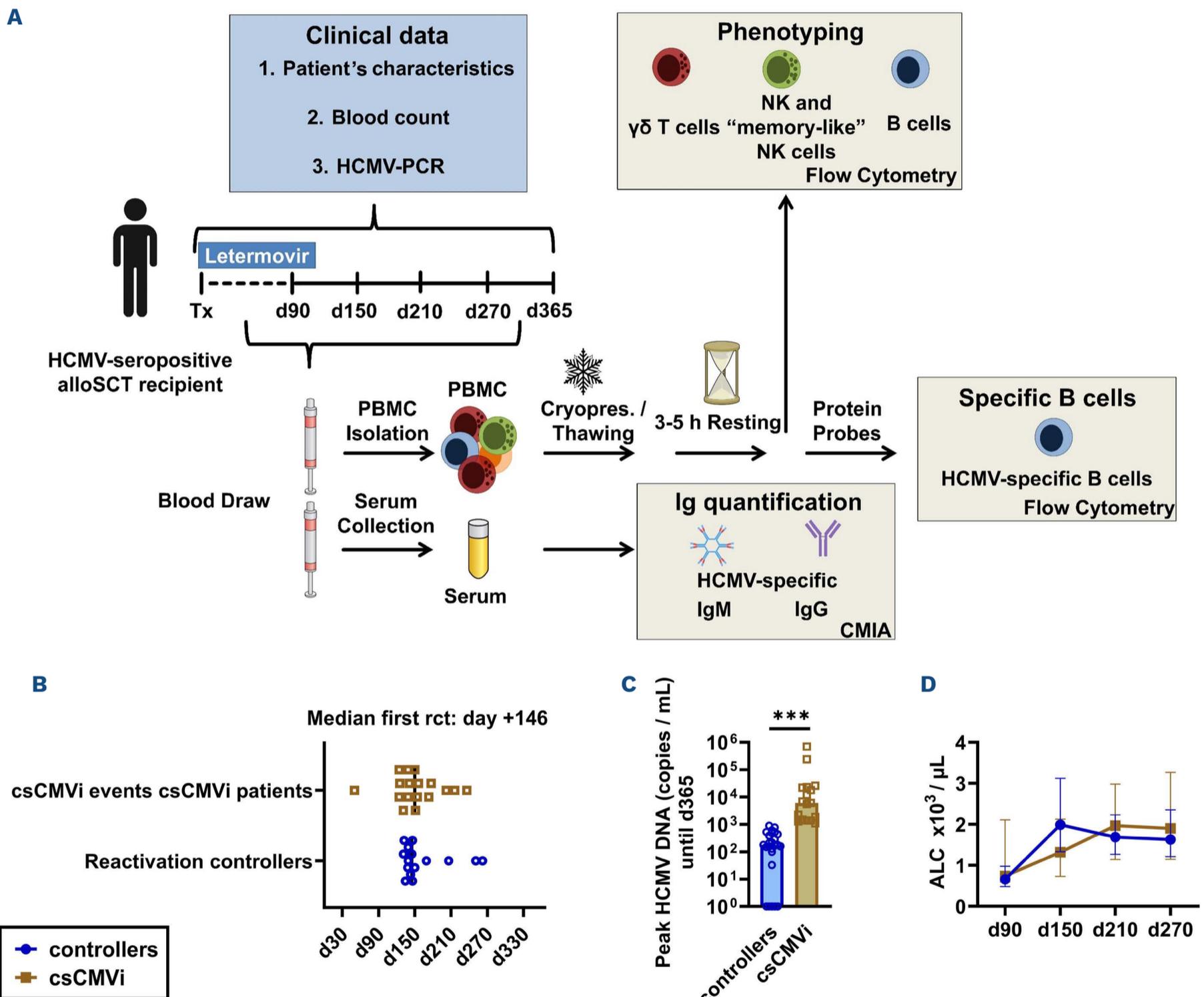


Figure 1. Human cytomegalovirus reactivation timing, viral load, and absolute lymphocyte counts. (A) Study flowchart. (B) Median day of first quantifiable human cytomegalovirus (HCMV) load in HCMV controllers (blue, N=25) and patients with clinically significant cytomegalovirus infection (csCMVi) (brown), as well as the day of csCMVi onset in affected patients (N=17). Transient low-level HCMV DNAemia (“blips”) occurred in some patients during letermovir prophylaxis but are not shown, as they likely reflect non-infectious viral DNA. No HCMV viral load was detected in five HCMV controllers throughout the observation period. (C) Peak HCMV DNA copies/mL until day 365 (measured by polymerase chain reaction). Mann-Whitney U test. (D) Absolute lymphocyte count kinetics for HCMV controllers and csCMVi patients. Mann-Whitney U test. * $P < 0.05$, *** $P < 0.001$. PCR: polymerase chain reaction; Tx: transplantation; alloSCT: allogeneic stem cell transplantation; PBMC: peripheral blood mononuclear cells; cryopres.: cryopreservation; CMIA: chemiluminescent microparticle immunoassay; NK: natural killer; IgM: immunoglobulins M; IgG: immunoglobulins G; ALC: absolute lymphocyte counts.

Methods

Ethics approval

This study was approved by the Ethics Committees of the University of Wuerzburg, Germany (protocol code 17/19-sc). Written informed consent was obtained from all patients.

Study population

Forty-two HCMV-seropositive alloSCT recipients (with either HCMV-positive or -negative donors) were enrolled at the University Hospital of Wuerzburg (Germany) between December 2019 and October 2023. All patients received letermovir prophylaxis with a dose of 480 mg (240 mg when co-administered with cyclosporine A) once daily from day 1 to day 100 after transplant. HCMV DNAemia was monitored weekly until day 100 after transplant and biweekly thereafter using a clinically validated real-time polymerase chain reaction on plasma samples, which is described in more detail in the *Online Supplementary Materials*. Preemptive systemic antiviral therapy with (val)ganciclovir was initiated whenever HCMV DNA levels exceeded 1,000 copies/mL. If this occurred before day 100, letermovir prophylaxis was discontinued. Additionally, cryopreserved samples from 15 patients from the pre-letermovir era without letermovir prophylaxis (2015-2019) were used for comparative analyses.

Definitions

During a 365-day follow-up, subjects were classified as HCMV controllers (DNA <1,000 copies/mL without antiviral therapy) or csCMVi patients (DNA >1,000 copies/mL requiring therapy). Refractory HCMV infection (Rf-csCMVi) was defined by Chemaly et al.¹⁶

Cell populations were compared before csCMVi (last available timepoint before therapy initiation) and after csCMVi (first available timepoint after HCMV DNA levels returned below 300 copies/mL, corresponding to the lower limit of quantification of the polymerase chain reaction assay). In controllers, pre-reactivation measurements were taken before DNA first rose into 1-1,000 copies/mL, and post-reactivation once levels returned to zero, reflecting a more stringent threshold used for this group due to their typically low-level, self-limiting DNAemia without antiviral therapy.

Immunoassays

EDTA blood and serum samples were collected on days 84-106 (day 90), 141-167 (day 150), 200-233 (day 210), and 262-309 (day 270) after transplant. Serum HCMV-specific IgM and IgG were measured using Architect CMV kits (Abbott Ireland). Peripheral blood mononuclear cells were isolated and cryopreserved for flow cytometry of $\gamma\delta$ T-, B-, and NK-cell phenotypes and quantification of HCMV-specific B cells via biotinylated glycoprotein B, trimer and pentamer protein probes. Detailed protocols and gating strategies (*Online Supplementary Figures S1-S4*) are provided in the *Online Supplementary Materials*. Frequencies of immune

populations were calculated using a combination of flow cytometry data and absolute lymphocyte counts.

Statistical analysis and software applications

Statistical significance was assessed using the Mann-Whitney U test, paired Wilcoxon test, Kruskal-Wallis test, or Fisher exact test as detailed in the figure legends. Data were compiled, visualized, and analyzed using Microsoft Excel (Microsoft, Redmond, WA, USA), Prism v.10.2 (GraphPad Software, La Jolla, California, USA), and R v.4.2.1 (R Core Team, Vienna, Austria).

Results

Patients' characteristics

Forty-two HCMV-seropositive alloSCT patients receiving letermovir prophylaxis until day 100 after transplant were included in the study. Of these, 25 (60%) controlled HCMV until day 365, while 17 (40%) developed csCMVi. The patients' characteristics were similar between controllers and csCMVi patients, except for a higher incidence of grade ≥ 2 acute graft-versus-host disease in csCMVi patients ($P=0.018$) (Table 1). To preclude a significant impact of pharmacological immunosuppression on our immunological analyses, we specifically analyzed the number and dosage of immunosuppressants administered during and after letermovir prophylaxis. Slightly more csCMVi patients than controllers (18% vs. 8%) received systemic high-dose glucocorticosteroids (2 mg/kg prednisolone), while use of non-glucocorticoid immunosuppressive agents was similar (Table 2).

The first HCMV reactivation occurred on a median of day 146. In five controllers, no quantifiable HCMV DNAemia was detected. Eight csCMVi patients and five controllers experienced minimal and transient HCMV DNAemia during letermovir prophylaxis. Except for one patient with csCMVi case who required preemptive therapy, these episodes likely do not represent true viral reactivation but rather the detection of non-infectious viral DNA, commonly referred to as "blips", a well-documented phenomenon during letermovir prophylaxis.¹⁷ Consequently, these events are not classified and shown as reactivations (Figure 1B). Peak median HCMV DNA loads were significantly higher in csCMVi patients than in controllers (6,100 copies/mL vs. 180 copies/mL, $P<0.001$) (Figure 1C). Notably, absolute lymphocyte counts were similar between controllers and csCMVi patients across all timepoints (Figure 1D).

Allogeneic stem cell transplant recipients in the letermovir era mount robust humoral responses by the time of human cytomegalovirus reactivation and develop memory B cells after reactivation events

Before the approval of letermovir, there was no routine prophylaxis against HCMV. HCMV reactivations mostly occurred early (median day 26 in our pre-letermovir cohort)

before B-cell reconstitution (CD20⁺), resulting in minimal humoral responses at the time of reactivation (Figure 2A, B). In contrast, in our contemporary cohort, HCMV reactivation occurred significantly later (median, day 146). By that time, we expectedly observed more readily reconstituted B-cell numbers (median, 97.5) and frequencies (median, 6.8% of total lymphocytes) (Figure 2A).

Unlike the historic cohort (Figure 2B), patients in the letermovir-era contemporary cohort showed increasing HCMV-specific IgG and IgM levels between days 150 and 210, coinciding with reactivation (Figure 2C). HCMV-specific IgM ($P=0.370$) and IgG ($P=0.506$) levels were comparable between the two cohorts on day 90, thereby precluding bias from differences in immunoglobulin levels. Notably, the characteristics of the patients in the pre-letermovir cohort were also largely comparable to those of our cohort from the letermovir era (*Online Supplementary Table S1*). Comparisons of pre- and post-reactivation measurements in the letermovir cohort further confirmed significant expansion of HCMV-specific IgG (131 vs. 809 arbitrary units [AU]/mL, $P=0.009$) and IgM (0.24 vs. 1.84 IgM Index, $P<0.001$) during reactivation (Figure 2D).

Total B-cell numbers and frequencies of csCMVi patients and controllers in the cohort from the letermovir era were largely comparable, except for higher frequencies in controllers at day 210 (Figure 2E). In both groups, IgM⁺ B cells decreased from day 90 to day 270, whereas IgG⁺ B cells increased, consistent with expected patterns of immune reconstitution and memory formation including antibody class switching (Figure 2F, G). Furthermore, the ratio of IgG⁺ to IgM⁺ memory B cells (MBC: CD20⁺IgD⁻) increased from pre- to post-HCMV events ($P=0.006$) (Figure 2H), a trend that was observed in both controllers ($P=0.040$) and csCMVi patients ($P=0.085$). Although global B-cell frequencies were largely comparable, the time-dependent evolution of B-cell phenotypes differed significantly between HCMV controllers and csCMVi patients. Specifically, those with csCMVi displayed significantly higher day-270 frequencies of MBC (14.0 vs. 5.7% among B cells, $P=0.032$) and lower frequencies of naïve B cells (CD20⁺IgD⁺, 84.8 vs. 93.0% among B cells, $P=0.018$) than controllers (Figure 2I-K).

In summary, alloSCT patients in the letermovir era mount robust HCMV-specific humoral responses by the time of HCMV reactivation, suggesting a potential novel role of the humoral response in anti-HCMV immunity. Furthermore, the time-dependent shift in B-cell phenotypes gives rise to the hypothesis that occurrence and severity of HCMV reactivations may shape the B-cell repertoire.

Human cytomegalovirus reactivation triggers the expansion of cytomegalovirus-specific B cells

To test this hypothesis, we longitudinally analyzed the magnitude and quality of HCMV-specific humoral response in our letermovir cohort in more detail. To do this, HCMV-specific MBC were detected using fluorescent protein probes target-

Table 1. Characteristics of allogeneic stem cell transplant recipients who successfully controlled human cytomegalovirus reactivation and those who developed clinically significant human cytomegalovirus infection.

Variables	Total N=42	HCMV controllers N=25	csCMVi N=17	P
Age, years, median (range)	62	62 (22-73)	61 (33-71)	0.995
Sex, N (%)				0.530
Male	21 (50)	14 (56)	7 (41)	
Female	21 (50)	11 (44)	10 (59)	
Underlying disease, N (%)				-
Chronic leukemia	1 (2)	1 (4)	0 (0)	
Multiple myeloma	2 (5)	1 (4)	1 (6)	
Acute leukemia	25 (60)	14 (56)	11 (65)	
Lymphoma	0 (0)	0 (0)	0 (0)	
Others	14 (33)	9 (36)	5 (29)	
HLA matching, N (%)				-
Matched related	5 (12)	1 (4)	4 (24)	
Matched unrelated	24 (57)	19 (76)	5 (29)	
Haploidentical	0 (0)	0 (0)	0 (0)	
Mismatch	13 (31)	5 (20)	8 (47)	
Stem cell source, N (%)				>0.999
Bone marrow	1 (2)	1 (4)	0 (0)	
PBSC	41 (98)	24 (96)	17 (100)	
Conditioning regimen, N (%)				>0.999
Reduced intensity	39 (93)	23 (92)	16 (94)	
Myeloablative	3 (7)	2 (8)	1 (6)	
Antithymocyte globulin, N (%)				0.140
No	5 (12)	1 (4)	4 (24)	
Yes	37 (88)	24 (96)	13 (76)	
HCT-CI, N (%)				-
0-2	30 (71)	19 (76)	11 (65)	
3-4	9 (21)	4 (16)	5 (29)	
≥5	3 (7)	2 (8)	1 (6)	
Acute GvHD, N (%)				0.018
0-1	29 (69)	21 (84)	8 (47)	
2-4	13 (31)	4 (16)	9 (53)	
Chronic GvHD, N (%)				>0.999
No	27 (64)	16 (64)	11 (65)	
Yes	15 (36)	9 (36)	6 (35)	
HCMV serostatus, N (%)				0.353
R+/D+	26 (62)	17 (68)	9 (53)	
R+/D-	16 (38)	8 (32)	8 (47)	
Vital status at 1 year, N (%)				>0.999
Alive	35 (83)	21 (84)	14 (82)	
Dead	7 (17)	4 (16)	3 (18)	

The Mann-Whitney U test or Fisher exact test was applied as appropriate. HCMV: human cytomegalovirus; csCMVi: clinically significant cytomegalovirus infection; HLA: human leukocyte antigen; PBSC: peripheral blood stem cells; HCT-CI: Hematopoietic Cell Transplantation Comorbidity Index; GvHD: graft-versus-host disease, R: recipient; D: donor. $P<0.05$ is considered statistically significant.

Table 2. Immunosuppressant administration during and after letermovir prophylaxis in patients who successfully controlled human cytomegalovirus reactivation and patients with clinically significant cytomegalovirus infection.

Immunosuppression	Total		HCMV controllers		csCMVi	
	on LVR	after LVR	on LVR	after LVR	on LVR	after LVR
Steroid use*, N (%)						
None	36 (86)	37 (88)	24 (96)	23 (92)	12 (71)	14 (82)
>0 to ≤1 mg/kg/day	2 (5)	0 (0)	1 (4)	0 (0)	1 (6)	0 (0)
2 mg/kg/day	4 (10)	5 (12)	0 (0)	2 (8)	4 (24)	3 (18)
Immunosuppressants#						
0	0 (0)	39 (93)	0 (0)	23 (92)	0 (0)	16 (94)
1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2	40 (95)	2 (5)	25 (100)	2 (8)	15 (88)	0 (0)
> 2	2 (5)	1 (2)	0 (0)	0 (0)	2 (12)	1 (6)

*Divided into 0, >0 to ≤1, or 2 mg/kg/day based on maximum steroid dose during the study period. #Not including steroids (e.g., ruxolitinib, tacrolimus, sirolimus). LVR: letermovir; HCMV: human cytomegalovirus; csCMVi: clinically significant HCMV infection.

ing three key HCMV-derived antigens eliciting neutralizing antibody responses, the gH/gL/gO trimer, the gH/gL/UL128/UL130/UL131A pentamer, and glycoprotein B (Figure 3A, B). Quantifying these antigens individually and in combination showed high specificity, with no HCMV-specific MBC detected in HCMV-seronegative alloSCT recipients (*Online Supplementary Figure S5A*).

Notably, csCMVi patients had higher counts and frequencies of glycoprotein B-, trimer-, and pentamer-specific MBC than controllers on day 270 (Figure 3C). Given the low frequencies of HCMV-specific MBC early after transplantation and limited cell availability, we combined the probes for all three antigens for subsequent longitudinal quantification. This approach revealed strongly increasing HCMV-specific MBC in both HCMV controllers and csCMVi patients between days 90 and 210. However, by day 270, csCMVi patients had developed higher HCMV-specific MBC counts (35.1 vs. 16.8, $P=0.064$) and frequencies (0.33% vs. 0.10%, $P=0.009$) than controllers (Figure 3D). To determine whether the increase in HCMV-specific MBC coincided with HCMV reactivation, we further compared their frequencies before and after reactivation in patients with detectable HCMV viral load, regardless of csCMVi status. Indeed, HCMV-specific MBC frequencies significantly increased from a median of 0.00% before reactivation to 0.13% after reactivation ($P=0.001$) (Figure 3E). Overall, these results suggest that HCMV reactivation drives HCMV-specific MBC development in seropositive alloSCT recipients.

Patients with clinically significant cytomegalovirus infection display an IgM-skewed human cytomegalovirus reactivation-specific response during reactivation and a delayed IgG response compared to controllers

Given the disparate affinity and antiviral effector responses of IgG and IgM,¹⁸ we next examined differences in HCMV-specific IgG and IgM serum concentration in controllers and csCMVi patients. On day 150, i.e., around the median time of HCMV

reactivation (Figure 1B), 100% of the detected HCMV-specific MBC in HCMV controllers showed an IgG phenotype. In contrast, HCMV-specific MBC in csCMVi patients were predominantly IgM-positive (70% IgM⁺, 30% IgG⁺) (Figure 3F). These differences were also reflected in longitudinal serum levels of HCMV-specific IgG and IgM. HCMV controllers showed a non-significant trend toward higher HCMV-specific IgG levels on day 150 (median 212 vs. 82 AU/mL, median-to-median ratio [MMR]=2.6) and day 210 (median 1,363 vs. 163 AU/mL, MMR=8.4). Conversely, csCMVi patients had significantly higher HCMV-specific IgM indices on day 210 (median 3.3 vs. 0.6, MMR=5.5, $P=0.029$) and day 270 (1.1 vs. 0.4, MMR=2.8, $P=0.046$) (Figure 3G).

Moreover, controllers showed a robust increase in HCMV-specific IgG levels between days 90 and 210 (147 vs. 1,363 AU/mL, $P<0.001$), but only a small increase in HCMV-specific IgM indices (0.2 vs. 0.6, $P<0.001$). Inversely, csCMVi patients mounted a strongly increasing HCMV-specific IgM response during that timeframe (0.3 vs. 3.3, $P=0.015$), but showed no significant increase in HCMV-specific IgG levels (130 vs. 164 AU/mL, $P=0.421$) (Figure 3H). Instead, csCMVi patients had a delayed rise in HCMV-specific IgG levels between day 210 and day 270, resulting in comparable IgG levels between the two groups by day 270 (median 1,523 vs. 1,500 AU/mL) (Figure 3I). Overall, time-dependent HCMV-specific B-cell responses differed significantly between controllers and csCMVi patients. Controllers displayed modest, early IgG-tilted development, whereas csCMVi patients exhibited a comparatively more IgM-skewed humoral response upon viral reactivation. This suggests a delayed IgM-to-IgG class switch in csCMVi patients.

Clinically significant cytomegalovirus infection events drive the expansion of Vδ1⁺ γδ T cells and shift the Vδ1⁺/Vδ2⁺ ratio

In addition to directly neutralizing virions, HCMV-specific IgG can enhance other determinants of immune defense.¹³⁻¹⁵ Specifically, immune cells with anti-HCMV properties, in-

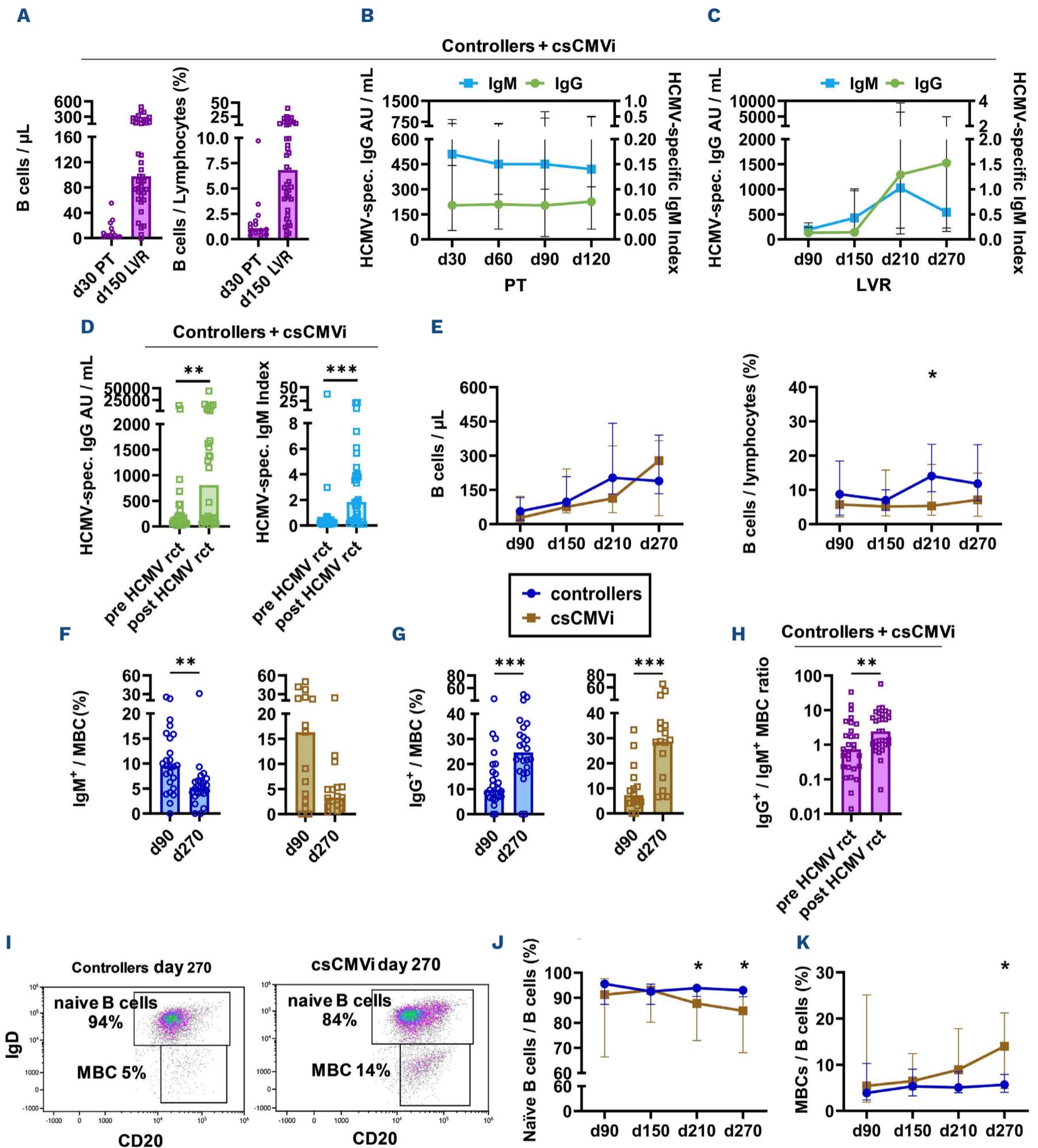


Figure 2. Human cytomegalovirus reactivation is associated with a robust humoral response and memory B-cell formation. Samples from allogeneic stem cell transplant recipients receiving letemovir prophylaxis (N=42) or historic samples from the pre-letemovir era (N=15, A and B only) were analyzed using flow cytometry for cellular markers and chemiluminescent microparticle immunoassay for serological markers. (A) B-cell counts (CD20⁺) and frequencies around the median time of first human cytomegalovirus (HCMV) reactivation in the pre-letemovir era (day 26) and in patients receiving letemovir prophylaxis (day 150). Mann-Whitney U test. (B-D) Kinetics of HCMV-specific IgM and IgG levels in the pre- (B) and post-letemovir era (C, D) around the time of HCMV reactivation. N=40. (E) B-cell counts and frequencies in HCMV controllers and patients with clinically significant

Continued on following page.

cytomegalovirus infection (csCMVi). Mann-Whitney U test. (F, G) Evolution of frequencies of IgM⁺ (F) and IgG⁺ (G) memory B cells (MBC; CD20⁺IgD⁻) between day 90 and day 270 in HCMV controllers and csCMVi patients. Paired Wilcoxon test. (H) Ratio of IgG⁺/IgM⁺ MBC frequencies before and after HCMV reactivation. Paired Wilcoxon test. (I) Representative flow cytometry plot showing differentiation of naïve B cells (CD20⁺IgD⁺) and MBC. (J, K) Kinetics of MBC (J) and naïve B-cell (K) frequencies in HCMV controllers and csCMVi patients. Mann-Whitney U test. **P*<0.05, ***P*<0.01, ****P*<0.001. Interquartile ranges are shown where applicable. D: day; PT: preemptive therapy; LVR: letermovir; AU: arbitrary units; IgG: immunoglobulin; rct: reactivation.

cluding $\gamma\delta$ T cells and memory-like NK cells, can interact in part with HCMV-specific IgG via the IgG receptor CD16.¹³⁻¹⁵ To investigate potential associations between humoral and cellular immunity, we also characterized the reconstitution of these immune cell populations.

Firstly, we evaluated the reconstitution of $\gamma\delta$ T cells (TCR $\gamma\delta$ ⁺), a cell population that is protective against HCMV reactivation in alloSCT patients (Figure 4A).¹⁹⁻²¹ Both HCMV controllers and csCMVi patients had similar $\gamma\delta$ T-cell kinetics and significantly expanded their total $\gamma\delta$ T-cell numbers (Figure 4B, C). However, sub-populations differed (Figure 4D). Specifically, controllers had higher V δ 1⁺ cell numbers (TCR $\gamma\delta$ ⁺TCRV δ 1⁺, 3.7 vs. 1.8 cells/ μ L, MMR=2.1) and frequencies (40.7 vs. 19.6% V δ 1⁺ cells among $\gamma\delta$ T cells, *P*=0.050) than csCMVi patients before letermovir cessation (day 90) (Figure 4E). In fact, high day 90 V δ 1⁺ cell frequencies were associated with subsequent HCMV control, as confirmed by receiver-operating characteristics analysis (area under the curve=0.688, *P*=0.049, 95% confidence interval: 0.4967-0.8793) (Figure 4F). Inversely, csCMVi patients showed higher V δ 1⁺ frequencies among $\gamma\delta$ T cells than controllers from day 210 onward (day 210: 72.0 vs. 56.0%, *P*=0.032; day 270: 73.6 vs. 59.9%, *P*=0.021) (Figure 4E). V δ 2⁻ (TCR $\gamma\delta$ ⁺TCRV δ 2⁻) cells followed the trend of V δ 1⁺ cells (*Online Supplementary Figure S5B-E*).

V δ 2⁺ frequencies (TCR $\gamma\delta$ ⁺TCRV δ 2⁺) showed minimal differences between HCMV controllers and csCMVi patients. Likewise, comparable numbers and frequencies of the V δ 2⁺V δ 9⁺ and V δ 2⁺V δ 9⁻ subpopulations were found in both groups (*Online Supplementary Figure S5F-Q*).

In csCMVi patients, these trends collectively led to a marked shift in V δ 1⁺/V δ 2⁺ ratios over time, which were initially higher (day 90: V δ 2⁺/V δ 1⁺ 4.0 vs. 1.2, *P*=0.038) and later lower (day 270: V δ 2⁺/V δ 1⁺ 0.2 vs. 0.4, *P*=0.015) than in controllers (Figure 4G). Both groups expanded V δ 1⁺ cells after reactivation/csCMVi, but csCMVi patients showed significantly greater expansion from day 90 to 210 than controllers (median fold changes, 22.3 vs. 4.3, *P*=0.007) (Figure 4H-J).

Furthermore, differences in V δ 1⁺ memory populations were observed between the two cohorts from day 210 onward. Specifically, csCMVi patients had significantly higher numbers of effector memory (day 270: 1.4 vs. 0.5 CD45RA⁻CD27⁻V δ 1⁺ cells/ μ L, *P*=0.003) and central memory V δ 1⁺ cells (day 210: 4.6 vs. 1.8 CD45RA⁻CD27⁺V δ 1⁺ cells/ μ L, *P*=0.023) than controllers (Figure 4K). In contrast, naïve and terminally differentiated effector cells remained comparable (*Online Supplementary Figure S5R, S*).

In summary, low V δ 1⁺ frequencies before letermovir cessation were a marker of increased csCMVi risk. csCMVi

events were, in turn, associated with significant expansion of V δ 1⁺ $\gamma\delta$ T cells, resulting in an increased V δ 1⁺/V δ 2⁺ ratio compared to that in controllers, partially due to expansion of effector and central memory phenotypes.

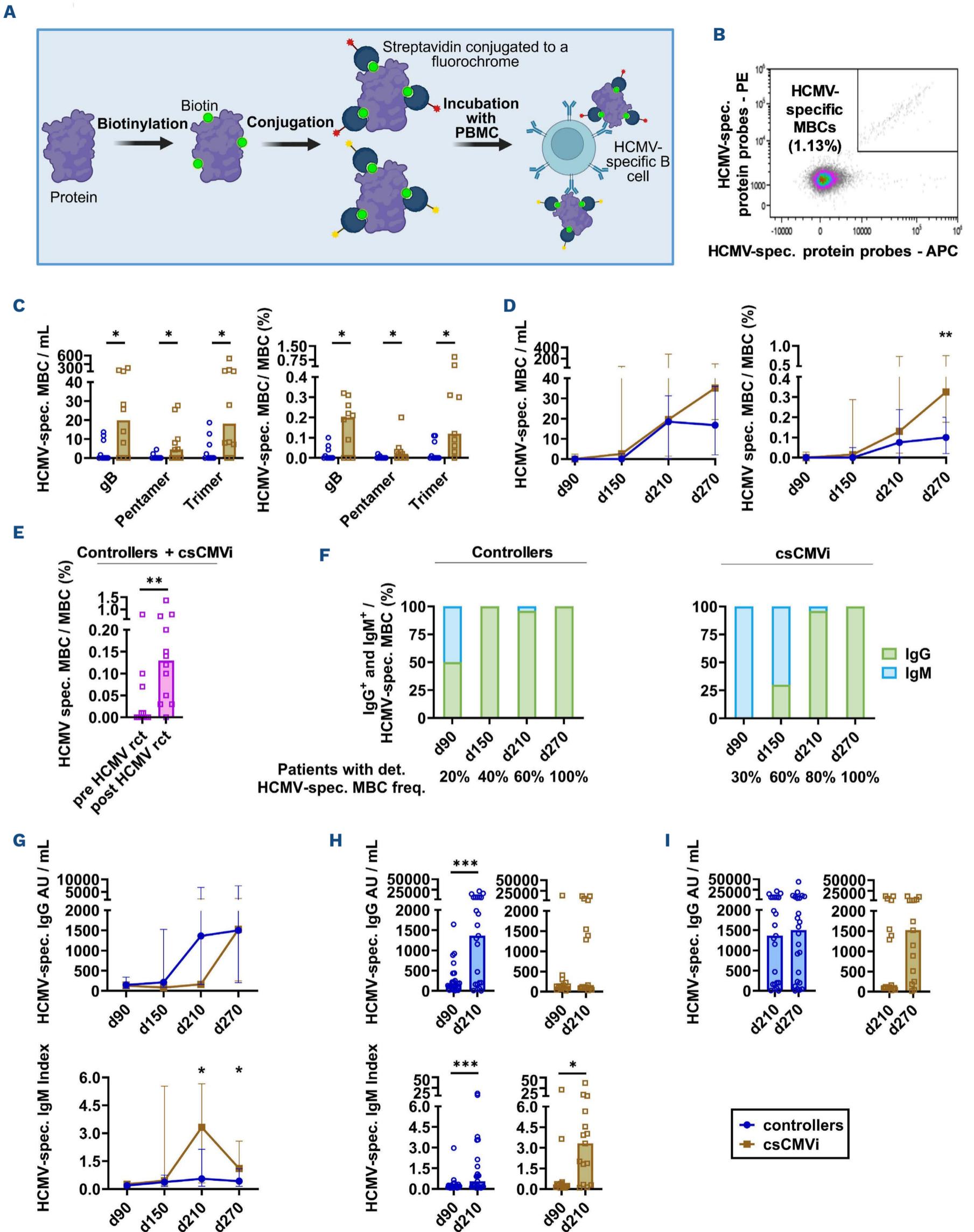
Lack of “memory-like” natural killer-cell expansion is associated with refractory clinically significant cytomegalovirus infection

NK and “memory-like” NK cells are key for HCMV control and IgG-dependent ADCC.^{14,22} Therefore, we also analyzed NK-cell reconstitution in our cohort. Consistent with prior evidence,⁸ there were no differences in total NK-cell numbers or distribution of CD56^{dim} and CD56^{bright} NK-cell subpopulations between HCMV controllers and csCMVi patients (Figure 5A, B). Likewise, there was no major change in the expansion of these cell populations before and after reactivation except for a minor shift toward CD56^{dim} (Figure 5C).

Next, we specifically focused on (NKG2C/CD159c⁺) “memory-like” NK-cell reconstitution (Figure 5D). Although not statistically significant due to considerable inter-individual variation, controllers consistently showed a trend toward higher “memory-like” CD56^{dim} (NK^{dim}) NK-cell numbers (MMR: 1.6-2.0) and frequencies (MMR: 1.2-1.4) than those in csCMVi patients (Figure 5E). Similarly, mature (CD57⁺) “memory-like” NK^{dim}-cell counts tended to be higher in controllers than in csCMVi patients from day 210 onwards (Figure 5F).

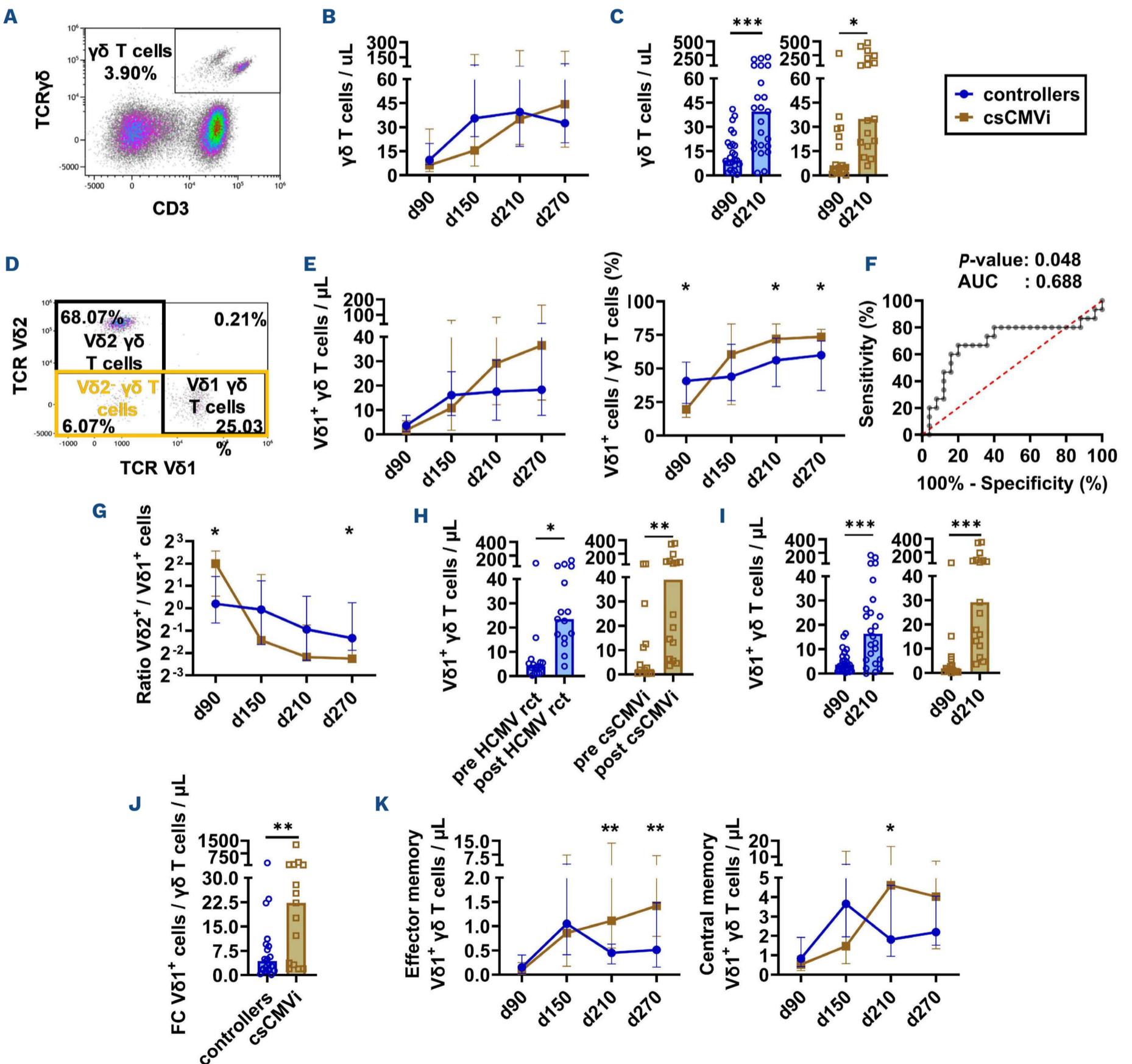
We previously reported that low numbers and frequencies of “memory-like” NK cells were associated with severe and refractory csCMVi events.⁸ To corroborate that trend in our current cohort, we again subdivided csCMVi patients into those with and without Rf-csCMVi. Indeed, patients with Rf-csCMVi had markedly lower numbers and frequencies of “memory-like” NK^{dim} cells and mature “memory-like” NK^{dim} cells compared to both no Rf-csCMVi patients and controllers (Figure 5G, H).

Notably, controllers demonstrated significant expansion of “memory-like” NK^{dim} cells from day 90 to day 210 (16.0 vs. 35.8, median fold change=2.2, *P*=0.006) and from before to after HCMV reactivation (15.4 vs. 50.0, median fold change=3.2, *P*=0.003). Although not reaching significance due to the smaller group size, similar changes were seen in non-Rf-csCMVi patients (day 90 to day 210: 8.9 vs. 36.3, median fold change=4.1, *P*=0.052; before to after csCMVi: 24.2 vs. 36.3, median fold change=1.5, *P*=0.206). In contrast, median “memory-like” NK^{dim}-cell counts remained consistently below 10 cells/ μ L in Rf-csCMVi patients (*Online*



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Figure 3. Patients with clinically significant cytomegalovirus infection show an IgM-skewed human cytomegalovirus-specific response with delayed IgG kinetics compared to cytomegalovirus controllers. Reconstitution of the human cytomegalovirus (HCMV)-specific humoral immune response was analyzed by flow cytometry and chemiluminescent microparticle immunoassay. (A) Flowchart illustrating the generation of HCMV-specific protein probes to detect HCMV-specific B cells. (B) Representative flow cytometry plot showing the detection of HCMV-specific memory B cells (MBC; CD20⁺IgD⁻). (C) Absolute numbers and frequencies of HCMV-specific MBC targeting glycoprotein B (gB), the pentamer complex (gH/gL/UL128/UL130/UL131A), and the trimer complex (gH/gL/gO) on day 270. N=20. Mann-Whitney U test. (D) Kinetics of absolute numbers and frequencies of combined gB-, pentamer-, and trimer-specific MBC. N=20. Mann-Whitney U test. (E) HCMV-specific MBC frequencies before and after HCMV reactivation in patients with detectable HCMV viral load. N=12. Paired Wilcoxon test. (F) Normalized median frequencies of IgG⁺ and IgM⁺ HCMV-specific MBC over time. N=20. (G) Kinetics of HCMV-specific IgG levels and IgM indices. N=40. Mann-Whitney U test. (H, I) Evolution of serological responses between days 90 and 210 (H) and days 210 and 270 (I). N=40; 25 HCMV controllers, 15 patients with clinically significant HCMV infection. Paired Wilcoxon test. **P*<0.05, ***P*<0.01, ****P*<0.001. Interquartile ranges are shown where applicable. PBMC: peripheral blood mononuclear cells; spec.; specific; PE: phycoerythrin; APC: allophycocyanin; d: day; rct: reactivation; AU: arbitrary units; Ig: immunoglobulin; csCMVi: clinically significant HCMV infection.



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Figure 4. Clinically significant cytomegalovirus infection events promote V δ 1⁺ $\gamma\delta$ T-cell expansion and alter the V δ 1⁺/V δ 2⁺ ratio.

Reconstitution of $\gamma\delta$ T cells in 42 allogeneic stem cell transplant recipients was analyzed by flow cytometry. (A) Representative flow cytometry plot for quantification of $\gamma\delta$ T cells. (B) Kinetics of $\gamma\delta$ T-cell (TCR $\gamma\delta$ ⁺) numbers. Mann-Whitney U test. (C) Comparison of $\gamma\delta$ T-cell numbers on days 90 and 210. Paired Wilcoxon test. (D) Representative flow cytometry plot illustrating $\gamma\delta$ T-cell subpopulations. (E) Kinetics of absolute numbers and frequencies of V δ 1⁺ $\gamma\delta$ T cells (TCR $\gamma\delta$ ⁺TCRV δ 1⁺). Mann-Whitney U test. (F) Receiver operating characteristic curve analysis for prediction of clinically significant cytomegalovirus infection (csCMVi) events based on V δ 1⁺ $\gamma\delta$ T-cell frequencies on day 90. (G) Longitudinal assessment of the V δ 2⁺ (TCR $\gamma\delta$ ⁺TCRV δ 2⁺)/V δ 1⁺ ratio. Mann-Whitney U test. (H) Quantification of V δ 1⁺ $\gamma\delta$ T cells before and after human cytomegalovirus reactivation/csCMVi events. Paired Wilcoxon test. (I) Comparison of V δ 1⁺ $\gamma\delta$ T-cell counts at days 90 and 210. Paired Wilcoxon test. (J) Intra-individual fold changes (FC) of V δ 1⁺ $\gamma\delta$ T-cell counts from day 90 until day 210. Mann-Whitney U test. (K) Kinetics of effector (CD45RA⁺CD27⁻) and central memory (CD45RA⁻CD27⁺) V δ 1⁺ $\gamma\delta$ T-cell counts. Mann-Whitney U test. * P <0.05, ** P <0.01, *** P <0.001. Interquartile ranges are shown where applicable. d: day; AUC: area under the curve; FC: fold changes; rct: reactivation.

Supplementary Figure S6A). Similar trends and differences between the groups were observed when specifically analyzing mature “memory-like” NK cells (Online Supplementary Figure S6B).

In summary, consistent with prior evidence, HCMV controllers had higher numbers and frequencies of (mature) “memory-like” NK cells compared to csCMVi patients, especially those with Rf-csCMVi events. These differences increased over time and after reactivation events due to significant expansion of these cells in controllers and some patients with non-Rf-csCMVi, contrasting very limited expansion in those with Rf-csCMVi.

Human cytomegalovirus reactivation-specific humoral IgG response correlates with the expansion of CD56^{dim} natural killer-cell subpopulations in virus controllers but not in patients with clinically significant cytomegalovirus infection

Lastly, we correlated HCMV-specific IgG levels with $\gamma\delta$ T cells and “memory-like” NK cells, both key IgG-interacting populations. Across all patients, correlations between HCMV-specific IgG levels and $\gamma\delta$ T-cell subpopulations were generally weak and non-significant. However, the V δ 1⁺ $\gamma\delta$ T-cell population showed a slight positive correlation with HCMV-specific IgG levels aggregated across all timepoints (ρ =0.22, P =0.005) (Online Supplementary Figure S7A, B). Notably, this correlation was confined to the effector V δ 1⁺ $\gamma\delta$ T-cell population, where weak to moderate correlations were observed both on aggregate across all timepoints (ρ =0.38, P <0.001) and specifically on day 150 (ρ =0.32, P =0.046) (Online Supplementary Figure S7A, C), shortly after the median time of HCMV reactivation (Figure 1B). These trends were largely comparable among controllers and csCMVi patients.

Interestingly, CD56^{dim} NK cells (ρ =0.27, P =0.008), “memory-like” NK^{dim} cells (ρ =0.27, P =0.007), and mature “memory-like” NK^{dim} cells (ρ =0.33, P =0.001) showed significant correlations with HCMV-specific IgG levels on aggregate across all timepoints only in controllers but not in csCMVi patients. A positive correlation between HCMV-specific IgG levels and these cell populations in controllers was most pronounced on day 150, near the median time of HCMV reactivation (CD56^{dim}: ρ =0.55, P =0.005; “memory-like” NK^{dim}

cells: ρ =0.31, P =0.136; mature “memory-like” NK^{dim} cells: ρ =0.41, P =0.046). In contrast, csCMVi patients showed an increasing disconnection between HCMV-specific IgG formation and memory-like” NK^{dim}-cell reconstitution, resulting in a marked negative correlation by day 270 (mature “memory-like” NK^{dim} cells: ρ =-0.47, P =0.076) (Figure 6A-C). Overall, HCMV-specific IgG production correlated weakly with $\gamma\delta$ T-cell reconstitution, with minimal differences between controllers and csCMVi patients. In contrast, CD56^{dim} NK-cell subpopulations, including (mature) “memory-like” NK cells correlated significantly with HCMV-specific IgG levels only in controllers, whereas these responses were disconnected in csCMVi patients.

Discussion

Extending letermovir prophylaxis from day 100 to day 200 did not substantially improve post-prophylaxis csCMVi incidence or survival.¹² Therefore, alternative management strategies and improved risk stratification are urgently needed. Late-onset reactivation is driven by slow anti-HCMV immune recovery.⁹ While T cells are well-recognized as central to HCMV immunity,⁸⁻¹⁰ other key effectors, including HCMV-specific B cells, antibodies, “memory-like” NK cells, and $\gamma\delta$ T cells,⁸ had been scarcely studied.

Before letermovir, early-onset HCMV reactivation often preceded significant B-cell reconstitution. In contrast, we hypothesized that more readily reconstituted humoral immunity might play a more important role in controlling late-onset HCMV reactivation in the letermovir era. Indeed, in our cohort, B cells were moderately reconstituted at the time of the first HCMV reactivation, with significant expansion of HCMV-specific humoral immunity between days 150 and 210. These observations suggest that letermovir creates a window for B-cell reconstitution, facilitating robust humoral responses following HCMV stimulation. Reactivation in csCMVi patients drove MBC expansion, exceeding that in controllers by the end of follow-up, likely in response to elevated viral replication and antigen presentation. These prominent humoral responses highlight the potential of future HCMV vaccines to leverage enhanced B-cell reconstitution, promoted by the shift to late-onset csCMVi in

alloSCT recipients receiving letermovir. Interestingly, we found a noticeable difference in HCMV-specific IgG and IgM response dynamics between HCMV controllers and csCMVi patients. Specifically, csCMVi patients showed a delayed onset of HCMV-specific IgG production and a stronger HCMV-specific IgM response than control-

lers. Consistent with this observation, others had reported that HCMV-specific IgG levels >400 mg/dL at the time of letermovir cessation were associated with reduced risk of HCMV reactivation.⁷ More frequent quantification of HCMV-specific IgM and IgG levels in future studies may enable a more detailed characterization of the humoral

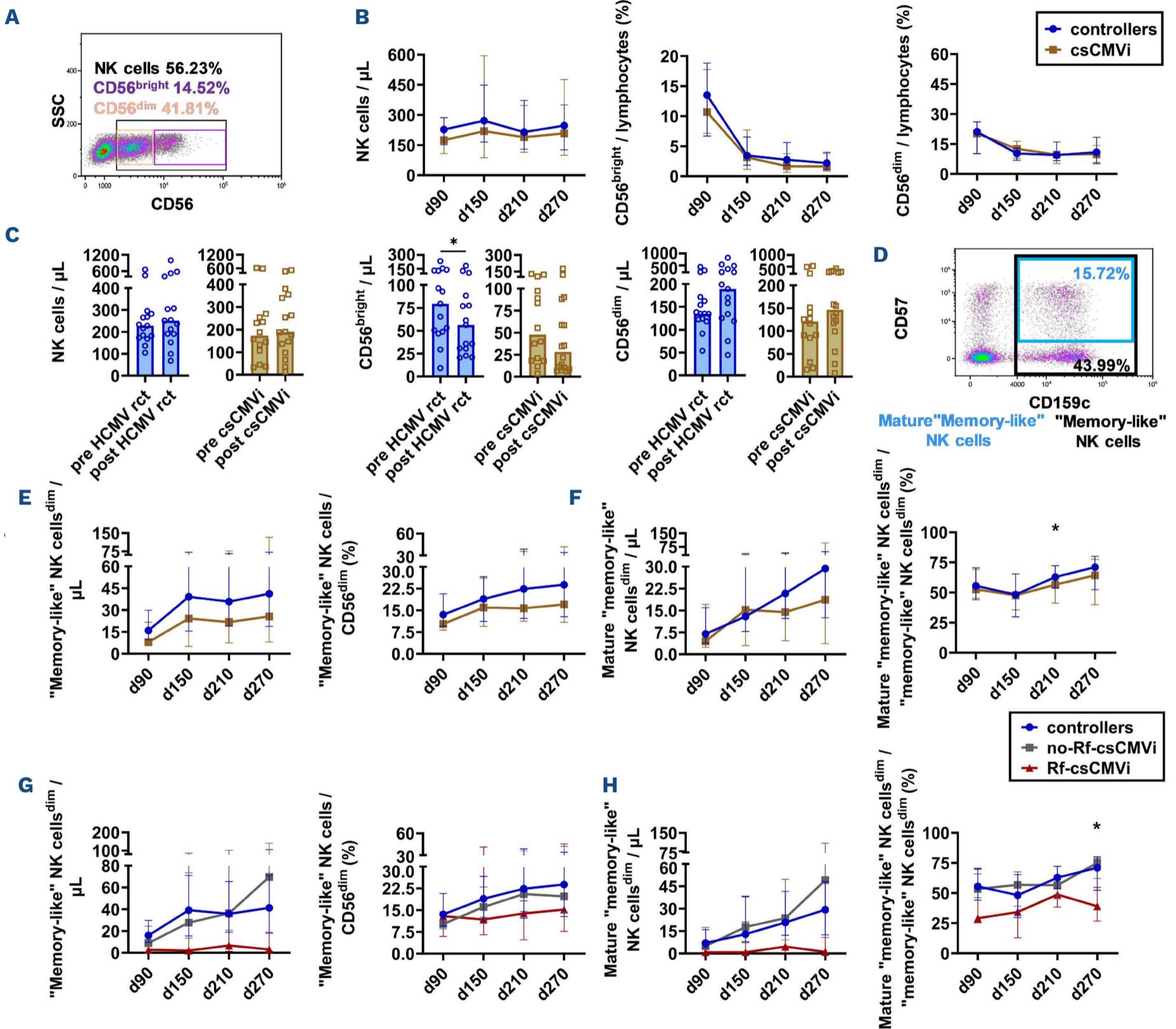


Figure 5. Failure to expand “memory-like” natural killer cells is linked to refractory clinically significant cytomegalovirus infection. Reconstitution of natural killer (NK) cells in allogeneic stem cell transplant recipients (N=42) was analyzed by flow cytometry. (A) Representative flow cytometry plot of NK-cell subpopulations. (B) Kinetics of total NK cells (CD56⁺) and the frequencies of CD56^{dim} and CD56^{bright} NK-cell subpopulations. Mann-Whitney U test. (C) Absolute numbers of NK cells and CD56^{dim} and CD56^{bright} NK-cell subpopulations before and after human cytomegalovirus (HCMV) reactivation or clinically significant CMV infection (csCMVi) events. Paired Wilcoxon test. (D) Representative flow cytometry plot showing the gating strategy for “memory-like” NK cells based on CD159c expression. (E, G) Kinetics of absolute numbers and frequencies of “memory-like” (CD159c⁺) NK cells^{dim} (CD56^{dim}) (F, H) and mature (CD57⁺) “memory-like” NK cells^{dim} according to HCMV control, using two-group comparisons of HCMV controllers and csCMVi patients (E, F, Mann-Whitney U test) or three-group comparisons of controllers, no Rf-csCMVi, and Rf-csCMVi patients (G, H, Kruskal-Wallis). *P<0.05, **P<0.01, ***P<0.001. Interquartile ranges are shown where applicable. SSC: side scatter; d: day; rct: reactivation; Rf-csCMVi: refractory clinically significant HCMV infection.

response, especially considering the short half-life of IgM.²³ The delayed switch from HCMV-specific IgM to IgG may be attributable to a deficiency of HCMV-specific T helper (Th) cells. Th cells are known to support class switch recombination through mechanisms such as CD40L-CD40 interaction and cytokine secretion.²⁴ We have previously shown that the kinetics of HCMV-specific Th-cell reconsti-

tution differ significantly between controllers and csCMVi patients. While controllers often develop HCMV-specific Th cells early, even during letermovir prophylaxis, csCMVi patients have significantly lower frequencies of these cells for an extended period following transplantation.⁸⁻¹⁰ Thus, the presence of HCMV-specific Th cells in controllers may facilitate an early Ig class switch, whereas in csCMVi

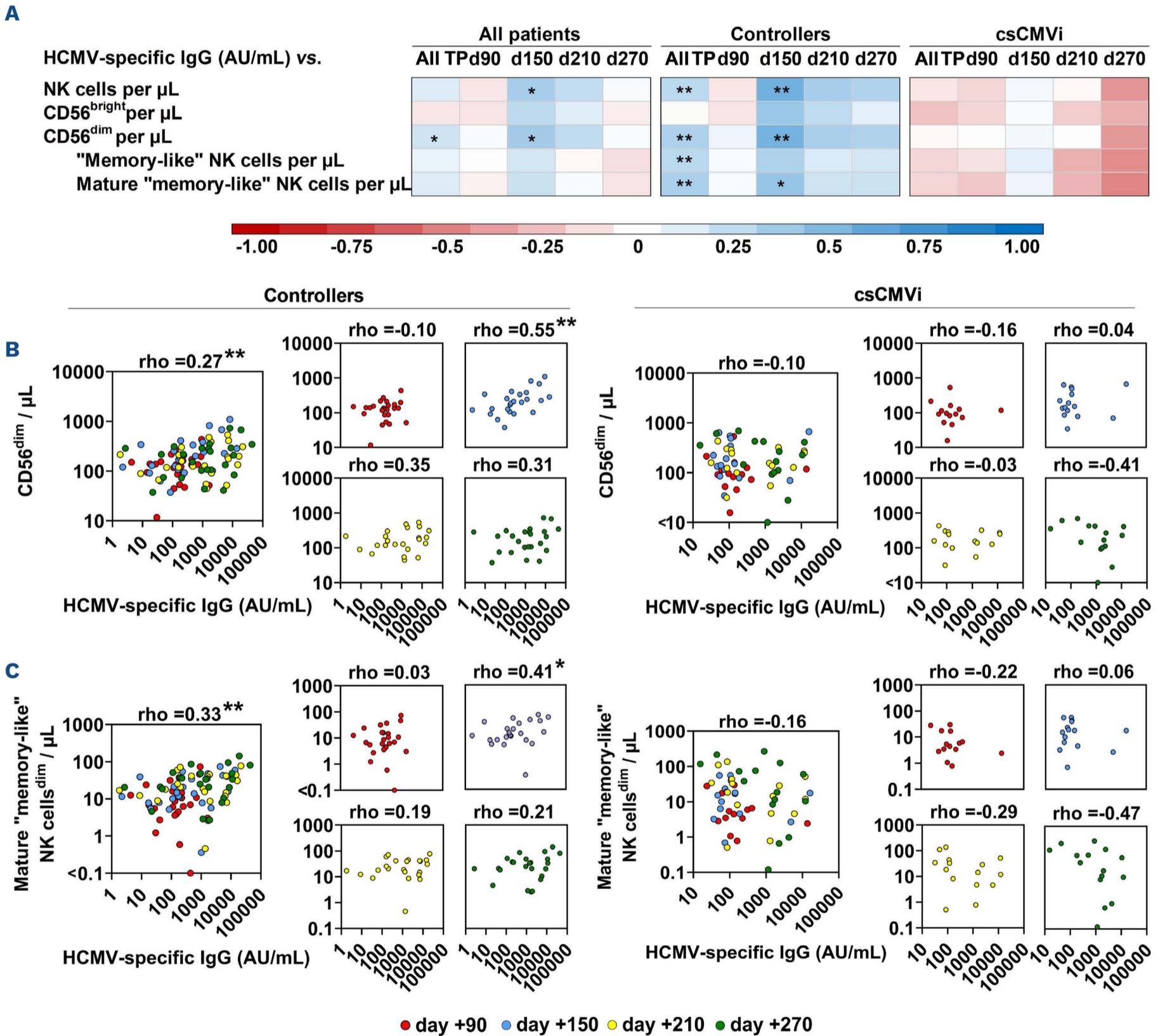


Figure 6. Human cytomegalovirus-specific humoral IgG response is associated with the expansion of CD56^{dim} natural killer-cell subpopulations in cytomegalovirus controllers but not in patients with clinically significant cytomegalovirus infection. Correlations between human cytomegalovirus (HCMV)-specific IgG and natural killer (NK) cells were analyzed across all timepoints and at individual timepoints. N=40 patients; 25 HCMV controllers, 15 patients with clinically significant HCMV infection (csCMVi). (A) Heatmap summarizing correlations of HCMV-specific IgG levels with numbers of NK cells and "memory-like" (CD159c⁺) NK cells. (B, C) Correlation of HCMV-specific IgG with total CD56^{dim} NK cells (B) and mature (CD57⁺) "memory-like" CD56^{dim} NK cells (C) across all timepoints (large panels), and at individual timepoints (small panels). Data for HCMV controllers (left) and csCMVi patients (right) are displayed separately. All analyses were performed using a Spearman rank correlation test. *P<0.05, **P<0.01, ***P<0.001. AU: arbitrary units; TP: timepoints; Ig: immunoglobulin.

patients, the switch may be delayed until these cells are reconstituted. Therefore, future research exploring the interaction between HCMV-specific Th responses and humoral immunity could inform strategies to enhance vaccine efficacy or develop targeted immunotherapies.

Humoral responses provide sustained antibody production, but effective viral control after alloSCT also depends on $\gamma\delta$ T cells²⁵ and “memory-like” NK cells.²² Both $V\delta 1^+$ and $V\delta 2^+$ $\gamma\delta$ T-cell subpopulations are protective against infections.²⁶ In the context of HCMV, $V\delta 1^+$ cells play a key role by lysing HCMV-infected cells and secreting interferon- γ ,¹⁹⁻²¹ while $V\delta 2^+$ cells appear to have limited anti-HCMV activity.²⁶ In the pre-letermovir era, HCMV reactivation primarily induced expansion of $V\delta 2^-$ cells, especially $V\delta 1^+$ cells.^{19,27} This resulted in an increased $V\delta 2^-/V\delta 2^+$ ratio, which may benefit anti-HCMV immunity.²⁸ Our new data suggest that this trend persists even in the letermovir era since both controllers and csCMVi patients showed an expansion of their $V\delta 2^-$ and $V\delta 1^+$ $\gamma\delta$ T-cell populations following letermovir cessation. However, $V\delta 1^+$ $\gamma\delta$ T-cell expansion was more pronounced in csCMVi patients, resulting in an increased $V\delta 1^+/V\delta 2^+$ ratio after reactivation events compared to that in controllers. This is likely a function of longer and more intense HCMV reactivation in csCMVi patients.

Notably, HCMV controllers already had elevated $V\delta 1^+$ $\gamma\delta$ T-cell frequencies prior to letermovir cessation, underscoring the significance of $V\delta 1^+$ $\gamma\delta$ T cells in effective HCMV control. While the predictive performance of $V\delta 1^+$ $\gamma\delta$ T-cell frequencies in our study would be insufficient for use as a standalone risk stratification marker, quantification of these cells might be of interest for combinatorial immune-guided risk stratification. However, the higher incidence of acute graft-*versus*-host disease and the associated immunosuppression in csCMVi patients may have influenced the reconstitution of key immune populations, such as $\gamma\delta$ T cells, and could have biased their predictive value at the end of letermovir prophylaxis.

HCMV also reshapes the NK-cell repertoire, leading to the emergence of “memory-like” NK cells.^{29,30} These cells have adaptive features, including clonal expansion, long-term persistence, and enhanced recall responses.^{22,31} Their significant anti-HCMV effects are well-documented^{22,31} and are further supported by the reported association of low numbers of “memory-like” NK cells with an increased risk of future HCMV reactivation in alloSCT recipients.³² In both our previous⁸ and current studies, controllers showed higher frequencies and counts of “memory-like” NK cells than csCMVi patients, particularly those with Rf-csCMVi. However, even patients with higher “memory-like” NK-cell frequencies can develop csCMVi, suggesting that these cells alone are insufficient for viral control. This conclusion is supported by data from kidney transplant recipients, indicating that “memory-like” NK cells can suppress HCMV replication after its onset but cannot prevent its initiation.³³ Both $\gamma\delta$ T cells and “memory-like” NK cells are linked to

the humoral immune response through their receptor CD16 (Fc γ RIII), an Fc receptor for IgG that mediates key immune functions such as ADCC.¹³⁻¹⁵ Consequently, we investigated whether the more advanced humoral response in the letermovir era correlated with an expansion of these immune cell populations. We found that HCMV-specific IgG positively correlated with the expansion of effector $\gamma\delta$ T cells in both controllers and csCMVi patients. CD16⁺ effector $\gamma\delta$ T cells are known to expand following stimulation by stress-induced signals from HCMV-infected cells and persist long-term,²⁰ a process potentially supported by HCMV-specific IgG. This likely enhances protection via interferon- γ production upon CD16 engagement with Ig-coated virions.¹³

The expansion of “memory-like” NK cells *in vitro* has been linked to polymorphic signal sequences of HCMV UL40 which can bind to HLA-E serving as a ligand for CD159c.³⁴ However, it appears that the most effective CD159c ligands of UL40 are relatively rare among clinical HCMV isolates.³⁴ Moreover, a link between the pool size of “memory-like” NK cells and HCMV-IgG was previously reported in healthy seropositive individuals.³⁵ Our observation of CD56^{dim} “memory-like” NK-cell expansion correlating with HCMV-specific IgG suggests that humoral immune responses may play a role in driving this NK-cell phenotype *in vivo*. Notably, “memory-like” NK cells have enhanced ADCC capacity and superior functionality compared to conventional NK cells, responding robustly to antibody-coated HCMV-infected cells.²² The observed differences in the co-evolution of these cells and humoral immunity between controllers and csCMVi patients suggest that IgG-dependent “memory-like” NK cell-mediated anti-HCMV immunity may constitute a thus far understudied protective mechanism against csCMVi in the letermovir era, warranting further investigation. While beyond the scope of the present study, future functional analyses are needed to characterize the interactions between patient-derived specific IgG and “memory-like” NK cells or $\gamma\delta$ T cells in greater detail.

This study has some limitations. Firstly, HCMV-specific IgM and IgG levels were measured by a commercial immunoassay without distinguishing neutralizing from non-neutralizing antibodies or identifying antigen-specific targets. Secondly, although we captured key timepoints in HCMV immune reconstitution, the 60-day intervals between our immune analyses led to variable gaps around reactivation or csCMVi events. Thirdly, the use of cryopreserved peripheral blood mononuclear cells may have influenced phenotypic analyses. Future studies should address these limitations and incorporate multivariate analyses integrating the combined impact of clinical and immunological variables on HCMV control in larger cohorts.

Despite these limitations, our study highlights how the shift from early- to late-onset HCMV reactivation driven by letermovir prophylaxis has increased the impact of humoral immune responses in anti-HCMV defense. We further provide

additional evidence for the involvement of “memory-like” NK cells and the first comprehensive analysis of $\gamma\delta$ T-cell reconstitution in the context of letermovir prophylaxis. Investigating the interplay between humoral and cellular immunity will be essential to optimize vaccination timing and develop effective immunotherapies. Moreover, early tracking of HCMV-specific B cells, immunoglobulins, and/or $\gamma\delta$ T-cell reconstitution may refine prognostic immune monitoring and risk stratification strategies beyond the assessment of type-1 T cells.

Disclosures

No conflicts of interest to disclose.

Contributions

The study was conceived by CDL, HE, SW and SK. SK was responsible for the patients' enrollment and clinical documentation. Experiments were planned and performed by CDL, HG, KK, BW and CK. Data were analyzed by CDL, HG, NI, MH, HH, SW and SK. Data were visualized by CDL. The project was administered and supervised by CDL, LD, HE, SW and SK. Funding was acquired by LD, HE and SK. The original draft was written by CDL, SW and SK. All co-authors reviewed, edited, and approved the manuscript.

Artificial intelligence

ChatGPT-4o (OpenAI, San Francisco, CA, USA) was used to assist with the language editing of this paper, improving its readability, and condensing text for clarity. Artificial intelli-

gence did not contribute to the study's conceptualization, analysis, or interpretation. The final responsibility for the text rests with the authors.

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Data-sharing statement

The datasets generated and analyzed in this study are available from the corresponding author upon reasonable request. Individual patient data will not be shared.

References

- Singh AK, McGuirk JP. Allogeneic stem cell transplantation: a historical and scientific overview. *Cancer Res.* 2016;76(22):6445-6451.
- Einsele H, Ljungman P, Boeckh M. How I treat CMV reactivation after allogeneic hematopoietic stem cell transplantation. *Blood.* 2020;135(19):1619-1629.
- Stern L, Withers B, Avdic S, et al. Human cytomegalovirus latency and reactivation in allogeneic hematopoietic stem cell transplant recipients. *Front Microbiol.* 2019;10:1186.
- Yan B, Sun G, Wu Y, et al. Letermovir prophylaxis reduced cytomegalovirus reactivation and resistance post umbilical cord blood transplantation. *Br J Haematol.* 2024;204(6):2378-2389.
- Mori Y, Harada T, Yoshimoto G, et al. Risk factors for late cytomegalovirus infection after completing letermovir prophylaxis. *Int J Hematol.* 2022;116(2):258-265.
- Gabanti E, Borsani O, Colombo AA, et al. Human cytomegalovirus-specific T-cell reconstitution and late-onset cytomegalovirus infection in hematopoietic stem cell transplantation recipients following letermovir prophylaxis. *Transplant Cell Ther.* 2022;28(4):211.e1-211.e9.
- Liu LW, Yn A, Gao F, et al. Letermovir discontinuation at day 100 after allogeneic stem cell transplant is associated with increased CMV-related mortality. *Transplant Cell Ther.* 2022;28(8):510.e1-510.e9.
- Lauruschkat CD, Muchsin I, Rein AF, et al. Impaired T cells and “memory-like” NK-cell reconstitution is linked to late-onset HCMV reactivation after letermovir cessation. *Blood Adv.* 2024;8(11):2967-2979.
- Lauruschkat CD, Muchsin I, Rein A, et al. CD4+ T cells are the major predictor of HCMV control in allogeneic stem cell transplant recipients on letermovir prophylaxis. *Front Immunol.* 2023;14:1148841.
- Zamora D, Duke ER, Xie H, et al. Cytomegalovirus-specific T-cell reconstitution following letermovir prophylaxis after hematopoietic cell transplantation. *Blood.* 2021;138(1):34-43.
- Teira P, Battiwalla M, Ramanathan M, et al. Early cytomegalovirus reactivation remains associated with increased transplant-related mortality in the current era: a CIBMTR analysis. *Blood.* 2016;127(20):2427-2438.
- Russo D, Schmitt M, Pilorge S, et al. Efficacy and safety of extended duration letermovir prophylaxis in recipients of haematopoietic stem-cell transplantation at risk of cytomegalovirus infection: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Haematol.* 2024;11(2):e127-e135.
- Couzi L, Pitard V, Sicard X, et al. Antibody-dependent anti-cytomegalovirus activity of human $\gamma\delta$ T cells expressing CD16 (Fc γ R11a). *Blood.* 2012;119(6):1418-1427.
- Muccio L, Bertaina A, Falco M, et al. Analysis of memory-like natural killer cells in human cytomegalovirus-infected children

- undergoing $\alpha\beta$ +T and B cell-depleted hematopoietic stem cell transplantation for hematological malignancies. *Haematologica*. 2016;101(3):371-381.
15. Coenon L, Villalba M. From CD16a biology to antibody-dependent cell-mediated cytotoxicity improvement. *Front Immunol*. 2022;13:913215.
16. Chemaly RF, Chou S, Einsele H, et al. Definitions of resistant and refractory cytomegalovirus infection and disease in transplant recipients for use in clinical trials. *Clin Infect Dis*. 2019;68(8):1420-1426.
17. 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(3):787-794.
18. Welten SPM, Redeker A, Toes REM, Arens R. Viral persistence induces antibody inflation without altering antibody avidity. *J Virol*. 2016;90(9):4402-4411.
19. Huang Y, Jiang C, Zhu J, et al. Expansion of effector memory V δ 2(neg) $\gamma\delta$ T cells associates with cytomegalovirus reactivation in allogeneic stem cell transplant recipients. *Front Immunol*. 2024;15:1397483.
20. Khairallah C, Dechanet-Merville J, Capone M. $\gamma\delta$ T cell-mediated immunity to cytomegalovirus infection. *Front Immunol*. 2017;8:105.
21. Prinz I, Thamm K, Port M, et al. Donor V δ 1+ $\gamma\delta$ T cells expand after allogeneic hematopoietic stem cell transplantation and show reactivity against CMV-infected cells but not against progressing B-CLL. *Exp Hematol Oncol*. 2013;2(1):14.
22. Zhang T, Scott JM, Hwang I, Kim S. Cutting edge: antibody-dependent memory-like NK cells distinguished by Fc γ deficiency. *J Immunol*. 2013;190(4):1402-1406.
23. Buchner J, Sitia R, Svilenov HL. Understanding IgM structure and biology to engineer new antibody therapeutics. *BioDrugs*. 2025;39(3):347-357.
24. Vinuesa CG, Linterman MA, Yu D, MacLennan IC. Follicular helper T cells. *Annu Rev Immunol*. 2016;34:335-368.
25. Arruda LCM, Gaballa A, Uhlin M. Impact of $\gamma\delta$ T cells on clinical outcome of hematopoietic stem cell transplantation: systematic review and meta-analysis. *Blood Adv*. 2019;3(21):3436-3448.
26. Lawand M, Dechanet-Merville J, Dieu-Nosjean MC. Key features of gamma-delta T-cell subsets in human diseases and their immunotherapeutic implications. *Front Immunol*. 2017;8:761.
27. Silva-Santos B, Serre K, Norell H. $\gamma\delta$ T cells in cancer. *Nat Rev Immunol*. 2015;15(11):683-691.
28. Gaballa A, Alagrafi F, Uhlin M, Stikvoort A. Revisiting the role of $\gamma\delta$ T cells in anti-CMV immune response after transplantation. *Viruses*. 2021;13(6):1031.
29. Gao F, Zhou Z, Lin Y, Shu G, Yin G, Zhang T. Biology and clinical relevance of HCMV-associated adaptive NK cells. *Front Immunol*. 2022;13:830396.
30. Guma M, Angulo A, Vilches C, Gomez-Lozano N, Malats N, Lopez-Botet M. Imprint of human cytomegalovirus infection on the NK cell receptor repertoire. *Blood*. 2004;104(12):3664-3671.
31. Newhook N, Fudge N, Grant M. NK cells generate memory-type responses to human cytomegalovirus-infected fibroblasts. *Eur J Immunol*. 2017;47(6):1032-1039.
32. Kheav VD, Busson M, Scieux C, et al. Favorable impact of natural killer cell reconstitution on chronic graft-versus-host disease and cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation. *Haematologica*. 2014;99(12):1860-1867.
33. Lopez-Botet M, De Maria A, Muntasell A, Della Chiesa M, Vilches C. Adaptive NK cell response to human cytomegalovirus: facts and open issues. *Semin Immunol*. 2023;65:101706.
34. Hammer Q, Ruckert T, Borst EM, et al. Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. *Nat Immunol*. 2018;19(5):453-463.
35. Manser AR, Scherenschlich N, Thons C, Hengel H, Timm J, Uhrberg M. KIR polymorphism modulates the size of the adaptive NK cell pool in human cytomegalovirus-infected individuals. *J Immunol*. 2019;203(8):2301-2309.