Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

e**Methods. Immune Assay Methods**

CMV-specific T-cell assays

Participant specimens

Peripheral blood mononuclear cells (PBMC) were isolated from sodium-heparinized blood by density centrifugation and cryopreserved in 90% fetal bovine serum (FBS; Gemini Bio-products, Sacramento, CA) and 10% DMSO (Sigma-Aldrich, St. Louis, MO). Serum was isolated from coagulated blood by centrifugation and stored at -80°C until use.

Neutralizing antibody assay

Cells. Human retinal pigmented epithelial (ARPE-19; ATCC CRL-2302, ATCC, Manassas, VA) cells were maintained in Dulbecco's Modified Eagle Media (DMEM; Gibco, Gaithersburg, MD) supplemented with 10% NuSerum (Corning, Corning NY), 1% L-glutamine, 10,000 IU/L penicillin, and 10 mg/L streptomycin (all Gibco) at 37°C with 5% CO_2. Cells were plated in 96-well flat-bottom plates one to two days before assay setup and used when confluent.

Virus. We used the *BADrUL131-Y4* laboratory strain of human CMV, originally modified from the AD169 genome by Wang and Shenk (2005) [1]. This strain expresses a functional UL131 protein and green fluorescent protein (GFP) cassette that allows for quantitation of viral entry and replication. Viral stocks were grown from infected HFF and suspended in DMEM and sterile skim milk and stored at -80 °C.

Assay. A method adapted from Cui et al. (2008)[2] was used. Briefly, 10 two-fold serial dilutions of participant plasma in DMEM were prepared in 96-well round-bottom plates from 1:8 – 1:4096. (120uL total initial volume). One row of the dilution plate was reserved for serum from a known CMV seropositive donor. The first and last wells of each row were reserved for media-

only and media + virus-only negative and positive controls, respectively. The serum dilutions were then mixed 1:1 with a *BADrUL131-Y4* virus solution diluted to yield a final concentration 1000 pfu/well and incubated for 1 hour (all incubations were at 37°C with 5% CO_2). Media was then aspirated from cells and 50uL/well of serum dilutions + virus was added to wells in duplicate. After 1 hour of incubation, the media was aspirated, 100 uL of fresh DMEM added to each well, and the plates incubated for 7 days. On day 7, fluorescence (485nm excitation/527nm emission) was measured using a Fluoroskan Ascent fluorimeter, (Thermo Lab Systems, Grand Rapids, OH). Raw GFP data was analyzed in R (R Foundation for Statistical Computing, Vienna, Austria)[5] using the "drc" [6] and "tidyverse" [7] packages. To estimate the negative-$\log_2$ serum concentration at which 50% virus neutralization was achieved (IC-50), a four-parameter logistic virus neutralization curve was fit to each sample's GFP data using an algorithm from the "drc" package. IC-50 values above 5.0 were considered positive based on minimum levels measured in CMV seropositive healthy donors (data not shown). When appropriate, samples with high neutralizing capacity were re-assayed at a greater initial dilution to more accurately determine the IC50.

Intracellular cytokine staining.

Cryopreserved PBMC were thawed in enriched media (RPMI-HEPES (Gibco, Gaithersburg, MD) with 10% FBS (Gemini Bio-products), 2mM L-glutamine (Gibco) and 1% antibiotic/antimycotic (Sigma-Aldrich)) and incubated for 6 hours at 37°C and 5% CO_2 at $1x10^6$ cells/well in 96-well round bottom plates with co-stimulatory antibodies anti-CD28 (1μg/mL) and anti-CD49d (1μg/mL) and Brefeldin A (10 μg/mL, Sigma-Aldrich)/Golgi stop. Cells were also stimulated with CMV pp65 overlapping peptide libraries (2μg/ml; JPT Peptide Technologies, Berlin, Germany), *Staphylococcus aureus* enterotoxin B (0.05 μg/mL; Sigma-Aldrich) or 1:1

DMSO/PBS as a vehicle control for the peptide mix. Samples were held overnight at 4^0C and then stained for flow cytometric analysis the following day.

After incubation with EDTA (20 mM), samples were incubated first for 30 minutes at room temperature in the dark with blue fixable viability dye (Invitrogen/Thermo Fisher, Waltham, MA) in 1X PBS, washed, and then surface-stained for 30 min under the same conditions with the following fluorescently labelled antibodies: CD14 BV605, CCR7 BV785, PD-1 PE-Dazzle594 (all Biolegend, San Diego, CA), CD57 APC-Vio770 (Miltenyi, Bergisch Gladbach, Germany), NKG2C AlexaFluor700 (R&D Systems, Minneapolis, MN), CD4 BUV496, CD45RA BUV737 and CD56 BV650 (all BD Biosciences, San Jose, CA). Cells were then washed twice with FACS wash buffer (0.5% FBS in 1X PBS), then incubated for 20 min under the same conditions with BD Cytofix/Cytoperm (BD Biosciences), washed with Perm/Wash buffer (BD Biosciences). Following permeabilization, cells were incubated for 30 minutes under the same conditions with the following antibodies: CD3 BUV395, CD8 BUV805, IL-2 PE, IFNɣ V450 CD154 APC (all BD Biosciences), TNFα FITC (Thermo Fisher Scientific, Waltham, MA), IL-4 PerCPCy5.5 and Perforin PECy7 (Biolegend). Finally, cells were washed twice with Perm/Wash buffer and fixed with 1% paraformaldehyde (Sigma-Aldrich); cell acquisition (100,000 to 400,000 events) was done on a Symphony flow cytometer (BD Biosciences) within 24 hours. All antibodies were titrated for optimum performance, and appropriate single color compensation and fluorescence minus one controls were run.
Data were analyzed with FlowJo Version 9 (FlowJo, LLC, Ashland OR). Gating strategy is shown in Supplementary e Figure 1. Not all measured markers are discussed in this manuscript. Absolute lymphocyte counts were obtained from clinical laboratory records and used to calculate CMV-specific cells/μL after background was subtracted. The primary response variables were

the magnitude of CD4+ and CD8+ cells, respectively, that expressed interferon-ɣ (IFN-ɣ) without regard for other markers or IFN-ɣ plus at least one other marker of functionality (a measure of polyfunctional response). Responses that were >0.05% above background of the relevant subset and at least three-fold greater than background were considered positive (Horton 2007; Nebbia 2008)[3,4].

eReferences

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3. Horton H, Thomas EP, Stucky JA, et al. Optimization and validation of an 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T cells induced by vaccination. J Immunol Methods 2007;323:39-54.

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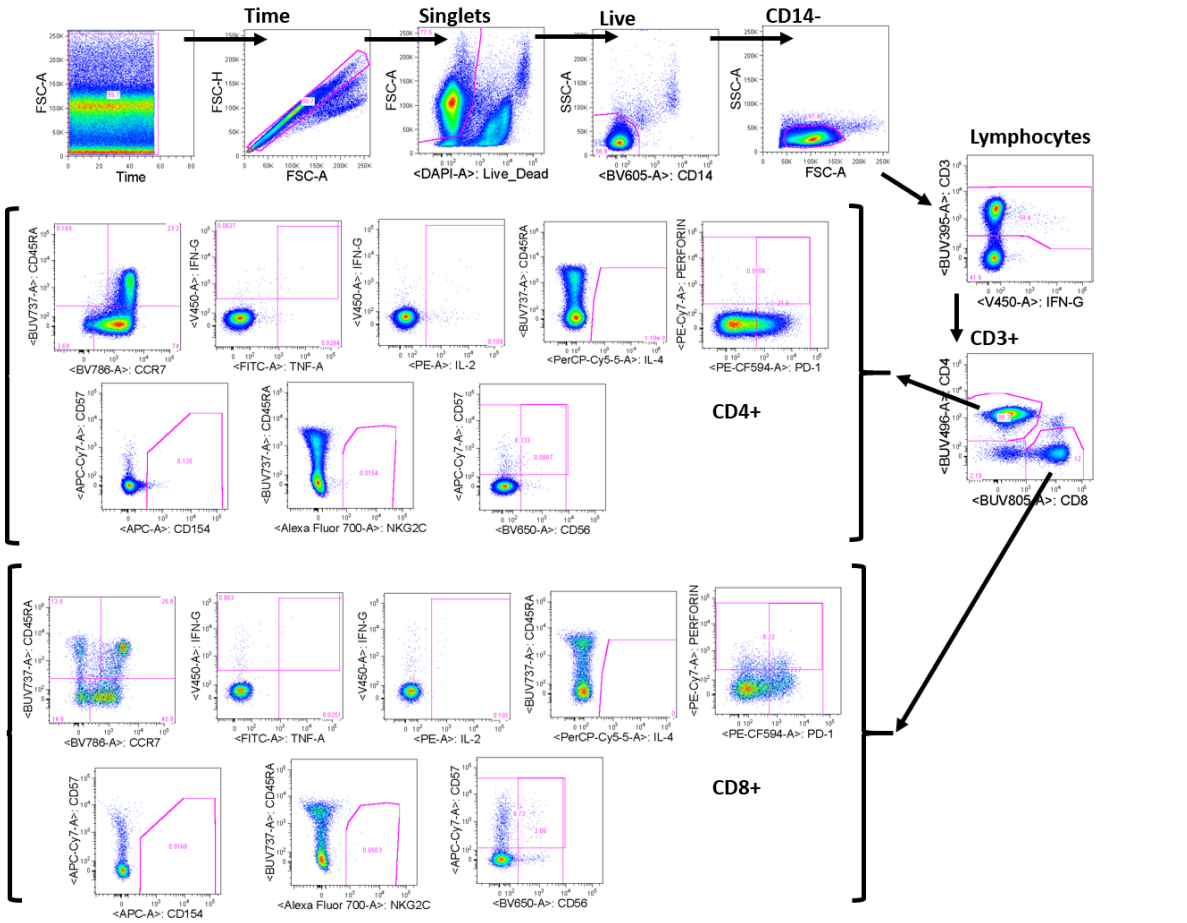
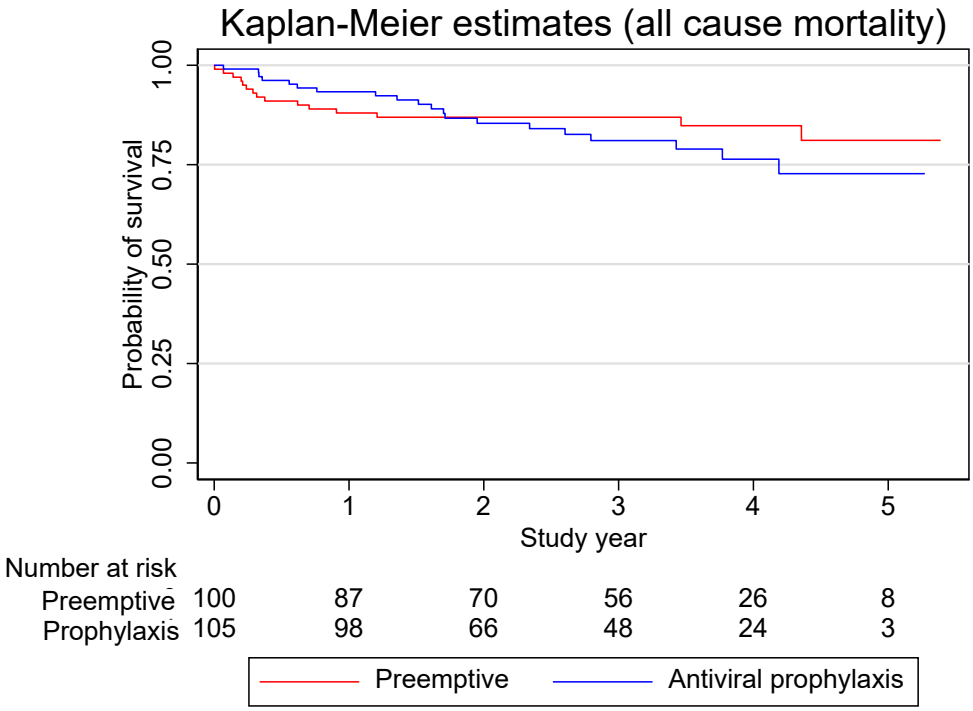
eFigure 1. Gating Strategy

A representative participant response to CMV pp65 is shown. A time gate was drawn to ensure stable collection of samples, followed by a singlets gate to eliminate doublets and then a gate on viable cells. Cells in the live gate were restricted by CD14- gate to remove monocytes and then CD3+ cells were gated, followed by selection of CD4+ and CD8+ subsets. CD4+ and CD8+ subsets were gated for cytokine expression (IFN-γ, TNF-α, IL-2 and IL-4), degranulation marker Perforin, and activation marker CD154. The following markers were also included but not evaluated in the present study: Memory subsets (CD45RA and CCR7), PD-1 (T cell exhaustion), CD57 (senescence), CD56 and NKG2C (NK-like T cells); these subsets will be discussed in future publications. Intermediate gates to remove antibody aggregates are not shown. Markers were displayed and gated against the appropriate channel to allow optimum gating while avoiding potential spread from overlapping channels.



eFigure 2. Kaplan-Meier Plot Showing Survival in the Study Groups. The survival estimates are not significantly different, *P*=.46.



eTable 1. Number and Percentage of Participants With CMV Disease Diagnoses Based on Each Criterion

Criteria	Number of participants (%) with respective criteria		
CMV syndrome[1] (n=16)			
	Preemptive therapy N=5	Antiviral prophylaxis N=11	Total N=16
Viremia and fever	3 (60%)	2 (18.2%)	5 (31.3%)
Viremia, new or worsening leukopenia, and severe malaise	2 (40%)	2 (18.2%)	4 (25.0%)
Viremia and new or worsening leukopenia	0	2 (18.2%)	2 (12.5%)
Viremia and severe malaise	0	2 (18.2%)	2 (12.5%)
Viremia, fever, and severe malaise	0	1 (9.1%)	1 (6.3%)
Viremia, fever, severe malaise, and new or worsening leukopenia	0	2 (18.2%)	2 (12.5%)
End organ CMV disease[2] (n=13)			
	Preemptive therapy N=4	Antiviral prophylaxis N=9	Total N=13
Histopatholog y with typical CMV inclusions and/or presence of CMV antigens by immunohistochemistry	4 (100%)	9 (100%)	13 (100%)
Liver allograft	2 (50%)	6 (67%)	8 (62%)
Gastrointestinal tract	2 (50%)	1 (11%)	3 (23%)
Multi-organ	0	2 (22%)	2 (15%)

[1] Viral load in participants with CMV syndrome was median 20,459 IU/mL (IQR 1,790 to 246,764 IU/mL); [2] Viral load in participants with end organ disease was median 34,930 IU/mL (IQR 7,000 to 48,500 IU/mL); [3] Involvement of 2 or more organ sites.

eTable 2. Adverse Events in the Study Participants

Variable	Preemptive N=100	Prophylaxis N=105	Total N=205
Hematologic toxicity			
Neutropenia (ANC)[1]<500	12 (12%)	10 (10%)	22 (11%)
Receipt of G-CSF[2]	6 (6%)	7 (7%)	13 (6%)
Other adverse events[3]			
Pericardial effusion	1	0	1 (<1%)
Kidney stones	1	0	1 (<1%)

Neutropenia was assessed in all participants during study drug administration period and for 7 days beyond.[1]ANC= Absolute neutrophil count; [2]G-CSF= Granulocyte colony stimulating factor; [3]Both events were considered serious adverse events.
An adverse event for the study was defined as any clinically important untoward medical occurrence in a participant receiving study drug that is not expected in the clinical course of a patient with a liver transplant OR any clinically important, untoward medical occurrence that is considered study related, regardless of the "expectedness" of the event for the course of a liver transplant recipient. Expected events for liver transplant are untoward clinical occurrences that are deemed to occur with reasonable frequency in the routine care of patients with a liver transplant (The Protocol Appendix lists the events considered to be part of the expected course of liver transplant).

eTable 3. Non-CMV Disease–Related Valganciclovir Use After Day 100 in Study Participants

Variable	Preemptive therapy (n=91)[1]	Antiviral prophylaxis (n=85)[1]	*P* value
Number of participants who received valganciclovir [2]	29 (32%)	21(26%)	.45
Total days of use in participants treated	61.1	68.7	.57
Total valganciclovir usage (valganciclovir usage per person)	1685g (58.1g)	1521g (61.1g)	.34
Valganciclovir use per person without CMV disease	18.5g	17.9g	.33

[1]Excludes participants requiring valganciclovir for the treatment of CMV disease; [2] Reasons for usage were treatment of viremia per local center practice, concurrent use for rejection, provider choice or other viral infections (herpes zoster).