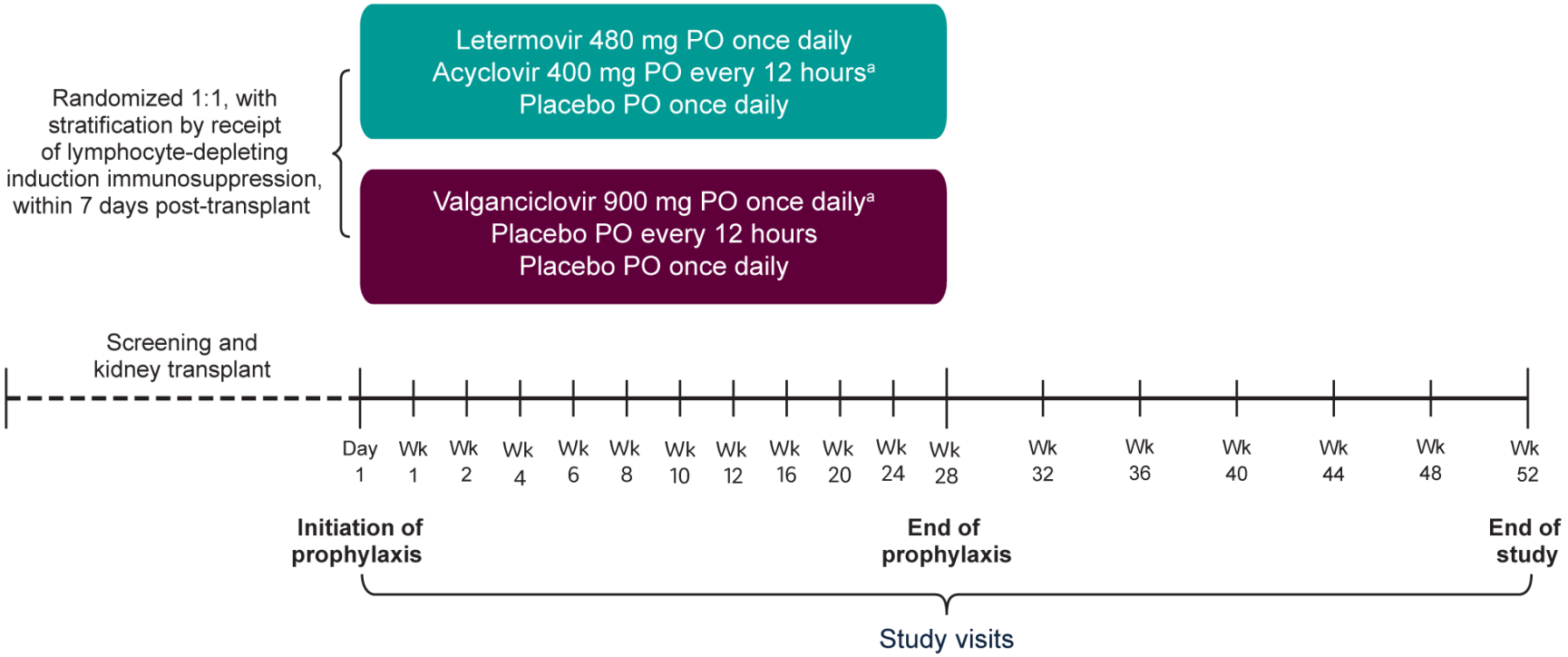
Supplemental Online Content

Limaye A, Budde K, Humar A, Vincenti F, Kuypers D, et al. Letermovir vs valganciclovir for prophylaxis of cytomegalovirus disease in high-risk kidney transplant recipients: a randomized clinical trial. *JAMA*. Published online June 6, 2023. doi:10.1001/jama.2023.9106

Supplement 2.

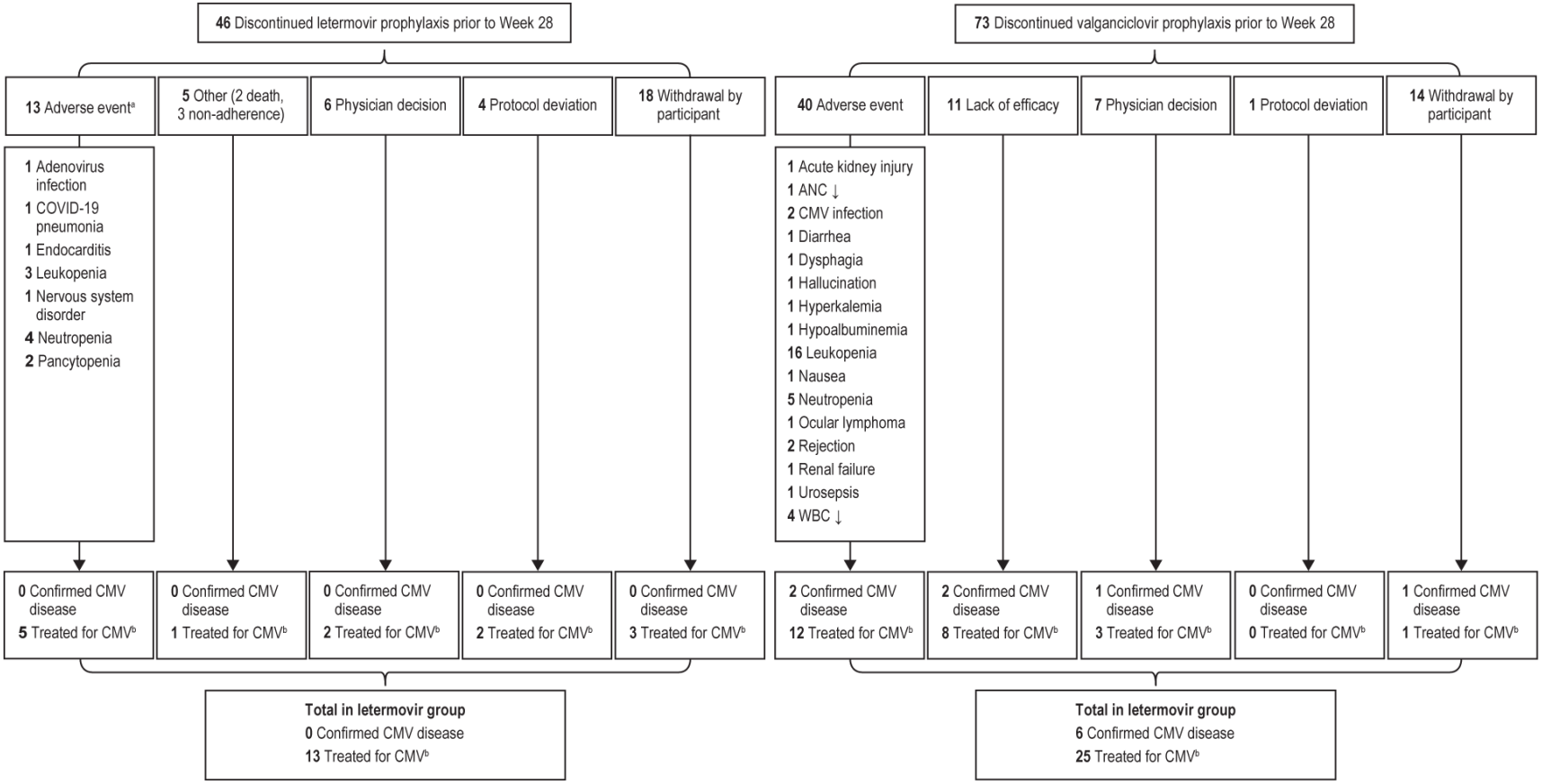
This supplemental material has been provided by the authors to give readers additional information about their work.

eFigure 1. Study Design and Timeline



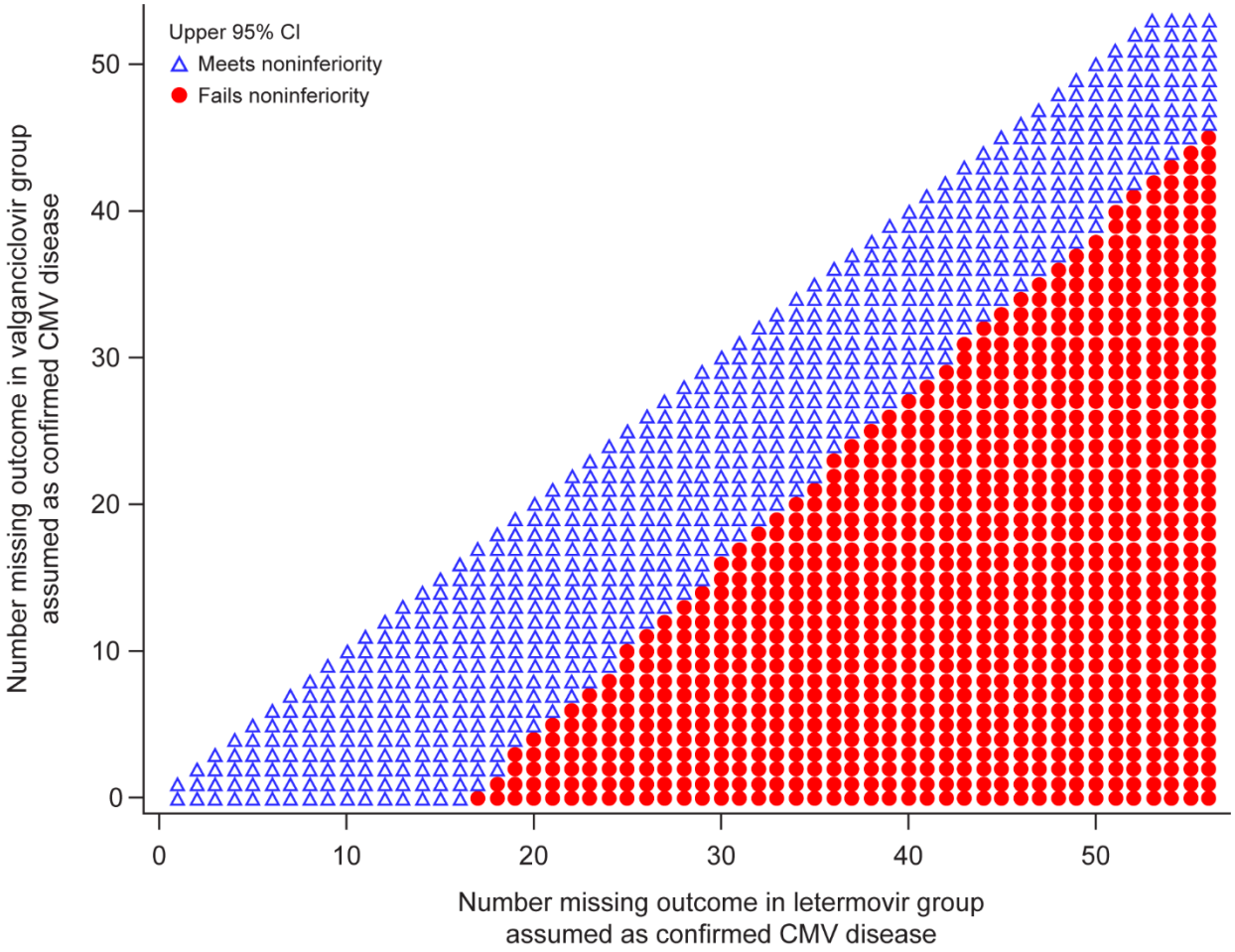
CrCl indicates creatinine clearance; PO, orally; Wk, week.
[a]Dose adjusted based on CrCl calculated with the Cockcroft-Gault formula.
Letermovir prescribed as 1-480 mg tablet or 2-240 mg tablets daily. 1-240 mg tablet daily prescribed for participants on cyclosporine A.
Acyclovir prescribed as 1-400 mg capsule every 12 hours for CrCl ≥30 mL/min or 1-400 mg capsule daily for CrCl >10 and <30 mL/min.
Valganciclovir prescribed as 2-450 mg tablets daily for CrCl ≥60 mL/min, 1-450 mg tablet daily for CrCl 40-59 mL/min, 1-450 mg tablet every 2 days for CrCl 25-39 mL/min, and 1-450 mg tablet twice weekly for CrCl 10-24 mL/min.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eFigure 2. Disposition of Participants Who Discontinued Prophylaxis Prior to Week 28



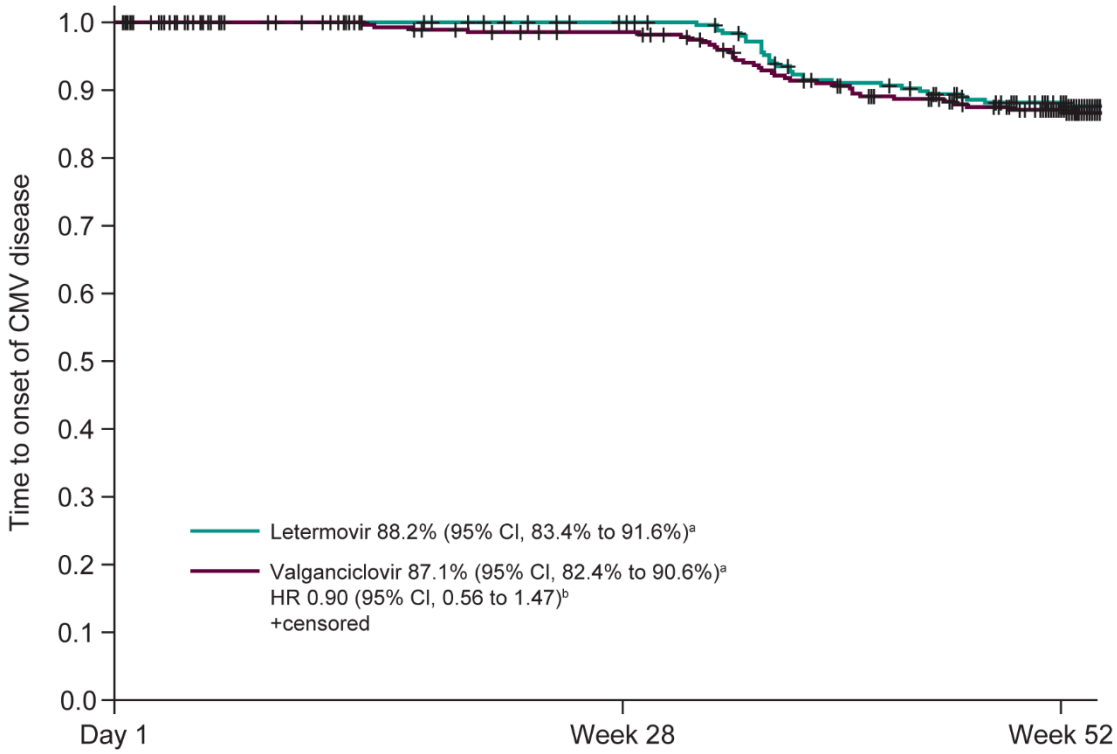
ANC indicates absolute neutrophil count; CMV, cytomegalovirus; WBC, white blood cell count; ↓, decreased.
[a]One participant developed glomerulonephritis (reported as an adverse event) that began the day after transplant and prior to initiating letermovir prophylaxis.
[b]Treated for CMV DNAemia, suspected CMV disease, or confirmed CMV disease.

eFigure 3. Tipping Point Analysis



CMV indicates cytomegalovirus.
In this analysis, occurrence of confirmed CMV disease was varied in those participants who either discontinued from the study before week 52 or were missing a result for CMV DNAemia at the week 52 visit window. The missing data in the valganciclovir group was set to 0 failures, and all possible combinations of values of strata were evaluated within the letermovir group. In all possible strata combinations, once 17 of those letermovir participants with missing outcome were assumed to be confirmed CMV disease cases, the study would fail to meet noninferiority. The result was shown to be insensitive to the distribution of failures among the strata, and subsequent analyses were executed randomly assigning the failures among strata. The x-axis represents the number of confirmed CMV disease cases (failures) in the participants in the letermovir group with missing data and the y-axis represents the number of confirmed CMV disease cases in the participants in the valganciclovir group with missing data. The area with the filled circles represents the outcomes in which the study would have failed to conclude noninferiority (the upper bound of the 95% confidence interval exceeded 10%). The open triangles show those outcomes where the study would conclude noninferiority. As noted, if no failures occurred in the valganciclovir group, 17 participants in the letermovir group would have had to fail for the study to fail. The delta decreased as the number of failures assumed in the valganciclovir group increased. The minimum difference for the study to fail occurred when 51 cases were assumed in the letermovir group and 40 were assumed in the valganciclovir group.

eFigure 4. Time to Onset of CMV Disease Through Week 52 in the Full Analysis Set



No. of participants at risk

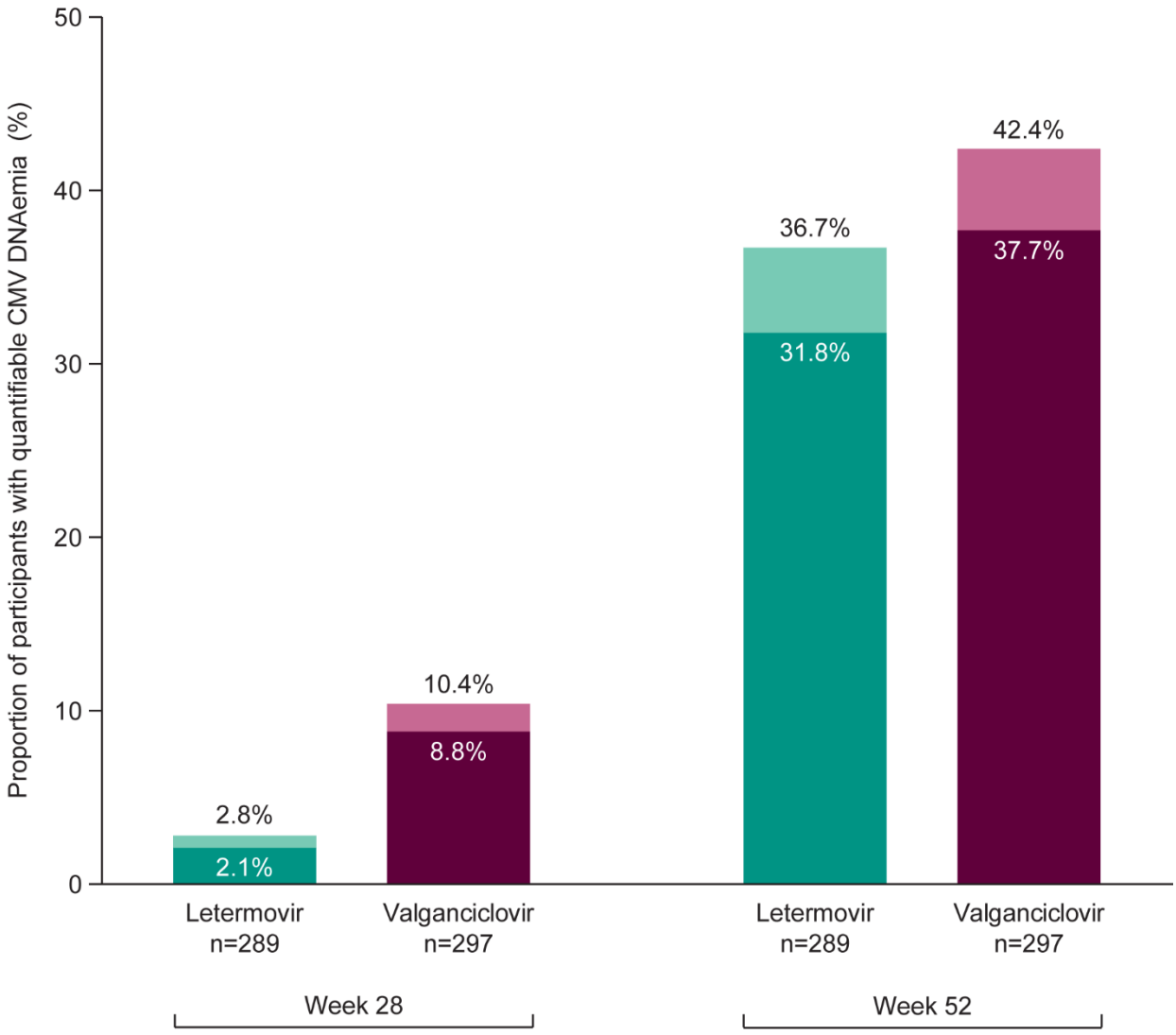
Letermovir	289	252	196
Valganciclovir	297	268	191

No. of events

Letermovir	0	29	1
Valganciclovir	4	30	1

CMV indicates cytomegalovirus; HR, hazard ratio.
[a]From product-limit (Kaplan-Meier) method for censored data. Data were censored at the time of last assessment.
[b]Based on Cox regression model with Efron's method of tie handling with study regimen as a covariate stratified by use/non-use of lymphocyte-depleting induction immunosuppression. Hazard ratio <1 favors the letermovir group.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eFigure 5. Quantifiable CMV DNAemia Through Weeks 28 and 52 in the Full Analysis Set



CMV indicates cytomegalovirus.
Quantifiable CMV DNAemia evaluated by the central laboratory represented by the darker colored bars. The lower limit of quantification for the central laboratory assay was 137 IU/mL. Quantifiable CMV DNAemia reported by local laboratories represented by the lighter colored bars.
Participants were only counted once at each time point.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 1. Prophylaxis Adherence Through Week 28 in the Safety Population

	No. (%)	
	Letermovir (n = 292)	**Valganciclovir (n = 297)**
<75%	1 (0.3)	34 (11.4)
≥75% to <90%	3 (1.0)	33 (11.1)
≥90% to <100%	107 (36.6)	133 (44.8)
100%	181 (62.0)	97 (32.7)

Percent adherence = (number of days the participant took prophylaxis / number of days participant should have taken prophylaxis) x 100.
'On-prophylaxis' day = day that participant took at least one dose of the study regimen.
For valganciclovir group, 0 dose days where reason = physician's decision to titrate did not count as non-adherence days.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 2. Sensitivity Analyses for Efficacy Through Week 52 in the Full Analysis Set

	No. (%)		
	Letermovir (n=289)	Valganciclovir (n=297)	Difference (95% CI), %[a]
Per-protocol[b]	**n=266**	**n=266**	
CMV disease[c]	29 (10.9)	33 (12.4)	-1.4 (-6.9 to 4.1)
Non-completer = failure approach[d]	**n=289**	**n=297**	
Failures	86 (29.8)	88 (29.6)	0.3 (-7.1 to 7.6)
CMV disease[c]	30 (10.4)	35 (11.8)	
Did not complete the study through week 52[e]	32 (11.1)	28 (9.4)	
Missing result for CMV DNAemia in week 52 visit window	24 (8.3)	25 (8.4)	

Abbreviations: CMV, cytomegalovirus.

[a]The 95% CIs for the differences in percent response were calculated using stratum-adjusted Mantel-Haenszel method with the difference weighted by the harmonic mean of sample size per group for each stratum (use/non-use of lymphocyte-depleting induction immunosuppression).

[b]Per-protocol population excluded participants due to important study deviations that could substantially affect the results of the efficacy endpoint. Participants who discontinued prematurely from the study for any reason were not considered failures.

[c]CMV disease confirmed by the independent blinded adjudication committee (CMV end-organ disease or CMV syndrome).

[d]Participants who developed CMV disease, did not complete the study through week 52, or had a missing result for CMV DNAemia in the week 52 visit window were considered failures (non-completer = failure approach). Categories of failure were mutually exclusive and based on the hierarchy of categories in the order listed.

[e]Reasons for discontinuation from the study in participants who did not have CMV disease included death, lost to follow-up, physician decision, other, and withdrawal by participant.

All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 3. Investigator-Reported and Adjudication Committee-Confirmed CMV Disease Through Week 52 in the Full Analysis Set

	No. (%)			
	Investigator-reported CMV disease[a]		Adjudication committee-confirmed CMV disease[b]	
	Letermovir (n = 289)	Valganciclovir (n = 297)	Letermovir (n = 289)	Valganciclovir (n = 297)
CMV disease	50 (17.3)	51 (17.2)	30 (10.4)	35 (11.8)
CMV syndrome	31 (10.7)	40 (13.5)	24 (8.3)[c]	34 (11.4)[c]
CMV end-organ disease[c]	33 (11.4)	28 (9.4)	6 (2.1)	1 (0.3)
GI disease	25 (8.7)	16 (5.4)	5 (1.7)	0 (0.0)
Pneumonia	3 (1.0)	3 (1.0)	1 (0.3)	1 (0.3)
Other end-organ disease	11 (3.8)[d]	12 (4.0)[d]	0 (0.0)	0 (0.0)

Abbreviations: CMV, cytomegalovirus.
[a]CMV disease identified by study investigators; categories were not mutually exclusive.
[b]CMV disease confirmed by the external independent blinded adjudication committee; categorized as either CMV end-organ disease or CMV syndrome based on diagnostic criteria.
[c]Participants had to have at least two CMV syndrome findings in addition to CMV DNAemia.
[d]Included investigator-reported CMV encephalitis/ventriculitis, hepatitis, pancreatitis, retinitis, and/or other.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 4. CMV Resistance-Associated Substitutions in Participants at the Time of Evaluation for Suspected CMV Disease or CMV DNAemia

	No. (%)	
	Letermovir (n = 52)	Valganciclovir (n = 66)
Letermovir resistance-associated substitution	0 (0)	0 (0)
pUL51	0 (0)	0 (0)
pUL56	0 (0)	0 (0)
pUL89	0 (0)	0 (0)
Valganciclovir resistance-associated substitution	2 (3.8)	8 (12.1)
pUL97	2 (3.8)	7 (10.6)
pUL54	0 (0)	2 (3.0)

Abbreviations: CMV, cytomegalovirus.
Amino acid variants detected at a frequency of ≥5%.
One participant in the valganciclovir group had a pUL97 and pUL54 resistance-associated substitution.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 5. Adverse Events ≥5% to <10% Through Week 28 in the Safety Population

	No. (%)		
	Letermovir (n = 292)	Valganciclovir (n = 297)	Difference (95% CI), %[a]
Pyrexia	21 (7.2)	15 (5.1)	2.1 (-1.8, 6.2)
Headache	20 (6.8)	19 (6.4)	0.5 (-3.7, 4.6)
Acute kidney injury	20 (6.8)	17 (5.7)	1.1 (-2.9, 5.2)
Abdominal pain	20 (6.8)	15 (5.1)	1.8 (-2.1, 5.8)
Hypotension	19 (6.5)	13 (4.4)	2.1 (-1.6, 6.0)
Anemia	18 (6.2)	29 (9.8)	-3.6 (-8.1, 0.8)
Vomiting	18 (6.2)	27 (9.1)	-2.9 (-7.4, 1.4)
Constipation	18 (6.2)	24 (8.1)	-1.9 (-6.2, 2.3)
Dyspepsia	18 (6.2)	13 (4.4)	1.8 (-1.9, 5.6)
Hypokalemia	17 (5.8)	10 (3.4)	2.5 (-1.0, 6.1)
Dysuria	15 (5.1)	13 (4.4)	0.8 (-2.8, 4.4)
Dizziness	15 (5.1)	9 (3.0)	2.1 (-1.2, 5.6)
Cough	14 (4.8)	20 (6.7)	-1.9 (-5.9, 1.9)
Back pain	12 (4.1)	15 (5.1)	-0.9 (-4.5, 2.6)
Hyperglycemia	10 (3.4)	17 (5.7)	-2.3 (-5.9, 1.2)
New onset diabetes after transplantation	10 (3.4)	15 (5.1)	-1.6 (-5.1, 1.7)
Muscle spasms	8 (2.7)	15 (5.1)	-2.3 (-5.7, 0.9)
Dyspnea	5 (1.7)	21 (7.1)	-5.4 (-9.1, -2.2)

[a]Based on Miettinen & Nurminen method.
MedDRA version 25.0 used in the reporting of this study.
All adverse events were collected from randomization (day 1) through 14 days after the prophylaxis period or early discontinuation of prophylaxis.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 6. Serious, Drug-Related, Serious Drug-Related, and Prophylaxis Discontinuations Due to Adverse Events Through Week 28 in the Safety Population

	No. (%)	
	Letermovir (n = 292)	Valganciclovir (n = 297)
Serious[a] adverse events in ≥2% of participants		
Acute kidney injury	7 (2.4)	9 (3.0)
Creatinine increased	6 (2.1)	3 (1.0)
Diarrhea	2 (0.7)	8 (2.7)
Leukopenia	2 (0.7)	9 (3.0)
Lymphocele	8 (2.7)	5 (1.7)
Pyrexia	7 (2.4)	1 (0.3)
Transplant rejection	5 (1.7)	9 (3.0)
Urinary tract infection	8 (2.7)	7 (2.4)
Drug-related[b] adverse events in ≥2% of participants		
Leukopenia	20 (6.8)	68 (22.9)
Neutropenia	6 (2.1)	24 (8.1)
White blood cell count decreased	3 (1.0)	12 (4.0)
Serious[a] drug-related[b] adverse events in ≥2 participants		
Febrile neutropenia	1 (0.3)	3 (1.0)
Leukopenia	2 (0.7)	6 (2.0)
Neutropenia	2 (0.7)	2 (0.7)
White blood cell count decreased	0 (0.0)	3 (1.0)
Discontinuations due to adverse events in ≥2 participants		
CMV infection	0 (0.0)	2 (0.7)
Leukopenia	3 (1.0)	16 (5.4)
Neutropenia	4 (1.4)	5 (1.7)
Pancytopenia	2 (0.7)	0 (0.0)
White blood cell count decreased	0 (0.0)	4 (1.3)

[a]An adverse event defined as serious if it resulted in death, was life-threatening, required inpatient hospitalization or prolonged an existing hospitalization, or resulted in persistent or significant disability or incapacity.
[b]Considered by the investigator to be related to the study drug.
MedDRA version 25.0 was used in the reporting of this study.
All adverse events were collected from randomization (day 1) through 14 days after the prophylaxis period or early discontinuation of prophylaxis.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.

eTable 7. Grade 3 or 4 Laboratory Values Worsening From Baseline Through Week 28

Worsening Grade From Baseline: Grade 3 or 4 in ≥1% Through Week 28		Letermovir n/m (%)	Valganciclovir n/m (%)
Alanine aminotransferase (IU/L)	Grade 3: 5 to <10 x ULN	6/284 (2.1)	1/293 (0.3)
Calcium, low (mg/dL)	Grade 3: 6.1 to <7.0	4/285 (1.4)	2/293 (0.7)
Creatinine (mg/dL)	Grade 3: >1.8 to <3.5 x ULN	13/285 (4.6)	14/293 (4.8)
	Grade 4: ≥3.5 x ULN	1/285 (0.4)	5/293 (1.7)
eGFR (mL/min/1.73 m^2)	Grade 3: 30 to 60 or 30 to 50% ↓ from baseline	30/258 (11.6)	36/277 (13.0)
	Grade 4: <30 or ≥50% ↓ from baseline	27/258 (10.5)	34/277 (12.3)
Potassium (mEq/L)	Grade 3: 6.5 to <7.0	0/285 (0.0)	3/293 (1.0)
Urea nitrogen (mg/dL)	Grade 3: >31	28/285 (9.8)	26/293 (8.9)
Hemoglobin (g/dL)	Grade 3: 7.0 to <9.0 (M) / 6.5 to <8.5 (F)	25/286 (8.7)	42/291 (14.4)
Lymphocytes (10^9/L)	Grade 3: 0.35 to <0.50	6/286 (2.1)	8/291 (2.7)
	Grade 4: <0.35	12/286 (4.2)	13/291 (4.5)
Neutrophils (10^9/L)	Grade 3: 0.400 to 0.599	2/286 (0.7)	10/291 (3.4)
	Grade 4: <0.400	4/286 (1.4)	13/291 (4.5)
Leukocytes (10^9/L)	Grade 3: 1.000 to 1.499	5/286 (1.7)	13/291 (4.5)
	Grade 4: <1.000	3/286 (1.0)	6/291 (2.1)

Abbreviations: eGFR, estimated glomerular filtration rate; F, female; M, male; ULN, upper limit of normal; ↓, decrease.
Participants were counted once per test in the highest grade reported. For inclusion in this analysis, both a baseline and at least one on-prophylaxis laboratory value had to be present. Only participants with a worsened grade from baseline were included. A participant was listed with a grade 3 or 4 event if their highest grade during prophylaxis was grade 3 or 4.
n=number of participants with post-baseline test results that met the predetermined criterion.
m=number of participants with at least one post-baseline test result; for the criteria that involved a comparison to baseline, a baseline value was also required.
Laboratory abnormalities were graded for intensity based on criteria from the Division of AIDS (DAIDS) 2017 Table for Grading the Severity of Adult and Pediatric Adverse Events.
All participants in the letermovir group received acyclovir for prophylaxis of herpes simplex and varicella-zoster virus.