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APPLICATION NUMBER:

209939Orig1s000
209940Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 209939 **SDN:** 000 Addendum **DATE REVIEWED:** September 22, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC, Eric F. Donaldson, Ph.D. & Anamaris M. Colberg Poley, Ph.D.

NDA #: 209939 and 209940

Supporting Document Numbers: 000 and 000
(Addendum to Original NDA Review)

Applicant Name and Address: Merck Sharp & Dohme Corp.
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Reviewers: Takashi E. Komatsu, Ph.D., RAC
Eric F. Donaldson, Ph.D.
Anamaris M. Colberg Poley, Ph.D.

Initial Submission Dates:

Correspondence Date: March 8, 2017
CDER Receipt Date: March 8, 2017
Reviewer Receipt Date: March 8, 2017
Review Complete Date: October 11, 2017
PDUFA Date: November 8, 2017

Amendments reviewed:

SDN	Date Received	Date Assigned
032	August 22, 2017	August 22, 2017
034	September 07, 2017	September 07, 2017
040	September 22, 2017	October 5, 2017
041	September 25, 2017	October 5, 2017
043	October 10, 2017	October 10, 2017
044	October 10, 2017	October 10, 2017
051	October 31, 2017	October 31, 2017
052	November 3, 2017	November 3, 2017

Related/Supporting Documents: IND 104706, IND 118361, NDA 209940, DMF (b) (4)

Product Name(s):

Proprietary: Prevymis®
Non-Proprietary/USAN: letermovir
Code Name/Number: MK-8228, AIC246, AIC090027, BAY 73-6327

Chemical Name: (S)-{8-fluoro-2-[4-(3-methoxyphenyl)-1-piperazinyl]-3-[2-methoxy-5-(trifluoromethyl)-phenyl]-3,4-dihydro-4-quinazolinyl} acetic acid

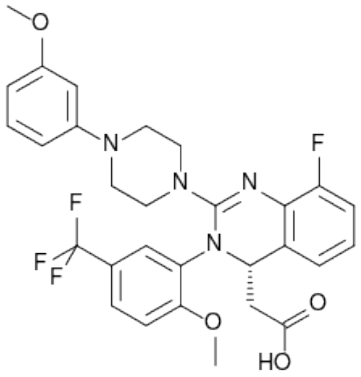
DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 209939 **SDN:** 000 Addendum **DATE REVIEWED:** September 22, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC, Eric F. Donaldson, Ph.D. & Anamaris M. Colberg Poley, Ph.D.

Structure:



LETERMOVIR

Molecular formula: $C_{29}H_{28}F_4N_4O_4$
Molecular weight: 572.56
Drug category: Antiviral
Indication(s): Prophylaxis of cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT)
Dosage Form(s): 480 mg administered once daily orally or as an intravenous (IV) infusion over 1 hour through 100 days post-transplant
Route(s) of Administration: Tablet: 240 mg; 480 mg or Injection: 240 mg/12 mL or 480 mg/24 mL in a single-dose vial for intravenous infusion
Recommended Dosage: 480 mg administered once daily
Dispensed: Rx _X_ OTC ___ (Discipline relevant)

Abbreviations: ASAT, all subjects as treated; BAC, bacterial artificial chromosome; CPE, cytopathic effect; EC_{50}, half maximal efficacy concentration; FAS, full analysis set; FC, fold change; GVHD, graft versus host disease; HCMV, human cytomegalovirus; HSCT, hematopoietic stem cell transplant; i.v., intravenous; LLoQ, lower limit of quantification; LOD, limit of detection; NGS, next-generation DNA sequencing; PET, preemptive therapy; SOT, solid organ transplant;

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NDA: 209939 **SDN:** 000 Addendum **DATE REVIEWED:** September 22, 2017
Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC, Eric F. Donaldson, Ph.D. & Anamaris M. Colberg Poley, Ph.D.

CLINICAL VIROLOGY REVIEW ADDENDUM

1. Introduction and Background

The purpose of this review addendum is to document the following:

- Final agreed labeling for Section 12.4 Microbiology
- Final agreed PMRs/PMCs related to Clinical Virology

2. Final Agreed Labeling for Section 12.4 Microbiology

The final agreed Microbiology section of the label (12.4 Microbiology) is shown below. Note that additional Clinical Virology-related edits such as the highlights and section 12.1 have been suggested and incorporated into other sections of the label as appropriate (reviewed in SDN 000).

12.4 Microbiology

Mechanism of Action

Letermovir inhibits the CMV DNA terminase complex (pUL51, pUL56, and pUL89) which is required for viral DNA processing and packaging. Biochemical characterization and electron microscopy demonstrated that letermovir affects the production of proper unit length genomes and interferes with virion maturation. Genotypic characterization of virus resistant to letermovir confirmed that letermovir targets the terminase complex.

Antiviral Activity

The median EC_{50} value of letermovir against a collection of clinical CMV isolates in a cell-culture model of infection was 2.1 nM (range = 0.7 nM to 6.1 nM, n = 74). There was no significant difference in EC_{50} value by CMV gB genotype (gB1=29; gB2=27; gB3=11; and gB4=3).

Combination Antiviral Activity

No antagonism of the antiviral activity was seen when letermovir was combined with CMV DNA polymerase inhibitors (cidofovir, foscarnet, or ganciclovir).

Viral Resistance

In Cell Culture

CMV mutants with reduced susceptibility to letermovir have been selected in cell culture and the resistance mutations map to UL56. Resistance-associated substitutions occur between amino acid positions pUL56 231 and 369 (V231A/L, V236L/M, E237D, L241P, T244K/R, L257I, F261C/L/S, Y321C, C325F/R/Y, M329T, R369G/M/S). EC_{50} values for virus expressing these substitutions are 13- to 5,870-fold higher than those for the wild-type reference virus.

In Clinical Studies

In a Phase 2b trial evaluating letermovir or placebo in 131 HSCT recipients, DNA sequence analysis of a select region of UL56 (amino acids 231 to 369) was performed on samples obtained from 12 letermovir-treated subjects who experienced prophylaxis failure and for whom on-treatment samples were available for analysis. One subject had a letermovir resistance substitution, pUL56 V236M.

In a Phase 3 trial (P001), DNA sequence analysis of the entire coding regions of UL56 and UL89 was performed on samples obtained from 28 letermovir-treated subjects who had received at least one dose of study drug and experienced prophylaxis failure and for whom samples were available for analysis. Two subjects were identified as having a letermovir-resistance substitution, pUL56 V236M or C325W.

These substitutions were identified from on-treatment samples [see Clinical Studies (14)]. A virus from a third subject who experienced prophylaxis failure had a pUL56 E237G substitution at low frequency (<5%), and while pUL56 E237D was associated with resistance in cell culture, the clinical significance of this substitution at this frequency is unknown.

Cross Resistance
Cross resistance is not likely with drugs outside of this class. Letermovir is fully active against viral populations with substitutions conferring resistance to CMV DNA polymerase inhibitors (cidofovir, foscarnet, and ganciclovir). These DNA polymerase inhibitors are fully active against viral populations with substitutions conferring resistance to letermovir.

3. Final Agreed PMRs

A. Conduct phenotypic analysis of letermovir against HCMV mutants carrying the following pUL56 and pUL89 substitutions using bacterial artificial chromosome technology:
   - pUL56 M3V, E237G, C325W, E485G, E485G + SNS445-447 deletion, S255L, Y575C, and R816W.
   - pUL89 I531T.

   Please include previously identified substitutions with a range of susceptibilities from low fold change (e.g. pUL56 L257I) to high fold change (e.g. pUL56 C325Y) as references.

   Draft Protocol Submission: February 2018
   Final Protocol Submission: May 2018
   Study/Trial Completion: October 2019
   Final Report Submission: February 2020

Note: The agreed upon list is shorter than what was originally proposed by this reviewer (reviewed in SDN 000). However, the sponsor is including the substitutions that this reviewer believes to be the most likely to confer resistance to letermovir (i.e., pUL56 E237G, C325W, and E485G + SNS445-447 deletion). Furthermore, the sponsor will be conducting a number of additional studies including (b) (4) , a study to evaluate the efficacy when treatment is extended to 200 days, (b) (4) The sponsor will also be asked to use the more sensitive genotypic assay evaluating pUL51, pUL56, and pUL89, which should provide more comprehensive data. (b) (4)

Final Agreed PMCs

B. Conduct a randomized, double-blind, placebo-controlled trial to assess the efficacy and safety of 100 days of letermovir prophylaxis versus 200 days of letermovir prophylaxis for the prevention of clinically significant cytomegalovirus (CMV) infection in CMV seropositive recipients of an allogeneic hematopoietic stem cell transplant (HSCT).

   Draft Protocol Submission: September 2018
   Final Protocol Submission: December 2018
   Study/Trial Completion: November 2022
   Final Report Submission:

4. Administrative

4.1. Reviewers' Signatures

_____
Takashi E. Komatsu, Ph.D., RAC
Clinical Virology Reviewer

_____
Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

_____
Anamaris M. Colberg Poley, Ph.D.
Clinical Virology Reviewer

4.2. Concurrence

_____
HFD-530/Clin.Virol.TL/J. O'Rear, Ph.D.

CC:
HFD-530/NDA # 209939
HFD-530/Division File
HFD-530/PM/Tyson

Appendix:
Sponsor's Responses to the Division's Labeling Recommendations
The Division's comments are in bold, 11 pt font, the sponsor's responses are in 10 pt font, and the Division's comments are underlined, in 10 pt font.

Sponsor's Responses included in SDN 032

1. The cell culture antiviral activity data were broken down by gB genotype.

Sponsor's response: A statistical analysis of the relationship between CMV UL55 (gB) genotype and susceptibility to letermovir found no significant difference in EC_{50} values as a function of gB genotype; therefore, Merck proposes the following language to describe the antiviral activity of letermovir in section 12.4 (Microbiology) of the USPI:

> "The median EC_{50} value of letermovir against a collection of clinical CMV isolates in a cellculture model of infection was 2.1 nM (range = 0.7 nM to 6.1 nM, n = 74). There was no significant difference in EC_{50} value by CMV gB genotype (b) (4)

A total of 74 clinical CMV isolates were assessed for susceptibility to letermovir in a cell-culture model of infection, using plaque or CPE reduction assays to determine the letermovir EC_{50} value for each isolate. The median EC_{50} value was 2.1 nM, with a range from 0.7 nM to 6.1 nM. A typographical error misidentified 0.14 nM as the lowest value in the study (see the entry for strain U-18 in Table 1 of [Ref. 4.3: 04KCKD]).The correct EC_{50} (sic) for strain U-18 is 1.4 nM, therefore 0.7 nM (the EC_{50} (sic) for strain U-5) is the lowest value in the study. The median was calculated using the corrected value. To determine the UL55 (gB) genotype of these isolates, PCR amplification and Sanger sequencing of a portion of the UL55 gene was performed, followed by alignment to reference sequences for each of the 4 major gB genotypes. The distribution of gB genotypes was 29/27/11/3/4 for gB 1/2/3/4/not determined, respectively. The F-Test from an ANOVA model for testing the null hypothesis that there is no mean difference in EC_{50} (sic) of letermovir susceptibility among any of the four gB subtypes yielded a p-value >0.5. All of the 95% confidence intervals for pairwise ratios between gB subtypes contain the null value of ratio = 1 [Ref. 4.3: 04KCKD].

Comment (not to be communicated to the sponsor): (b) (5)

Comment: We believe that the number of isolates that were evaluated for each gB genotype should be included for transparency.

2. These substitutions were added as they were observed on-treatment at known resistanceassociated positions. These substitutions were added as this combination was observed in 2 subjects while on-treatment.

Sponsor's response: Under the heading of Viral Resistance: In Clinical Studies" in section 12.4 (Microbiology) of the USPI, Merck proposes the text below:
"In a Phase 3 trial (P001), DNA sequence analysis of the entire coding regions of UL56 and UL89 was performed on samples obtained from (b) (4) letermovir-treated subjects (b) (4)

The rationale for the text proposed by Merck is as follows:
(b) (4)

(b) (4)

[redacted]

Comment (not to be communicated to the sponsor): (b) (5)

(b) (5)

[redacted]

Comment: We do not agree (b) (4)

[redacted]

We agree [redacted (b) (4)]. However, this combination should be evaluated in your phenotypic analyses.

Sponsor's Responses included in SDN 040

1. FDA requested to add the GV E237G to the list of letermovir-resistance substitutions.

Sponsor's response: The Applicant does not agree to include E237G in the list of letermovir-resistance substitutions. Two lines of evidence (in addition to points raised in a previous response to labelling comments Response 14, SN 029, submitted August 22, 2017) indicate that the E237G substitution seen in this sample is an artifact. First, the CMV DNA concentration in this plasma sample is too low (≤150 copies/mL) to enable reliable identification of a variant in only 4.1% of the CMV DNA population. At this frequency, the template containing the E237G substitution would be present at less than a single copy in the DNA sample used for PCR amplification and would therefore be undetectable. Second, in replicate genotypic analysis of 22 possible substitutions that had been observed in ≥5% but < 99% of NGS reads, none of the candidates with low NGS reads in samples with low CMV DNA copy number (like E237G) were confirmed. The only two GVs that repeated were from samples with higher levels of CMV DNA (70% NGS reads, 1076 copies/mL and 7% NGS reads, 2067 copies/mL). Among the 22 replicate-tested candidate GVs, all three that were detected in plasma with ≤150 copies of CMV DNA/mL were identified as artifacts.

The Applicant will perform phenotypic analysis on the pUL56 E237G substitution, and the results will be provided to the Agency by June 2018. However, in the setting of prophylaxis, there is no clinical action that can be taken based on knowledge of a letermovir-resistant GV; therefore, there is no urgency to include unconfirmed substitutions in the USPI. Inclusion of the pUL56 E237G substitution in the list of letermovir-resistant substitutions prior to phenotypic confirmation is not considered by the Applicant to be warranted.

The proposed text for Section 12.4 Microbiology: Viral Resistance: In Clinical Studies (Phase 3 study) is below:

> In a Phase 3 trial (P001), DNA sequence analysis of the entire coding regions of UL56 and UL89 was performed on samples obtained from 28 letermovir-treated subjects [redacted (b) (4)] letermovir-resistance substitution, pUL56 V236M or C325W. These substitutions were identified from on-treatment samples [see Clinical Studies (14)].

Comment (not to be communicated to the sponsor): [redacted (b) (5)]

Sponsor's Responses included in SDN 051

2. Conduct phenotypic analysis of letermovir against HCMV mutants carrying the following pUL56 and pUL89 substitutions using bacterial artificial chromosome technology:

[redacted (b) (4)]

Please include previously identified substitutions with a range of susceptibilities from low fold change (e.g. pUL56 L257I) to high fold change (e.g. pUL56 C325Y) as references.

Sponsor's response: The Applicant agrees to perform phenotypic analysis of the following HCMV mutants using bacterial artificial chromosome technology:

- pUL56: M3V, E237G, C325W, E485G, E485G + SNS445-447 deletion, S255L, Y575C, and R816W
- pUL89: I531T

(b) (4)

(b) (4)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

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NDA: 209939 **SDN:** 000 Addendum **DATE REVIEWED:** September 22, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC, Eric F. Donaldson, Ph.D. & Anamaris M. Colberg Poley, Ph.D.

(b) (4)

[redacted]

Comment (not to be communicated to the sponsor): (b) (5)

[redacted]

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TAKASHI E KOMATSU
11/06/2017

ERIC F DONALDSON
11/06/2017

ANAMARIS M COLBERG POLEY
11/06/2017

JULIAN J O REAR
11/07/2017

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 209939 **SDN:** 000 **DATE REVIEWED:** August 8, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Anamaris M. Colberg Poley, Ph.D.

NDA #: 209939 and 209940 **Supporting Document Numbers:** 000 and 000

Applicant Name and Address: Merck Sharp & Dohme Corp.
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North Wales, PA 19454-2505
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Reviewers' Name: Takashi E. Komatsu, Ph.D., RAC
Anamaris M. Colberg Poley, Ph.D.

Initial Submission Dates:

Correspondence Date: March 8, 2017
CDER Receipt Date: March 8, 2017
Reviewer Receipt Date: March 8, 2017
Review Complete Date: August 8, 2017
PDUFA Date: November 8, 2017

Amendments reviewed:

SDN	Date Received	Date Assigned
003	March 8, 2017	March 10, 2017
006	March 24, 2017	March 27, 2017
009	April 24, 2017	April 24, 2017
010	May 12, 2017	May 12, 2017
015	June 7, 2017	June 7, 2017
016	June 8, 2017	June 8, 2017
022	July 14, 2017	July 14, 2017
NDA 209940 SDN 005	June 8, 2017	June 8, 2017

Related/Supporting Documents: IND 104706, IND 118361, NDA 209940, DMF (b) (4)

Product Name(s):

Proprietary: Prevymis®
Non-Proprietary/USAN: letermovir
Code Name/Number: MK-8228, AIC246, AIC090027, BAY 73-6327

Chemical Name: (S)-{8-fluoro-2-[4-(3-methoxyphenyl)-1-piperazinyl]-3-[2-methoxy-5-(trifluoromethyl)-phenyl]-3,4-dihydro-4-quinazolinyl} acetic acid

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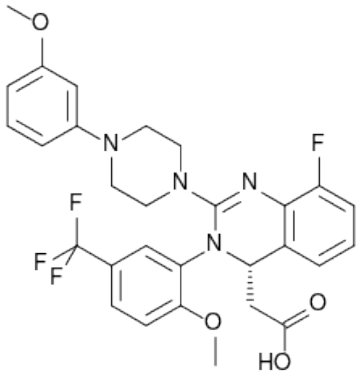
NDA: 209939 **SDN:** 000 **DATE REVIEWED:** August 8, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Anamaris M. Colberg Poley, Ph.D.

Structure:



LETERMOVIR

Molecular formula: $C_{29}H_{28}F_4N_4O_4$
Molecular weight: 572.56
Drug category: Antiviral
Indication(s): Prophylaxis of cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT)
Dosage Form(s): 480 mg administered once daily orally or as an intravenous (IV) infusion over 1 hour through 100 days post-transplant
Route(s) of Administration: Tablet: 240 mg; 480 mg or Injection: 240 mg/12 mL or 480 mg/24 mL in a single-dose vial for intravenous infusion
Recommended Dosage: 480 mg administered once daily
Dispensed: Rx _X_ OTC ___ (Discipline relevant)

Abbreviations: AAG, α-1-acid glycoprotein; ACV, acyclovir; AIDS, acquired immune deficiency syndrome; ATP, adenosine triphosphate; ATV, atazanavir; BAC, bacterial artificial chromosome; BAL, bronchoalveolar lavage; BCV, brincidofovir; BID, dose twice daily; CDV, cidofovir; CPE, cytopathic effect; CsA, cyclosporine A; d4T, stavudine; ddI, didanosin; dpi, days post infection; DRV, darunavir; EBV, Epstein-Barr virus; EC_{50}, half maximal efficacy concentration; EC_{90}, 90% effective concentration; EFV, efavirenz; ETR, etravirine; FAS, full analysis set; FC, fold change; FOS, foscarnet; FTC, emtricitabine; gB, glycoprotein B; GCV, ganciclovir; GFP, green fluorescent protein; GVHD, graft versus host disease; HAART, highly active antiretroviral regimen; HAdV, human adenovirus; HBeAg, hepatitis B e antigen; HBPC, human blood precursor cell; HBsAg, hepatitis B s antigen; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HFFs, human foreskin fibroblasts; HHV, human herpesvirus; HIV, human immunodeficiency virus; hpi, hours post infection; HS, human serum; HSA, human serum albumin; HSCT, hematopoietic stem cell transplant; HUVEC, human umbilical vein endothelial cells; ITT, intent-to-treat; IVIg, intravenous immunoglobulin; LLoQ, lower limit of quantification; LOD, limit of detection; LPV, lopinavir; MBV, maribavir; MCMV, murine cytomegalovirus; MOI, multiplicity of infection; MT-4, human T cell line; NGS, next-generation DNA sequencing; NHDF, normal human dermal fibroblasts; NHLF, normal human lung fibroblasts; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; NVP, nevirapine; ORF, open reading frame; PET, pre-emptive therapy; PFGE, pulsed field gel electrophoresis; pfu, plaque forming units; PIs, protease inhibitors; PJP, *Pneumocystis jirovecii* pneumonia; PO, dose orally; PTLD, post-transplant lymphoproliferative disease; QC, quality control; QD, dose once daily; RAL, raltegravir; RBV, ribavirin; RCMV, rat cytomegalovirus; RSV, respiratory syncytial virus; RTV, ritonavir; SEAP, secreted alkaline phosphatase; SOT, solid organ transplant; SVC, shell vial culture; TB40/E, clinical endothelial and macrophage tropic HCMV isolate from a bone marrow transplant recipient; $TCID_{50}$, 50% cell culture infectious dose; TDF, tenofovir; TI, therapeutic index; UL, unique long; US, unique short; vGCV, valganciclovir; VZV, varicella zoster virus;

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DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 209939 **SDN:** 000 **DATE REVIEWED:** August 8, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Anamaris M. Colberg Poley, Ph.D.

EXECUTIVE SUMMARY

This Original NDA submission (209930 and 209940, received March 8, 2017) is for Prevymis® (letermovir) to be used in the prophylaxis of cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT). The clinical development to support the efficacy of letermovir included evaluations in 3 completed studies. Two Phase 2 studies, MK-8228-019 (AIC001-2-001 non-IND study) and MK-8228-020 (NCT01063829), and a pivotal Phase 3 study, MK-8228-001 (NCT02137772) were conducted by the sponsor. These studies support the approval of Prevymis®.

Letermovir is an inhibitor of the pUL51/pUL56/pUL89 HCMV terminase complex necessary for the generation of unit length DNA genomes from viral DNA concatemers for packing and subsequent virion maturation. The median EC_{50} value of letermovir in cell culture assays was 1.9 nM (range 0.1 nM-5.8 nM, n = 29), 2.0 nM (range 0.7 nM-6.1 nM, n = 27), 2.3 nM (range 1.5 nM-3.4 nM, n = 11), and 2.9 nM (range 2.6 nM-3.2 nM, n = 3) against HCMV gB genotypes 1, 2, 3, and 4, respectively.

The combination of letermovir with other approved drugs using in the treatment of HCMV, acyclovir (ACV), cidofovir (CDV), foscarnet (FOS), or ganciclovir (GCV), was not antagonistic at their respective EC_{50} values. The lack of antagonism between letermovir and these compounds indicates that inhibition of HCMV DNA polymerase does not antagonize concurrent inhibition of HCMV DNA terminase in a cell-culture model of infection.

Selection in cell culture of HCMV resistant to letermovir resulted in the pUL56 amino acid substitutions L51M, V231A/L, V236L/M, E237D, L241P, T244K/R, L257I, F261C/L/S, Y321C, C325F/R/Y, M329T, and R369G/M/S. In addition, the pUL89 A345S substitution was observed. To date, there are no amino acid substitutions detected in pUL51, which represents the other component of the HCMV terminase complex or in the associated protein, pUL104. All of the pUL56 amino acid substitutions conferred ≥2-fold reduced susceptibility to letermovir, with several >30-fold. Furthermore, all of the pUL56 mutants including those with 3 amino acid substitutions displayed growth comparative to WT virus indicating that these amino acid substitutions do not impact cell culture fitness. HCMV mutants resistant to letermovir were still sensitive to CDV, FOS, or GCV as is expected based on their different mechanisms of action. Conversely, HCMV mutants resistant to CDV, FOS, or GCV were still sensitive to letermovir.

Clinical Virology Assessment of the Clinical Studies

Study MK-8228-019 (AIC001-2-001) was a non-IND Phase 2a proof-of-concept open label study in which 26 kidney and kidney/pancreas transplant subjects and 1 bone marrow transplant subject with positive HCMV DNaemia (preemptive therapy) were treated with daily doses of 80 mg letermovir or an active control, which was the local standard of care of the investigational site (valganciclovir) for 14 days. The majority of subjects were kidney transplant recipients (25 subjects [92.6%]). One subject was a kidney/pancreas transplant recipients (3.7%) and another subject was a bone marrow transplant recipient (3.7%). The primary efficacy endpoint was the reduction in HCMV DNA load (assessed by PCR) from Baseline to Day 15 using the (b) (4) central laboratory HCMV PCR assay. In the active control (valganciclovir) group, a sharp decline in the plasma viral load mean change from Baseline was seen at Day 4 and the viral load remained similar between Days 4 and 15. In the letermovir treatment groups, the viral load remained similar between Days 1 and 11 and a decline in the viral load mean change from Baseline was seen between Days 11 and 15. At Day 15, a statistically significant viral load decline from Baseline was seen in both the letermovir and active control groups (40 mg BID: P = 0.031; 80 mg QD: P = 0.018; standard of care: P = 0.001), with a larger (though not statistically significantly larger) decline observed in the active control group.

Study MK-8228-020 (AIC246-01-II-02, NCT01063829) was a Phase 2, multi-center, randomized, double-blind, placebo-controlled, dose-ranging study to investigate the safety and efficacy of 3 different doses (60, 120, or 240 mg/day) of letermovir given orally for 84 days in comparison to matching placebo for the prevention of human cytomegalovirus (HCMV) active replication by re-infection or reactivation in HCMV seropositive allogeneic human blood precursor cell (HBPC) recipient subjects. The primary endpoints were incidence and time to onset of "HCMV prophylaxis failed" during the 84-day treatment period. The total number of subjects included in the analysis set in the placebo and letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups were 33, 33, 33, and 34, respectively. The number (%) of subjects who discontinued study medication intake before day 84 was 21 (63.6%), 16 (48.5%), 10 (30.3%), and 10 (29.4%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively. Prophylaxis failure was defined as evidence of HCMV replication (confirmed) leading to initiation of preemptive therapy or evidence of HCMV disease. Failure also includes subjects who discontinued treatment prior to Day 84 for reasons other than HCMV prophylaxis failure. Subjects in the 120 mg/day and 240 mg/day letermovir treatment groups were significantly less likely to fail than subjects in the placebo group (p=0.014 and p=0.007, respectively). The number (%) of subjects who failed HCMV prophylaxis (not including those who discontinued for other reasons prior to Day 84) was 13 (n=33, 39.4%), 7 (n=33, 21.2%), 6 (n=33, 18.2%), and 2 (n=34, 5.9%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively. The incidence of failure decreased with increasing letermovir dose. There was no subject who reached the definition of HCMV end-organ disease alone during the 84 days (12 weeks) of study drug administration.

Study MK-8228-P001 (NCT02137772) was a Phase 3, randomized, placebo-controlled, multi-site, double-blind study designed to evaluate the efficacy and safety of letermovir versus placebo, dosed for 14 weeks, for prevention of clinically significant HCMV infection in adult, HCMV-seropositive allogeneic HSCT recipients (R+), a population at high risk for HCMV infection and/or disease. The primary endpoint for this study, clinically significant HCMV infection at Week 24 post-transplant, was defined as the occurrence of either one of the following outcomes: 1) onset of HCMV end-organ disease; or 2) initiation of anti-HCMV pre-emptive therapy based on documented HCMV DNaemia in the subject's plasma samples (detection of HCMV viral DNA as measured by the central laboratory) and the clinical condition of the subject.

The primary population for efficacy evaluations in the study was the FAS population, which consisted of all randomized subjects who received at least one dose of study medication and had no detectable HCMV viral DNA (measured by the central laboratory) on Day 1 (when study medication was initiated). At Week 24, the proportion of subjects with clinically significant HCMV infection in the letermovir group (37.5%, 122/325) was lower than that in the placebo group (60.6%, 103/170). The estimated difference (95% CI) was -23% (-31.7% to -13.8%). This difference between the two groups was statistically significant (one-sided p-value <0.0001). Of importance, these differences were still observed regardless of the risk stratification (high vs. low), stem cell source (bone marrow vs. peripheral blood), or donor HCMV serostatus (positive vs. negative). Of note, the difference was smaller between the groups in the cord blood recipients (58.3%, 7/12 vs 60%, 6/10, in the letermovir and placebo arms, respectively). The cumulative incidence of all-cause mortality was also lower in the letermovir group (10.8%, 35/325) compared to the placebo group (16.5%, 28/170) through Week 24 post-transplant in the FAS population. Importantly, these differences were still observed when stratified by different subgroup categories, although the numbers were not powered to demonstrate statistical differences. The incidence of HCMV end-organ disease was low for both treatment arms primarily due to PET initiation. The Kaplan-Meier method also resulted in a statistically significant difference in the incidence of all-

cause mortality in favor of the letermovir arm for the FAS population with the nominal two-sided stratified log-rank p-value = 0.033 if only the mortality that was observed within the study was considered. The incidence of all-cause mortality was still in favor of the letermovir arm for the FAS population with the nominal two-sided stratified log-rank p-value = 0.40 when all confirmed mortality regardless of whether it was observed within the study was included. The statistical significance was maintained using all confirmed deaths at Week 24 based on the statistical reviewer's analysis. The median time to PET in the FAS population was 146 days and 42 days in the letermovir and placebo arms, respectively, for subjects whose death occurred by Week 24. Given that there were 28 subjects who experienced relapse in the letermovir arm after 14 weeks of treatment and the median time to death was 104 days, longer treatment with letermovir beyond 14 weeks may be beneficial. Indeed, extending prophylaxis with vGCV to 200 days was demonstrated to be significantly better than 100 days for the prevention of HCMV disease in high risk (donor HCMV seropositive [D+]/recipient HCMV seronegative [R-]) adult kidney transplant recipients (Humar et al., 2010).

Letermovir Resistance

An analysis of amino acid substitutions was conducted pooling data from the 55 subjects who had detectable HCMV DNaemia in the Phase 2 and Phase 3 prophylaxis studies. The following amino acid substitutions were observed in pUL56 more frequently in letermovir-treated subjects compared to placebo: L134V, E157G, S227I, Q228H, V236M, E237G, S255L, I313V, C325W, A366P, R410G, D414N, A425V/A, G430V, E495Q, Y575C, L658S, S705F, R816W, and P846L. The pUL56 V236M amino acid substitution is a known letermovir resistance-associated substitution that has previously been selected in cell culture and phenotypically characterized (~45-fold reduced susceptibility to letermovir). While the pUL56 C325W substitution has not previously been reported, pUL56 C325F, C325R, and C325Y substitutions have been selected in cell culture (these substitutions confer >3,000-fold reduced susceptibility to letermovir). In addition, pUL56 E237G substitution was detected in one subject who failed letermovir treatment. This substitution was observed at 4% and thus was not reported by the sponsor (note: The sponsor used a 5% cut-off for their analyses). While the pUL56 E237G substitution has not previously been reported, pUL56 E237D substitution has been selected in cell culture (10-fold reduced susceptibility to letermovir). The pUL56 V236M, E237G, C325W, and C325Y should be included in the clinical resistance section of the label. In addition, the pUL56 S445/N446/S447 deletion seen in combination with an E485G substitution, although at polymorphic positions, occurred at high frequency (>70%) in 2 subjects who failed while on letermovir and should also be considered for the label. The following pUL56 substitutions at conserved amino acid positions were each observed in one virologic failure while on letermovir: pUL56 L134V, S227I, Q228H, A366P, R410G, D414N, A425V/A, G430V, and E495Q. The following pUL56 substitutions at conserved amino acid positions were each observed in one virologic failure who relapsed/failed off-treatment: pUL56 E157G, S255L, I313V, Y575C, L658S, S705F, R816W, and P846L.

The following amino acid substitutions at conserved amino acid positions were observed in pUL89 more frequently in letermovir-treated subjects compared to placebo: N74S, P176S, D309D/V, D309G, M406V, L522P, A532T, and Q625*(stop). The following pUL89 substitutions were each observed in a virologic failure while on letermovir: pUL89 L522P and Q625* (stop). The following pUL89 substitutions at conserved amino acid positions were each observed in a virologic failure who relapsed/failed off-treatment: pUL89 N74S, P176S, D309D/V, D309G, M406V, and A532T.

Amongst the subjects with complete sequencing data in the letermovir arm in the Phase 3 study, there were 37.5% (3/8) and 0% (0/22) in the on-treatment virologic failures and off-treatment virologic failures, respectively, who had *previously identified* resistance-associated substitutions. A 100 day vs

200 day prophylaxis study similar to what was conducted with vGCV in SOT recipients would be reasonable based on the current rates of resistance in the off-treatment virologic failures.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability:

This Original NDA for prophylaxis of cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT) is approvable from a Clinical Virology perspective. Additionally, the data support the use of the primary endpoint, which is mostly driven by the HCMV DNaemia, to grant traditional approval.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable:

These reviewers recommend the following post-marketing commitments or requirements, as appropriate:

1. Please conduct phenotypic analysis of letermovir against HCMV mutants carrying the following pUL56 and pUL89 substitutions:

(b) (4)

2. Summary of OND Virology Assessments

2.1. Nonclinical Virology

Letermovir is an inhibitor of the HCMV terminase complex necessary for the generation of unit length DNA genomes from viral DNA concatemers for packing and subsequent virion maturation. The median EC_{50} value of letermovir in cell culture assays was 1.9 nM (range 0.1 nM-5.8 nM, n = 29), 2.0 nM (range 0.7 nM-6.1 nM, n = 27), 2.3 nM (range 1.5 nM-3.4 nM, n = 11), and 2.9 nM (range 2.6 nM-3.2 nM, n = 3) against HCMV gB genotypes 1, 2, 3, and 4, respectively.

The combination of letermovir with other approved drugs using in the treatment of HCMV, acyclovir (ACV), cidofovir (CDV), foscarnet (FOS), or ganciclovir (GCV), was not antagonistic at their respective EC_{50} values. The lack of antagonism between letermovir and these compounds indicates that inhibition of HCMV DNA polymerase does not antagonize concurrent inhibition of HCMV DNA terminase in a cell-culture model of infection.

Selection in cell culture of HCMV resistant to letermovir resulted in the pUL56 amino acid substitutions L51M, V231A/L, V236L/M, E237D, L241P, T244K/R, L257I, F261C/L/S, Y321C, C325F/R/Y, M329T, and R369G/M/S. In addition, the pUL89 A345S substitution was observed. To date, there are no amino acid substitutions detected in the pUL51 or pUL104, which represent the other components of the HCMV terminase complex. All of the pUL56 amino acid substitutions conferred ≥2-fold reduced susceptibility to letermovir, with several >30-fold. Furthermore, all of the pUL56 mutants including those with 3 amino acid substitutions displayed growth comparative to WT virus indicating that these amino acid substitutions do not impact cell culture fitness. HCMV mutants resistant to letermovir were still

sensitive to CDV, FOS, or GCV as is expected based on their different mechanisms of action. Conversely, HCMV mutants resistant to CDV, FOS, or GCV were still sensitive to letermovir.

2.2. Clinical Virology

The clinical development to support the efficacy of letermovir included evaluations in 3 completed studies. Two Phase 2 studies, MK-8228-019 (AIC001-2-001 non-IND study) and MK-8228-020 (NCT01063829), were designed and conducted by AiCuris GmbH & Co. KG. Following the completion of the Phase 2 program, letermovir was licensed to Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. (the sponsor of this NDA submission). A pivotal Phase 3 study, MK-8228-001 (NCT02137772), was designed and conducted by the sponsor.

Study MK-8228-019 (AIC001-2-001) was a non-IND Phase 2a proof-of-concept open label study in which 26 kidney and kidney/pancreas transplant subjects and 1 bone marrow transplant subject with positive HCMV DNaemia (preemptive therapy) were treated with daily doses of 80 mg letermovir or an active control, which was the local standard of care of the investigational site (valganciclovir) for 14 days. The majority of subjects were kidney transplant recipients (25 subjects [92.6%]). One subject was a kidney/pancreas transplant recipients (3.7%) and another subject was a bone marrow transplant recipient (3.7%). The primary efficacy endpoint was the reduction in HCMV DNA load (assessed by PCR) from Baseline to Day 15 using the (b) (4) central laboratory HCMV PCR assay. In the active control (valganciclovir) group, a sharp decline in the plasma viral load mean change from Baseline was seen at Day 4 and the viral load remained similar between Days 4 and 15. In the letermovir treatment groups, the viral load remained similar between Days 1 and 11 and a decline in the viral load mean change from Baseline was seen between Days 11 and 15. At Day 15, a statistically significant viral load decline from Baseline was seen in both the letermovir and active control groups (40 mg BID: P = 0.031; 80 mg QD: P = 0.018; standard of care: P = 0.001), with a larger (though not statistically significantly larger) decline observed in the active control group.

Study MK-8228-020 (AIC246-01-II-02, NCT01063829) was a Phase 2, multi-center, randomized, double-blind, placebo-controlled, dose-ranging study to investigate the safety and efficacy of 3 different doses (60, 120, or 240 mg/day) of letermovir given orally for 84 days in comparison to matching placebo for the prevention of human cytomegalovirus (HCMV) active replication by re-infection or reactivation in HCMV seropositive allogeneic human blood precursor cell (HBPC) recipient subjects. The primary endpoints were incidence and time to onset of "HCMV prophylaxis failed" during the 84-day treatment period. The total number of subjects included in the analysis set in the placebo and letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups were 33, 33, 33, and 34, respectively. The number (%) of subjects who discontinued study medication intake before day 84 was 21 (63.6%), 16 (48.5%), 10 (30.3%), and 10 (29.4%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively. Prophylaxis failure was defined as evidence of HCMV replication (confirmed) leading to initiation of preemptive therapy or evidence of HCMV disease. Failure also includes subjects who discontinued treatment prior to Day 84 for reasons other than HCMV prophylaxis failure. Subjects in the 120 mg/day and 240 mg/day letermovir treatment groups were significantly less likely to fail than subjects in the placebo group (p=0.014 and p=0.007, respectively). The number (%) of subjects who failed HCMV prophylaxis (not including those who discontinued for other reasons prior to Day 84) was 13 (n=33, 39.4%), 7 (n=33, 21.2%), 6 (n=33, 18.2%), and 2 (n=34, 5.9%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively. The incidence of failure decreased with increasing letermovir dose. There was no subject who reached the definition of HCMV end-organ disease alone during the 84 days (12 weeks) of study drug administration.

Study MK-8228-P001 (NCT02137772) was a Phase 3, randomized, placebo-controlled, multi-site, double-blind study designed to evaluate the efficacy and safety of letermovir versus placebo, dosed for 14 weeks, for prevention of clinically significant HCMV infection in adult, HCMV-seropositive allogeneic HSCT recipients (R+), a population at high risk for HCMV infection and/or disease. The primary endpoint for this study, clinically significant HCMV infection at Week 24 post-transplant, was defined as the occurrence of either one of the following outcomes: 1) onset of HCMV end-organ disease; or 2) initiation of anti-HCMV pre-emptive therapy based on documented HCMV DNaemia in the subject's plasma samples (detection of HCMV viral DNA as measured by the central laboratory) and the clinical condition of the subject.

The primary population for efficacy evaluations in the study was the FAS population, which consisted of all randomized subjects who received at least one dose of study medication and had no detectable HCMV viral DNA (measured by the central laboratory) on Day 1 (when study medication was initiated). At Week 24, the proportion of subjects with clinically significant HCMV infection in the letermovir group (37.5%, 122/325) was lower than that in the placebo group (60.6%, 103/170). The estimated difference (95% CI) was -23% (-31.7% to -13.8%). This difference between the two groups was statistically significant (one-sided p-value <0.0001). Of importance, these differences were still observed regardless of the risk stratification (high vs. low), stem cell source (bone marrow vs. peripheral blood), or donor HCMV serostatus (positive vs. negative). Of note, the difference was smaller between the groups in the cord blood recipients (58.3%, 7/12 vs 60%, 6/10, in the letermovir and placebo arms, respectively). While the numbers are too small to make any conclusions, this observation is consistent with reports that the risk of complications due to infection, including HHV-6 encephalitis and HCMV diseases, is higher in patients who received cord blood transplantation (Walker et al., 2007). The cumulative incidence of all-cause mortality was also lower in the letermovir group (10.8%, 35/325) compared to the placebo group (16.5%, 28/170) through Week 24 post-transplant in the FAS population. Importantly, these differences were still observed when stratified by different subgroup categories, although the numbers were not powered to demonstrate statistical differences. The incidence of HCMV end-organ disease was low for both treatment arms primarily due to PET initiation. Of note, one of the subjects in each of the letermovir and placebo arms who died also developed HCMV end-organ disease. The Kaplan-Meier method also resulted in a statistically significant difference in the incidence of all-cause mortality in favor of the letermovir arm for the FAS population with the nominal two-sided stratified log-rank p-value = 0.033. The statistical significance was maintained using all confirmed deaths at Week 24 based on the statistical reviewer's analysis. The median time to PET in the FAS population was 146 days and 42 days in the letermovir and placebo arms, respectively, for subjects whose death occurred by Week 24. Given that there were 28 subjects who experienced relapse in the letermovir arm after 14 weeks of treatment and the median time to death was 104 days, longer treatment with letermovir beyond 14 weeks may be beneficial. Indeed, extending prophylaxis with vGCV to 200 days was demonstrated to be significantly better than 100 days for the prevention of HCMV disease in high risk (donor HCMV seropositive [D+]/recipient HCMV seronegative [R-]) adult kidney transplant recipients (Humar et al., 2010).

Utility of the Primary Endpoint for Granting Traditional Approval

A regulatory approval pathway is needed for products intended for prophylaxis of subjects infected with HCMV in HSCT recipients. Such pathways have already been established for products in other antivirals such as those for the treatment of HIV-1 infection as well as other therapeutic areas such as oncology and autoimmune diseases. Clinical studies of HCMV treatment, prophylaxis and diagnostics in transplant recipients have traditionally used symptomatic HCMV disease as the primary end-point. However, in current clinical practice, rates of HCMV disease are often low, in part due to prolonged

prophylaxis, but also due to early detection of DNaemia and initiation of antiviral therapy before definitive symptoms attributable to HCMV infection are evident. In addition, currently, the most common form of HCMV disease after organ transplant is viral syndrome. Although definitions for viral syndrome exist, it represents a spectrum of illness, which shares many overlapping features with other infectious and non-infectious etiologies. For other chronic viral illnesses, such as HIV, HBV, and HCV, viral load has become a well-accepted surrogate marker for use in clinical studies and regulatory submissions.

At Week 24, there was a statistically significant benefit in favor of letermovir for both the HCMV DNaemia and mortality endpoints. However, the effect on mortality was modest (p=0.033 or p=0.040 depending on whether the deaths outside of the study were not or were included, respectively). Furthermore, there are no definitive data at this time to show that suppression of HCMV DNaemia predicts improved survival. The rate of HCMV end-organ disease was similar and low in both arms (1.5% and 1.8% in the letermovir and placebo arms, respectively). Thus, it is unclear how letermovir is reducing mortality if not through impact on HCMV end-organ disease and whether the difference in mortality is directly due to letermovir. There is circumstantial evidence that may support the association of HCMV DNaemia and mortality.

With respect to the incidence of HCMV end-organ disease in subjects who died, there were 1 and 3 subjects in the letermovir and placebo arms, respectively, which numerically favors the letermovir arm. Furthermore, the timing of when the subject developed HCMV end-organ disease is important as not every HCMV end-organ disease leads to death. All 3 subjects in the placebo arm who developed HCMV end-organ disease and died occurred within 18 days, 2 of whom died within a week whereas the subject in the letermovir arm had at least 3 months between the events.

With respect to the timing of the HCMV-related mortality, the all-cause mortality in subjects who had detectable HCMV was lower in the letermovir arm. The difference was maintained even if the window between positive DNaemia and death was restricted to events within 39 days. The difference in all-cause mortality in subjects who had detectable HCMV DNA between the two arms was smaller in deaths after Week 24. These results indicate that the impact of letermovir on HCMV-related mortality was greater through the first 24 weeks compared to after Week 24.

The incidence of non-relapse death through Week 24 in the FAS population was 6.8% (22/325) and 11.2% (19/170) in the letermovir and placebo arms, respectively (∆4.4%). The incidence of relapse death through Week 24 in the FAS population was 4% (13/325) and 5.9% (10/170) in the letermovir and placebo arms, respectively (∆1.9%). The differences were smaller between the treatment arms after Week 24, consistent with risk of HCMV related mortality decreasing over time.

The proportion of subjects in the FAS population with serious adverse events related to opportunistic infections within 14 days till death by Week 24 was 2.8% (9/325) and 6.5% (11/170) in the letermovir and placebo arms, respectively.

Through Week 48, the proportion of subjects who developed GVHD was numerically lower in the letermovir group, compared to the placebo group. Subjects with ≥Grade II of GVHD also comprised a numerically lower proportion of subjects in the letermovir group compared to the placebo group. In the placebo arm, through Week 48, the rate of mortality was higher in subjects with GVHD who had detectable HCMV DNaemia (34.8%, 16/46) compared to those who did not (14%, 8/57). While this trend was not observed in the letermovir arm, overall these data support the hypothesis that the presence of HCMV and GVHD were associated with worse outcomes.

The range in viral load at the time of PET was 137-39,396 IU/mL and 137-97,571 IU/mL in the letermovir and placebo arms, respectively. The viral load cutoff for PET initiation was evaluated to see if higher HCMV viral load resulted in worse outcomes. At Week 24, in the FAS group, the mortality in subjects with <913 IU/mL at time of PET was 5.3% (2/38) and 15.4% (6/39) in the letermovir and placebo arms, respectively. In comparison, the mortality in subjects with ≥913 IU/mL at time of PET was higher at 7.1% (1/14) and 27.6% (8/29) in the letermovir and placebo arms, respectively. Furthermore, the mortality in subjects with ≥9,130 IU/mL at time of PET was 25% (1/4) and 50% (4/8) in the letermovir and placebo arms, respectively. Similar trends were observed at Week 48. These data support the hypothesis that higher HCMV viral load resulted in worse clinical outcomes.

Overall, the data support the use of the primary endpoint, which is mostly driven by the HCMV DNaemia, to grant traditional approval. The primary endpoint was robust ($p<0.0001$), which meets the criteria for using a single study to support an NDA. Furthermore, while the endpoint was at the end-of-treatment, this endpoint was statistically significant at even lower doses in the sponsor's Phase 2b study. The use of HCMV DNaemia is already used in clinical practice and is viewed by the treating community as clinically meaningful. In addition to the mortality benefit that was observed at Week 24, letermovir may have also prevented unintended consequences of HCMV treatment such as reduction in immunosuppression that could lead to GVHD or treatment with GCV/vGCV, which has toxicity concerns. Although prolonged survival is considered the most reliable endpoint with clinical benefit in oncology studies, the FDA has accepted non-survival endpoints such as tumor response rates as the basis for both regular and accelerated approval. For supportive care drugs, such as for GVHD prophylaxis, the FDA typically does not use mortality as an efficacy endpoint as there are so many other causes of death after Day 180. In studies of patients with serious or life-threatening diseases, accelerated approval status permits the use of non-survival endpoints if they are reasonably likely to provide clinical benefit. Post-marketing studies are usually required to confirm clinical benefit (Johnson et al., 2003; McCaul et al., 2000). The challenges inherent in assessing response to treatment of HCMV infection in the context of the complex and variable manifestations of the disease suggest the need for a more standardized and clinically meaningful approach to clinical study design.

Letermovir Resistance

An analysis of amino acid substitutions was conducted pooling data from subjects who had detectable HCMV DNaemia in the Phase 2 and Phase 3 prophylaxis studies. (note: in the sponsor's Phase 2 study, the sponsor did not genotype the complete pUL56 nor the pUL89.) The following amino acid substitutions were observed in pUL56 more frequently in letermovir-treated subjects compared to placebo: L134V, E157G, S227I, Q228H, V236M, E237G, S255L, I313V, C325W, A366P, R410G, D414N, A425V/A, G430V, E495Q, Y575C, L658S, S705F, R816W, and P846L. The pUL56 V236M amino acid substitution is a known letermovir resistance-associated substitution that has previously been selected in cell culture and phenotypically characterized (~45-fold reduced susceptibility to letermovir). While the pUL56 C325W substitution has not previously been reported, pUL56 C325F, C325R, and C325Y substitutions have been selected in cell culture (these substitutions confer >3,000-fold reduced susceptibility to letermovir; Chou 2015; Goldner et al., 2014). In addition, pUL56 E237G substitution was detected in one subject who failed letermovir treatment. This substitution was observed at 4% and thus was not reported by the sponsor (note: The sponsor used a 5% cut-off for their analyses) (please see the review of Eric Donaldson, Ph.D. for more details). While the pUL56 E237G substitution has not previously been reported, pUL56 E237D substitution has been selected in cell culture (10-fold reduced susceptibility to letermovir; Chou 2015). The pUL56 V236M, E237G, C325W, and C325Y (detected in cell culture with >3000-fold reduced susceptibility to letermovir;

Goldner et al., 2014) should be included in the clinical resistance section of the label. In addition, the pUL56 S445/N446/S447 deletion seen in combination with an E485G substitution, although at polymorphic positions, occurred at high frequency (>70%) in 2 subjects who failed while on letermovir and should also be considered for the label. The following pUL56 substitutions at conserved amino acid positions were each observed in one virologic failure while on letermovir (b) (4) pUL56 L134V, S227I, Q228H, A366P, R410G, D414N, A425V/A, G430V, and E495Q. The following pUL56 substitutions at conserved amino acid positions were each observed in one virologic failure who relapsed/failed off-treatment (b) (4) pUL56 E157G, S255L, I313V, Y575C, L658S, S705F, R816W, and P846L.

The following amino acid substitutions at conserved amino acid positions were observed in pUL89 more frequently in letermovir-treated subjects compared to placebo: N74S, P176S, D309D/V, D309G, M406V, L522P, A532T, and Q625*(stop). The following pUL89 substitutions were each observed in a virologic failure while on letermovir (b) (4) pUL89 L522P and Q625* (stop). The following pUL89 substitutions at conserved amino acid positions were each observed in a virologic failure who relapsed/failed off-treatment (b) (4) pUL89 N74S, P176S, D309D/V, D309G, M406V, and A532T.

Amongst the subjects with complete sequencing data in the letermovir arm, there were 37.5% (3/8) and 0% (0/22) in the on-treatment virologic failures and off-treatment virologic failures, respectively, who had *previously identified* resistance-associated substitutions. A 100 day vs 200 day prophylaxis study similar to what was conducted with vGCV in SOT recipients would be reasonable based on the current rates of resistance in the off-treatment virologic failures.

3. Administrative

3.1. Reviewers' Signatures

_____
Takashi E. Komatsu, Ph.D., RAC
Clinical Virology Reviewer

_____
Anamaris M. Colberg Poley, Ph.D.
Clinical Virology Reviewer

3.2. Concurrence

_____
HFD-530/Clin.Virol.TL/J. O'Rear, Ph.D.

CC:
HFD-530/NDA # 209939
HFD-530/Division File
HFD-530/PM/Tyson

OND VIROLOGY REVIEW

1. Introduction and Background

1.1. Important milestones in product development

Letermovir (MK-8228) is an HCMV terminase complex inhibitor. The Original IND 104706 (SDN 000), sponsored by AiCuris GmbH & Co. KG, was submitted on February 19, 2009. The IND was transferred to Merck Sharp & Dohme Corp. on April 9, 2013. An IND for intravenous letermovir (118361 SDN 000) was opened by the sponsor on August 22, 2013.

Letermovir was granted Fast Track Designation on May 25, 2011, Orphan Drug Designation on December 12, 2011, and FDA Breakthrough Therapy Designation on February 27, 2017 for prevention of cytomegalovirus (HCMV) infection and/or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT) (reviewed in IND 118361 SDN 057).

A pre-NDA meeting to discuss the current application was held with the sponsor on December 14, 2016 (reviewed in IND 104706 SDN 167). The Division requested at the meeting that the sponsor include a method to discriminate between viral DNA polymerase errors and the background of their assays.

1.2. Overview of HCMV

1.2.1. HCMV General Virology

Cytomegaloviruses are members of the Herpesviridae family, which contain large, linear, double-stranded DNA genomes, and are capable of causing a variety of acute, latent, and recurrent infections in humans and animals. HCMV, also designated as the human herpesvirus 5, is the prototype of the betaherpesvirus group (Roizman et al., 1981). Like other herpesviruses, HCMV is able to establish latent infections, which can subsequently recur to an active infection state. HCMV replicates in endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts. Latency occurs in cells of the myeloid lineage.

Seroepidemiologic surveys have demonstrated HCMV infection in every population tested. The HCMV seroprevalence is estimated between 50% and 80% in the US (40% globally). The prevalence of HCMV infection increases with age and is higher in developing countries and among the lower socioeconomic groups in developed countries. Primary infection usually occurs during the first decades of life and humans are believed to be the only host of the virus. Transmission sources of HCMV include saliva, urine, semen, cervical and vaginal secretions, milk, stool, blood, cells, tissues, and organ transplants. Primary infection in immunocompetent subjects is mainly asymptomatic or it may be associated with a self-limited mononucleosis-like syndrome and leads to a life-long latency. However, HCMV infections are symptomatic and associated with increased morbidity and mortality in patients with immature or compromised immune systems. These groups of patients include congenitally infected newborns, patients with acquired immune deficiency syndrome (AIDS), primary immunodeficiencies and transplant recipients (hematopoietic stem cell and solid organ transplants). This overview focuses primarily on the antiviral drugs and mechanism of drug resistance in the setting of HCMV infection of adults with compromised immune systems. Unfortunately, despite the major public health need (Dollard et al., 2007), no antiviral drugs are approved for the prevention or treatment of congenital HCMV infection.

HCMV is a common infection in patients with advanced human immunodeficiency (HIV-1) disease, acquired immune deficiency syndrome (AIDS). In the era before the availability of highly active antiretroviral regimen (HAART), it was estimated that up to 45% of patients with AIDS acquired HCMV disease (Masur et al., 1996). HCMV retinitis is by far the most common manifestation of HCMV disease

in AIDS patients, accounting for 85% of the cases (Gallant et al., 1992). However, the introduction of HAART has decreased the incidence of HCMV diseases in AIDS patients by more than 80% (Jabs et al., 2007). Currently approved drugs for the treatment of HCMV retinitis in HIV infected patients include intravenous ganciclovir (GCV), oral ganciclovir, ganciclovir intraocular implant, oral valganciclovir (vGCV), intravenous foscarnet (FOS), intravenous cidofovir (CDV), and intravenous fomivirsen. The choice for the initial treatment of HCMV retinitis is based on the severity of the disease and other factors such as the ability to adhere to treatment. In most of the cases, oral valganciclovir is the preferred drug for initial treatment.

Primary HCMV infection commonly occurs in childhood, and the immune system of infected individuals thereafter sustains continuous suppression of viral replication. In patients undergoing allogeneic hematopoietic cell transplantation (allo-HSCT) or solid organ transplantation (SOT), this important suppressive immunity is temporarily lost or attenuated, leading to the reactivation of viral replication and dissemination between different tissues. Due to recent advances in HCMV detection and treatment strategies, the incidence of HCMV disease (i.e., symptomatic end-organ infection) has decreased, but has not been eliminated, in patients undergoing allo-HSCT and SOT. In allo-HSCT, the standard approach to HCMV management in the post-transplant setting is to initiate pre-emptive antiviral therapy upon detection of significant HCMV DNaemia, whereas it is more common to administer prophylactic antiviral therapy following SOT. The difference in strategies between allo-HSCT and SOT is primarily driven by the ability of most SOT patients to tolerate myelosuppression associated with the most commonly used antiviral agents, valganciclovir and ganciclovir, whereas myelosuppression is undesirable in the early post-allo-HSCT setting (Panagou et al., 2016). In spite of pre-emptive management of HCMV reactivation/infection in allo-HSCTs, HCMV-seropositive patients continue to experience poorer outcomes than HCMV-seronegative patients through increased non-relapse mortality and decreased overall survival (Broers et al., 2000; Schmidt-Hieber et al., 2013). However in SOT recipients, HCMV disease risk is highest when primary HCMV infection occurs in an SOT recipient with no preexisting HCMV-specific immunity, such as the HCMV donor-seropositive, recipient-seronegative (D+R-) patient (Razonable and Humar, 2013). HCMV disease, though rare, still occurs and can unfortunately be quite devastating. Recognition of HCMV disease, which typically occurs with DNaemia but may occur without it, remains important for allo-HSCT patients. Due to the deleterious impact of HCMV infection on allo-HSCT outcomes and toxicity concerns with currently approved antiviral drugs, new strategies for preventing and controlling HCMV reactivation in transplant recipients remain a crucial goal of several lines of clinical investigation. Because of the increased morbidity and mortality associated with HCMV infection in transplant patients, it has been recognized that prevention of HCMV infection may be a better strategy than treatment of established infection.

Preemptive therapy and prophylactic therapy are the two major strategies used for prevention for transplant recipients. Currently FDA-approved drugs for prophylaxis and/or the treatment of HCMV in the transplant setting include cidofovir, foscarnet, and ganciclovir/valganciclovir. Pre-emptive treatment and prophylaxis therapy using one or more of these drugs currently represent the main treatment strategies to prevent HCMV disease in HSCT or SOT subjects, respectively. The incidence of resistance to anti-HCMV drugs using either strategy is poorly characterized, especially for oral valganciclovir, and it is conceivable that the incidence of resistance may differ between the two strategies. With pre-emptive therapy, treatment is given in the setting of active HCMV replication measured by DNaemia. For prophylaxis therapy, lower doses of valganciclovir are used in the absence of HCMV replication, and the main concern relates to the risk of resistance development during episodes of low level DNaemia. The approval of letermovir would represent the first drug indicated for

prophylaxis in HSCT recipients, and since letermovir has different resistance pathways, pre-emptive therapy would still be possible.

Without pre-emptive therapy, approximately 80% of HCMV-seropositive patients experience HCMV re-activation after allo-HSCT. Current preventive strategies have decreased the incidence of HCMV disease, which had historically occurred in 20% to 35% of these patients (Lönnqvist et al., 1986). The main disease in HSCT patients is HCMV pneumonitis. The typical median time of presentation of HCMV pneumonitis is between 50 to 60 days after transplant. It had a high attributed mortality (≥70%) and was the cause of the majority of HCMV-deaths (Camara 2016). Today, with the use of preemptive GCV therapy, HCMV disease has become a more significant problem after day 100 following allogeneic HSCT (Bowden et al., 1995; Winston et al., 2003; Alain et al., 2013). Late HCMV recurrent infection is strongly associated with nonrelapse mortality (note: nonrelapse mortality refers to mortality that is not related to the underlying condition leading to transplant) (Winston et al., 2003).

1.2.2. HCMV Reactivation and Disease

HCMV reactivation, or secondary infection, occurs when HCMV replicates within an individual and, thereby, virus-derived nucleic acids or proteins become detectable in the body fluids or tissues, generally as a consequence of immune suppressive therapy (Ljungman et al., 2017). The incidence of HCMV reactivation is roughly 50% (reported range 40–80%) in HCMV-seropositive allo-HSCT recipients not receiving anti-viral prophylaxis (i.e., most allo-HSCT recipients) (Ljungman et al., 2011; Takenaka et al., 2015; Manjappa et al., 2014; Jang et al., 2015; Nichols et al., 2002; Sousa et al., 2014). In addition, roughly 30% of HCMV seronegative recipients of grafts from seropositive donors also develop primary HCMV infection (Ljungman et al., 2011), which is clinically indistinguishable from HCMV reactivation in seropositive recipients. HCMV DNaemia may be asymptomatic or accompanied by constitutional symptoms such as pyrexia; however, reactivation is most frequently diagnosed in the absence of symptoms during routine surveillance after allo-HSCT. HCMV DNaemia is now commonly quantified by real-time PCR, but the conversion to this technology occurred within the past 10 years, and some institutions have continued to use bloodborne HCMV antigen (pp65) quantification.

A subset of individuals with reactivation develop HCMV disease, defined as the isolation of HCMV from an appropriate tissue specimen along with clinical signs and symptoms of compatible end-organ dysfunction (Ljungman et al., 2017), which may manifest in various organs, sometimes with fatal complications. However, as stated above, rare cases of localized HCMV disease without DNaemia do occur (Martin, 1994; Avsar et al., 2014). When treated with pre-emptive antiviral therapies, <5% of cases with HCMV reactivation progress to HCMV disease (Boeckh et al., 2003). High initial viral loads (>20,000 copies/mL) in blood and leukopenia (white blood cell count <3,000/mL) at the time of HCMV DNaemia diagnosis correlate with the likelihood of HCMV disease (Jang et al., 2012). Furthermore, refractory HCMV infection, defined as HCMV DNaemia lasting greater than two weeks despite anti-HCMV treatment, occurs in half (50.6%) of patients experiencing HCMV DNaemia, and is associated with increased risk for HCMV disease and treatment-related mortality when it occurs within the first 100 days (Liu et al., 2015). In the setting of refractory HCMV infection, the toxicities of prolonged treatment that may contribute to negative outcomes include myelosuppression, renal impairment, and in rare instances, multi-drug resistant HCMV disease (Takahata et al., 2015; Emery et al., 2013; Pollack et al., 2011; Herling et al., 2016).

1.2.3. HCMV management in the HSCT setting

Serial HCMV surveillance for viral DNA or pp65 antigen in peripheral blood and pre-emptive anti-viral treatment is the current standard approach for HCMV management following allo-HSCT (Emery et al.,

2013; Pollack et al., 2011). An optimal frequency for HCMV DNaemia surveillance has not been rigorously studied, but it is common in most transplant centers and in most HCMV therapeutic trials to test weekly through Day +100 post-HSCT and less frequently thereafter in a patient-tailored fashion (Green et al., 2016). Additional testing may be performed at the discretion of the physician based on the patient's clinical condition. Primary anti-HCMV prophylaxis is not generally recommended because drug-related toxicities outweigh the benefits in allo-HSCT patients.

While HCMV viral load thresholds for initiating pre-emptive treatment vary between institutions, the goal of this threshold is to effectively identify and treat patients with the greatest risk of rapid HCMV replication while minimizing exposure to the toxicities of unnecessary treatment. Due to the lower sensitivity of HCMV antigen (pp65) testing, pre-emptive treatment using this method is typically initiated upon detection of any level of HCMV antigenemia. Meanwhile, for HCMV real-time PCR assays, the recommended threshold for initiating pre-emptive treatment is typically 500 to 1,000 copies/mL, although it has been reported to range from 500 copies/mL to 10,000 copies/mL, in standard recipients of marrow or mobilized peripheral blood stem cell grafts (Emery et al., 2013). For high risk patients receiving T-cell depleted or umbilical cord blood grafts, it is common to maintain a lower threshold for treating HCMV DNaemia (100 copies/mL to 1,000 copies/mL, with some variance between different transplant centers). A definite cut-off for HCMV viral load cannot be established at the present time. The cut-off is likely to vary between different patients and according to how the sample processing is performed as well as the assay used for HCMV DNA quantitation.

Approved drugs used for HCMV prophylaxis and/or treatment include cidofovir, foscarnet, ganciclovir/valganciclovir, HCMV hyperimmune globulin, and intravenous immunoglobulin (IVIg). Ganciclovir/valganciclovir is the front-line pre-emptive treatment typically preferred by most transplant centers (Takahata et al., 2015; Pollack et al., 2011). While GCV/vGCV is well-tolerated in the solid organ transplant population and is commonly used for prophylaxis (Paya et al., 2004), its myelosuppressive effects make this approach unsuitable for the majority of allo-HSCT recipients, thus necessitating pre-emptive therapy strategies. Approximately 30% of patients treated with vGCV or GCV develop treatment-associated neutropenia, which leads to greater incidence of bacterial and fungal infections and subsequent treatment-related mortality, as well as increased costs of care related to hospitalizations for infections and use of granulocyte growth factors to counteract myelosuppression.

The primary endpoint for studies of anti-HCMV therapies in the past has been incidence of HCMV disease (Marty and Boeckh, 2011). The currently available HCMV therapies were developed at a time when the overall incidence of HCMV disease in the post-transplant population was high in some populations (as much as 45% to 96%; Fiala et al., 1975; Lowance et al., 1999; Merigan et al., 1992) and there was no approved therapy so a placebo comparator could be used. Thus, the sample sizes required to show a statistically significant improvement in disease incidence in clinical trials were relatively small. The standard of care today for post-transplant HSCT subjects includes prevention of HCMV disease by preemptive antiviral therapy (Atkinson and Emery, 2011; Boeckh et al., 1992; Gerna et al., 2011). In the current era of preemptive anti-HCMV therapy, the incidence of HCMV disease in HSCT subjects is much lower (approximately 2.5% to 9%; Marty and Boeckh, 2011; Ljungman et al., 1998) although higher incidences may be observed in Donor+/Recipient- (D+/R-) solid organ transplant recipients (Kotton et al., 2010; Atkinson and Emery, 2011). Given the current background incidence of HCMV disease, the samples sizes required to show statistically significant improvement in disease incidence in clinical trials is high. Marty et al. (2011) reported a sample size of 1900 subjects to demonstrate a statistically significant decrease of 50% in incidence of HCMV disease for a population of HSCT subjects with an incidence of HCMV disease of 2.5%. Furthermore, earlier studies were able

to observe placebo control groups for prolonged periods of time post-transplantation (e.g., Winston et al., 1993; Ljungman et al., 2001), whereas the current standard of care prohibits the use of this study design today.

1.2.4. Relationship between HCMV DNaemia and HCMV Disease

The ability to predict onset of HCMV disease from viremia, HCMV DNaemia or HCMV antigenemia in plasma or leukocytes has been studied. The measurement of HCMV infection by HCMV DNaemia is already used routinely to customize subject care in the post-transplant period (Atkinson and Emery, 2011; Harter and Michel 2012; Gerna et al., 2011). It is becoming clearer that HCMV disease is driven by HCMV replication, with subjects who have high peak viral loads or with a high cumulative viral load being at increased risk of developing HCMV disease (Atkinson and Emery, 2011).

Some researchers in the field have proposed that incidence of HCMV disease is an inappropriate endpoint to use in the study of anti-HCMV therapies, proposing instead that virologic endpoints (such as antigenemia or number of HCMV DNA copies in plasma or leukocytes) should be adopted (Marty and Boeckh, 2011; Stachel et al., 2008; Snydman, 2011; Murray et al., 1999). The research above indicates that virologic endpoints may be good surrogates for HCMV disease endpoints in future studies of anti-HCMV therapies.

The use of virologic endpoints in clinical trials of antiviral agents is not a new concept. Early studies of anti-human immunodeficiency virus type 1 (HIV-1) therapies were conducted using endpoints of AIDS disease incidence. However, with the advent of more effective therapies and a lower incidence of AIDS symptoms, plasma HIV-1 RNA level was adopted as an acceptable primary endpoint in HIV trials (Murray et al., 1999; FDA - Antiretroviral Drugs Using Plasma HIV RNA Measurements - Clinical Considerations for Accelerated and Traditional Approval 2002). In the case of hepatitis B virus (HBV) infection, clinical endpoints like development of cirrhosis, end-stage liver disease, and hepatocellular carcinoma typically take years or decades to occur and are therefore impractical targets for clinical studies which last only 1-2 years. As a result, surrogate biomarkers that are believed to correlate with long-term outcome are used to evaluate therapy. Loss of hepatitis B virus e antigen (HBeAg) has been the traditional therapeutic endpoint; however, the indefinite durability off treatment and the emergence of HBeAg-negative disease have made it inadequate as the sole goal of therapy. Loss of hepatitis B virus surface antigen is associated with improved clinical outcomes, but it is rarely achieved with current therapies. Suppression of viral replication, as measured by serum HBV DNA levels, has become the major goal of therapy. Unfortunately, viral levels also fluctuate and the significance of a given viral level depends greatly on the stage of disease, degree of liver damage, and the type of therapy being used (i.e., oral nucleoside analogs versus peginterferon) (Feld et al., 2009). Clearly, none of the currently available biomarkers is an ideal measure of treatment efficacy on its own. Perhaps for this reason, approval of new therapies for the treatment of hepatitis B virus infection by licensing authorities has usually depended on demonstration of significant improvements in two or more surrogate markers of disease progression with treatment. Typically, the surrogates are (1) biochemical (aminotransferase levels), (2) virological (HBV DNA levels, HBeAg, HBsAg), and (3) histological (based on histological scoring systems).

Boeckh et al. (1992) found that there was a correlation between HCMV antigenemia and HCMV disease in a population of 59 HCMV seropositive HSCT subjects. In this study, subjects were followed for 100 days after allogenic bone marrow transplantation and researchers found that antigenemia was present in 21 of 22 subjects with culture-proven HCMV infection and in 3 of 37 without culture-proven HCMV infection (sensitivity 95%, specificity 91%). Among subjects who developed HCMV disease

without preceding cultures, antigenemia was detected in all subjects with HCMV pneumonia (N = 6) and in 2 of 3 subjects with HCMV gastrointestinal disease by a median of 10 and 7 days, respectively, before the onset of disease (P = 0.0002). Goodrich et al. (1991) showed that in a study of IV GCV vs. placebo in 72 bone marrow transplant recipients who were shedding HCMV virus, suppressing viral shedding with GCV significantly reduced the incidence of HCMV disease and overall mortality. Emery et al. (2000) studied a group of 359 transplant recipients (162 liver, 87 renal, and 110 bone marrow) who were prospectively monitored for HCMV DNA in blood. HCMV viral load in the initial phase of active infection and the rate of increase in viral load both correlated with HCMV disease in transplant recipients. Gor et al. (1998) used quantitative competitive PCR to monitor the quantity of HCMV DNA in blood samples from bone marrow transplant (BMT) recipients. DNaemia was detected in 49/110 (45%) of the subjects, of whom 15/49 experienced HCMV disease. Peak virus load during surveillance was elevated in symptomatic vs asymptomatic subjects (P = 0.002); odds ratio for disease per 0.25 $\log_{10}$ increase in viral load was 1.43 (P = 0.004). The viral load curve showed a rapid increase in disease risk at viral loads between 3.8 and 5.5 $\log_{10}$ genomes/mL in blood. Sia et al. (2000) analyzed HCMV DNA load as a marker for relapse of HCMV infection in 24 solid organ transplant subjects with HCMV infection or disease who received a fixed 14-day course of intravenous ganciclovir. Relapse of HCMV infection or disease occurred in 8 (33.3%) of 24 subjects after completing the 14-day course of IV ganciclovir therapy for the initial episode of HCMV infection or disease. During the initial episode, 10 (41.6%) of 24 subjects presented with asymptomatic infection and 14 (58.3%) presented with HCMV disease. The clinical presentation (asymptomatic infection or disease) did not discriminate subjects who subsequently had relapses (P = 1.0). Therefore, a pretreatment HCMV load >23,100 copies/10^6 leukocytes conveyed 81% sensitivity and 75% specificity for the prediction of a subsequent relapse. Conversely, a pretreatment HCMV DNA level <5,750 copies/10^6 leukocytes was 56% sensitive and 100% specific for the absence of future relapse. Stachel et al. (2008) reported that HCMV viral load is still a predictor of transplant-related mortality, even with current medical practice including pre-emptive anti-HCMV therapy. Investigators analyzed data from 2,896 subjects following a first HSCT over 100 days; 1,481 of the subjects were HCMV seropositive. Preemptive antiviral therapy was given for any pp65 result. Initial and peak viral load and viral AUC were both independently associated with transplant-related mortality and overall survival in allogeneic HSCT recipients receiving preemptive antiviral therapy. The peak viral load showed the strongest association with transplant-related mortality and overall survival. The authors concluded that their data further support the use of parameters of viral dynamics as primary endpoints for studies that evaluate immune augmentation or drug prevention strategies. Other researchers who have also reported a relationship between HCMV viral load and the development of HCMV disease include Cope et al. (1997a; 1997b), Cortez et al. (2003), Hassan-Walker et al. (1999), and Roberts et al. (1998).

Researchers have also reported that a high rate of HCMV replication and incidence is correlated with HCMV disease in other populations of immunosuppressed subjects. Emery et al. (1999) found that the doubling time of HCMV in the blood of acquired immune deficiency syndrome (AIDS) subjects with active HCMV disease was approximately 1 day, and that upon treatment with ganciclovir, the half-life of decline of HCMV viral load in blood was 0.98 days.

There are few clinical studies that evaluated the treatment of HCMV disease. In the VICTOR study (NCT00431353), IV GCV was compared to oral vGCV for the treatment of HCMV disease in 321 solid organ transplant recipients. Valganciclovir was non-inferior to ganciclovir in both the clearance of HCMV DNaemia and the clinical resolution of HCMV disease (Asberg et al., 2007; Razonable et al., 2013). Of the 267 subjects, 251 had HCMV disease resolution by Day 49 of treatment. Subjects with pre-treatment HCMV DNA <18,200 IU/mL had a faster time to disease resolution (p = 0.001) and

patients with HCMV viral load suppression at days 7, 14, and 21 had faster times to clinical disease resolution (p = 0.005, <0.001, and <0.001, respectively). There are several studies that assessed how viral load kinetics influenced the likelihood of HCMV disease. The rate of increase in HCMV viral load between the last PCR-negative and first PCR positive sample was significantly faster in patients with HCMV disease (log_{10} 0.33 versus log_{10} 0.19 copies/mL daily, p<0.001) (Emery et al., 2000). In multivariate-regression analyses, both initial HCMV viral load and rate of viral load increase were independent risk factors for HCMV disease. Similar results where a rapid viral load increase correlated with the development of HCMV disease were reported (Tong et al., 2000; Eid et al., 2010; Hadaya et al., 2003; Michaelides et al., 2001).

Active HCMV disease does not always correlate with viral load detection (Ruell et al., 2007). In more than 500 subjects undergoing HSCT at a single center, the introduction of HCMV PCR monitoring and preemptive therapy has been associated with a low incidence of HCMV end-organ disease and related mortality, with the incidence of HCMV pneumonia reduced particularly. Fifty-one subjects in this study had HCMV reactivation. Notably, the authors observed negative HCMV PCR in peripheral whole blood in association with active HCMV organ disease in half of the eight affected subjects, all high risk, although reactivation had been observed previously in these subjects. Two of these 4 subjects were later PCR positive for HCMV DNA in the peripheral whole blood. One subject had an adenovirus infection. These findings indicate the correlation between serum HCMV PCR and active disease may be limited in high risk subjects and highlight a need for caution when relying on virus detection prior to and at the time of HCMV disease. The PCR assay used in this study may not have had the sensitivity due to primer/probe sequence mismatch of these particular virus isolates, especially at low copy numbers, or the virus may not have been circulating in the blood cells. These findings are similar to what were reported by Wiita et al. (2012).

In a single-center observational study, 65 out of 128 lung transplant recipients discontinued valganciclovir prophylaxis. There were six cases of HCMV disease identified (4.7%), with no significant difference between those who were on continuous prophylaxis or not (4.6% vs. 4.9%; P = non-significant). HCMV disease was defined as patients with characteristic clinical signs and symptoms of either HCMV syndrome or tissue-invasive HCMV disease in conjunction with laboratory-detected HCMV (BAL PCR, BAL shell vial culture [SVC], other tissue SVC, plasma PCR, plasma antigenemia). However, those who discontinued prophylaxis showed an increased incidence of laboratory-detected HCMV infection (40.7% vs. 12.7%; P = 0.001). High-risk D+/R- patients did not demonstrate a significantly increased incidence of HCMV disease (8.1% vs. 3.3% other serotypes). Despite a higher incidence of HCMV DNA detection in those who discontinued prophylaxis (23.8% vs. 6.3%), the incidence of HCMV disease was similar (4.6% vs 4.9%). All of the subjects who developed HCMV disease had detectable HCMV DNA. Of note, every subject who developed HCMV disease in the cohort that was on continuous prophylaxis had detection of HCMV with GCV resistance-associated substitutions; whereas, there was none in the cohort that discontinued valganciclovir prophylaxis. Therefore, according to the authors, bronchoalveolar lavage HCMV viral load was not predictive of subsequent disease development. However, the authors did note that there was a correlation between bronchoalveolar lavage viral loads >100,000 copies/mL and the diagnosis of HCMV pneumonitis. Therefore, the correlation between HCMV DNA detection and HCMV disease may be a function of the amount detected or the assay used. A limitation of this study was the relatively small number of patients who manifested HCMV disease during the study (3 patients in each cohort).

Quantification of HCMV viral load is not straightforward. Prior to July 5, 2012 there were no FDA–approved laboratory tests for the quantification of HCMV DNA. Therefore, most HCMV viral load tests

are considered laboratory-developed tests that are developed and validated by an individual laboratory to the standard of the laboratory-inspecting agencies. A multicenter study conducted to assess the variability of HCMV viral load testing across 33 laboratories in the United States, Europe, and Canada showed that the variability in viral load values for individual samples ranged from 2.0 $\log_{10}$ copies/mL to 4.3 $\log_{10}$ copies/mL (Pang et al., 2009). These findings reinforce the fact that clinicians cannot compare test results from 2 laboratories nor can clinically relevant cutoffs developed using 1 test be applied to results from another test, unless the 2 tests have been rigorously compared and the relationship between them is well understood. In November 2010, an international standard for HCMV composed of a standardized quantity of HCMV was developed and approved by the World Health Organization. This international standard will allow laboratories and manufacturers to assess the accuracy of viral load values and to calibrate different assays. On July 5, 2012, the COBAS® AmpliPrep/COBAS® TaqMan® CMV Test (Roche) was approved by CDRH. The availability and widespread use of FDA-approved tests will further improve inter-laboratory agreement. While the variability in HCMV DNA results reported on individual samples has been reduced by the WHO International Standard ongoing clinically relevant variability persists. The variance for individual HCMV DNA-positive samples was greater for clinical samples (median, 1.50 [range, 1.22-2.82] $\log_{10}$ IU/mL) than for the International Standard dilutions (median, 0.94 [range, 0.69-1.35] $\log_{10}$ IU/mL) (P <0.001); 58.9% of all clinical sample results and 93.6% of the International Standard dilution results fell within ±0.5 $\log_{10}$ IU/mL of the mean viral load of each sample. Result variability was not impacted by either genotype or quantitative levels of HCMV DNA (Preiksaitis et al., 2016). Testing procedure differences significantly influenced results, even when analyte-specific reagents were identical. Sample type is another important preanalytical variable. Quantitative HCMV testing is typically performed on whole blood or plasma. Each specimen type has strengths and limitations. HCMV DNA is detected more frequently and viral load values are often higher in whole blood compared with plasma because both cell-free and intracellular viruses are detected in the former. A recent study showed that viral load values in most subjects are about 10-fold higher in whole blood compared with plasma (Lisboa et al., 2011). However, in some subjects the difference was as great as 100-fold, and occasionally plasma viral load was found to be higher than whole blood viral load (Lisboa et al., 2011). Furthermore, the HCMV decay kinetics in the whole blood may be different where in whole blood samples there appears to be two phases in the viral decay (Emery et al., 2012). In a separate study, virus was still detectable in subjects treated with either oral vGCV or i.v. GCV by Day 21 in 154 of 219 (70.3%) subjects with the whole blood versus 105 of 219 (52.1%; P<0.001) subjects with the plasma assay. The use of plasma instead of whole blood in these tests requires rapid separation to keep the DNA from degrading with resultant fragmentation (Razonable et al., 2002).

Given that there is no definitive finding at this time to show that suppression of HCMV DNaemia predicts improved survival as well as the challenges of the viral load assays, the Division has recommended using HCMV DNaemia in plasma as a surrogate endpoint in studies designed to support accelerated approval. For accelerated approvals, the drugs need to 1) treat a serious condition, 2) offer meaningful advantage over therapies, and 3) demonstrate an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit (see 21.CFR314 Subpart H, Accelerated Approval). The sponsors must subsequently complete post-marketing confirmatory studies that validate the surrogate endpoint as being predictive of disease to receive traditional approval.

1.2.5. HCMV Viral Replication

HCMV has a double-stranded, DNA genome about 230 kbp in size, which encodes more than 250 open reading frames. It has unique long (U_L) and unique short (U_S) sequences that have numerous open reading frames encoding structural and functional proteins. Two of them, UL97, encoding a

protein kinase, and UL54, encoding the viral DNA polymerase, are relevant in the context of currently approved antiviral therapeutics that target viral DNA replication.

During HCMV replication, viral DNA for each emerging virion is not produced separately. Instead, it is synthesized in the nucleus as a long DNA chain containing multiple repeated genome sequences (known as concatemers). Each genome unit (monomer) constitutes the genetic material for one virion. The concatemeric DNA will therefore need to undergo cleavage into multiple unit-length monomers that will be individually packaged into a viral capsid prior to its release as an infectious virion. This maturation, packaging, and termination process is performed by a group of proteins collectively known as the "terminase complex" (Bogner, 2002; Bogner, 2010). Inhibition of the termination process is the proposed mechanism of antiviral activity by the novel drug letermovir (Buerger et al., 2001; Bogner et al., 1998).

The HCMV terminase complex is comprised of three proteins, pUL51, pUL56, and pUL89 (Hwang and Bogner, 2002; Borst et al., 2013; Neuber et al., 2017). The main function of the terminase complex is to cleave HCMV concatemers into single units of functional HCMV monomers (Griffiths and Emery, 2014). The terminase complex further interacts with the pUL104 portal (viral capsid) protein to facilitate DNA translocation into the capsid. The structure of the terminase complex is conserved across members of herpesviruses, but there is no known human homologue. Consequently, at least in theory, drugs that specifically interact with this complex are virus-selective and would be predicted to have minimal off-target effects in human cells.

pUL56, which is encoded by the UL56 gene, participates in the DNA packaging process in three ways: it recognizes the specific sites where viral DNA will be cleaved; it hydrolyzes the adenosine triphosphate (ATP) necessary for DNA translocation; and it interacts with capsid proteins to allow translocation of DNA into the viral capsid (Bogner, 2002). Its C-terminal end also serves as a potential site for interaction with pUL56, thereby enhancing overall nuclease activity that cleaves the HCMV DNA concatemer into monomeric unit-length pieces (Couvreux et al., 2010; Scheffczik et al., 2002). The cleavage sites are indicated by two conserved motifs located at the end of each unit-length DNA monomer (Bogner et al., 1998; Hwang and Bogner, 2002). pUL51 has recently been identified as a third component of the HCMV terminase, as it forms a complex with pUL56 and pUL89 in infected cells and was found to be crucial for viral genome cleavage-packaging and their interaction stabilizes the terminase complex (Borst et al., 2013; Neuber et al., 2017).

The pUL51, pUL56, and pUL89 terminase subunits are synthesized in the cytoplasm of HCMV-infected cells. Hence, they need to be transported into the nucleus to exert their vital functions. pUL56 translocation into the nucleus appears to be mediated by interaction of the "nuclear localization signal" (a short amino acid segment in the carboxyl-terminus of pUL56) and translocation-protein importin *a* (Giesen et al., 2000). pUL56 and importin *a* form a stable complex with importin *b*, and the complex interacts with filaments of the nuclear pore for its translocation to the nucleus. Once inside, importin subunits dissociate, leaving pUL56 available for packaging of HCMV DNA (Giesen et al., 2000; Weis et al., 1996). The association of pUL56 with pUL51 and pUL89 results in enhanced stability, nuclear import, and endonuclease activity of the terminase complex (Hwang and Bogner, 2002; Couvreux et al., 2010; Borst et al., 2013; Neuber et al., 2017).

After the viral DNA concatemer undergoes cleavage, it is transported inside an empty viral procapsid (Bogner et al., 1998). This process is orchestrated by the interaction of the terminase complex and "portal proteins" found on the surface of viral procapsids (Bogner et al., 1998; Dittmer et al., 2005). The

portal proteins are large macromolecules that are arranged in rings and associate with procapsid proteins, forming a "pore" that serves as a port of entry for viral DNA. The DNA translocation process requires the functional interaction among portal proteins, viral pro-capsid and the terminase complex subunits. In particular, pUL56 forms a stable structure with viral procapsid and interacts with portal protein pUL104, forming part of the ring structure that allows DNA translocation (Dittmer et al., 2005). The energy required for DNA translocation is provided by pUL56 through hydrolysis of ATP (Scholz et al., 2003). To complete the termination process, there is a second DNA cleavage that liberates the unit-length monomeric genome from the remaining concatemeric DNA chain (Nadal et al., 2010).

The proteins pUL52, pUL77, pUL93, and pUL104 are believed to be associated with the terminase complex or to participate in the HCMV DNA packaging process (Borst et al., 2013; Neuber et al., 2017). pUL56 binds to the capsid portal protein pUL104 (Dittmer et al., 2005). pUL52, whose specific role is not defined yet, as well as pUL77 and pUL93, which are capsid constituents presumably building the capsid vertex specific complex, are also necessary for HCMV genome encapsidation (Borst et al., 2008; Borst et al., 2016; Koppen-Rung et al., 2016; DeRussy and Tandon, 2015). Other proteins that may also be important to the termination process include pUL46 (minor capsid protein binding protein), pUL47/48 (smallest capsid protein), pUL80a (proteinase precursor protein), pUL80.5 (assembly protein), pUL85 (minor capsid protein), and pUL86 (major capsid protein) (Buerger et al., 2001; Bogner et al., 1998; Giesen et al., 2000). Collectively, viral termination appears to be a highly complex process that is facilitated by numerous interacting protein molecules.

1.3. Approved Drugs for HCMV

As stated above, there is currently no antiviral drug approved for HCMV management in the HSCT setting. However, there are several antiviral drugs that are approved for the treatment of HCMV retinitis and/or HCMV management in the SOT setting.

Cytovene® (ganciclovir, NDA 19661, link to label), a non-prodrug, injectable version of the active deoxynucleoside analog moiety of valganciclovir, was approved for the treatment of HCMV retinitis in adult subjects with AIDS in 1989. After uptake by cells infected with HCMV, ganciclovir is phosphorylated to the active moiety, ganciclovir triphosphate, initially by the virally encoded pUL97 kinase and subsequently by cellular kinases. Ganciclovir triphosphate is the active inhibitor of viral DNA synthesis catalyzed by the viral DNA polymerase pUL54.

The antiviral activity of GCV is dependent on its initial phosphorylation by the virally encoded pUL97 kinase. Interestingly, the UL97 gene encodes amino acid sequence motifs characteristic of protein kinases, and has homologies with kinase genes in other herpesviruses (Michel and Mertens, 2004). Several pUL97 amino acid substitutions conferring resistance to GCV have been detected in clinical isolates. Resistance can also develop by amino acid substitutions in the viral encoded pUL54 DNA polymerase and high level resistance can develop due to substitutions in both pUL97 and pUL54.

Valcyte® (valganciclovir hydrochloride tablets, NDA 21304, link to label) was approved for the treatment of HCMV retinitis in adult subjects with AIDS in March, 2001. The indication for Valcyte® tablets was further expanded in September, 2003 to include the prevention of HCMV disease in adult solid organ transplant (SOT) patients. Valcyte® oral solution (valganciclovir hydrochloride) was approved on August 28, 2009 for the prevention of HCMV disease in pediatric kidney and heart transplant subjects ≥4 months of age at high risk of developing HCMV. Valganciclovir is an L-valyl ester prodrug of ganciclovir. After oral administration, valganciclovir is rapidly absorbed and hydrolyzed to ganciclovir. The majority

of the hydrolysis occurs during pre-systemic absorption, with only 1%-2% of the absorbed prodrug valganciclovir appearing as valganciclovir in the plasma, the remainder being found as ganciclovir.

Vistide® (cidofovir, NDA 20638, link to label), an acyclic cytidine deoxynucleoside analog phosphonate, received US marketing approval in 1996 for treatment of HCMV retinitis in AIDS patients. CDV is available only as an IV formulation; its oral bioavailability is less than 5%. CDV has activity against herpesviruses and other DNA viruses, such as adenovirus (De Clercq and Holý, 2005). Host kinases convert CDV to the active diphosphoryl form, and cidofovir diphosphate then acts as a competitive inhibitor of the viral DNA polymerase, causing premature chain termination in viral DNA synthesis. The intracellular half-life of CDV-DP is reported to be >24 hours, and antiviral activity in both animal models and in humans can be achieved with infrequent dosing (De Clercq and Holý, 2005).

Foscavir® (foscarnet, NDA 20068, link to label) was approved for treatment of HCMV retinitis in AIDS patients in 1991. Foscavir®, or foscarnet sodium, is the trisodium salt of phosphormophonic acid, a pyrophosphonate analogue and is the second drug approved for HCMV retinitis. Foscarnet (FOS) inhibits the activity of the viral DNA polymerase by binding to the pyrophosphate binding site and blocking cleavage of pyrophosphate from the terminal nucleoside triphosphate added to the growing DNA chain. FOS is considered second-line therapy, but is the preferred drug for patients who are failing GCV therapy, presumably due to viral resistance, or those who cannot be treated with GCV due to dose-limiting neutropenia or leucopenia (Razonable and Emery, 2004). In one study, FOS was compared to IV GCV as a preemptive therapy in a large, prospective, randomized, open-label study in HSCT patients. FOS and IV GCV were equally effective in prevention of HCMV disease and mortality within 180 days of HSCT (Reusser et al., 2002). FOS has also been used in combination with IV GCV, each at half dose, and the combination was compared to IV GCV alone in SOT patients. The outcome was unfavorable for the combination in terms of virologic response and toxicities (Mattes et al., 2004). In cell culture, the combination of FOS and GCV was not antagonistic at the respective EC_{50} values (Manion et al., 1996; Cai et al., 2014).

Vitravene® (fomivirsen, NDA 20961, link to label) is an antisense phosphorothioate oligonucleotide that blocks the replication of HCMV mRNA. It was licensed by the FDA for treatment of HCMV retinitis in 1998. It is 21 nucleotides long, comprised of a sequence that is complementary to the mRNA transcribed from the major immediate-early transcriptional unit of HCMV (Mulamba et al., 1998). It is used in the treatment of HCMV retinitis in immunocompromised patients, including those with AIDS. Fomivirsen was the first antisense antiviral approved by the FDA. A human cytomegalovirus mutant that was isolated for resistance (10-fold) to fomivirsen (ISIS 2922) exhibited cross-resistance to a modified derivative of fomivirsen with an identical base sequence but little or no resistance to an oligonucleotide with an unrelated sequence (Mulamba et al., 1998). No changes in the mutant's DNA corresponding to the fomivirsen target sequence were found.

1.4. Methodology

This section summarizes clinical virology procedures that were used for the Phase 2 and Phase 3 studies.

HCMV viral load assessments

For the Phase 1b/2a study (MK-8228-019), plasma samples were collected for monitoring HCMV DNaemia using the (b) (4) central laboratory HCMV PCR assay. The lower limit of quantification (LLoQ) for this assay is 500 copies/mL (Note: It is unclear how the copies relate to the IU standard).

For the Phase 2b study (MK-8228-020), plasma samples were collected for monitoring HCMV DNaemia using the CE marked Qiagen/Artus CMV® assay tested by the central laboratory (note: a new version of this assay was recently FDA approved; assay package insert). The lower limit of quantification (LLoQ) for this assay is 54 IU/mL which is ~43 copies/mL (using a conversion factor of 0.8 copies/IU as per the sponsor's performance qualification report).

For the Phase 3 study (MK-8228-001), plasma samples were collected for monitoring HCMV DNaemia using the FDA approved Roche COBAS® AmpliPrep/COBAS TaqMan® (CAP/CTM) assay tested by the central laboratory. The lower limit of quantification (LLoQ) for this assay is 137 IU/mL which is ~150 copies/mL (using a conversion factor of 1.1 copies/IU as per the assay package insert).

HCMV nucleotide sequence analysis

Phase 2 Study

The genotypic analysis was conducted by (b) (4) The gene encoding pUL56 was amplified using nested PCR (note: the sponsor did not genotype the UL89 ORF for this study). The reference sequences used are from the HCMV Merlin strain (GenBank accession number: CMV_Merlin_NC006273_rev2). The limit of detection (LOD) value is 42 copies/mL (Note: It is unclear how the copies relate to the IU standard).

Phase 3 Study

To perform HCMV UL56/UL89 Genotypic Analysis, total DNA was isolated from plasma, and the UL56 and UL89 protein coding regions were amplified and subjected to DNA sequence analysis. Two different methods were used to perform HCMV UL56 and UL89 DNA sequence analysis.

(b) (4)

The HCMV UL56 and UL89 genes were amplified by PCR using five and ten HCMV-specific primer sets, respectively. PCR products are analyzed by fluorescent dye terminator dideoxy sequencing on an ABI 3730xl capillary DNA Sequencer. The DNA sequences were base-called and assembled into contigs, and the deduced UL56 or UL89 amino acid sequences were aligned with the appropriate reference sequence from the HCMV AD169 isolate (obtained from American Type Culture Collection, Manassas, Virginia, United States). All of the UL56 and UL89 amplicons have a measured LOD value of at least 1,875 copies/mL (Note: It is unclear how the copies relate to the IU standard).

(b) (4)

The genes encoding UL56 and UL89 were amplified in 3 different nested PCR assays. The amplicons generated by the nested PCR were analyzed by next-generation DNA sequencing (NGS) using the Illumina MiSeq system. QC analysis and variant calling compared to a reference sequence was performed using the 'Athena' pipeline, which is custom software developed by (b) (4) specifically for the analysis of amplicon-based deep sequencing data. The reference sequences used are from the HCMV Merlin strain (GenBank accession number: HCMV_Merlin_NC006273_rev2). The LOD value is 108 copies/mL for UL56, 37 copies/mL for UL89A, and 92 copies/mL for UL89B (Note: It is unclear how the copies relate to the IU standard). For the sponsor's analyses, differences detected at a frequency of ≥5% at a given position were identified as HCMV genotypic variants.

1.5. Prior FDA Virology reviews

Clinical Virology reviews of IND submissions for oral letermovir (IND 104706) and i.v. letermovir (IND 118361) were conducted by Takashi Komatsu, Ph.D. The original IND submissions for IND 104706 and IND 118361 were submitted on February 18, 2009 and August 22, 2013, respectively.

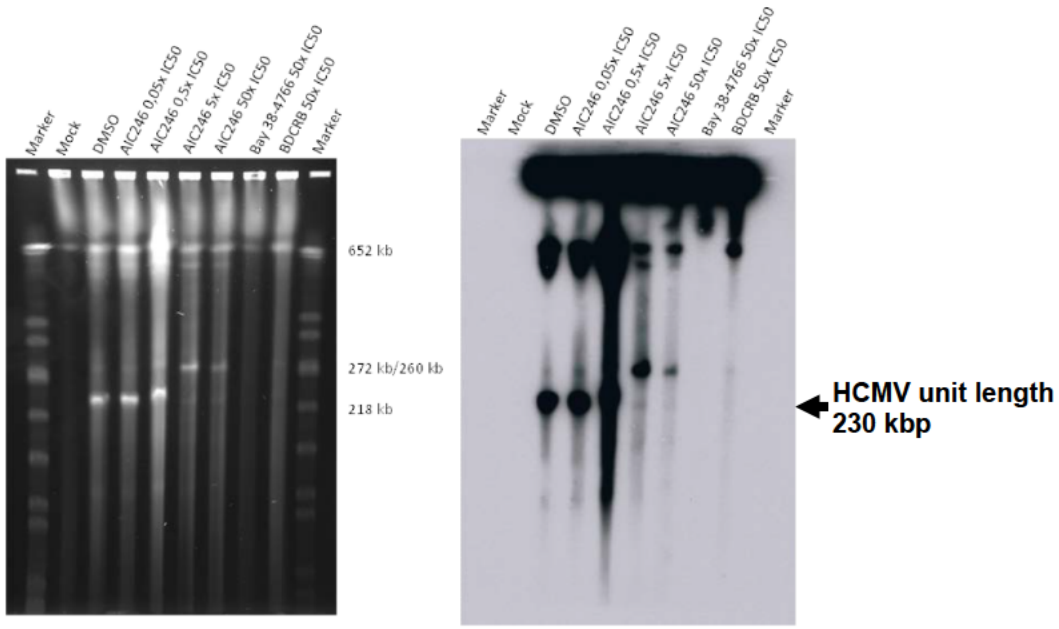
2. Nonclinical Virology

2.1. Mechanism of Action (Study Reports MK-8228-PD005, MK-8228-PD012, MK-8228-PD014)

Letermovir (AIC246, AIC090027, AIC001, Bay 73-6327, EX000030, MK-8228) is a non-deoxynucleoside inhibitor of human cytomegalovirus (HCMV) growth. Letermovir targets the HCMV terminase complex (Goldner et al., 2011; Lischka et al., 2010). The terminase complex is comprised of pUL56, pUL51 and pUL89 and is essential for the cleavage of replicated HCMV DNA concatemers for the production and packaging of unit length viral DNA genomes during virion maturation (Bogner, 2002; Bogner, 2010; Borst et al., 2013; Neuber et al., 2017). pUL56 associates with the pUL104 portal protein, which mediates unit-length genome entry into the capsid (Bogner, 2010; Neuber et al., 2017). Due to the absence of a human counterpart of viral terminase, letermovir has low cytotoxicity in normal primary human cells.

The sponsor conducted whole cell time of addition studies to determine if letermovir inhibited early or late viral functions. Letermovir inhibits HCMV (strain AD169 RV-HG) growth when added either 2 hours before (EC_{50} value = 0.0029 µM) or after (EC_{50} value = 0.0025 µM) infection (Study Report MK-8228-PD014). A similar time course of inhibition of HCMV growth was observed with ganciclovir (GCV), a deoxynucleotide analog inhibitor of HCMV DNA polymerase (pUL54), whose EC_{50} value is about 1,000 fold higher (EC_{50} value = 2-2.5 µM) than that of letermovir (EC_{50} value = 0.0025-0.0029 µM). However, in contrast to GCV, letermovir does not reduce (as determined using real-time quantitative PCR) HCMV DNA replication at early (24 hours post infection, hpi) or late (96 hpi) times of infection (Study Report MK-8228-PD012). Nonetheless, letermovir reduces virus progeny production at later times of infection (5 dpi) of HCMV (strain TB40/E) infected human umbilical vein endothelial cells (HUVEC) and of HCMV (strain AD169) in primary human foreskin fibroblasts (HFFs) (Study Report MK-8228-PD005).

To verify its mechanism of action, the sponsor used pulsed field gel electrophoresis (PFGE) to resolve large molecular mass DNA extracted from human foreskin fibroblasts (Psf5) infected at a multiplicity of infection (moi) of 1 with HCMV (recombinant virus RV-HB5 derived from strain AD169) (Study Report, MK-8228-PD012). The sponsor found that letermovir treatment (5xEC_{50} and 50xEC_{50} value, not serum-adjusted) inhibits in a dose-dependent manner the cleavage of HCMV DNA concatemers to the 230 kbp unit length genome at 96 hpi (Figure 1). Of note the C_{min} value of letermovir in the sponsor's Phase 3 study was 41 nM which is ~4x the serum adjusted EC_{50} value (please see the clinical pharmacology review of Mario Sampson, Ph.D. for more details). Although an unidentified larger HCMV genome cleavage product (~270 kbp) appears following treatment with intermediate letermovir concentrations, most of the partially cleaved HCMV genome appears in larger molecular mass size range of unprocessed concatemeric DNA (650 kbp or larger). Controls for these experiments included treatment of HCMV-infected cells in parallel with BAY 38-4766 or BDCRB, two other anti-HCMV drugs that block viral DNA cleavage (Buerger et al., 2001; Krosky et al., 1998). These drugs similarly inhibited the production of HCMV unit length genomes (230 kbp) in the infected cells (Figure 1). These results are consistent with inhibition of HCMV terminase activity by letermovir and other terminase inhibitors, sulfonamide BAY 38-4766 and benzimidazole ribonucleoside BDCRB.



Sponsor's note: Due to insufficient mixing, a higher amount of DNA was loaded in lane AIC246, 0.5xIC$_{50}$
DMSO=dimethylsulfoxide, kb=kilobases

Figure 1. Ethidium bromide staining (left) and Southern blot (right) of PFGE gel (Figure 7-2B, Study Report, MK-8228-PD012, page 18)

2.2. Antiviral Activity in Cell Culture (Study Reports MK-8228-PD001, MK-8228-PD002, MK-8228-PD004, MK-8228-PD006, MK-8228-PD009, MK-8228-PD011)

Letermovir inhibits replication of HCMV laboratory strains AD169 and Davis as well as multiple clinical HCMV isolates with EC$_{50}$ values ranging from 1 nM to 6 nM (**Study Report MK-8228-PD001**), using visual inspection of the reduction of HCMV cytopathic effect (CPE) compared to untreated, HCMV-infected normal diploid human foreskin fibroblasts (NHDFs).

Furthermore, letermovir (labeled EX000030 in these experiments) treatment reduced a laboratory HCMV strain (AD169) (EC$_{50}$ value = 1 nM) and 17 clinical isolates (EC$_{50}$ values = 0.8-3.1 nM) CPE or plaque formation grown in HFFs; whereas, GCV reduced infectivity of HCMV strain AD169 (EC$_{50}$ value = 1.8 µM) and the 17 clinical HCMV isolates (EC$_{50}$ values = 0.9-3.0 µM) at ~1000-fold higher doses (Study Report MK-8228-PD006 and Marschall et al., 2012). Similarly, letermovir treatment reduced plaque formation by clinical isolates (14-4B, Coffman, E. Mann, C9207, and C9208) with EC$_{50}$ values of 2.21-13.5 nM; while GCV inhibited these clinical isolates with EC$_{50}$ values of 4.25 to 12.1 µM (**Study Report MK-8228-PD008**).

The antiviral activity of letermovir against primary isolates was determined using more than 70 primary isolates, stratified into the four glycoprotein B subtypes of HCMV (Lischka et al., 2016). To that end, the sponsor determined the letermovir EC$_{50}$ values for 50 uncharacterized low passage clinical HCMV isolates using a standard plaque reduction assay in normal human dermal fibroblasts (NHDF). These values were combined with previously determined EC$_{50}$ values for other HCMV clinical isolates (Lischka et al., 2010; Marschall et al., 2012). Based upon their results (Lischka et al., 2016), we determined the median EC$_{50}$ values as well as the minimum and maximum EC$_{50}$ values for each gB subtype (Table 1). The median EC$_{50}$ values of letermovir against clinical HCMV isolates were 1.9 nM for subtype gB1 (range 0.1-5.8 n M, N=29), 2 nM for subtype gB2 (range 0.7-6.1 nM, N=27), 2.3 nM for subtype gB3

(range 1.5-3.4 nM, N=11), and 2.9 µM for subtype gB4 (range 2.6-3.2 nM, N=3). Together with the results above, these results show that letermovir is active against many regional and temporally different isolates of HCMV.

Table 1. Letermovir Median EC_{50} Values for HCMV Primary Isolates, Stratified by Glycoprotein B (gB) Subtype (derived from data in Lischka et al., 2016)

	Letermovir EC_{50} values		
gB subtype (number of isolates)	**Median µM**	**Minimum µM**	**Maximum µM**
gB1 (29)	0.0019	0.0001	0.0058
gB2 (27)	0.002	0.0007	0.0061
gB3 (11)	0.0023	0.0015	0.0034
gB4 (3)	0.0029	0.0026	0.0032

Letermovir specifically has activity against the HCMV terminase complex. Letermovir does not inhibit other human herpesvirus, HSV-1, HSV-2 (in Vero cells), VZV (in HFFs), HHV-6A (strain GS in HSB-2 cells) and EBV (B95-8 in 293T cells), rat CMV (EC_{50} value >10 µM), or unrelated viruses such human adenovirus, hepatitis B virus, HIV-1, influenza A virus or hepatitis C virus (EC_{50} values range from >10 to >32 µM) (Study Reports MK-8228-PD004, MK-8228-PD009, MK-8228-PD011; Marschall et al., 2012).

2.3. Antiviral Activity in Cell Culture in the Presence of Serum and Serum Proteins (Study Reports MK-8228-PD002)

To determine if letermovir is significantly bound by human serum proteins and to predict a therapeutically effective concentration, the sponsor measured the effects of human serum and human serum proteins on the EC_{50} values of letermovir and of control maribavir (MBV) and ganciclovir (GCV) using a fluorescence reduction assay (Table 2). NHDFs were infected with HCMV AD169-tagged with green fluorescent protein (GFP) at an moi of 0.1, treated with letermovir, MBV, or GCV in the presence or the absence of human serum (range from 5 to 40%) or individual human serum proteins, which are the major proteins involved the binding of drugs (human serum albumin, HSA, 45 mg/ml, or α-1-acid glycoprotein, AAG, 1 mg/ml), and the corresponding EC_{50} values were determined using fluorescence reduction of GFP units at 7 dpi using a charge coupled-device camera fluorescence detector. The letermovir EC_{50} value increased 4.2-fold in the presence of 40% human serum; whereas, MBV and GCV increased 3-fold and 2.7-fold, respectively. Although HSA did not affect the letermovir EC_{50} value, AAG increased the letermovir EC_{50} value 5.9-fold.

Table 2. Effect of Human Serum or Serum Proteins on Antiviral Activity of Letermovir, MBV and GCV (Table 4, Study Report MK8228-PD002, page 17)

		EC_{50} µM (fluorescence reduction)		
		Letermovir	**Maribavir**	**Ganciclovir**
A)	**/**	0.0025 +/- 0.0032 (4)	0.22 +/- 0.17 (3)	3.19 +/- 3.18 (4)
	5% HS	0.0035 +/- 0.0017 (4)	0.24 +/- 0.05 (3)	3.07 +/- 2.39 (4)
	10% HS	0.0047 +/- 0.0017 (4)	0.33 +/- 0.12 (3)	3.75 +/- 2.42 (4)
	20% HS	0.0056 +/- 0.0053 (4)	0.52 +/- 0.14 (3)	5.73 +/- 4.02 (4)
	40% HS	0.0107 +/- 0.0034 (3)	0.66 +/- 0.43 (3)	8.74 +/- 7.80 (4)
	100% HS (predicted)	0.0224 µM	1.4 µM	17.6 µM
B)	**/**	0.0035 +/- 0.0007 (3)	0.26 +/- 0.17 (3)	3.66 +/- 0.62 (3)
	AAG	0.0208 +/- 0.0005 (3)	0.75 +/- 0.20 (3)	1.14 +/- 1.40 (3)
	HSA	0.0021 +/- 0.0006 (3)	0.09 +/- 0.04 (3)	2.62 +/- 2.24 (3)

The number of separate experiments is shown in parentheses.

2.4. Cytotoxicity/Therapeutic Index (Study Reports MK-8228-PD008, MK-8228-PD013; Lischka et al., 2016)

Using the letermovir CC_{50} value reported for NHDFs (>30 µM) (**Study Report MK-8228-PD013 and Table 4 below**), we calculated the corresponding letermovir therapeutic indices (TIs) for the HCMV primary isolates stratified by gB subtypes and studied by Lischka et al., 2016 (Table 3). The TI values for letermovir against these 70 HCMV primary isolates, which were stratified by gB subtype and grown in NHDFs, ranged from >10345 to >15789.

Table 3. Therapeutic Index (TI) Values of Letermovir for HCMV Primary Isolates, Stratified by Glycoprotein B Subtype, Grown in NDHFs (based upon the results in Lischka et al., 2016 and the NDHF CC_{50} value reported in Study Report MK-8228-PD013, page 12)

gB subtype (isolates)	**Letermovir Median EC_{50} µM**	**NDHF CC_{50} µM**	**TI (CC_{50}/EC_{50})**
gB1 (29)	0.0019	>30	>15789
gB2 (27)	0.002	>30	>15000
gB3 (11)	0.0023	>30	>13043
gB4 (3)	0.0029	>30	>10345

To test the effects of letermovir on a broad representation of cell lines of diverse lineages, the sponsor used a fluorescent oxidoreduction indicator dye, AlamarBlue®, to quantify cell viability (Study Report MK-8228-PD013) in the presence of different concentrations of letermovir (Table 4). The cell lines tested include mouse monocyte-macrophage (J774A.1), human liver epithelial (HepG2), mouse embryo fibroblast (BALB/3T3 clone A31), mouse neuroblastoma (N18TG2), rat heart muscle (H9c2), rat kidney epithelial (NRK-52/E), human monocyte (THP-1), human cutaneous T lymphocyte (H9), human hepatoma cell line (Huh7), and human dermal fibroblasts (NHDFs). Cycloheximide, an inhibitor of protein synthesis, was used as a positive control. The CC_{50} values for letermovir were >10 µM and

most were close to the highest concentration tested (30 µM). In contrast, cycloheximide was cytotoxic to most cell lines (with the exception of THP1 cells) with CC_{50} values of <0.03 to 2.1 µM.

However, it is notable that most cell lines tested by the sponsor are transformed and, therefore, have rates of cell division and cell metabolism that are different from those of corresponding primary human cells, which are biologically relevant for HCMV growth in patients. Furthermore, five of the studied cell lines are of rat or mouse origin, in which HCMV cannot grow. While the low cytotoxicity in these cell lines is reassuring, testing of these cell lines (except for the NHDFs) is not biologically relevant to determining the cytotoxicity of letermovir in primary human cells.

Table 4. Cytotoxicity Effects of Letermovir on Different Cell Lines (extracted from Table 1, Study Report MK-8228-PD013, page 12)

Cell line (Passage number)	Letermovir CC_{50} (µM)	Cycloheximide CC_{50} (µM)
J774A.1 (P12)	29 (±1.9)	<0.03
HepG2 (P88)	27 (±1.0)	0.38 (±0.49)
BALB/3T3.A31 (P74)	>30	<0.03
N18TG2 (P7)	28 (±0.96)	<0.03
H9C2 (P11)	>30	0.15 (±0.078)
NRK52E (P10)	28 (±0.11)	<0.03
THP1 (P6)	>30	>67/ 35
H9 (P4)	>30	2.1 (±18)
HUH7 (P136)	30/ >30	0.22 (±0.042)
NHDF (P12)	>30	1.4 (±0.37)

2.5. Combination Antiviral Activity in Cell Culture (Study Report MK-8228-PD-010, Wildum et al., 2015)

Letermovir with approved anti-HCMV drugs.
The effects of letermovir combined with other anti-HCMV drugs, including acyclovir (ACV), cidofovir (CDV), foscarnet (FOS), and ganciclovir (GCV), on GFP expressing RV-HG infected NHDFs (moi = 0.2) were tested using fluorescence reduction assay at 7 dpi (Table 5). The antiviral interactions between letermovir and ACV, CDV, or GCV are not antagonistic when combined at their respective EC_{50} values

and appear to be the same for letermovir with FOS although there is some discrepancy with the Bliss and Loewe models (Wildum et al., 2015). These results indicate that combined use of letermovir with approved anti-HCMV drugs will not inhibit their antiviral activities.

Table 5. Analysis of the Combination of Letermovir with Approved Anti-HCMV Drugs by Use of the Bliss Independence Model (Wildum et al., 2015, Table 1)

Drug combination	Mean vol (μM²%)[a]		Antiviral effect
	Synergy	Antagonism	
Letermovir + ACV	0.5 ± 0.6	-12.4 ± 7.7	Additive
Letermovir + CDV	3.3 ± 4.7	-12.6 ± 4.1	Additive
Letermovir + FOS	5.1 ± 5.8	-15.2 ± 14.3	Additive
Letermovir + GCV	15.6 ± 13.8	-5.1 ± 6.5	Additive

[a]Antiviral activities of drug combinations were determined in a checkerboard fashion using a GFP-based fluorescence reduction assay. MacSynergy II software was used to calculate the synergy and antagonism volumes of the antiviral effects of two-drug combinations at the 95% confidence level. The positive and negative values from five cell culture replicates were individually summed to give the mean synergy and antagonism volumes. Datasets from at least 3 independent experiments were combined, and arithmetic means were calculated for each drug-drug combination. Volumes of synergy or antagonism greater than ±50 μM²% can be considered significant and may be important *in vivo*.

Combination of letermovir and anti-HIV drugs.
The anti-HIV activities of an integrase strand transfer inhibitor (raltegravir, RAL), non-nucleoside reverse transcriptase inhibitors (NNRTIs: efavirenz, EFV; etravirine, ETR; and nevirapine, NVP), nucleoside reverse transcriptase inhibitors (NRTIs: emtricitabine, FTC and tenofovir, TDF), and protease inhibitors (PIs: atazanavir, ATV; darunavir, DRV; lopinavir, LPV; and ritonavir, RTV) in combination with letermovir were tested using 50% cell culture infectious dose ($TCID_{50}$) of HIV-1 (clone LAI) in the human T cell line, MT-4. Control combinations included azidothymidine (AZT) with AZT for additivity, ribavirin (RBV) with stavudine (d4T) for antagonism, and RBV with didanosine (ddI) for synergism. Given that none of the tested HIV-1 drugs has antiviral activity against HCMV and letermovir is not active against HIV-1, the maximum clinically therapeutic levels of the "inactive" drug (i.e., C_{max} values) were used. The sponsor found that the anti-HIV drugs do not affect the antiviral activity of letermovir and that letermovir did not affect the activities of the anti-HIV drugs (Wildum et al., 2015).

Thus, letermovir had no antagonistic effect on any of the anti-HIV drugs tested. Furthermore, none of the drug combinations showed synergistic cytotoxicity in the range of the drug concentrations tested (Study Report MK-8228-PD-010). These results establish that the combined use of letermovir with anti-HIV therapies will not adversely affect their antiviral activities.

2.6. Resistance Development in Cell Culture (Study Reports MK-8228-PD015, MK-8228-PD018, Goldner et al., 2011; Goldner et al., 2014; Chou, 2015)
To identify amino acid substitutions in the HCMV genome that are associated with letermovir resistance, the sponsor selected letermovir resistant isolates from HCMV (strain AD169) using two approaches: (1) a single step selection (letermovir at 10x EC_{50} value, 50 nM, moi = 0.03) or (2)

increasing concentrations of letermovir following the appearance of HCMV cytopathic effect (CPE) at each dose and up to ≥128-fold EC_{50} value (moi = 0.01 to 0.03) (**Study Report MK-8228-PD-015**, Goldner et al., 2011; Goldner et al., 2014).

Ten letermovir resistant isolates were generated and the pUL51, pUL56, pUL89, and pUL104 open reading frames (ORFs) were sequenced in each of the isolates. No amino acid substitutions were detected in the pUL51, pUL89, and pUL104 ORFs although the variant pUL89 S345 was present in the HCMV isolates (**Study Report MK-8228-PD015**). Of note, while the sponsor concludes that the pUL89 A345S is an interstrain variation and is not associated with letermovir resistance, the sponsor has not independently phenotyped this substitution to make this claim. Polymorphic amino acid positions should not be excluded. The FDA regards polymorphic amino positions as equally concerning with respect to resistance as conserved sites. For example, the polymorphic amino acid positions HCV NS3 Q80 and NS5A M28, Q30, L31 and Y93 are well established to impact efficacy both as treatment-emergent substitutions and baseline polymorphisms. Furthermore, pUL89 Q256E substitution was selected with a close analog indicating that substitutions in pUL89 may confer resistance to letermovir (reviewed in IND104706 SDN 000). The sponsor should phenotypically characterize pUL89 Q256E and A345S substitutions. All of the substitutions detected in the letermovir resistant viruses were in the UL56 ORF and not in the UL51, UL89 or UL104 genes (Table 6). The pUL56 substitutions were transferred to an HCMV bacterial artificial chromosome (BAC) carrying HCMV AD169 pHG and the fluorescence reduction assay was used to measure the susceptibility of the resulting recombinant viruses to letermovir. The EC_{50} values ranged from 0.06 µM to 27 µM. Thus, these pUL56 substitutions caused decreased susceptibility to letermovir in the range of 13-fold to 5870-fold (Table 6).

Table 6. Summary of the Phenotypic and Genotypic Characteristics of Letermovir-Resistant HCMV Strain AD169 Isolates (Study Report MK-8228 PD015, Table 7-1, page 2)

HCMV Strain	EC_{50} [µM][a]		FC[b]	AA substitution[c]			
	letermovir	GCV		UL56[d]	UL89[d]	UL104[d]	UL51[d]
AD169	0.0046 ± 0.0019	3.6 ± 1.4	1	n.a.[e]	n.a.	n.a.	n.a.
Selected mutants[f]							
rAIC246-1[g]	1.23 ± 0.32	1.2 ± 0.2	268	L241P	–[h]	–	–
rAIC246-2[g]	0.37 ± 0.07	4.0 ± 0.9	81	R369S	A345S[i]	–	–
rAIC246-3	27 ± 3.27	3.0 ± 2.4	5870	C325Y	–	–	–
rAIC246-4	0.13 ± 0.01	4.2 ± 1.3	28	V231L	–	–	–
rAIC246-5	0.11 ± 0.01	5.0 ± 0.4	23	R369M	–	–	–
rAIC246-6	0.08 ± 0.02	2.9 ± 0.9	17	R369M	–	–	–
rAIC246-7	0.92 ± 0.12	2.2 ± 0.6	200	L241P	–	–	–
rAIC246-8	25 ± 5.53	2.2 ± 1.2	5413	C325Y	–	–	–
rAIC246-9	0.06 ± 0.04	1.7 ± 0.2	13	R369G	–	–	–
rAIC246-10	0.09 ± 0.02	1.4 ± 0.4	19	V236M	A345S[i]	–	–

[a]EC_{50} values were determined by a CPE reduction assay. Data are means from at least three independent experiments and are expressed with standard deviation. [b]Fold change (FC) is the letermovir EC_{50} value for mutant virus divided by the letermovir EC_{50} value for wild-type virus. [c]Amino acid substitution identified by HCMV

genotyping. [d]HCMV genes involved in cleavage/packaging of viral progeny DNA. [e]not applicable, [f]HCMV strain AD169 isolates obtained in cell culture under selective pressure with letermovir. [g]previously published (Goldner et al., 2011), [h]no amino acid substitution. [i]According to the sponsor, this interstrain variation is not associated with letermovir resistance.

Figure 2 shows the HCMV pUL56 ORF and its substitutions, which confer reduced sensitivity to letermovir and were identified in cell culture by the sponsor.

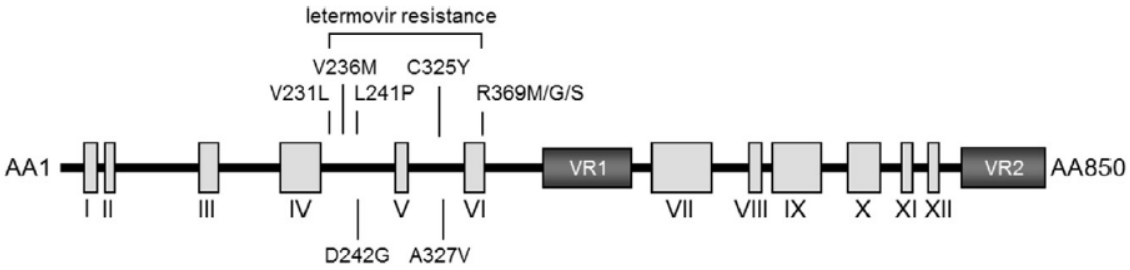


Figure 2 (Figure 1 in Goldner et al., 2015). Schematic representation of the UL56 ORF with the substitutions that confer decreased sensitivity to letermovir. Conserved regions are indicated gray boxes (I-XII); variable regions (VRI and VR2) are shown as black boxes. Amino acids associated with letermovir resistance identified in cell culture assays are indicated. Two pUL56 polymorphisms that are not associated with letermovir resistance are shown below the gene.

Chou also isolated letermovir resistant HCMV viruses in cell culture (Chou, 2015). His approach was to select resistant viruses using an HCMV (strain AD169) mutant carrying an error-prone UL54 exonuclease domain II and culturing the infected cells in letermovir containing medium starting with concentrations close to the EC_{50} value (5 nM). Following the appearance of CPE, letermovir concentrations were sequentially increased (1.5-4-fold) and the subsequent appearance of HCMV CPE was monitored. The pUL56 ORF (amino acids 1-500) was determined to screen for substitutions during passage of the virus. At the final passage, the pUL51, pUL56, pUL89 ORF sequences were determined. The novel pUL56 substitutions were transferred to BAC clones of HCMV AD169 carrying a reporter gene secreted alkaline phosphatase (SEAP) and changes in the susceptibility to letermovir were determined using inhibition of viral SEAP growth (Chou, 2015). The updated list of pUL56 substitutions which confer decreased sensitivity to letermovir and determined by the sponsor and Chou labs are shown in Table 7. It is notable that several positions, V231, V236, T244, F261, C325, and R369, have multiple amino acids which can confer decreased sensitivity to letermovir. Moreover the fold changes of some substitutions (particularly C325F/R/Y) are >3000. Together, these results indicate the key roles of these pUL56 residues in terminase activity. Further, HCMV carrying the substitutions that confer decreased sensitivity to letermovir do not affect the growth of the recombinant HCMV in cell culture, indicating that these pUL56 substitutions do not significantly impact the fitness of virus growth in cell culture.

Table 7. pUL56 Substitutions that Confer Decreased Sensitivity to Letermovir Isolated in Cell Culture (from Study Report MK-8228-PD-015, Table 7-1, page 21, Goldner et al., 2011; Goldner et al., 2014; Chou, 2015)

Substitution	Fold Change	References
V231A/L	FC = 2.1-267/FC = 5-28	Goldner et al., 2014; Chou, 2015; Study Report MK-8228-PD-015
V236L/M	FC = 14/FC = 45	Goldner et al., 2014; Chou, 2015
E237D	FC = 10	Chou, 2015
L241P	FC = 160-268	Goldner et al., 2011; Goldner et al., 2014; Chou, 2015; Study Report MK-8228-PD-015

T244K/R	FC = 3.3	Chou, 2015
L257I	FC = 4.9	Chou, 2015
F261C/L/S	FC = 4.4/FC = 2.8	Chou, 2015
Y321C	FC = 4.6	Chou, 2015
C325F/R/Y	FC = >3000/FC = >3000/FC = >3000-8796	Goldner et al., 2014; Chou, 2015; Study Report MK-8228-PD-015
M329T	FC = 4.4	Chou, 2015
R369M/G/S	FC =13-23/FC =13-44/FC = 48	Goldner et al., 2011; Goldner et al., 2014; Chou, 2015; Study Report MK-8228-PD-015

In Study Report MK-8228-PD018, the sponsor sequenced the HCMV UL56 and UL89 ORFs from the plasma of patients who met the primary endpoint in MK-8228 P001 trial and compared these HCMV sequences and publically available HCMV genomic sequences to those of the HCMV (strain Merlin, the NCBI RefSeq standard and first WHO International Standard for HCMV) (Dolan et al., 2004). They identified new UL56 and UL89 genotypic variants in the HCMV UL56 and UL89 ORFs. Nonetheless, the sponsor has not verified the sufficiency of these novel genotypic variants for decreased sensitivity to letermovir by marker transfer.

2.7. Cross-resistance (Study Reports MK-8228-PD007, MK-8228-PD001)

Decreased susceptibility to letermovir does not confer cross-resistance to ACV, CDV, FOS, or GCV (Study Report MK-8228-PD015, Table 7-2). Using a panel of recombinant HCMV carrying pUL56 substitutions that confer decreased susceptibility to letermovir (pUL56 V231L, V236M, L241P, C325Y, R369M/G), the sponsor determined that these pUL56 substitutions do not confer cross-resistance to ACV, CDV, FOS, GCV, BDCRB, or to BAY38-4766 with the exception of pUL56 R369M). Conversely, HCMV isolates that are resistant to anti-HCMV DNA polymerase drugs that inhibit HCMV DNA replication are susceptible to letermovir inhibition. Specifically, letermovir inhibits growth of an HCMV DNA polymerase mutant, HCMV isolates resistant to ganciclovir (GCV), benzimidavir (maribavir, an inhibitor of pUL97), BAY 38-4766 (another terminase inhibitor), or BAY 64-8030 (Table 8). Nonetheless, growth of an HCMV isolate resistant to BAY 66-6047 (pUL56 Y321C substitution), a predecessor compound of letermovir, is not inhibited by letermovir (EC_{50} value >62.5 µM).

Table 8. Letermovir Inhibition of HCMV Isolates Resistant to Anti-HCMV Drugs against other HCMV Targets or Against the HCMV Terminase Complex (extracted from Study Report MK-8228-PD-001, Table 2, page 14)

HCMV strain	Letermovir EC_{50} value (µM)
Resistant strains	
AD169 polymerase res.	0.005
AD169 GCV res.	0.004
AD169 benzimidavir res.	0.006
Hellebrand BAY 38-4766 res.	0.004
AD169 BAY 64-8030 res.	0.005
AD169 BAY 66-6047 res.	>62.5

These results are consistent with the sponsor's findings of letermovir inhibition of three HCMV mutants GDGrXbaF4 (UL97 del590-593, CDV^S, FOS^S, $GCV^{R/S}$), GDGrP53 (UL54A987G, CDV^R, FOS^S, GCV^R), and 759rD10 (UL97del590-593 and UL54 A987G, CDV^R, FOS^S, GCV^R) with known pUL54 and pUL97 resistance substitutions (Table 9, **Study Report MK-8228-PD-007**). The sponsor tested plaque formation by these viruses in HFFs at various concentrations of letermovir and control drugs, CDV, FOS, and GCV to calculate EC_{50} values for each mutant.

Table 9. Letermovir Inhibition of the Growth of HCMV pUL97 and pUL54 Mutants (extracted from Study Report MK-8228-PD007, Tables 1, 2, and 3, page 9)

	EC_{50} values (μM)			
HCMV mutant	**Letermovir**	**CDV**	**FOS**	**GCV**
GDGrXbaF4 (UL97 del590-593)	0.0026 ± 0.0001	0.103 ± 0.01	142.3 ± 3.58	4.48 ± 1.98
GDGrP53 (UL54A987G)	0.0029 ± 0.0002	11.3 ± 10.46	55.678 ± 3.68	6.46 ± 0.694
759rD100 (UL97 del590-593 /UL54A987G)	0.0024 ± 0.0009	>1.1	27.6 ± 6.8	>10

The sponsor also reports letermovir inhibition of other HCMV isolates with reduced sensitivity to GCV (pUL97 M460I; A594V; del590-593), GCV/cidofovir (CDV) cross resistance (pUL54 K513E/pUL97 M460I; pUL54 A987G; pUL54 A987G/pUL97 del590-593) with EC_{50} values in the range of 1.6 to 3.9 nM (Marschall et al., 2012). Together with the reports above, these results show that HCMV isolates with decreased sensitivity to approved HCMV DNA polymerase inhibitors, CDV, FOS, or GCV, are not cross-resistant to letermovir. Nonetheless, an HCMV isolate with resistance to BAY 66-6047, a predecessor compound of letermovir, is cross resistant to letermovir.

2.8. Activity of Letermovir in Animal Models (Study Reports MK-8228-PD003)

To study the effects of letermovir in an animal model, the sponsor tested the effects of daily letermovir treatment of Fox Chase NOD SCID mice implanted with Gelfoam® xenograft of NHDFs infected with HCMV (strain Davis, moi of 0.03) (Figure 3). Control mice were treated daily with placebo or with the approved anti-HCMV drug, valcyte (100 mg/kg/day). The mice were treated for 9 days and virus titers in the explanted tissues were determined. Letermovir treatment (10 mg/kg/day, 30 mg/kg/day and 100 mg/kg/day) of the immunodeficient mice carrying the HCMV infected NHDF xenografts were active in reducing HCMV growth.

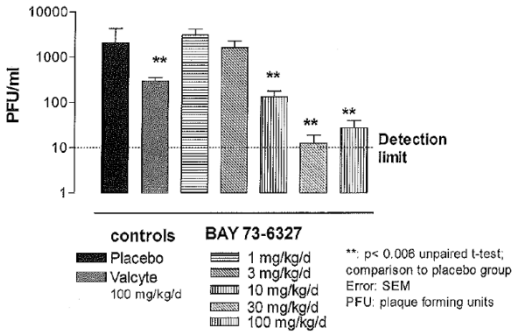


Figure 3. Activity of letermovir in the engineered mouse Gelfoam® xenograft model (Study Report MK-8228-PD003, Figure 5-2, page 8). NHDFs were infected with HCMV (strain Davis), loaded on collagen sponges and transplanted into immunodeficient mice. Mice were treated daily by gavage for 9 days with letermovir, Valcyte or placebo at the indicated doses. Virus titers in the explanted tissues were determined.

2.9. Simulation of HCMV growth in a letermovir PK/PD model (Study Report MK-8228-PD001)

In order to estimate an efficacious dose for humans, the sponsor used a pharmacodyanmic model (Study Report MK-8228-PD001). For this model, the sponsor assumed that the EC_{90} value needs to be covered throughout the entire dosing interval and that the free concentration in plasma equals the free concentration at the intracellular site of infection. The letermovir EC_{90} value (5.1 nM) corresponds to a total plasma concentration of 150 nM (86.1 ng/mL). Trough levels exceeding 150 nM were reached at 40 mg bid or 80 mg bid. Using a pharmacodynamic simulation of letermovir plasma concentration curves after bid dosing of 7.7 mg (0.11 mg/kg), 15.4 mg (0.22 mg/kg), 30.8 mg (0.44 mg/kg), 38.5 mg (0.55 mg/kg), 77 mg (1.1 mg/kg), and 123 mg (1.76 mg/kg), HCMV growth was simulated (Figure 4). Even though the trough levels exceeded the EC_{90} values needed to inhibit HCMV growth were reached at 40 mg or 80 mg bid, HCMV growth is predicted to be inhibited only at the highest concentration (123 mg, 1.4 mg/kg). However, the animal model study (Study Report MK-8228-PD-003) showed that HCMV growth in impanted human fibroblasts was inhibited at 10 mg/kg/day, 30 mg/kg/day and 100 mg/kg/day.

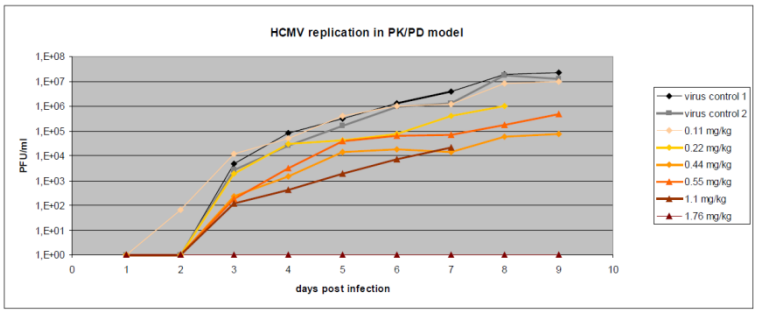


Figure 4. PK/PD Assessment of Letermovir (AIC090027) in Hollow Fiber Experiment (Figure 1, MK8228-PD001, page 16)

3. Clinical Virology

3.1. Summary of Key Efficacy Studies

The clinical development to support the efficacy of letermovir included evaluations in 3 completed studies. Two Phase 2 studies, MK-8228-019 (AIC001-2-001 non-IND study) and MK-8228-020 (NCT01063829), were designed and conducted by AiCuris GmbH & Co. KG. Following the completion of the Phase 2 program, letermovir was licensed to Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. (the sponsor of this NDA submission). A pivotal Phase 3 study, MK-8228-001 (NCT02137772), was designed and conducted by the sponsor.

3.2. Study MK-8228-019 (AIC001-2-001)

Study MK-8228-019 (AIC001-2-001) was a non-IND Phase 2a proof-of-concept open label study in which 26 kidney and kidney/pancreas transplant subjects and 1 bone marrow transplant subject with positive HCMV DNaemia (preemptive therapy) were treated with daily doses of 80 mg letermovir or an active control which was the local standard of care of the investigational site (valganciclovir) for 14 days. The majority of subjects were kidney transplant recipients (25 subjects [92.6%]) or kidney/pancreas transplant recipients (1 subject [3.7%]). One subject was a bone marrow transplant recipient (1 subject [3.7%]). There was discordance in the HCMV viral load data between the local and central laboratory that made interpretation of the results difficult. Furthermore, the HCMV serology status was not equally balanced across the treatment groups (Table 10, study report P019, pg. 79). Overall, the HCMV serology status of donor [D] positive, recipient [R] negative was the most unbalanced, with 1 subject (11.1%) enrolled in the AIC-001 40-mg BID group, 3 subjects (33.3%) enrolled in the AIC-001 80-mg QD group, and 6 subjects (66.7%) enrolled in the observational control group.

Table 10: Summary of Transplantation Type and Serology Status

	Letermovir 40mg BID (n=9)	Letermovir 80mg QD (n=9)	Control (vGCV) (n=9)
Type of Transplantation			
Kidney	88.9% (8/9)	88.9% (8/9)	100% (9/9)
Kidney/Pancreas	0% (0/9)	11.1% (1/9)	0% (0/9)
Bone marrow (autologous or allogeneic)	11.1% (1/9)	0% (0/9)	0% (0/9)
Serology Status			
D+/R-	11.1% (1/9)	33.3% (3/9)	66.7% (6/9)
D+/R+	44.4% (4/9)	44.4% (4/9)	33.3% (3/9)
D-/R-	22.2% (2/9)	0% (0/9)	0% (0/9)
D-/R+	11.1% (1/9)	11.1% (1/9)	0% (0/9)
Missing	11.1% (1/9)	11.1% (1/9)	0% (0/9)

The primary efficacy endpoint was the reduction in plasma HCMV DNA load (assessed by PCR) from Baseline to Day 15 using the (b) (4) central laboratory HCMV PCR assay. In the active control (valganciclovir) group, a sharp decline in the viral load mean change from Baseline was seen at Day 4 and the viral load remained similar between Days 4 and 15 (Figure 5, study report P019, pg. 83). In the letermovir treatment groups, the viral load remained similar between Days 1 and 11 and a decline in the viral load mean change from Baseline was seen between Days 11 and 15. At Day 15, a statistically significant viral load decline from Baseline was seen in both the letermovir and active control groups (40 mg BID: P = 0.031; 80 mg QD: P = 0.018; standard of care: P = 0.001), with a larger (though not statistically significantly larger) decline observed in the active control group. Differences in the time course of HCMV viral DNA decrease were identified between the active control (valganciclovir) and letermovir treatment groups during the 2-week treatment period. In the active control (valganciclovir) group, a decline in HCMV DNA values was seen by Day 4 (the first data point after baseline), whereas in both letermovir groups, the decline occurred later, mainly after Day 11. The observed delayed reduction in DNaemia in the letermovir treatment groups could be due to the difference in the mechanism of action where letermovir interferes with viral DNA maturation and packaging of monomeric genome lengths at a later stage in the viral replication cycle. The formation of the infectious viral particles is inhibited by this mode of action, but viral DNA might still be measured for some time via the standard biomarkers. Alternatively, a potential suboptimal drug exposure is responsible for, or contributes to, the observed phenomenon.

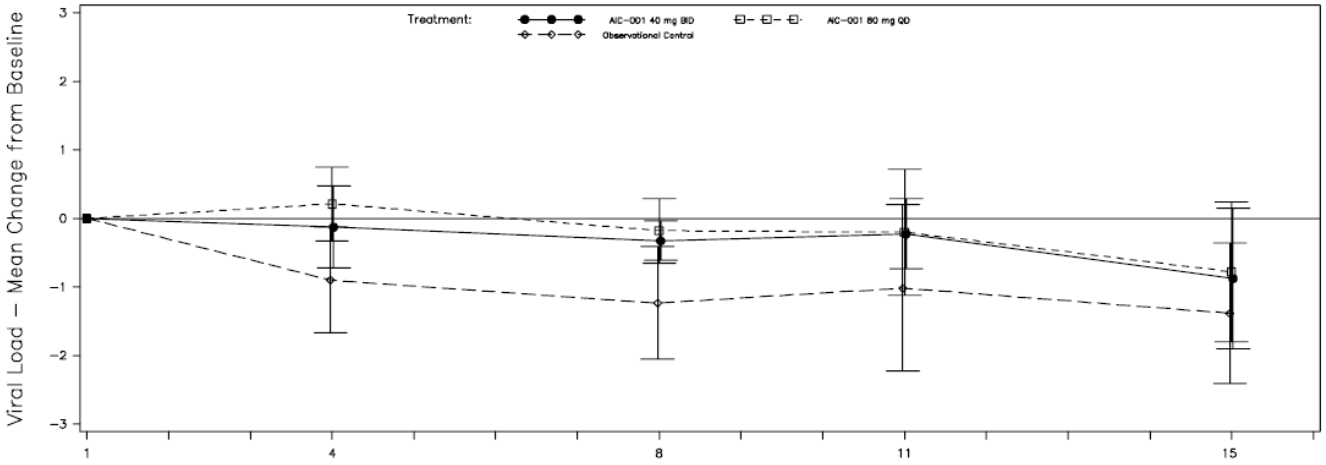


Figure 5: HCMV PCR Plasma Viral Load Change from Baseline (Log_{10} copies/mL) Means plus or minus Standard Deviations (PP Population Subgroup: Excludes Patients with Either a Zero or BLQ Value at Day 1).

3.3. Study MK-8228-020 (AIC246-01-II-02, NCT01063829)

This was a Phase 2, multi-center, randomized, double-blind, placebo-controlled, dose-ranging study to investigate the safety and efficacy of 3 different doses (60, 120, or 240 mg/day) of letermovir given orally for 84 days in comparison to matching placebo for the prevention of human cytomegalovirus (HCMV) active replication by re-infection or reactivation in HCMV seropositive allogeneic human blood precursor cell (HBPC) recipient subjects. The primary endpoints were incidence and time to onset of "HCMV prophylaxis failed" during the 84-day treatment period, with "HCMV prophylaxis failed" defined as:

- Development of systemic detectable HCMV replication, OR
- Development of HCMV End-Organ Disease, OR
- Non-HCMV-related reasons (AE, death, protocol non-compliance, withdrew consent, other)

Note: The subjects were not followed beyond the 84-treatment period so the durability of the drug response could not be determined.

The total number of subjects included in the analysis set in the placebo and letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups were 33, 33, 33, and 34, respectively. The number (%) of subjects who discontinued study medication intake before day 84 was 21 (63.6%), 16 (48.5%), 10 (30.3%), and 10 (29.4%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively (Table 11, FDA analysis). Prophylaxis failure was defined as evidence of HCMV replication (confirmed) leading to initiation of preemptive therapy or evidence of HCMV disease. Failure also includes subjects who discontinued treatment prior to Day 84 for reasons other than HCMV prophylaxis failure. Subjects in the 120 mg/day and 240 mg/day letermovir treatment groups were significantly less likely to fail than subjects in the placebo group (p=0.014 and p=0.007, respectively). The number (%) of subjects who failed HCMV prophylaxis (not including those who discontinued for other reasons prior to Day 84) was 13 (n=33, 39.4%), 7 (n=33, 21.2%), 6 (n=33, 18.2%), and 2 (n=34, 5.9%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively. The incidence of failure decreased with increasing letermovir dose. There were no subjects who reached the definition of HCMV end-organ disease alone during the 84 days (12 weeks) of study drug administration. Note: The statistics that were conducted here and below were conducted by this reviewer using the Newcombe-Wilson hybrid

score procedure without continuity correction (Newcombe, 1998). Please see the review of Biometrics Reviewer Fraser Smith, Ph.D., for more thorough statistical analyses as well as for the confirmation of the sponsor's analyses.

Table 11: Clinically Significant HCMV Infection within the 84-day Treatment Period (ITT)

	60 mg of Letermovir QD	120 mg of Letermovir QD	240 mg of Letermovir QD	Placebo
Total	48.5% (16/33)	30.3% (10/33)	29.4% (10/34)	63.6% (21/33)
p-value*	0.21	0.0067	0.005	
Initiation of PET based on documented HCMV DNaemia	21.2% (7/33)	18.2% (6/33)	5.9% (2/34)	39.4% (13/33)
HCMV end-organ disease	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)
Death	6.1% (2/33)	0% (0/33)	2.9% (1/34)	3.0% (1/33)
Other discontinuations	27.3% (9/33)	12.1% (4/33)	20.6% (7/34)	24.2% (8/33)
Stem cell source				
Bone marrow	100% (1/1)	0% (0/0)	0% (0/1)	50% (1/2)
Peripheral blood	46.9% (15/32)	30.3% (10/33)	30.3% (10/33)	64.5% (20/31)

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

A sensitivity analysis was conducted to stratify the subjects based on the presence or absence of baseline HCMV DNA. The number (%) of subjects with negative HCMV DNA at baseline who developed clinically significant HCMV infection was 18 (n=29, 62.1%), 14 (n=29, 48.3%), 5 (n=27, 18.5%), and 6 (n=30, 20%), in the placebo, letermovir 60 mg/day, 120 mg/day, and 240 mg/day groups, respectively. The treatment benefit was not observed in subjects who had HCMV DNA at baseline (Table 12, FDA analysis).

Table 12: Clinically Significant HCMV Infection by Presence of Baseline HCMV DNA

	60 mg of Letermovir QD	120 mg of Letermovir QD	240 mg of Letermovir QD	Placebo
Median Baseline Viral Load (IU/mL)	200.5 (range 40-414)	62.5 (range 46-109)	416.5 (range 34-11902)	400 (range 113-593)
Clinically Significant HCMV Infection				
Total	48.5% (16/33)	30.3% (10/33)	29.4% (10/34)	63.6% (21/33)
Subjects with Positive DNA at Baseline	50% (2/4)	83.3% (5/6)	100% (4/4)	3/4 (75%)
Subjects with Negative DNA at Baseline	48.3% (14/29)	18.5% (5/27)	20% (6/30)	62.1% (18/29)
Initiation of PET based on documented HCMV DNaemia				
Total	21.2% (7/33)	18.2% (6/33)	5.9% (2/34)	39.4% (13/33)
Subjects with Positive DNA at Baseline	66.7% (2/3)	66.7% (4/6)	25% (1/4)	75% (3/4)
Subjects with Negative DNA at Baseline	17.2% (5/29)	7.4% (2/27)	3.3% (1/30)	34.5% (10/29)

3.4. Study MK-8228-P001 (NCT02137772)

MK-8228-P001 was a Phase 3, randomized, placebo-controlled, multi-site, double-blind study designed to evaluate the efficacy and safety of letermovir versus placebo, dosed for 14 weeks, for prevention of clinically significant HCMV infection in adult, HCMV-seropositive allogeneic HSCT recipients (R+), a population at high risk for HCMV infection and/or disease.

Subjects were randomized in a 2:1 ratio to receive letermovir or placebo beginning anytime from the day of transplant until 28 days post-transplant. Study medication continued through Week 14 (~100 days) post-transplant, the period of highest risk for HCMV infection and/or disease in HSCT recipients. Both oral (tablet) and IV formulations were used in this study. The dose of letermovir used was 480 mg administered once daily, with dose adjustment to 240 mg once daily when co-administered with

cyclosporine A. Subjects had follow-up assessments through Week 24 post-transplant (the primary study period). Following completion of the primary study period, all subjects were to remain in the study through Week 48 post-transplant in order to continue collecting information on (1) HCMV disease, (2) health outcomes data and (3) quality of life.

The primary endpoint for this study, clinically significant HCMV infection at Week 24 post-transplant, was defined as the occurrence of either one of the following outcomes: 1) onset of HCMV end-organ disease; or 2) initiation of anti-HCMV pre-emptive therapy based on documented HCMV DNaemia in the subject's plasma samples (detection of HCMV viral DNA as measured by the central laboratory) and the clinical condition of the subject. The criteria for initiation of anti-HCMV preemptive treatment were as follows:

- During the study medication period:
  - High risk subjects: viral DNA ≥137 IU/mL
  - Low risk subjects: viral DNA >274 IU/mL
- After Week 14 post-transplant:
  - High risk subjects: viral DNA >274 IU/mL
  - Low risk subjects: viral DNA >274 IU/mL

3.4.1. Clinically Significant HCMV Infection/Disease

As the definition of the primary efficacy endpoint in this study included initiation of pre-emptive therapy based on detectable HCMV DNaemia, the full analysis set (FAS) excluded subjects who were viremic at baseline in order to assess the prophylactic effect of letermovir on HCMV DNaemia. The primary population for efficacy evaluations in the study was the FAS population which consisted of all randomized subjects who received at least one dose of study medication and had no detectable HCMV viral DNA (measured by the central laboratory) on Day 1 (when study medication was initiated). FAS population consisted of 495 subjects, 325 (65.6%) in the letermovir group and 170 (34.3%) in the placebo group. The Non-Completer=Failure (NC=F) approach was used for imputing missing data in this analysis. In this approach, subjects who discontinued from the study prior to the Week 24 follow-up visit were considered as failures regardless of reason for discontinuation. Subjects missing the HCMV DNA measurement needed for efficacy assessment in the Week 24 window were also considered as failures. At Week 24, the proportion of subjects with clinically significant HCMV infection in the letermovir group (37.5%, 122/325) was lower than that in the placebo group (60.6%, 103/170) (Table 13, FDA analysis). The estimated difference (95% CI) was -23% (-31.7% to -13.8%). This difference between the two treatment groups was statistically significant (one-sided p-value <0.0001). Of importance, these differences were still observed regardless of the risk stratification (high vs. low), stem cell source (bone marrow vs. peripheral blood), or donor HCMV serostatus (positive vs. negative). Of note, the difference was smaller between the treatment groups in the cord blood recipients (58.3%, 7/12 vs 60%, 6/10, in the letermovir and placebo arms, respectively). While the numbers are too small to make any conclusions, this observation is consistent with reports that the risk of infectious complications, including HHV-6 encephalitis and HCMV diseases, is higher in patients who received cord blood transplantation (Walker et al., 2007).

Table 13: Clinically Significant HCMV Infection/Disease by Week 24 (FAS) – Primary Endpoint

	Letermovir (n=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	37.5% (122/325)	60.6% (103/170)	23.0	(13.8, 31.7)	<0.0001
Initiation of PET based on documented HCMV DNaemia	16% (52/325)	40% (68/170)	24.0	(15.7, 32.3)	<0.0001
HCMV end-organ disease	1.5% (5/325)	1.8% (3/170)	0.2	(-2.1, 3.6)	0.85
Discontinued from study before	17.2% (56/325)	15.9% (27/170)	-1.3	(-7.9, 5.9)	0.7

	Letermovir (n=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Week 24					
Missing outcome in Week 24 visit window	2.8% (9/325)	2.9% (5/170)	0.2	(-2.8, 4.2)	0.91
Risk Stratification					
High Risk	42.6% (43/101)	72.7% (32/44)	30.2	(12.6, 44.4)	0.00084
Low Risk	35.3% (79/224)	56.3% (71/126)	21.1	(10.2, 31.3)	0.00013
Stem cell source					
Bone marrow	43.1% (31/72)	67.4% (29/43)	24.4	(5.5, 40.6)	0.011
Cord blood	58.3% (7/12)	60% (6/10)	1.7	(-34.7, 36.8)	0.94
Peripheral blood	34.9% (84/241)	58.1% (68/117)	23.3	(12.3, 33.6)	<0.0001
Donor HCMV Serostatus					
Negative	46.3% (57/123)	66.7% (48/72)	20.3	(5.9, 33.3)	0.006
Positive	32.2% (64/199)	56.1% (55/98)	24.0	(12, 35.2)	<0.0001
Unknown	33.3% (1/3)	0% (0/0)			
Initiation of PET based on documented HCMV DNaemia using Protocol-recommended viral load thresholds	8.9%, 29/325	28.8%, 49/170	19.9	(12.7, 27.6)	<0.0001
Initiation of PET based on documented HCMV DNaemia including Local Laboratory results	17.8%, 58/325	41.2%, 70/170	23.4	(14.9, 31.7)	<0.0001
Initiation of PET based on documented HCMV DNaemia using Local and Central Laboratory results as well as those subjects who met the Viral Load threshold but did not receive PET	19.7% (64/325)	42.9% (73/170)	23.2	(14.7, 31.7)	<0.0001

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

Of note, the specific thresholds for initiating PET were not mandated per protocol as a subject's risk status and clinical condition may have changed during the course of the study and was best assessed by the investigator taking care of the subject. Furthermore, sites were permitted to initiate PET based on local laboratory test results as there are no universally accepted guidelines for viral load thresholds for PET initiation and institutional practice varies widely across sites. Additionally, there were several subjects who had achieved threshold HCMV viral load but still did not receive PET:

- 8228-001_001700011 (low risk, placebo): Week 6=440 IU/mL, Week 8=1827 IU/mL
- 8228-001_003200004 (low risk, placebo): Week 4=751 IU/mL, Week 5=184 IU/mL
- 8228-001_003300007 (low risk, placebo): Week 6=222 IU/mL, Week 7=881 IU/mL
- 8228-001_003400004 (high risk, letermovir): Week 22=786 IU/mL, Week 24=1329 IU/mL
- 8228-001_007300004 (low risk, letermovir): Week 8=881 IU/mL, Week 9=2190 IU/mL
- 8228-001_011300003 (high risk, letermovir): Week 20=1832 IU/mL, Week 22=1754 IU/mL
- 8228-001_014800002 (high risk, letermovir): Week 2=669 IU/mL, Week 3=257 IU/mL

Finally, there were two subjects (8228-001_011000009 and 8228-001_016500008, both in the letermovir arm) who received PET based on the co-medication report but were not accurately counted as treatment failures. Therefore sensitivity analyses were performed to evaluate efficacy in the subset of subjects in whom PET was initiated at or above the protocol-recommended viral load thresholds as well as including HCMV DNaemia results from the local laboratory. A lower proportion of subjects initiated PET using the protocol recommended thresholds in the letermovir group (8.9%, 29/325) compared to the placebo group (28.8%, 49/170) through Week 24 post-transplant, which was still statistically significant (one-sided p-value <0.0001) (Table 13). There was also a lower proportion of subjects initiating PET that included the local laboratory results in the letermovir group (17.8%, 58/325)

compared to the placebo group (41.2%, 70/170) through Week 24 post-transplant, which was still was statistically significant (one-sided p-value <0.0001) (Table 13). Even if all of the subjects who achieved that viral load threshold but did not receive PET as well as the two additional subjects who were miscategorized are counted as failures, there was a lower proportion of subjects initiating PET in the letermovir group (19.7%, 64/325) compared to the placebo group (42.9%, 73/170) through Week 24 post-transplant, which was still was statistically significant (one-sided p-value <0.0001) (Table 13). The results of the sensitivity analysis were supportive of the results of the primary analysis.

A key secondary efficacy endpoint was the proportions of subjects who developed clinically significant HCMV infection through Week 14 post-transplant (the treatment period). At Week 14, the proportion of subjects with clinically significant HCMV infection in the letermovir group (19.1%) was lower than that in the placebo group (50.0%) using the NC=F approach (Table 14, FDA analysis). The estimated difference and 95% CI, -31.3% (-39.9% to -22.6%), adjusted for the stratification factor (high vs. low risk), was statistically significant (one-sided p-value <0.0001). Of note there were 24 subjects (n=325) who experienced virologic failure while on-treatment and an additional 28 subjects who experienced relapse in the letermovir arm. In comparison, there were 65 subjects (n=170) who had documented HCMV DNaemia during the treatment phase and an additional 3 subjects who had documented HCMV DNaemia after the treatment phase in the placebo arm.

Table 14: Clinically Significant HCMV Infection/Disease by Week 14 (FAS)

	Letermovir (n=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	19.1% (62/325)	50% (85/170)	30.9	(22.2, 39.3)	<0.0001
Initiation of PET based on documented HCMV DNaemia	7.4% (24/325)	38.2% (65/170)	30.8	(23.1, 38.7)	<0.0001
HCMV end-organ disease	0.3% (1/325)	1.2% (2/170)	0.9	(-0.8, 3.9)	0.24
Discontinued from study before Week 14	10.2% (33/325)	9.4% (16/170)	-0.7	(-5.9, 5.3)	0.79
Missing outcome in Week 14 visit window	1.2% (4/325)	1.2% (2/170)	-0.1	(-2.1, 3)	0.96
Risk Stratification					
High Risk	20.8% (21/101)	63.6% (28/44)	42.8	(25.6, 57.1)	<0.0001
Low Risk	18.3% (41/224)	45.2% (57/126)	26.9	(16.8, 36.7)	<0.0001
Stem cell source					
Bone marrow	27.8% (20/72)	55.8% (24/43)	28	(9.5, 44.5)	0.0028
Cord blood	25% (3/12)	30% (3/10)	5	(-29.2, 39.3)	0.79
Peripheral blood	16.2% (39/241)	49.6% (58/117)	33.4	(23.1, 43.2)	<0.0001
Donor HCMV Serostatus					
Negative	18.7% (23/123)	55.6% (40/72)	36.9	(23, 49.3)	<0.0001
Positive	19.6% (39/199)	45.9% (45/98)	26.3	(15, 37.3)	<0.0001
Unknown	0% (0/3)	0% (0/0)			

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

Overall, the incidence of HCMV end-organ disease was low through both the Week 14 and Week 24 post-transplant time points, with only 8 subjects in the FAS population adjudicated through Week 24 post-transplant. The rates of HCMV end-organ disease were comparable between the groups at both time points.

Amongst the 70 subjects who were viremic at baseline (i.e., non-FAS population), 63 subjects (44/48 and 19/22 in the letermovir and placebo arms, respectively) had just 137 IU/mL (median = 137 IU/mL, range = 137-654 IU/mL and median 137 IU/mL, range 137-231 IU/mL in the letermovir and placebo arms, respectively) (see Table A1-8 & Table A1-9 in the Appendix for individual subject data). Given

that the viral load for the majority of these subjects was just barely detectable, a separate analysis was conducted in this population to determine whether letermovir was efficacious. Interpretation of this population is challenging given the study protocol's criteria for initiation of preemptive treatment is low which resulted in many of these subjects taken off of the study early by the investigator. Despite the challenges, there was a statistically significant difference in favor of letermovir in most of the groups (Table 15, FDA analysis). Similar results were also seen for the all-subjects-as-treated population (ITT population, n=465) where there was a statistically significant difference in favor of the letermovir arm (Table 16 & Figure 6, FDA analysis).

As was the case in the FAS population, the incidence of HCMV end-organ disease was low through Week 24 post-transplant, with only 11 subjects in the ITT population adjudicated through Week 24 post-transplant. The rates of HCMV end-organ disease were comparable between the groups at both time points. Of note, all 11 subjects had detectable HCMV DNA (Appendix Table A1-10).

Table 15: Clinically Significant HCMV Infection/Disease by Week 24 (non-FAS; subjects with viremia at baseline)

	Letermovir (n=48)	Placebo (n=22)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	64.6% (31/48)	90.9% (20/22)	26.3	(4.1, 41.9)	0.021
Initiation of PET based on documented HCMV DNaemia	43.8% (21/48)	77.3% (17/22)	33.5	(8.5, 51.7)	0.009
HCMV end-organ disease	4.2% (2/48)	4.5% (1/22)	0.4	(-10.1, 17.9)	0.94
Discontinued from study before Week 14	16.7% (8/48)	13.6% (3/22)	-3.0	(-18.7, 18.2)	0.75
Missing outcome in Week 14 visit window	2.1% (1/48)	0% (0/22)	-2.1	(-10.9, 12.9)	0.5
Risk Stratification					
High Risk	78.9% (15/19)	100% (8/8)	21.1	(-13.7, 43.3)	0.16
Low Risk	55.2% (16/29)	85.7% (12/14)	30.5	(0.1, 50.9)	0.049
Stem cell source					
Bone marrow	80% (8/10)	100% (4/4)	20.0	(-31, 51)	0.33
Cord blood	0% (0/0)	100% (1/1)			
Peripheral blood	60.5% (23/38)	88.2% (15/17)	27.7	(1.2, 45.6)	0.04
Donor HCMV Serostatus					
Negative	62.5% (10/16)	100% (6/6)	37.5	(-5.9, 61.4)	0.079
Positive	63.3% (19/30)	87.5% (14/16)	24.2	(-3.6, 44.1)	0.083
Unknown	100% (2/2)	0% (0/0)			

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

Table 16: Clinically Significant HCMV Infection/Disease by Week 24 (ITT; includes subjects with viremia at baseline)

	Letermovir (n=373)	Placebo (n=192)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	41% (153/373)	64.1% (123/192)	23	(14.4, 31.1)	<0.0001
Initiation of PET based on documented HCMV DNaemia	19.6% (73/373)	44.3% (85/192)	24.7	(16.6, 32.7)	<0.0001
HCMV end-organ disease	1.9% (7/373)	2.1% (4/192)	0.2	(-2.1, 3.5)	0.87
Discontinued from study before Week 14	17.2% (64/373)	15.6% (30/192)	-1.5	(-7.6, 5.2)	0.64
Missing outcome in Week 14 visit window	2.7% (10/373)	2.6% (5/192)	-0.1	(-2.7, 3.5)	0.96
Risk Stratification					
High Risk	48.3% (58/120)	76.9% (40/52)	28.6	(12.8, 41.4)	0.00051
Low Risk	37.5% (95/253)	59.3% (83/140)	21.7	(11.4, 31.4)	<0.0001
Stem cell source					

	Letermovir (n=373)	Placebo (n=192)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Bone marrow	47.6% (39/82)	70.2% (33/47)	22.7	(4.9, 37.9)	0.013
Cord blood	58.3% (7/12)	63.6% (7/11)	5.3	(-30.7, 39.1)	0.79
Peripheral blood	38.4% (107/279)	61.9% (83/134)	23.6	(13.3, 33.1)	<0.0001
Donor HCMV Serostatus					
Negative	48.2% (67/139)	69.2% (54/78)	21	(7.3, 33.3)	0.0028
Positive	36.2% (83/229)	60.5% (69/114)	24.3	(13.1, 34.6)	<0.0001
Unknown	60% (3/5)				

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

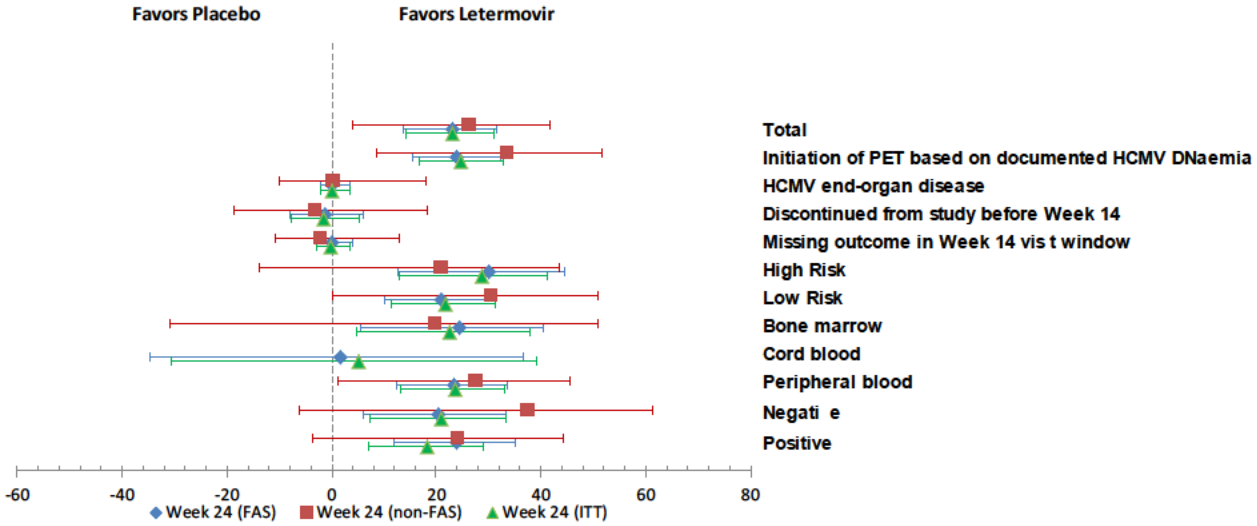


Figure 6: Forest Plot of Clinically Significant HCMV Infection/Disease by Week 24

The time to initiation of preemptive treatment for HCMV DNaemia through Week 24 post-transplant was different between the treatment groups (Table 17, FDA analysis). In the FAS group, the median day to initiation of preemptive treatment was 116 days and 36 days in the letermovir and placebo arms, respectively. The median viral load at the time of initiation of preemptive treatment was 258 IU/mL and 617 IU/ml in the letermovir and placebo arms, respectively. A similar trend was observed in the ITT population.

Table 17: Subjects Who Required PET by Week 24

	FAS					
	Letermovir			Placebo		
	N	Median Day to PET	Median Viral Load at PET (IU/mL)	N	Median Day to PET	Median Viral Load at PET (IU/mL)
Risk Stratification						
High Risk	24	120 (range 4 - 156)	327 (range 137 - 9974)	23	29 (range 12 - 101)	604 (range 137 - 10813)
Low Risk	28	108 (range 7 - 174)	239 (range 137 - 39396)	45	41 (range 15 - 162)	630 (range 137 - 97571)
Stem cell source						
Bone marrow	16	65.5 (range 7 - 143)	190 (range 137 - 19706)	18	29.5 (range 12 - 101)	458 (range 137 - 10298)
Cord blood	4	120.5 (range 50 - 156)	653 (range 137 - 3384)	3	37 (range 29 - 43)	212 (range 137 - 10813)
Peripheral blood	32	124 (range 4 - 174)	337 (range 137 - 39396)	47	42 (range 15 - 162)	747 (range 137 - 97571)
Donor HCMV Serostatus						
Negative	22	128 (range 7 - 174)	337 (range 137 - 25524)	32	31.5 (range 12 - 101)	886 (range 137 - 97571)
Posi ive	30	102.5 (range 4 - 174)	239 (range 137 - 39396)	36	41 (range 14 - 162)	362 (range 137 - 36421)
Unknown	0	0 (range 0 - 0)	0 (range 0 - 0)	0	0 (range 0 - 0)	0 (range 0 - 0)

Total	**52**	**116 (range 4 - 174)**	**258 (range 137 - 39396)**	**68**	**36 (range 12 - 162)**	**617 (range 137 - 97571)**
ITT						
	Letermovir			Placebo		
	N	Median Day to PET	Median Viral Load at PET (IU/mL)	N	Median Day to PET	Median Viral Load at PET (IU/mL)
Risk Stratification						
High Risk	35	58 (range 4 - 156)	387 (range 137 - 25661)	30	25.5 (range 8 - 101)	463 (range 137 - 15101)
Low Risk	38	101 (range 4 - 174)	290 (range 137 - 39396)	55	38 (range 12 - 162)	630 (range 137 - 97571)
Stem cell source						
Bone marrow	22	47 (range 7 - 143)	249 (range 137 - 19706)	21	36 (range 15 - 62)	623 (range 137 - 97571)
Cord blood	4	120.5 (range 50 - 156)	653 (range 137 - 3384)	4	33 (range 14 - 43)	251 (range 137 - 10813)
Peripheral blood	47	105 (range 4 - 174)	379 (range 137 - 39396)	60	36 (range 8 - 162)	795 (range 137 - 97571)
Donor HCMV Serostatus						
Negative	29	121 (range 7 - 174)	337 (range 137 - 25524)	36	29.5 (range 12 - 101)	852 (range 137 - 97571)
Posi ive	42	65.5 (range 4 - 150)	272 (range 137 - 39396)	49	36 (range 8 - 162)	367 (range 137 - 36421)
Unknown	2	56 (range 4 - 108)	3111 (range 658 - 5564)	0	0 (range 0 - 0)	0 (range 0 - 0)
Total	**73**	**92 (range 4 - 174)**	**325 (range 137 - 39396)**	**85**	**33 (range 8 - 162)**	**604 (range 137 - 97571)**

A key study design was that initiation of letermovir treatment could be initiated between day 0 and day 28 post-transplant. Therefore, a sensitivity analysis was conducted to determine whether there was a difference in response depending on the time of treatment initiation. There was no clear difference in failure rate regardless of when the treatment was initiated (Table 18, FDA analysis).

Table 18: Clinically Significant HCMV Infection by Time of Treatment Initiation

	Treatment Start Day 0-7		Treatment Start Day 8-14		Treatment Start Day 15-21		Treatment Start Day 22-28	
	Letermovir	**Placebo**	**Letermovir**	**Placebo**	**Letermovir**	**Placebo**	**Letermovir**	**Placebo**
FAS	59/157 (37.6%)	53/78 (67.9%)	31/75 (41.3%)	17/39 (43.6%)	12/43 (27.9%)	20/24 (83.3%)	20/50 (40%)	13/29 (44.8%)
ITT	71/172 (41.3%)	58/84 (69%)	35/82 (42.7%)	22/45 (48.9%)	17/53 (32.1%)	25/29 (86.2%)	30/66 (45.5%)	18/34 (52.9%)

3.4.2. All-cause Mortality through Week 24

The cumulative incidence of all-cause mortality was also lower in the letermovir group (10.8%, 35/325) compared to the placebo group (16.5%, 28/170) through Week 24 post-transplant (Table 19, FDA analysis) in the FAS population. Importantly, these differences were still observed when stratified by different subgroup categories, although the numbers were not powered to detect statistical differences. As stated above, the incidence of HCMV end-organ disease was low for both treatment arms primarily due to PET initiation. Of note, one of the subjects in each of the letermovir and placebo arms who died also developed HCMV end-organ disease. Similar trends were also seen for the all-subjects-as-treated population (ITT) (Table 20, FDA analysis). Of note, these differences were not observed in the non-FAS population (Table 21, FDA analysis). The sponsor has also conducted a statistical analysis using the Kaplan-Meier method. According to the sponsor, the Kaplan-Meier method resulted in a statistically significant difference in the incidence of all-cause mortality in favor of the letermovir arm for the FAS population with the nominal two-sided stratified log-rank p-value = 0.033 (please see the review of the statistical reviewer for confirmation of this p-value). However, the sponsor conducted this analysis using only the mortality that was observed within the study (note: this reviewer's analyses included all confirmed mortality regardless of whether it was observed within the study). The statistical significance was maintained using all confirmed deaths at Week 24 based on the statistical reviewer's analysis. Furthermore, the Division has requested that the sponsor make an effort to determine if subjects who prematurely withdrew from the trial were alive or dead at Weeks 24 and 48 post-transplantation and if a subject died, determine the date of death. In response to the Division's request, the vital statuses were obtained for 58 of the 76 subjects who prematurely withdrew from the study and submitted during the NDA review. The cumulative incidence of all-cause mortality which includes these additional deaths was also lower in the letermovir group (12.3%, 40/325) compared to the placebo group (18.8%, 32/170)

through Week 24 post-transplant (p = 0.051). The statistical analysis using the Kaplan-Meier method resulted in a statistically significant difference (p = 0.0401; please see the review of the statistical reviewer for confirmation of this p-value).

Table 19: All-cause Mortality at Week 24 (FAS)

	Letermovir (N=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	10.8% (35/325)#	16.5% (28/170)#	5.7	(-0.5, 12.7)	0.071
Risk Stratification					
High Risk	13.9% (14/101)	25% (11/44)	11.1	(-2, 26.6)	0.1
Low Risk	9.4% (21/224)	13.5% (17/126)	4.1	(-2.6, 11.8)	0.23
Stem cell source					
Bone marrow	12.5% (9/72)	9.3% (4/43)	-3.2	(-14.3, 10.4)	0.6
Cord blood	16.7% (2/12)	30% (3/10)	13.3	(-20.7, 45.9)	0.46
Peripheral blood	10% (24/241)	17.9% (21/117)	8	(0.6, 16.5)	0.032
Donor HCMV Serostatus					
Negative	13.8% (17/123)	22.2% (16/72)	8.4	(-2.4, 20.4)	0.13
Positive	9% (18/199)	12.2% (12/98)	3.2	(-3.8, 11.8)	0.39
Unknown	0% (0/3)	0% (0/0)			
Total Deaths including Additional Deaths Identified by the Sponsor	12.3% (40/325)	18.8% (32/170)	6.5	(0, 13.8)	0.051

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One of the subjects developed HCMV end-organ disease.

Table 20: All-cause Mortality at Week 24 (ITT)

	Letermovir (n=373)	Placebo (n=192)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	11% (41/373)#	15.6% (30/192)#	4.6	(-1.1, 11.1)	0.12
Risk Stratification					
High Risk	13.3% (16/120)	23.1% (12/52)	9.7	(-2.1, 23.7)	0.11
Low Risk	9.9% (25/253)	12.9% (18/140)	3	(-3.3, 10.2)	0.37
Stem cell source					
Bone marrow	12.2% (10/82)	8.5% (4/47)	-3.7	(-13.9, 9)	0.52
Cord blood	16.7% (2/12)	27.3% (3/11)	10.6	(-22.5, 42.2)	0.54
Peripheral blood	10.4% (29/279)	17.2% (23/134)	6.8	(-0.1, 14.7)	0.052
Donor HCMV Serostatus					
Negative	12.9% (18/139)	20.5% (16/78)	7.6	(-2.4, 18.8)	0.14
Positive	9.6% (22/229)	12.3% (14/114)	2.7	(-3.9, 10.6)	0.45
Unknown	20% (1/5)	0% (0/0)			

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One of the subjects developed HCMV end-organ disease.

Table 21: All-cause Mortality at Week 24 (non-FAS)

	Letermovir (n=48)	Placebo (n=22)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	12.5% (6/48)	9.1% (2/22)	-3.4	(-17.3, 16.5)	0.68
Risk Stratification					
High Risk	10.5% (2/19)	12.5% (1/8)	2	(-21.3, 37.4)	0.88
Low Risk	13.8% (4/29)	7.1% (1/14)	-6.7	(-24.4, 19.1)	0.52
Stem cell source					
Bone marrow	10% (1/10)	0% (0/4)	-10	(-40.4, 39.7)	0.51
Cord blood	0/0 (0%)	0% (0/1)			
Peripheral blood	13.2% (5/38)	11.8% (2/17)	-1.4	(-17.9, 22.4)	0.89
Donor HCMV Serostatus					

	Letermovir (n=48)	Placebo (n=22)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Negative	6.3% (1/16)	0% (0/6)	-6.3	(-28.3, 33.1)	0.53
Positive	13.3% (4/30)	12.5% (2/16)	-0.8	(-19.5, 24)	0.94
Unknown	1/2 (50%)	0/0 (0%)			

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

The median time to PET in the FAS population was 146 days and 42 days in the letermovir and placebo arms, respectively, for subjects whose death occurred by Week 24 (Table 22, FDA analysis). Based on the sponsor's stratified log-rank test, the distribution of time to initiation of PET was statistically significant (two-sided p-value = 0.0003; please see the review of the statistical reviewer for confirmation of this value). Additionally, the median viral load around the time of PET initiation in the FAS population for subjects whose death occurred by Week 24 was lower in the letermovir group (696 IU/mL) compared to the placebo group (1051.7 IU/mL). In contrast the median time to death was similar between the two groups, likely due to the complexity of this population where there are many different causes of death. Similar observations were made in the ITT population. Interestingly, the median time to PET in the FAS population was 98 days and 37 days in the letermovir and placebo arms, respectively, for subjects whose death occurred after Week 24. Furthermore, the median time to PET was longer in subjects whose death occurred before Week 24 as compared to those that occurred after Week 24. A similar trend was observed in the ITT population. The significance of this difference is unclear. Furthermore, given that there were 28 subjects who experienced relapse in the letermovir arm after 14 weeks of treatment and the median time to death was 104 days, longer treatment of letermovir beyond 14 weeks may be beneficial. Indeed, extending prophylaxis with vGCV to 200 days was demonstrated to be significantly better than 100 days for the prevention of HCMV disease in high risk (donor HCMV seropositive [D+]/recipient HCMV seronegative [R-]) adult kidney transplant recipients (Humar et al., 2010).

Table 22: Median Day to Death

Death by Week 24 (FAS)					
Letermovir (n=35)			Placebo (n=28)		
Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)	Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)
104 (range 15-171)	146 (range 25-148)	696 (range 137-9975)	112.5 (range 16-174)	42 (range 24-85)	1051.7 (range 137-36422)
Death by Week 24 (ITT)					
Letermovir (n=41)			Placebo (n=30)		
Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)	Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)
104 (range 15-171)	127 (range 25-148)	3130 (range 137-9975)	112.5 (range 16-174)	42 (range 24-85)	730.2 (range 137-36422)
Death after Week 24 through Week 48 (FAS)					
Letermovir (n=33)			Placebo (n=14)		
Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)	Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)
241 (range 185-387)	98 (range 58-128)	327.4 (range 137-39397)	228.5 (range 183-368)	37 (range 16-54)	331.5 (range 137-10299)
Death after Week 24 through Week 48 (ITT)					
Letermovir (n=38)			Placebo (n=17)		
Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)	Median Day to Death	Median Day to PET	Median VL around time to PET (IU/mL)
245 (range 185-387)	102.5 (range 46-140)	399.6 (range 137-39397)	238 (range 183-368)	36 (range 16-54)	331.5 (range 137-10299)

3.4.3. HCMV-related Mortality

As stated above, there are no definitive data at this time to prove suppression of HCMV DNaemia predicts improved survival. Therefore, the Division has recommended using HCMV DNAemia as a surrogate endpoint in studies designed to support accelerated approval. The sponsor's study has met the primary endpoint using the unvalidated surrogate endpoint of HCMV DNaemia. Therefore, the sponsor's study has met the criteria for accelerated approval. However, given that there was a statistically significant reduction in mortality, the Division is considering whether letermovir can be given a traditional approval. The challenge is the HCMV DNaemia is an unvalidated surrogate which has not consistently predicted HCMV disease. Furthermore, the rate of HCMV end-organ disease was similar and low in both arms (1.5% and 1.8% in the letermovir and placebo arms, respectively, see Table 13). Therefore, it is unclear how letermovir is reducing mortality if not through impact on HCMV end organ disease and whether the difference in mortality is directly due to letermovir.

To explore whether the differences that were observed in the virologic endpoints and the mortality endpoints are related, several sub-group analyses were conducted. The incidence of all-cause mortality in subjects who met the primary endpoint (i.e., subjects who received PET) was 0.9% (3/325) and 8.2% (14/170) in the letermovir arm and placebo arm, respectively, in the FAS population (Table 23, FDA analysis). The difference was statistically significant ($p<0.0001$). There were several subjects who had detectable HCMV DNA around the time of all-cause mortality but did not receive PET (see Table A1-1 & Table A1-2 in the Appendix). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study was 1.8% (6/325) and 10.0% (17/170) in the letermovir arm and placebo arm, respectively, which was also statistically significant ($p<0.0001$). The incidence of all-cause mortality excluding the subjects who had detectable HCMV DNA was comparable between the letermovir (8.9%, 29/325) and placebo arms (6.5%, 11/170).

This is a very sick population with various co-morbidities and their death could be the result of other co-morbidities. Using the detection of HCMV DNA only may be an overestimate of the number of subjects whose death is associated with HCMV given the study protocol's low threshold for initiating PET. Indeed, there are several subjects whose date of PET initiation and date of death are several weeks apart. For example, subject 8228-001_001800018 initiated PET on Day 62; the subject's HCMV viral load was 350 IU/mL and 914 IU/mL on days 60 and 64, respectively. Upon receiving PET, the subject achieved HCMV viral load TND by Day 82. The subject's HCMV viral load remained TND until the subject's death on day 136. Given the time gap between these events, this subject's death is less likely due to the direct associated with HCMV. Therefore, the subjects whose day of PET and day of death was within 39 days were evaluated in order to account for subjects whose death is most likely directly attributable to HCMV. The window of 39 days was chosen because there were 6 subjects whose death occurred whose window was between 28 days and 39 days (Table A1-1 & Table A1-2 in the Appendix). The next window after this group was 74 days between day of PET and day of death. The incidence of all-cause mortality in subjects whose day of PET and day of death was within 39 days was 0.6% (2/325) and 2.9% (5/170) in the letermovir arm and placebo arm, respectively ($p=0.037$). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study and day of all-cause mortality was within 39 days was 1.5% (5/325) and 4.7% (8/170) in the letermovir arm and placebo arm, respectively ($p=0.036$). The incidence of all-cause mortality excluding these subjects was comparable between the letermovir (9.2%, 30/325) and placebo arms (11.8%, 20/170). As stated above, there was one subject in each of the letermovir and placebo arms who died and also developed HCMV end-organ disease.

Table 23: HCMV-related Mortality at Week 24 (FAS)

	Letermovir (N=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	10.8% (35/325)#	16.5% (28/170)#	5.7	(-0.5, 12.7)	0.071
Death and Positive DNaemia (PET)	0.9% (3/325)	8.2% (14/170)	7.3	(3.6, 12.5)	<0.0001
Death and Positive DNaemia (all)	1.8% (6/325)	10% (17/170)	8.2	(3.9, 13.7)	<0.0001
Death and PET within 39 days	0.6% (2/325)	2.9% (5/170)	2.3	(0, 6.1)	0.037
Death and Positive DNaemia within 39 days	1.5% (5/325)	4.7% (8/170)	3.2	(0.1, 7.6)	0.036
Death without DNaemia (PET)	9.8% (32/325)	8.2% (14/170)	-1.6	(-6.6, 4.2)	0.56
Death without DNaemia (all)	8.9% (29/325)	6.5% (11/170)	-2.5	(-7, 3)	0.34
Death excluding PET within 39 days	10.2% (33/325)	13.5% (23/170)	3.4	(-2.4, 10)	0.26
Death excluding Positive DNaemia within 39 days	9.2% (30/325)	11.8% (20/170)	2.5	(-2.9, 8.8)	0.37

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One of the subjects developed HCMV end-organ disease.

Similar trends were observed in the ITT population (Table 24, FDA analysis). The incidence of all-cause mortality in subjects who met the primary endpoint (i.e., subjects who received PET) was 1.1% (4/373) and 8.3% (16/192) in the letermovir arm and placebo arm, respectively (p<0.0001). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study was 2.1% (8/373) and 9.9% (19/192) in the letermovir arm and placebo arm, respectively (p<0.0001).

The incidence of all-cause mortality in subjects whose day of PET and day of death was within 39 days was 0.8% (3/373) and 3.1% (6/192) in the letermovir arm and placebo arm, respectively (p=0.037). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study and day of death was within 39 days was 1.9% (7/373) and 4.7% (9/192) in the letermovir arm and placebo arm, respectively (p=0.056). The incidence of all-cause mortality excluding these subjects was comparable between the letermovir (9.1%, 34/373) and placebo arms (10.9%, 21/192).

Table 24: HCMV-related Mortality at Week 24 (ITT)

	Letermovir (n=373)	Placebo (n=192)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	11% (41/373)#	15.6% (30/192)#	4.6	(-1.1, 11.1)	0.12
Death and Positive DNaemia (PET)	1.1% (4/373)	8.3% (16/192)	7.3	(3.7, 12.1)	<0.0001
Death and Positive DNaemia (all)	2.1% (8/373)	9.9% (19/192)	7.8	(3.7, 12.9)	<0.0001
Death and PET within 39 days	0.8% (3/373)	3.1% (6/192)	2.3	(0, 5.9)	0.037
Death and Positive DNaemia within 39 days	1.9% (7/373)	4.7% (9/192)	2.8	(-0.1, 6.9)	0.056
Death without DNaemia (PET)	9.9% (37/373)	7.3% (14/192)	-2.6	(-7.1, 2.7)	0.3
Death without DNaemia (all)	8.8% (33/373)	5.7% (11/192)	-3.1	(-7.3, 1.8)	0.19
Death excluding PET within 39 days	10.2% (38/373)	12.5% (24/192)	2.3	(-3, 8.4)	0.4
Death excluding Positive DNaemia within 39 days	9.1% (34/373)	10.9% (21/192)	1.8	(-3.1, 7.6)	0.49

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One of the subjects developed HCMV end-organ disease.

There were no obvious trends in the non-FAS population (Table 25, FDA analysis).

Table 25: HCMV-related Mortality at Week 24 (non-FAS)

	Letermovir (n=48)	Placebo (n=22)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	12.5% (6/48)	9.1% (2/22)	-3.4	(-17.3, 16.5)	0.68
Death and Positive DNaemia (PET)	2.1% (1/48)	9.1% (2/22)	7	(-4, 25.8)	0.18

	Letermovir (n=48)	Placebo (n=22)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Death and Positive DNaemia (all)	4.2% (2/48)	9.1% (2/22)	4.9	(-6.9, 23.9)	0.41
Death and PET within 39 days	2.1% (1/48)	4.5% (1/22)	2.5	(-7.1, 19.8)	0.57
Death and Positive DNaemia within 39 days	4.2% (2/48)	4.5% (1/22)	0.4	(-10.1, 17.9)	0.94
Death without DNaemia (PET)	10.4% (5/48)	0% (0/22)	-10.4	(-22.2, 5.6)	0.12
Death without DNaemia (all)	8.3% (4/48)	0% (0/22)	-8.3	(-19.6, 7.4)	0.16
Death excluding PET within 39 days	10.4% (5/48)	4.5% (1/22)	-5.9	(-18.2, 12.4)	0.42
Death excluding Positive DNaemia within 39 days	8.3% (4/48)	4.5% (1/22)	-3.8	(-15.6, 14.2)	0.57

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

The complete data through Week 48 post-transplant are not yet available as this study is ongoing. The sponsor provided all available data through database lock (when ~90% of subjects had completed the trial or discontinued early).

The cumulative incidence of all-cause mortality was numerically lower in the letermovir group (20.9%, 68/325) compared to the placebo group (24.7%, 42/170) through Week 48 post-transplant in the FAS population (Table 26, FDA analysis). Importantly, these differences were still observed when stratified by different subgroup categories, although the numbers were not powered to detect statistical differences. Similar trends were also seen for the all-subjects-as-treated population (ITT) (Table 27, FDA analysis). Of note, there were 3 and 1 additional subjects in the letermovir and placebo arms, respectively, who died after Week 48. Importantly, one of the subject in the letermovir arm also developed HCMV end-organ disease. As stated above, in response to the Division's request, the vital statuses were obtained for 58 of the 76 subjects who prematurely withdrew from the study and were submitted during the NDA review. The cumulative incidence of all-cause mortality which includes these additional deaths at Week 48 was numerically lower in the letermovir group (24.3%, 79/325) compared to the placebo group (28.2%, 48/170) through Week 48 post-transplant (p = 0.34).

While the difference in incidence of all-cause mortality was statistically significant at Week 24, it was not maintained through Week 48 with the p-value = 0.18 for the FAS population. The sponsor has also conducted a statistical analysis using the Kaplan-Meier method. According to the sponsor, the Kaplan-Meier method also did not result in a statistically significant difference in the incidence of all-cause mortality for the FAS population with the nominal two-sided stratified log-rank p-value = 0.12 (please see the review of the statistical reviewer for confirmation of this p-value). Of note, there were 1 and 3 subjects in the letermovir and placebo arms, respectively, who developed HCMV end-organ disease through Week 48. In this reviewer's opinion, a lack of statistically significant differences in all-cause mortality at Week 48 is not a major concern given that there are many causes of deaths in this patient population. For this reason, the Division of Hematology Products generally does not use all-cause mortality as an efficacy endpoint for supportive care drugs such as GVHD prophylaxis (per discussion between the clinical review team and the Division of Hematology Products; please see the clinical review of Aimee Hodowanec, M.D. for more details).

Table 26: All-cause Mortality at Week 48 (FAS)

	Letermovir (N=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	20.9% (68/325)#	24.7% (42/170)#	3.8	(-3.8, 11.9)	0.34
Risk Stratification					
High Risk	24.8% (25/101)	31.8% (14/44)	7.1	(-7.9, 23.6)	0.38
Low Risk	19.2% (43/224)	22.2% (28/126)	3	(-5.5, 12.3)	0.5

	Letermovir (N=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Stem cell source					
Bone marrow	26.4% (19/72)	18.6% (8/43)	-7.8	(-22.1, 8.8)	0.34
Cord blood	16.7% (2/12)	40% (4/10)	23.3	(-13.1, 54.5)	0.22
Peripheral blood	19.5% (47/241)	25.6% (30/117)	6.1	(-2.8, 15.8)	0.18
Donor HCMV Serostatus					
Negative	21.1% (26/123)	34.7% (25/72)	13.6	(0.8, 26.7)	0.037
Positive	20.1% (40/199)	17.3% (17/98)	-2.8	(-11.5, 7.3)	0.57
Unknown	66.7% (2/3)	0% (0/0)			
Total Deaths including Additional Deaths Identified by the Sponsor	24.3% (79/325)	28.2% (48/170)	3.9	(-4, 12.3)	0.34

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One and three of the subjects in the letermovir and placebo arms, respectively, developed HCMV end-organ disease.

Table 27: All-cause Mortality at Week 48 (ITT)

	Letermovir (n=373)	Placebo (n=192)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	21.2% (79/373)#	24.5% (47/192)#	3.3	(-3.8, 10.9)	0.37
Risk Stratification					
High Risk	24.2% (29/120)	28.8% (15/52)	4.7	(-8.8, 19.7)	0.52
Low Risk	19.8% (50/253)	22.9% (32/140)	3.1	(-5.1, 11.9)	0.47
Stem cell source					
Bone marrow	25.6% (21/82)	19.1% (9/47)	-6.5	(-20, 9.2)	0.4
Cord blood	16.7% (2/12)	36.4% (4/11)	19.7	(-15.5, 50.4)	0.28
Peripheral blood	20.1% (56/279)	25.4% (34/134)	5.3	(-3, 14.4)	0.22
Donor HCMV Serostatus					
Negative	20.1% (28/139)	33.3% (26/78)	13.2	(1.2, 25.7)	0.031
Positive	21% (48/229)	18.4% (21/114)	-2.5	(-10.9, 6.9)	0.58
Unknown	60% (3/5)	0% (0/0)			

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One and three of the subjects in the letermovir and placebo arms, respectively, developed HCMV end-organ disease..

The incidence of all-cause mortality in subjects who received PET in the FAS population was 2.8% (9/325) and 13.5% (23/170) in the letermovir arm and placebo arm, respectively (p<0.0001) (Table 28, FDA analysis). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study was 4.0% (13/325) and 15.3% (26/170) in the letermovir arm and placebo arm, respectively (p<0.0001).

The incidence of all-cause mortality in subjects whose day of PET and day of death was within 39 days was 0.6% (2/325) and 2.9% (5/170) in the letermovir arm and placebo arm, respectively (p=0.037). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study and day of death was within 39 days was 1.8% (6/325) and 4.7% (8/170) in the letermovir arm and placebo arm, respectively (p=0.068). The incidence of all-cause mortality excluding these subjects was comparable between the letermovir (19.1%, 62/325) and placebo arms (20%, 34/170).

Table 28: HCMV-related Mortality at Week 48 (FAS)

	Letermovir (N=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	20.9% (68/325)#	24.7% (42/170)#	3.8	(-3.8, 11.9)	0.34
Death and Positive DNaemia (PET)	2.8% (9/325)	13.5% (23/170)	10.8	(5.8, 16.9)	<0.0001
Death and Positive DNaemia (all)	4% (13/325)	15.3% (26/170)	11.3	(5.9, 17.7)	<0.0001

	Letermovir (N=325)	Placebo (n=170)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Death and PET within 39 days	0.6% (2/325)	2.9% (5/170)	2.3	(0, 6.1)	0.037
Death and Positive DNaemia within 39 days	1.8% (6/325)	4.7% (8/170)	2.9	(-0.3, 7.3)	0.068
Death without DNaemia (PET)	18.2% (59/325)	11.2% (19/170)	-7	(-13, -0.2)	0.043
Death without DNaemia (all)	16.9% (55/325)	9.4% (16/170)	-7.5	(-13.2, -1)	0.024
Death excluding PET within 39 days	20.3% (66/325)	21.8% (37/170)	1.5	(-5.8, 9.3)	0.7
Death excluding Positive DNaemia within 39 days	19.1% (62/325)	20% (34/170)	0.9	(-6.1, 8.6)	0.81

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
#One and three of the subjects in the letermovir and placebo arms, respectively, developed HCMV end-organ disease.

The incidence of all-cause mortality in subjects who received PET in the ITT population was 3.8% (14/373) and 14.1% (27/192) in the letermovir arm and placebo arm, respectively (p<0.0001) (Table 29, FDA analysis). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study was 5.1% (19/373) and 16.1% (31/192) in the letermovir arm and placebo arm, respectively (p<0.0001).

The incidence of all-cause mortality in subjects whose day of PET and day of death was within 39 days was 0.8% (3/373) and 3.1% (6/192) in the letermovir arm and placebo arm, respectively (p=0.037). The incidence of all-cause mortality in subjects who had detectable HCMV at any time during the study and day of death was within 39 days was 2.1% (8/373) and 5.2% (10/192) in the letermovir arm and placebo arm, respectively (p=0.05). The incidence of all-cause mortality excluding these subjects was comparable between the letermovir (19%, 71/373) and placebo arms (19.3%, 37/192).

Table 29: HCMV-related Mortality at Week 48 (ITT)

	Letermovir (n=373)	Placebo (n=192)	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Total	21.2% (79/373)#	24.5% (47/192)#	3.3	(-3.8, 10.9)	0.37
Death and Positive DNaemia (PET)	3.8% (14/373)	14.1% (27/192)	10.3	(5.4, 16.1)	<0.0001
Death and Positive DNaemia (all)	5.1% (19/373)	16.1% (31/192)	11.1	(5.8, 17.2)	<0.0001
Death and PET within 39 days	0.8% (3/373)	3.1% (6/192)	2.3	(0, 5.9)	0.037
Death and Positive DNaemia within 39 days	2.1% (8/373)	5.2% (10/192)	3.1	(0, 7.3)	0.05
Death without DNaemia (PET)	17.4% (65/373)	10.4% (20/192)	-7	(-12.5, -0.8)	0.027
Death without DNaemia (all)	16.1% (60/373)	8.3% (16/192)	-7.8	(-12.9, -1.9)	0.011
Death excluding PET within 39 days	20.4% (76/373)	21.4% (41/192)	1	(-5.8, 8.3)	0.79
Death excluding Positive DNaemia within 39 days	19% (71/373)	19.3% (37/192)	0.2	(-6.3, 7.4)	0.95

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).
One and three of the subjects in the letermovir and placebo arms, respectively, developed HCMV end-organ disease.

The incidence of all-cause mortality after Week 24 in the ITT population was comparable between the letermovir (10.2%, 38/373) and placebo arms (8.9%, 17/192) (Table 30, FDA analysis). The incidence of all-cause mortality after Week 24 in the FAS population was also comparable between the letermovir (10.2%, 33/325) and placebo arms (8.2%, 14/170). The difference in the incidence of all-cause mortality in subjects who received PET between the treatment arms was smaller (7.3% at Week 24 [Table 23] vs 3.4% after Week 24). Importantly, there were no subjects in either arm whose day of PET and day of death was within 39 days. These results indicate that the impact of letermovir on HCMV-related mortality was greater through the first 24 weeks compared to the 24 weeks after. These results are consistent with reports that the risk of HCMV related disease/death is highest in the first few months after transplant and decrease over time (Boeckh et al., 2003; Ljungman et al., 2011).

Table 30: All-cause Mortality after Week 24

	Letermovir	Placebo	Δ (%) [Placebo-Letermovir]	95% CI	p value*
Death after Week 24 (ITT)					
Total	10.2% (38/373)	8.9% (17/192)	-1.3	(-6.1, 4.2)	0.61
Death and Positive DNaemia (PET)	2.7% (10/373)	5.7% (11/192)	3	(-0.3, 7.5)	0.07
Death and Positive DNaemia (all)	2.9% (11/373)	6.3% (12/192)	3.3	(-0.2, 7.8)	0.06
Death and PET within 39 days	0% (0/373)	0% (0/192)	0	(-1, 2)	
Death and Positive DNaemia within 39 days	0.3% (1/373)	0.5% (1/192)	0.3	(-1.1, 2.6)	0.63
Death without DNaemia (PET)	7.5% (28/373)	3.1% (6/192)	-4.4	(-7.9, -0.2)	0.038
Death without DNaemia (all)	7.2% (27/373)	2.6% (5/192)	-4.6	(-8.1, -0.6)	0.024
Death excluding PET within 39 days	10.2% (38/373)	8.9% (17/192)	-1.3	(-6.1, 4.2)	0.61
Death excluding Positive DNaemia within 39 days	9.9% (37/373)	8.3% (16/192)	-1.6	(-6.3, 3.9)	0.54
Death after Week 24 (FAS)					
Total	10.2% (33/325)	8.2% (14/170)	-1.9	(-6.9, 3.9)	0.49
Death and Positive DNaemia (PET)	1.8% (6/325)	5.3% (9/170)	3.4	(0.2, 8)	0.034
Death and Positive DNaemia (all)	2.2% (7/325)	5.9% (10/170)	3.7	(0.3, 8.5)	0.031
Death and PET within 39 days	0% (0/325)	0% (0/170)	0	(-1.2, 2.2)	
Death and Positive DNaemia within 39 days	0.3% (1/325)	0.6% (1/170)	0.3	(-1.2, 3)	0.64
Death without DNaemia (PET)	8.3% (27/325)	2.9% (5/170)	-5.4	(-9.3, -0.8)	0.021
Death without DNaemia (all)	8% (26/325)	2.4% (4/170)	-5.6	(-9.4, -1.3)	0.012
Death excluding PET within 39 days	10.2% (33/325)	8.2% (14/170)	-1.9	(-6.9, 3.9)	0.49
Death excluding Positive DNaemia within 39 days	9.8% (32/325)	7.6% (13/170)	-2.2	(-7.1, 3.5)	0.42

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

HCMV DNaemia has also been reported to be associated with non-relapse mortality (Green et al., 2016). Therefore, if the difference in mortality that was observed in this study was due to the difference that was observed in the HCMV DNaemia, one would expect a greater impact in non-relapse mortality. Non-relapse mortality in this study is defined as mortality not related to the underlying condition leading to transplant. Based on the primary reason for transplant and AE which led to death, the sponsor identified the relapse deaths and those deaths not identified as relapse are defined as non-relapse. The incidence of non-relapse death through Week 24 in the FAS population were 6.8% (22/325) and 11.2% (19/170) in the letermovir and placebo arms, respectively, resulting in a difference of 4.4% between the treatment arms (Table 31, FDA analysis). In comparison, the incidence of relapse death through Week 24 in the FAS population were 4% (13/325) and 5.9% (10/170) in the letermovir and placebo arms, respectively, resulting in a difference of 1.9% between the treatment arms. That the differences was smaller between the treatment arms after Week 24 is also consistent with risk of HCMV related mortality decreasing over time. Of note, the distribution of death due to relapse and non-relapse changed between death through Week 24 and death after Week 24 in the letermovir arm but not in the placebo arm (Table 32, FDA analysis). However, these numbers are too small to make any formal conclusions.

Table 31: Rate of Relapse and Non-relapse Mortality

	Letermovir		Placebo	
FAS				
	Non-relapse Death	**Death due to Relapse**	**Non-relapse Death**	**Death due to Relapse**
Death by Week 24	6.8% (22/325)	4% (13/325)	11.2% (19/170)	5.9% (10/170)
Death between Week 24 and Week 48	5.5% (18/325)	4.6% (15/325)	4.7% (8/170)	2.9% (5/170)
ITT				
	Non-relapse Death	**Death due to Relapse**	**Non-relapse Death**	**Death due to Relapse**
Death by Week 24	6.7% (25/373)	4.3% (16/373)	9.9% (19/192)	5.7% (11/192)
Death between Week 24 and Week 48	5.6% (21/373)	4.6% (17/373)	5.2% (10/192)	3.6% (7/192)

Table 32: Proportions of Relapse and Non-relapse Mortality

	Letermovir		Placebo	
FAS				
	Non-relapse Death	**Death due to Relapse**	**Non-relapse Death**	**Death due to Relapse**
Death by Week 24	62.9% (22/35)	37.1% (13/35)	64.3% (18/28)	35.7% (10/28)
Death between Week 24 and Week 48	54.5% (18/33)	45.5% (15/33)	64.3% (9/14)	35.7% (5/14)
ITT				
	Non-relapse Death	**Death due to Relapse**	**Non-relapse Death**	**Death due to Relapse**
Death by Week 24	61% (25/41)	39% (16/41)	63.3% (19/30)	36.7% (11/30)
Death between Week 24 and Week 48	55.3% (21/38)	44.7% (17/38)	58.8% (10/17)	41.2% (7/17)

3.4.4. Incidence of Opportunistic Infections

In this study, an opportunistic infection was considered to be any infection that, in the opinion of the investigator, would be considered an opportunistic infection in the HSCT setting. This included, but was not limited to, serious bacterial or invasive fungal infections, Epstein-Barr virus post-transplant lymphoproliferative disease (EBV PTLD), respiratory syncytial virus (RSV) pneumonia, parainfluenza virus pneumonia, adenovirus disease, *Pneumocystis jirovecii* pneumonia (PJP), human herpes virus (HHV)-6 encephalitis and toxoplasmosis.

The proportion of subjects in the FAS population with adverse events likely due to bacterial, fungal, and viral opportunistic infections was similar for the letermovir (70.5%, 229/325) and placebo (63.5%, 108/170) groups through Week 24 post-transplant (Table 33, FDA analysis). The proportions were also similar for the subjects with adverse events likely due to opportunistic infections who died by Week 24 post-transplant (8% [26/325] and 10.6% [18/170] in the letermovir and placebo arms, respectively). As the overall list of all reported opportunistic infections contained a number of infections that may not be clinically relevant, a subgroup analysis of only serious adverse events due to opportunistic infections was conducted. The proportion of subjects in the FAS population with serious adverse events due to opportunistic infections was similar for the letermovir (24%, 78/325) and placebo (22.4%, 38/170) groups through Week 24 post-transplant. The proportion of subjects in the FAS population with serious adverse events likely due to opportunistic infections was 6.5% (21/325) and 7.1% (12/170) for the letermovir and placebo arms, respectively, through Week 24 post-transplant. Similar trends were observed in the ITT population. The individual subject data are summarized in Appendix Table A1-6 & Table A1-7.

Table 33: Summary of the Proportion of Subjects with Adverse Events Likely Due to Bacterial and/or Fungal and/or Viral Opportunistic Infections through Week 24 Post Transplant

	Letermovir	Placebo	Δ (%) [Placebo-Letermovir]	95% CI	p value*
FAS					
Subjects with Opportunistic Infections	70.5% (229/325)	63.5% (108/170)	-6.9	(-15.7, 1.7)	0.12
Subjects with Opportunistic Infections and Death by Wk 24	8% (26/325)	10.6% (18/170)	2.6	(-2.5, 8.6)	0.34
Subjects with Opportunistic Infections and Death after Wk 24	9.2% (30/325)	7.1% (12/170)	-2.2	(-6.9, 3.4)	0.41
Subjects with Serious Opportunistic Infections	24% (78/325)	22.4% (38/170)	-1.6	(-9.1, 6.4)	0.68
Subjects with Serious Opportunistic Infections and Death by Wk 24	6.5% (21/325)	7.1% (12/170)	0.6	(-3.8, 5.9)	0.8
Subjects with Serious Opportunistic Infections and Death after Wk 24	5.5% (18/325)	2.9% (5/170)	-2.6	(-6.1, 1.7)	0.19
Subjects with Opportunistic Infections within 14 days till death by Wk 24	4% (13/325)	6.5% (11/170)	2.5	(-1.4, 7.5)	0.22
Subjects with Opportunistic Infections within 14 days till death after Wk 24	2.2% (7/325)	0.6% (1/170)	-1.6	(-3.8, 1.3)	0.19
Subjects with Serious Opportunistic Infections within 14 days till death by Wk 24	2.8% (9/325)	6.5% (11/170)	3.7	(0, 8.6)	0.047
Subjects with Serious Opportunistic Infections within 14 days till death after Week 24	2.2% (7/325)	0.6% (1/170)	-1.6	(-3.8, 1.3)	0.19
ITT					
Subjects with Opportunistic Infections	69.2% (258/373)	62.5% (120/192)	-6.7	(-15, 1.5)	0.11
Subjects with Opportunistic Infections and Death by Wk 24	8.3% (31/373)	10.4% (20/192)	2.1	(-2.7, 7.8)	0.41
Subjects with Opportunistic Infections and Death after Wk 24	9.4% (35/373)	7.3% (14/192)	-2.1	(-6.5, 3.1)	0.4
Subjects with Serious Opportunistic Infections	24.7% (92/373)	22.4% (43/192)	-2.3	(-9.3, 5.3)	0.55
Subjects with Serious Opportunistic Infections and Death by Wk 24	6.7% (25/373)	6.8% (13/192)	0.1	(-4, 5)	0.98
Subjects with Serious Opportunistic Infections and Death after Wk 24	5.9% (22/373)	3.1% (6/192)	-2.8	(-6.1, 1.3)	0.15
Subjects with Opportunistic Infections within 14 days till death by Wk 24	4.6% (17/373)	5.7% (11/192)	1.2	(-2.4, 5.7)	0.54
Subjects with Opportunistic Infections within 14 days till death after Wk 24	2.4% (9/373)	0.5% (1/192)	-1.9	(-4, 0.7)	0.11
Subjects with Serious Opportunistic Infections within 14 days till death by Wk 24	3.5% (13/373)	5.7% (11/192)	2.2	(-1.2, 6.7)	0.21
Subjects with Serious Opportunistic Infections within 14 days till death after Week 24	2.4% (9/373)	0.5% (1/192)	-1.9	(-4, 0.7)	0.11

*Differences between proportions and their 95% confidence intervals were calculated without adjustments using the Newcombe-Wilson hybrid score procedure without continuity correction (Newcombe, 1998).

The use of acyclovir, valacyclovir and famciclovir at prophylactic doses for herpes simplex virus (HSV) or varicella zoster virus (VZV) infections was allowed in this study. Sites were instructed to specifically report the use of these antivirals for HSV/VZV prophylaxis. Acyclovir at doses >3200 mg PO per day or 25 mg/kg IV per day, valacyclovir at doses >3000 mg PO per day and famiciclovir at dose >1500 mg PO per day, were prohibited if the subject received these medications within 7 days of screening or planned to receive them during the study. The distribution of use of these agents across treatment groups was similar between the two treatment groups (Table 34, adapted from Table 14.1-14 of study report p001v01, pg. 524). The most frequently used antiviral agent for HSV/VZV prophylaxis was acyclovir. That the distribution of the use of acyclovir is similar between the two treatment groups is of particular importance as acyclovir has demonstrated to have some antiviral activity against HCMV in cell culture (EC_{50} value = 44.4 μM - 111 μM; Meyers et al., 1983).

Table 34: Antivirals used For Prior HSV Prophylaxis

	Letermovir	Placebo
FAS		
HSV/VZV Prophylaxis	96.6% (314/325)	93.5% (159/170)
Acyclovir	83.7% (272/325)	78.8% (134/170)
Famciclovir	2.8% (9/325)	1.8% (3/170)
Valacyclovir	27.1% (88/325)	25.3% (43/170)
ITT		
HSV/VZV Prophylaxis*	96.8% (361/373)	88.5% (170/192)
Acyclovir	83.4% (311/373)	79.2% (152/192)
Famciclovir	2.4% (9/373)	2.1% (4/192)
Valacyclovir	26.8% (100/373)	24.5% (47/192)

*Every subject is counted a single time for each applicable specific medication. Medication may be reported at any time during the study.

3.4.5. Graft-versus-host Disease (GVHD)

HCMV and GVHD frequently go together and is reported to be associated with worse outcomes overall (Cantoni et al., 2010). The management of HCMV often requires reduction of immunosuppression to help clear the infection. Conversely, for GVHD, the standard treatment is increased immunosuppression which exacerbates HCMV. The hypothesis of whether letermovir may have prevented the indirect effects of HCMV infection and the unintended consequences of HCMV treatment such as reduction in immunosuppression which could lead to GVHD was explored. The Glucksberg grading system (Glucksberg et al., 1974) was used by investigators for grading of acute GVHD in this study. Through Week 48 in the FAS group, the proportion of subjects who developed GVHD was numerically lower in the letermovir group (57.2%, 186/325), compared to the placebo group (60.6%, 103/170) (Table 35, FDA analysis). Subjects with ≥Grade II of GVHD comprised a numerically lower proportion of subjects in the letermovir group (29.8%, 97/325) compared to the placebo group (32.4%, 55/170). Similar trends were observed in the ITT group. In the placebo arm, through week 48, the rate of mortality was higher in subjects with GVHD who had detectable HCMV DNaemia (34.8%, 16/46) compared to those who did not (14%, 8/57) which supports the hypothesis. However, in the letermovir arm, through week 48, the rate of mortality was similar in subjects with GVHD who had detectable HCMV DNaemia (18.2%, 6/33) compared to those who did not (20.3%, 31/153). A possible explanation is that while the median viral load was similar between the two arms in subjects who had detectable HCMV DNaemia, there were numerically more subjects with higher viral load in the placebo arm which would have require longer PET treatment and/or changes to immunosuppression. The difference in viral load was even more apparent in subjects with ≥Grade II of GVHD.

Table 35: Summary of the Proportion of Subjects with Graft-Versus-Host-Disease (GVHD)

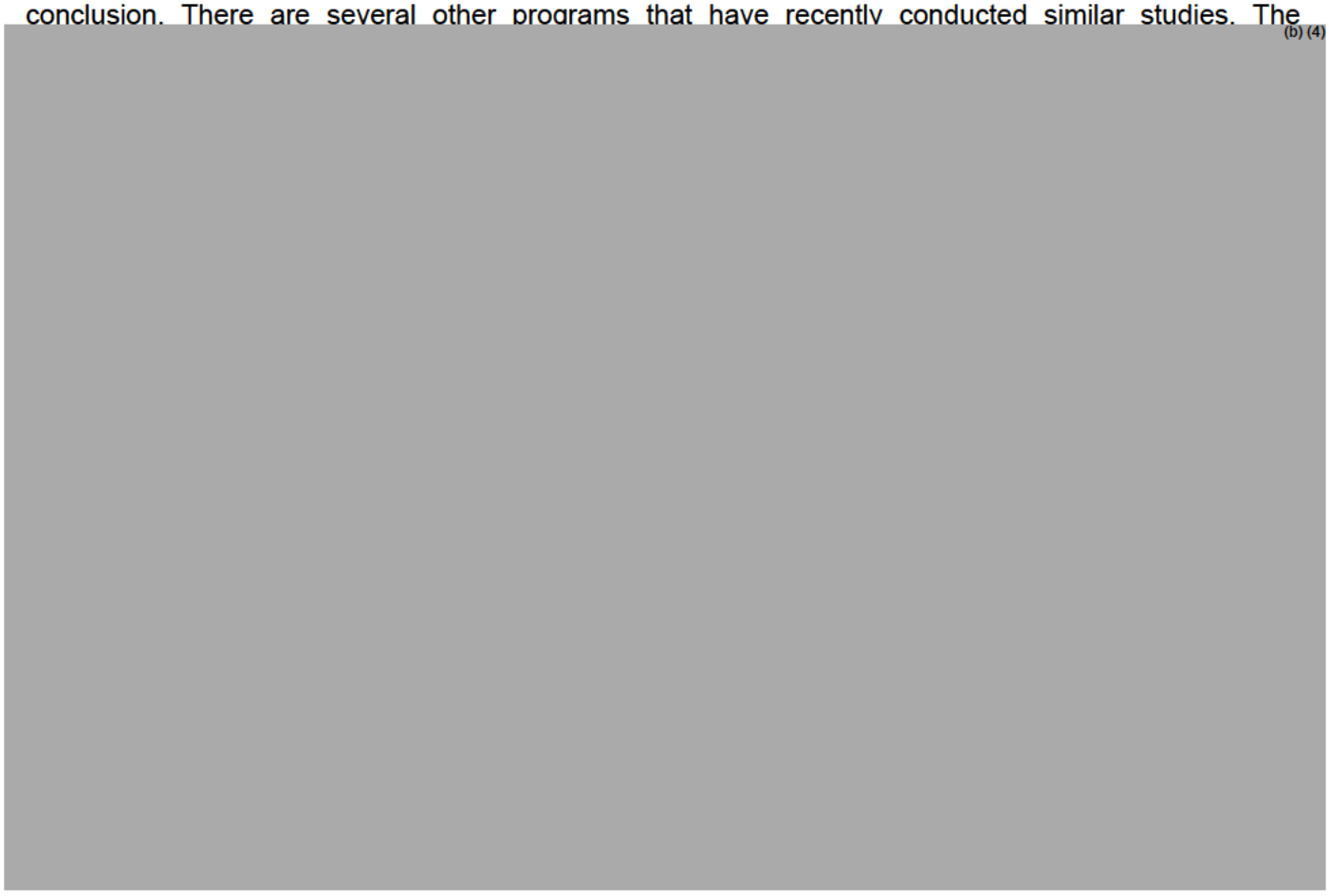
	Letermovir	Placebo	Letermovir	Placebo
All GVHD				
	At Week 24 (FAS)		**At Week 24 (ITT)**	
GVHD	49.2% (160/325)	54.7% (93/170)	46.4% (173/373)	53.1% (102/192)
Death and GVHD	11.3% (18/160)	16.1% (15/93)	12.1% (21/173)	15.7% (16/102)
GVHD and no PET	80.6% (129/160)	52.7% (49/93)	77.5% (134/173)	49% (50/102)
Death in GVHD and no PET	12.4% (16/129)	8.2% (4/49)	13.4% (18/134)	8% (4/50)
GVHD and PET	19.4% (31/160)	47.3% (44/93)	22.5% (39/173)	51% (52/102)
Death in GVHD and PET	6.5% (2/31)	25% (11/44)	7.7% (3/39)	23.1% (12/52)
	At Week 48 (FAS)		**At Week 48 (ITT)**	
GVHD	57.2% (186/325)	60.6% (103/170)	54.4% (203/373)	59.4% (114/192)
Death and GVHD	19.9% (37/186)	23.3% (24/103)	20.7% (42/203)	22.8% (26/114)
GVHD and no PET	82.3% (153/186)	55.3% (57/103)	78.8% (160/203)	50.9% (58/114)
Death in GVHD and no PET	20.3% (31/153)	14% (8/57)	20.6% (33/160)	13.8% (8/58)
GVHD and PET	17.7% (33/186)	44.7% (46/103)	21.2% (43/203)	49.1% (56/114)
Death in GVHD and PET	18.2% (6/33)	34.8% (16/46)	20.9% (9/43)	32.1% (18/56)
Median Viral Load	481 (range 137-9974	362 (range 137-36421	401 (range 137-9974	456 (range 137-36421

	Letermovir	Placebo	Letermovir	Placebo
	IU/mL)	IU/mL)	IU/mL)	IU/mL)
	GVHD Grade 2 or above			
	At Week 24 (FAS)		At Week 24 (ITT)	
GVHD	28% (91/325)	32.4% (55/170)	27.1% (101/373)	31.8% (61/192)
Death and GVHD	16.5% (15/91)	21.8% (12/55)	17.8% (18/101)	21.3% (13/61)
GVHD and no PET	74.7% (68/91)	52.7% (29/55)	72.3% (73/101)	49.2% (30/61)
Death in GVHD and no PET	20.6% (14/68)	13.8% (4/29)	21.9% (16/73)	13.3% (4/30)
GVHD and PET	25.3% (23/91)	47.3% (26/55)	27.7% (28/101)	50.8% (31/61)
Death in GVHD and PET	4.3% (1/23)	30.8% (8/26)	7.1% (2/28)	29% (9/31)
	At Week 48 (FAS)		At Week 48 (ITT)	
GVHD	29.8% (97/325)	32.4% (55/170)	28.7% (107/373)	32.3% (62/192)
Death and GVHD	27.8% (27/97)	32.7% (18/55)	29% (31/107)	32.3% (20/62)
GVHD and no PET	76.3% (74/97)	52.7% (29/55)	73.8% (79/107)	48.4% (30/62)
Death in GVHD and no PET	32.4% (24/74)	24.1% (7/29)	32.9% (26/79)	23.3% (7/30)
GVHD and PET	23.7% (23/97)	47.3% (26/55)	26.2% (28/107)	51.6% (32/62)
Death in GVHD and PET	13% (3/23)	42.3% (11/26)	17.9% (5/28)	40.6% (13/32)
Median Viral Load	267 (range212-695 IU/mL)	367 (range 137-36421 IU/mL)	397 (range 212-5564 IU/mL)	546 (range 137-36421 IU/mL)

3.4.6. Comparisons with other Development Programs

Another key review issue with the development of drugs in this population is that the basis of approval will be dependent on a single Phase 3 study. While the sponsor's results are consistent with an association between HCMV DNaemia and mortality, caution needs to be taken to make any formal conclusion. There are several other programs that have recently conducted similar studies. The

(b) (4)



(b) (4)

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Table 36: Comparisons with Other Development Programs in the HSCT Recipients

(b) (4)

	Letermovir	Placebo	Δ (%) [Placebo-Letermovir]
Clinically Significant HCMV Infection	37.5% (122/325)	60.6% (103/170)	23%
HCMV Disease	1.5% (5/325)	1.8% (3/170)	0.3%
Initiation of PET	16% (52/325)	40% (68/170)	24%
Initiation of PET based on HCMV DNaemia	16% (52/325)	40% (68/170)	24%
Mortality	9.8% (32/325)	15.9% (27/170)	6.1%
HCMV DNaemia above 137 IU/mL			
Clinically Significant HCMV Infection	19.1% (62/325)	50% (85/170)	30.9%
HCMV Disease	0.3% (1/325)	1.2% (2/170)	0.9%
Initiation of PET	7.4% (24/325)	38.2% (65/170)	30.8%
Initiation of PET based on HCMV DNaemia	7.4% (24/325)	38.2% (65/170)	30.8%
Mortality	5.2% (17/325)	7.1% (12/170)	1.9%
HCMV DNaemia above 137 IU/mL			

3.4.7. Utility of the Primary Endpoint for Granting Traditional Approval

A regulatory approval pathway is needed for products intended for prophylaxis of subjects infected with HCMV in HSCT recipients. Such pathways have already been established for products in other antivirals such as those for the treatment of HIV-1 infection as well as other therapeutic areas such as oncology and autoimmune diseases. The overall goal of clinical study is to provide direct evidence of clinical benefit for a treatment. Although improved survival would provide persuasive evidence of benefit in a HCMV prophylaxis study, experience has shown that successful control of HCMV does not necessarily lead to improved survival. In HCMV prophylaxis studies, differences in the magnitude of

response and survival effects are likely related to complications such as opportunistic infection, regimen-related toxicity, recurrent malignancy, GVHD, and pre-existing conditions unrelated to HCMV. Even though most HCMV prophylaxis are not likely to produce a survival benefit, survival remains as an appropriate secondary endpoint to consider in these studies.

Clinical studies of HCMV treatment, prophylaxis and diagnostics in transplant recipients have traditionally used symptomatic HCMV disease as the primary end-point. However, in current clinical practice, rates of HCMV disease are often low, in part due to prolonged prophylaxis, but also due to early detection of DNaemia and initiation of antiviral therapy before definitive symptoms attributable to HCMV are evident. In addition, currently, the most common form of HCMV disease after organ transplant is viral syndrome. Although definitions for viral syndrome exist, it represents a spectrum of illness which shares many overlapping features with other infectious and non-infectious etiologies. For other chronic viral illnesses, such as HIV, HBV, and HCV, viral load has become a well-accepted surrogate marker for use in clinical studies and regulatory submissions.

At Week 24, there was a statistically significant benefit in favor of letermovir for both the HCMV DNaemia and mortality endpoints. However, the effect on mortality was modest (P=0.040). Furthermore, there are no definitive data at this time to show that suppression of HCMV DNaemia predicts improved survival. The rate of HCMV end-organ disease was similar and low in both arms (1.5% and 1.8% in the letermovir and placebo arms, respectively). Thus, it is unclear how letermovir is reducing mortality if not through impact on HCMV end organ disease and whether the difference in mortality is directly due to letermovir. There is circumstantial evidence that may support the association of HCMV DNaemia and mortality. If the HCMV DNaemia and mortality are associated:

- The trend should be in favor of letermovir, even if the overall incidence of HCMV end-organ disease is low.
- The risk of HCMV related disease/death is highest in the first few months after transplant and decreases over time. Therefore, the impact of letermovir on HCMV-related mortality would be expected to be greater through the first 24 weeks compared to that after 24 weeks.
- HCMV DNaemia has been reported to be associated with non-relapse mortality (Green et al., 2016). Therefore, the impact of letermovir would be expected to be greater in the non-relapse mortality.
- HCMV has been associated with increased risk of bacteremia and invasive fungal infections (Falagas et al., 1996; George et al., 1997) in SOT recipients. Furthermore, GCV-induced neutropenia has been associated with increased rates of bacterial sepsis and invasive fungal infections in HSCT recipients (Salzberger et al., 1997; Boeckh et al., 1996). Therefore, the expectation would be for there to be more opportunistic infections in the subjects who died in the placebo arm.
- HCMV and GVHD frequently go together and is reported to be associated with worse outcomes overall (Cantoni et al. 2010).
- If HCMV viral load was predictive of clinical outcome, subjects whose HCMV viral load was higher at the time of PET would be expected to have worse outcome.

With respect to the incidence of HCMV end-organ disease in subjects who died, there were 1 and 3 subjects in the letermovir and placebo arms, respectively, which numerically favors the letermovir arm. Furthermore, the timing of when the subject developed HCMV end-organ disease is important as not every HCMV end-organ disease leads to death. The following are the timing of the HCMV end-organ disease and time of deaths:

- Subject 8228-001_004100002 (placebo arm) developed HCMV end-organ disease on day 12 and died on day 18.
- Subject 8228-001_001800030 (placebo arm) developed HCMV end-organ disease on day 204 and died on day 222.
- Subject 8228-001_006400012 (placebo arm) developed HCMV end-organ disease on day 312 and died on day 316.
- Subject 8228-001_014000011 (letermovir arm) developed HCMV end-organ disease on day 37 and died on day 129.

There was one additional subject in the letermovir arm (8228-001_009100020) who developed HCMV end-organ disease on day 141 and died on day 374 (post-study). Therefore, all 3 subjects in the placebo arm who developed HCMV end-organ disease and died occurred within 18 days, 2 of whom died within a week whereas the subjects in the letermovir arm had at least 3 months between the events.

With respect to the timing of the HCMV-related mortality, the all-cause mortality in subjects who had detectable HCMV was lower in the letermovir arm. The difference was maintained even if the window between positive DNaemia and death was restricted to within 39 days. The difference in all-cause mortality in subjects who had detectable HCMV DNA between the two arms was smaller in deaths after Week 24. These results indicate that the impact of letermovir on HCMV-related mortality was greater through the first 24 weeks compared to after Week 24.

The incidence of non-relapse death through Week 24 in the FAS population was 6.8% (22/325) and 11.2% (19/170) in the letermovir and placebo arms, respectively (Δ4.4%) (Table 31). The incidence of relapse death through Week 24 in the FAS population was 4% (13/325) and 5.9% (10/170) in the letermovir and placebo arms, respectively (Δ1.9%) (Table 31). The differences were smaller between the treatment arms after Week 24, consistent with risk of HCMV related mortality decreasing over time.

The proportion of subjects in the FAS population with serious adverse events related to opportunistic infections within 14 days till death by Week 24 was 2.8% (9/325) and 6.5% (11/170) in the letermovir and placebo arms, respectively (Table 33).

Through Week 48, the proportion of subjects who developed GVHD was numerically lower in the letermovir group, compared to the placebo group. Subjects with ≥Grade II of GVHD also comprised a numerically lower proportion of subjects in the letermovir group compared to the placebo group. In the placebo arm, through week 48, the rate of mortality was higher in subjects with GVHD who had detectable HCMV DNaemia (34.8%, 16/46) compared to those who did not (14%, 8/57) (Table 35). While this trend was not observed in the letermovir arm, overall these data support the hypothesis that HCMV and GVHD was associated with worse outcomes.

The range in viral load at the time of PET was 137-39,396 IU/mL and 137-97,571 IU/mL in the letermovir and placebo arms, respectively. The viral load cutoff for PET initiation was evaluated to see if higher HCMV viral load resulted in worse outcomes. At Week 24, in the FAS group, the mortality in subjects with <913 IU/mL at time of PET was 5.3% (2/38) and 15.4% (6/39) in the letermovir and placebo arms, respectively (Table 37, FDA analysis). In comparison, the mortality in subjects with ≥913 IU/mL at time of PET was higher at 7.1% (1/14) and 27.6% (8/29) in the letermovir and placebo arms, respectively. Furthermore, the mortality in subjects with ≥9,130 IU/mL at time of PET was 25% (1/4) and 50% (4/8) in the letermovir and placebo arms, respectively. Similar trends were observed at Week 48. These data support the hypothesis that higher HCMV viral load resulted in worse clinical outcomes.

Table 37: HCMV Viral Load at Time of PET and Clinical Outcomes

	Letermovir	Placebo	Letermovir	Placebo
	At Week 24 (FAS)		At Week 24 (ITT)	
PET at <913 IU/mL (1000 copies/mL)				
Death at Week 24	5.3% (2/38)	15.4% (6/39)	3.8% (2/53)	16% (8/50)
HCMV Disease at Week 24	0% (0/38)	0% (0/39)	0% (0/53)	0% (0/50)
PET at ≥913 IU/mL (1000 copies/mL)				
Death at Week 24	7.1% (1/14)	27.6% (8/29)	10% (2/20)	22.9% (8/35)
HCMV Disease at Week 24	0% (0/14)	0% (0/29)	5% (1/20)	2.9% (1/35)
PET at ≥9130 IU/mL (10000 copies/mL)				
Death at Week 24	25% (1/4)	50% (4/8)	20% (1/5)	36.7% (4/11)
HCMV Disease at Week 24	0% (0/4)	0% (0/8)	0% (0/5)	0% (0/11)
	At Week 48 (FAS)		At Week 48 (ITT)	
PET at <913 IU/mL (1000 copies/mL)				
Death at Week 48	15.8% (6/38)	28.2% (11/39)	15.1% (8/53)	30% (15/50)
HCMV Disease at Week 48	2.6% (1/38)	0% (0/39)	1.9% (1/53)	0% (0/50)
PET at ≥913 IU/mL (1000 copies/mL)				
Death at Week 48	21.4% (3/14)	41.4% (12/29)	30% (6/20)	34.3% (12/35)
HCMV Disease at Week 48	14.3% (2/14)	3.4% (1/29)	10% (2/20)	8.6% (3/35)
PET at ≥9130 IU/mL (10000 copies/mL)				
Death at Week 24	50% (2/4)	50% (4/8)	40% (2/5)	36.7% (4/11)
HCMV Disease at Week 24	0% (0/4)	0% (0/8)	0% (0/5)	0% (0/11)

Overall, the data support the use of the primary endpoint, which is mostly driven by the HCMV DNaemia, to grant traditional approval. The primary endpoint was robust ($p<0.0001$) which meets the criteria for using a single study to support an NDA. Furthermore, while the endpoint was at the end-of-treatment, this endpoint was statistically significant at even lower doses in the sponsor's Phase 2b study. The use of HCMV DNaemia is already used in clinical practice and is viewed by the treating community as clinically meaningful. In addition to the mortality benefit that was observed at Week 24, letermovir may have also prevented unintended consequences of HCMV treatment such as reduction in immunosuppression that could lead to GVHD or treatment with GCV/vGCV which has toxicity concerns. Although prolonged survival is considered the most reliable endpoint with clinical benefit in oncology studies, the FDA has accepted non-survival endpoints such as tumor response rates as the basis for both regular and accelerated approval. For supportive care drugs such as for GVHD prophylaxis, the FDA typically does not use mortality as an efficacy endpoint as there are so many other causes of death after day 180. In studies of patients with serious or life-threatening diseases, accelerated approval status permits the use of non-survival endpoints if they are reasonably likely to provide clinical benefit. Post-marketing studies are usually required to confirm clinical benefit (Johnson et al., 2003; McCaul et al., 2000). The challenges inherent in assessing response to treatment of HCMV infection in the context of the complex and variable manifestations of the disease suggest the need for a more standardized and clinically meaningful approach to clinical study design.

4. Clinical Virology Review of Drug Resistance

An analysis of amino acid substitutions was conducted pooling data from subjects who had detectable HCMV DNaemia in the Phase 2 and Phase 3 prophylaxis studies. (note: in the sponsor's Phase 2 study, the sponsor did not genotype the complete pUL56 nor the pUL89.) A challenge for conducting resistance analysis in these studies is that in prophylaxis studies there is a lack of baseline sequences that can be used to easily identify treatment-emergent substitutions. Another issue is that the sponsor initially used (b) (4) to conduct the resistance analysis for their Phase 3 study. The (b) (4) assay was not very sensitive; during a blinded analysis of the performance of the (b) (4) genotypic assays on 56 subject samples, it was revealed that these assays generated 0/56 and 2/56 complete deduced amino acid sequences for the pUL56 and pUL89 coding regions, respectively. Many plasma

samples had a low HCMV DNA copy number due to the study design which recommends that pre-emptive therapy be initiated in high risk patients when the HCMV DNA level is ≥137 IU/mL and in low risk patients when the HCMV DNA level is ≥274 IU/mL. The low viral load likely contributed to the difficulties in performing genotypic analysis of UL56 and UL89. Given these issues, the sponsor switched to using (b) (4) for conducting the genotypic analysis. Unfortunately, this led to several subjects where there were no remaining plasma samples available for genotypic analysis. Overall, there were 40/73 (28 of which used the (b) (4) assay) and 62/85 (42 of which used the (b) (4) assay) subjects with genotypic data in the letermovir and placebo arms, respectively. Additionally, there were 15 subjects from the sponsor's Phase 2 study with genotypic data (note: AiCuris did not genotype the pUL89).

The following amino acid substitutions in pUL56 were observed using next generation sequence analysis at higher frequencies in letermovir-treated subjects compared to placebo: L134V (n=1), E157G (n=1), S227I (n=1), Q228H (n=1), V236M (n=2), S255L (n=1), I313V (n=1), C325W (n=1), A366P (n=1), R410G (n=1), D414N (n=2), A425V/A (n=1), G430V (n=1), S445DEL (n=2), S445G (n=1), N446S (n=5), S447DEL (n=2), E485G (n=2), C493S (n=1), E495Q (n=1), Y575C (n=2), L658S (n=1), S705F (n=1), T775I (n=1), R816W (n=1), P846L (n=1) (Table 38, FDA analysis). The pUL56 V236M amino acid substitution is a known letermovir resistance-associated substitution that has previously been selected in cell culture and phenotypically characterized (~45-53-fold reduced susceptibility to letermovir). While the pUL56 C325W substitution has not previously been reported, pUL56 C325F, C325R, and C325Y substitutions have been selected in cell culture (both substitutions >3,000-fold reduced susceptibility to letermovir; Chou 2015; Goldner et al., 2014). In addition, pUL56 E237G substitution was detected in one subject who failed letermovir treatment. This substitution was observed at 4% and thus was not reported by the sponsor (note: The sponsor used a 5% cut-off for their analyses) (please see the review of Eric Donaldson, Ph.D. for more details). While the pUL56 E237G substitution has not previously been reported, pUL56 E237D substitution has been selected in cell culture (10-fold reduced susceptibility to letermovir; Chou 2015). The pUL56 L134V, E157G, S227I, Q228H, V236M, S255L, I313V, C325W, A366P, R410G, D414N, A425V/A, G430V, E495Q, Y575C, L658S, S705F, R816W, and P846L substitutions were observed at conserved positions. Conserved here is defined as 100% identity at the amino acid position based on the sponsor's analysis of 187 unique HCMV whole genome sequences available from NCBI (study report PD018). The pUL56 445DEL, S445G, E485G, C493S, and T775I substitutions developed at polymorphic positions. However, these positions have 99% identity and none of these have previously been reported so these should also be considered for analysis. The pUL56 N446S and E485G are known polymorphisms at these positions. Furthermore, pUL56 N446S was also observed in the placebo cohort. Therefore, these substitutions are of lower priority. Polymorphic was defined as the sponsor as observation of more than one unique amino acid residue, even if observed in only 1 of 187 sequences (0.5%). As stated above, this reviewer used 99% conservation as the threshold.

In the sponsor's resistance analysis of the Phase 2 study, sequencing analyses were performed on virus DNA isolated from 4 whole blood samples from 4 subjects. Substitutions pUL56 V425A, A464T, E495Q, and N586D were identified from virus in whole blood samples but not from the plasma samples of the same sample day (Table A1-11). This further illustrates that sample type is an important preanalytical variable. As stated above, quantitative HCMV testing is typically performed on whole blood or plasma. Each specimen type has strengths and limitations. HCMV DNA is detected more frequently and viral load values are often higher in whole blood compared with plasma because both cell-free and intracellular viruses are detected in the former. A recent study showed that viral load values in most subjects are about 10-fold higher in whole blood compared with plasma (Lisboa et al., 2011). However, in some subjects the difference was as great as 100-fold, and occasionally plasma

viral load was found to be higher than whole blood viral load (Lisboa et al., 2011). Furthermore, the HCMV decay kinetics in the whole blood may be different (Emery et al., 2012). The use of plasma instead of whole blood in these tests requires rapid separation to keep the DNA from degrading with resultant fragmentation (Razonable et al., 2002).

The pUL56 V236M, E237G, and C325W substitutions should be included in the clinical resistance section of the label. In addition, the pUL56 S445/N446/S447 deletion in combination with E485G, although at polymorphic positions, occurred at high frequency (>70%) in 2 subjects who failed while on letermovir and should also be considered for the label. The following pUL56 substitutions at conserved amino acid positions were each observed in a virologic failure while on letermovir (b) (4) pUL56 L134V, S227I, Q228H, A366P, R410G, D414N, A425V/A, G430V, and E495Q. The following pUL56 substitutions at conserved amino acid positions were each observed in a virologic failure who relapsed/failed off-treatment (b) (4) : pUL56 E157G, S255L, I313V, Y575C, L658S, S705F, R816W, and P846L.

Table 38: Substitutions in pUL56

Amino Acid Substitution	% Identity*	Polymorphic Amino Acid at this Position#	Letermovir (n=55)	Placebo (n=62)	Isolate Date
N004D	100	N	0	2	28, 42
A061A/V	100	A	0	1	42
Q062Q/P	100	Q	0	1	42
L070P	100	L	0	1	36
V132A	100	V	0	1	72
S133N	100	S	0	2	20, 24
L134V	100	L	1	0	8
N138K	100	N	0	1	23
I140V	100	I	0	1	36
E157G	100	E	1	0	128
E161G	100	E	0	1	49
A164V	100	A	1	1	23, 128
A180V	100	A	0	1	36
T189M	*96.8*	*T, M*	*0*	*3*	*28, 36, 41*
S227I	100	S	1	0	8
Q228H	100	Q	1	0	8
V236M	100	V	2	0	50, 62
*Q238**	*100*	*Q*	*0*	*1*	*28*
R246C	98.9	R, C	0	1	55
R246S	98.9	R, C	0	1	17
S255L	100	S	1	0	160
H268R	100	H	0	1	54
I313V	100	I	1	0	162
C325W	100	C	1	0	46
A366P	100	A	1	0	9
R410G	100	R	1	0	35
D414N	100	D	2	0	36
A425V/A	100	A	1	0	95
G430V	100	G	1	0	63
M442T	86.1	M, T, L	0	3	10, 49, 112
S445DEL	95.7	S, N, A, M	2	0	62, 66
S445G	95.7	S, N, A, M	1	0	145
S445N	95.7	S, N, A, M	4	11	10, 15, 24, 33, 36, 48, 49, 54, 126, 130, 162, 175
N446DEL	95.7	N, S, T	5	4	12, 14, 56, 62, 66, 72, 128, 131,

Amino Acid Substitution	% Identity*	Polymorphic Amino Acid at this Position#	Letermovir (n=55)	Placebo (n=62)	Isolate Date
					147
N446S	95.7	N, S, T	5	2	8, 23, 46, 121, 131, 147
S447DEL	98.9	S, A	2	0	66
T452I	89.3	T, I	0	3	10, 49, 112
S454N	90.4	S, N	0	3	10, 49, 112
A464T	96.3	A, T, V	6	6	8, 12, 14, 23, 46, 56, 72, 121, 128, 131, 147
D480N	100	D	0	1	64
E485G	99.5	E, G	2	0	62, 66
E485K	99.5	E, G	0	1	56
C493S	99.5	C, Y	1	0	13
E495Q	100	E	1	0	7
E497G	100	E	0	1	34
I535V	100	I	1	1	55 125
T545N	100	T	0	1	10
Y575C	100	Y	2	0	6, 120
Q577R	99.5	Q, R	0	1	12
D580G	100	D	0	1	72
V584A	100	V	0	1	36
D586N/D	100	D	0	1	24
L600P	100	L	0	1	72
P606P/L	100	P	0	1	54
T627S	100	T	0	1	30
C629*	100	C	0	1	40
Y643N	100	Y	0	1	39
H653L	100	H	0	1	64
L658S	100	L	1	0	132
M677V	100	M	0	1	72
Q692Q/H	100	Q	0	1	54
L704P	100	L	0	1	56
S705F	100	S	1	0	146
S718P	100	S	0	1	22
H727L/P	100	H	0	1	40
L742DEL	100	L	0	1	120
R745R/L	100	R	0	1	54
S749N	79.1	S, N	1	11	10, 14, 15, 33, 40, 41, 49, 54, 131, 175
S765T	100	S	0	1	41
P767L	100	P	0	1	8
T775I	99.5	T, R	1	0	66
V778A	90.9	V, A	1	6	10, 36, 42, 49, 64, 112, 146
A779V	99.5	A, V	2	3	12, 14, 72, 121, 128
R799*	100	R	0	1	12
P800L	87.2	P, L	2	11	10, 15, 20, 24, 28, 29, 34, 36, 41, 42, 57, 135, 147
R816W	100	R	1	0	145
T821A	100	T	0	1	41
R827H	100	R	0	1	10
A828V	100	A	0	1	112

Amino Acid Substitution	% Identity*	Polymorphic Amino Acid at this Position#	Letermovir (n=55)	Placebo (n=62)	Isolate Date
Q842E	100	Q	0	1	10
P846L	100	P	1	0	281
L849P	100	L	0	1	131

yellow: Substitutions that were numerically higher in letermovir arm.
green: Substitutions at positions that were identified in cell culture selection studies.
*Conserved is defined as 100% identity at the amino acid position based on the sponsor's analysis of 187 unique HCMV whole genome sequences available from NCBI (study report PD018).
#Polymorphic is defined as observation of more than one unique amino acid residue.

The following amino acid substitutions in pUL89 were observed using next generation sequence analysis at higher frequencies in letermovir-treated subjects compared to placebo: N74S (n=1), P176S (n=1), D309D/V (n=1), D309G (n=1), M406V (n=1), L522P (n=1), A532T (n=1), Q625* (n=1), D665E (n=4) (Table 39, FDA analysis). The pUL89 N74S, P176S, D309D/V, D309G, M406V, L522P, A532T, and Q625* (stop) substitutions were observed at conserved positions. Of note, the pUL89 substitution observed in cell culture using a close analog to letermovir was Q256E. The following pUL89 substitutions at conserved amino acid positions were observed in virologic failures while on letermovir treatment (b) (4) pUL89 L522P and Q625* (stop). The following pUL89 substitutions at conserved amino acid positions were observed in virologic failures who relapsed/failed off-treatment (b) (4) pUL89 N74S, P176S, D309D/V, D309G, M406V, and A532T.

Table 39: Amino Acid Substitutions in pUL89

Amino Acid Substitution	% Identity*	Polymorphic Amino Acid at this Position#	Letermovir (n=40)	Placebo (n=62)	Isolate Date
T037A	100	T	10	22	9, 10, 15, 17, 21, 23, 24, 28, 30, 33, 34, 36, 40, 42, 48, 53, 55, 57, 85, 89, 95, 125, 131, 132, 135, 144, 145, 147, 157
H047Y	100	H	0	1	33
N074S	100	N	1	0	175
D094N	85	D, N	5	6	12, 33, 46, 49, 54, 64, 95, 120, 144, 147, 162
K095E	100	K	10	19	9, 10, 15, 17, 21, 24, 28, 30, 33, 34, 36, 40, 42, 48, 55, 57, 85, 89, 95, 125, 131, 132, 135, 144, 145, 147, 157
T097A	100	T	0	1	41
T097M	100	T	0	1	29
A145T	100	A	0	1	36
D149G	100	D	0	1	54
I164V	100	I	0	1	72
F168L	100	F	0	1	140

Amino Acid Substitution	% Identity*	Polymorphic Amino Acid at this Position#	Letermovir (n=40)	Placebo (n=62)	Isolate Date
P176S	100	P	1	0	132
Q184R	100	Q	0	1	140
I187P	100	I	0	1	24
Q191H	100	Q	0	1	48
Q191R	100	Q	0	1	55
Y240C	100	Y	0	1	162
A242T	100	A	0	1	42
Q244*	100	Q	0	1	57
I298V	100	I	0	1	28
D309D/V	100	D	1	0	6
D309G	100	D	1	0	175
H312R	100	H	0	1	42
F348L	100	F	0	2	23 162
V362A	100	V	0	1	36
M406V	100	M	1	0	132
I417V	100	I	0	1	56
T431A	100	T	0	1	42
L441S	100	L	0	1	120
R442H	100	R	0	1	29
R484Q	100	R	0	1	15
L500P	100	L	0	1	24
E502K	100	E	0	1	48
A512T	100	A	0	1	55
L522P	100	L	1	0	95
I531V	96.8	I, V	1	1	42
A532T	100	A	1	0	146
N536S	100	N	0	1	22
L558I	100	L	0	1	10
S613G	100	S	0	1	14
Q625*	100	Q	1	0	66
A633V	98.9	A, V	2	5	8, 23, 46, 56, 72, 121, 162
M658V	100	M	0	1	28
D665E	91.4	D, E	4	0	62, 66, 108, 146

yellow: Substitutions that were numerically higher in letermovir arm.
*Conserved is defined as 100% identity at the amino acid position based on the sponsor's analysis of 187 unique HCMV whole genome sequences available from NCBI (study report PD018).
#Polymorphic is defined as observation of more than one unique amino acid residue.

Amongst the subjects with complete sequencing data in the letermovir arm, there were 37.5% (3/8) and 0% (0/22) in the on-treatment virologic failures and off-treatment virologic failures, respectively, who had *previously identified* resistance-associated substitutions.

There are some caveats which may change these rates:

- There are a number of uncharacterized amino acid substitutions in the pUL56 and pUL89 (PMR).
- Reports of GCV/vGCV resistance-associated substitutions detected in specific compartments exclusively and not in blood (Gohring et al., 2015), i.e., may need to look at specific compartments to identify letermovir resistance.
- Possible substitutions in other HCMV terminase complex (e.g. pUL51 or pUL104).

While there are limitations to the interpretation of these results due to the above-mentioned caveats, there are a couple of interesting preliminary observations. The 100 day vs 200 day prophylaxis study similar to what was conducted with vGCV in SOT recipients would be reasonable based on the current rates of resistance in the off-treatment virologic failures. Additionally, the rate of development of resistance to prophylaxis of GCV/vGCV in SOT recipients have been reported to be ~3-15% (Martin et

al., 2007; Boivin et al., 2009; James and Prichard, 2013; LePage et al., 2013). While the emergence of GCV/vGCV-resistant viruses in HSCT recipients has also been reported (Marfori et al., 2007), several reports suggest that it is not as common in this population as in SOT recipients (Bhorade et al., 2002; Nichols et al., 2001; Gilbert et al., 2001). More data will be needed to establish the rate of development of resistance to letermovir. However, that the rate of development of resistance to letermovir in on-treatment virologic failures is higher compared to what has been reported for GCV/vGCV would not be surprising given the lower genetic barrier to resistance observed in cell culture studies.

5. Local vs. Central Laboratory Data
In the event that test results from the central laboratory were not available within the timeframe the investigator wished to initiate anti-HCMV therapy, the investigator could use a positive local laboratory test (HCMV DNA PCR or pp65 antigen only) result in order to make the decision. However, as described above, plasma samples for HCMV DNA PCR testing also had to be sent to the central laboratory. In such instances, the local laboratory result also had to be reported.

A summary of local laboratory data (pp65 and PCR) and central laboratory data (PCR) from the sponsor's Phase 2 study (MK-8228-020) is presented in Table A1-16. The majority of local laboratory results were consistent with central laboratory findings, with the exception of Sites 101 and 109 which reported notably fewer negative pp65 results than the central laboratory (63.6% and 66.7% local pp65 negative/central PCR positive, respectively) and Site 108 which reported notably more positive PCR results than the central laboratory (45.5% local PCR positive/central PCR negative). The pp65 antigenemia assay can be impacted by the time between when the sample was obtained and assayed.

A summary of local laboratory data (pp65 and PCR) and central laboratory data (PCR) from the sponsor's Phase 2 study (MK-8228-020) is presented in Table A1-5.

6. Changes to Immunosuppression
The most frequently used concomitant immunosuppressant medications overall were calcineurin inhibitors (353/373 [94.6%] and 179/192 [93.2%] in the letermovir arm and placebo arm, respectively; Table 40, adapted from Table 10-13 of study report p001v01, pg. 168). Cyclosporine A (CsA) and tacrolimus were the most commonly used calcineurin inhibitors. Systemic corticosteroid steroid were used in 246/373 [66%] and 122/192 [63.5%] in the letermovir arm and placebo arm, respectively. There was no imbalance in the categories of immunosuppressants used between the treatment groups. The types and frequencies of these medications were comparable in the letermovir and placebo groups. The median time to changes in any immunosuppressive regimen dose were 10 days (range 1-262 days) and 10.5 days (range 1-298 days) in the letermovir and placebo arms respectively.

Table 40: Summary of Concomitant Immunosuppressive Regimen Use (ITT Population)

	Letermovir	Placebo
Calcineurin Inhibitors	94.6% (353/373)	93.2% (179/192)
Cyclosporine A	51.7% (193/373)	52.1% (100/192)
Tacrolimus	46.6% (174/373)	44.8% (86/192)
Selected Immunosuppressants	43.2% (161/373)	42.2% (81/192)
Everolimus	1.9% (7/373)	1.6% (3/192)
Leflunomide	0.3% (1/373)	0% (0/192)
Mycophenolate	37.3% (139/373)	34.9% (67/192)
Sirolimus	9.7% (36/373)	14.1% (27/192)
Systemic Corticosteroid	66% (246/373)	63.5% (122/192)

7. Conclusion
This Original NDA is approvable from a Clinical Virology perspective for the prophylaxis of

cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT). Overall, the data also support the use of the primary endpoint, which is mostly driven by the HCMV DNaemia, to grant traditional approval.

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)

VIROLOGY REVIEW

NDA: 209939 **SDN:** 000 **DATE REVIEWED:** August 8, 2017

Clinical Virology Reviewers: Takashi E. Komatsu, Ph.D., RAC & Anamaris M. Colberg Poley, Ph.D.

Microbiology Package Insert

Clinical virology-related sections of the proposed drug label and suggested edits are shown below. The words with strikethroughs are the text the sponsor was requested to delete and the words in blue are recommended insertions.

Indications and Usage

TRADEMARK is a CMV DNA terminase complex inhibitor (pUL51/pUL56/pUL89) [(b) (4)] indicated for:

12.1 Mechanism of Action

TRADEMARK is an antiviral drug against CMV *[see Microbiology (12.4)]*.

12.4 Microbiology

Mechanism of Action

Letermovir inhibits the CMV DNA terminase complex containing pUL51, pUL56, and pUL89, which is required for viral DNA [(b) (4)] processing and packaging. Biochemical characterization and electron microscopy demonstrated that letermovir affects the production [(b) (4)] of proper unit length genomes and interferes with virion maturation. Genotypic characterization of virus resistant to letermovir confirmed that letermovir targeted the terminase complex.

Antiviral Activity

The median [(b) (4)] EC_{50} values of letermovir against ~~a collection of~~ [(b) (4)] clinical CMV isolates in a cell-culture model of infection were 1.9 nM (range 0.1 nM-5.8 nM, n = 29), 2.0 nM (range 0.7 nM-6.1 nM, n = 27), 2.3 nM (range 1.5 nM-3.4 nM, n = 11), and 2.9 nM (range 2.6 nM-3.2 nM, n = 3) against CMV gB genotypes 1, 2, 3, and 4, respectively [(b) (4)]

Viral Resistance

In Cell Culture

[(b) (4)]. CMV mutants with reduced susceptibility to letermovir have been selected in cell culture ~~.and T~~the resistance mutations map to UL56. [(b) (4)] Resistance-associated substitutions occur [(b) (4)] amino acid [(b) (4)] positions [(b) (4)] pUL56 231 an 69 (V231A [(b) (4)] /L, V236L [(b) (4)] /M, E237D, L241P, T244K [(b) (4)] /R, L257I, F261C~~,~~ [(b) (4)] /L~~,~~ [(b) (4)] /S, Y321C, C325F, [(b) (4)] /R, M329T, R369G~~,~~ [(b) (4)] /M, [(b) (4)] /S). EC_{50} values for virus expressing these substitutions [(b) (4)] are 13- to 5,870-fold higher than those for the wild-type reference virus. [(b) (4)]

In Clinical Studies

In a Phase 2b trial evaluating letermovir [(b) (4)] or placebo [(b) (4)] in 131 HSCT recipients, DNA sequence analysis of a select region of UL56 (amino acids 231 to 369) was performed on samples obtained from 12 letermovir-treated subjects who experienced prophylaxis failure and for whom on-treatment samples were available for analysis. One subject [(b) (4)] had a letermovir resistance substitution, pUL56 [(b) (4)] V236M~~)~~.

In a Phase 3 trial (P001), DNA sequence analysis of the entire coding regions of UL56 and UL89 was performed on samples obtained from [(b) (4)] letermovir-treated subjects, [(b) (4)] experienced prophylaxis failure and for whom samples were available for analysis. Three [(b) (4)] subjects who failed while on-treatment had a letermovir resistance substitution, pUL56 [(b) (4)], E237G, or C325W~~)~~. In addition, the pUL56 445-447 SNS deletion in combination with the E485G

substitution was detected on-treatment by next generation sequencing in 2 subjects who experienced prophylaxis failure. The clinical significance of these substitutions is not known.

Cross Resistance
Cross resistance is not likely with drugs outside of this class. Letermovir is fully active against viral populations with substitutions conferring resistance to CMV DNA polymerase inhibitors (cidofovir, foscarnet, and ganciclovir-(b) (4)). These DNA polymerase inhibitors are fully active against viral populations with substitutions conferring resistance to letermovir.

Clean Version
Indications and Usage
TRADEMARK is a CMV DNA terminase complex inhibitor (pUL51/pUL56/pUL89) indicated for:

12.1 Mechanism of Action
TRADEMARK is an antiviral drug against CMV *[see Microbiology (12.4)]*.

12.4 Microbiology
Mechanism of Action
Letermovir inhibits the CMV DNA terminase complex containing pUL51, pUL56, and pUL89, which is required for viral DNA processing and packaging. Biochemical characterization and electron microscopy demonstrated that letermovir affects the production of proper unit length genomes and interferes with virion maturation. Genotypic characterization of virus resistant to letermovir confirmed that letermovir targeted the terminase complex.

Antiviral Activity
The median EC_{50} values of letermovir against (b) (4) clinical CMV isolates in a cell-culture model of infection were 1.9 nM (range 0.1 nM-5.8 nM, n = 29), 2.0 nM (range 0.7 nM-6.1 nM, n = 27), 2.3 nM (range 1.5 nM-3.4 nM, n = 11), and 2.9 nM (range 2.6 nM-3.2 nM, n = 3) against CMV gB genotypes 1, 2, 3, and 4, respectively.

Viral Resistance
In Cell Culture
CMV mutants with reduced susceptibility to letermovir have been selected in cell culture and the resistance mutations map to UL56. Resistance-associated substitutions occur between amino acid positions pUL56 231 and 369 (V231A/L, V236L/M, E237D, L241P, T244K/244R, L257I, F261C/L/S, Y321C, C325F/R, M329T, R369G/M/S). EC_{50} values for virus expressing these substitutions are 13- to 5,870-fold higher than those for the wild-type reference virus.

In Clinical Studies
In a Phase 2b trial evaluating letermovir (b) (4) or placebo (b) (4) in 131 HSCT recipients, DNA sequence analysis of a select region of UL56 (amino acids 231 to 369) was performed on samples obtained from 12 letermovir-treated subjects who experienced prophylaxis failure and for whom on-treatment samples were available for analysis. One subject (b) (4) had a letermovir resistance substitution, pUL56 V236M.

In a Phase 3 trial (P001), DNA sequence analysis of the entire coding regions of UL56 and UL89 was performed on samples obtained from (b) (4) letermovir-treated subjects, (b) (4) experienced prophylaxis failure and for whom samples were available for analysis. Three subjects who

failed while on-treatment had a letermovir resistance substitution, pUL56 V236M, E237G, or C325W. In addition, the pUL56 445-447 SNS deletion in combination with the E485G substitution was detected on-treatment by next generation sequencing in 2 subjects who experienced prophylaxis failure. The clinical significance of these substitutions is not known.

Cross Resistance
Cross resistance is not likely with drugs outside of this class. Letermovir is fully active against viral populations with substitutions conferring resistance to CMV DNA polymerase inhibitors (cidofovir, foscarnet, and ganciclovir. These DNA polymerase inhibitors are fully active against viral populations with substitutions conferring resistance to letermovir.

Appendices

Table A1-1: Subjects Whose Death Occurred by Week 24 in Letermovir Arm (ITT)

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
8228-001_000400005	Low Risk	Y	Peripheral blood	Negative	137							
8228-001_001400001	Low Risk	Y	Peripheral blood	Negative	30							
8228-001_001700001	High Risk	Y	Peripheral blood	Negative	155	151	9974	148	151	9974		
8228-001_001700009	High Risk	Y	Cord blood	Positive	138	135	704					Day 124 944 IU/mL
8228-001_001700013	High Risk	Y	Cord blood	Positive	41							
8228-001_001800009	Low Risk	Y	Peripheral blood	Negative	137							
8228-001_001800011	High Risk	Y	Bone marrow	Negative	90							
8228-001_001800013	High Risk	N	Bone marrow	Positive	17							
8228-001_001800037	Low Risk	Y	Peripheral blood	Positive	121							
8228-001_001800047	High Risk	Y	Bone marrow	Positive	29							
8228-001_001800050	Low Risk	Y	Peripheral blood	Negative	101							
8228-001_001800051	Low Risk	Y	Peripheral blood	Positive	115							
8228-001_001900003	High Risk	Y	Bone marrow	Negative	116							
8228-001_002000018	High Risk	Y	Peripheral blood	Positive	46							
8228-001_003400005	High Risk	Y	Peripheral blood	Positive	116							
8228-001_004100005	Low Risk	Y	Peripheral blood	Positive	63							
8228-001_004700010	Low Risk	Y	Peripheral blood	Negative	66							
8228-001_005800007	High Risk	Y	Peripheral blood	Negative	104	99	137	25	22	137		Continued shedding virus through day 99
8228-001_006100006	Low Risk	Y	Peripheral blood	Positive	146							
8228-001_006300003	High Risk	Y	Peripheral blood	Positive	16							
8228-001_006300006	Low Risk	Y	Peripheral blood	Positive	147							
8228-001_007800008	Low Risk	N	Peripheral blood	Negative	54	15	137					Day 8 784 IU/mL
8228-	High Risk	Y	Peripheral	Positive	149	105	862					Day 91 356 IU/mL

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
001_010000002			blood									
8228-001_010200004	Low Risk	N	Peripheral blood	Positive	127							
8228-001_010800006	Low Risk	N	Peripheral blood	Positive	116							
8228-001_010800027	Low Risk	Y	Peripheral blood	Positive	69							
8228-001_011000004	High Risk	Y	Peripheral blood	Positive	171	152	695	146	152	695		
8228-001_011300001	Low Risk	Y	Peripheral blood	Positive	60							
8228-001_011600001	Low Risk	Y	Bone marrow	Negative	155	147	1448					Day 133 137 IU/mL
8228-001_011600004	High Risk	Y	Bone marrow	Negative	106							
8228-001_011600018	Low Risk	Y	Bone marrow	Negative	72							
8228-001_011600042	Low Risk	Y	Bone marrow	Negative	59							
8228-001_011700004	Low Risk	Y	Peripheral blood	Negative	142							
8228-001_012200003	Low Risk	N	Peripheral blood	Unknown	163	137	201	108	108	5564		Continued shedding through day 137
8228-001_012300007	High Risk	Y	Peripheral blood	Positive	80							
8228-001_012600003	Low Risk	Y	Bone marrow	Negative	92							
8228-001_013100017	Low Risk	Y	Peripheral blood	Positive	15							
8228-001_014000011	Low Risk	Y	Peripheral blood	Negative	131						37	
8228-001_014200005	Low Risk	Y	Peripheral blood	Negative	138							
8228-001_014700011	High Risk	N	Peripheral blood	Positive	30							
8228-001_016400004	Low Risk	Y	Bone marrow	Positive	18							

Table A1-2: Subjects Whose Death Occurred by Week 24 in Placebo Arm (ITT)

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
8228-001_003000016	High Risk	Y	Bone marrow	Positive	16	15	621					

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
8228-001_001700003	Low Risk	Y	Peripheral blood	Negative	21							
8228-001_002000012	High Risk	Y	Peripheral blood	Positive	21							
8228-001_004200002	High Risk	Y	Peripheral blood	Negative	26							
8228-001_013100011	Low Risk	Y	Peripheral blood	Negative	28							
8228-001_004100002	Low Risk	Y	Peripheral blood	Positive	39	15	6996				12	
8228-001_000400023	High Risk	Y	Peripheral blood	Negative	60							
8228-001_010800017	High Risk	Y	Peripheral blood	Negative	66	57	137	24	24	1568		Continued shedding through day 57
8228-001_000300002	Low Risk	N	Peripheral blood	Positive	82	62	137	48	48	546		
8228-001_014000009	Low Risk	Y	Peripheral blood	Positive	84	78	601	54	54	367		Continued shedding through day 78
8228-001_014700015	High Risk	Y	Cord blood	Negative	96	93	1945					Day 86 141 IU/mL
8228-001_007500006	Low Risk	Y	Peripheral blood	Positive	97							
8228-001_001800015	Low Risk	Y	Peripheral blood	Negative	99							
8228-001_018500001	Low Risk	Y	Bone marrow	Negative	102	49	27096	26	20	1212		Continued shedding through day 49
8228-001_004200004	Low Risk	Y	Peripheral blood	Positive	107	91	673	79	77	137		Shedding day 77 and 83, 137 IU/mL
8228-001_001700012	Low Risk	Y	Bone marrow	Negative	118							
8228-001_012600002	High Risk	Y	Cord blood	Negative	118							
8228-001_014200003	Low Risk	Y	Peripheral blood	Positive	123							
8228-001_014200007	Low Risk	Y	Peripheral blood	Positive	126	86	6694	64	64	36421		No data after day 86, continued shedding through day 86
8228-001_002000019	Low Risk	Y	Peripheral blood	Positive	141	121	137	85	78	137		Continued shedding through day 121
8228-001_001700005	High Risk	Y	Cord blood	Negative	143	138	519	43	42	10813		Continued shedding through day 138
8228-001_001800018	Low Risk	Y	Peripheral blood	Negative	152			62	64	914		
8228-001_001800004	Low Risk	Y	Bone marrow	Positive	153			41	33	358		
8228-001_003400003	High Risk	N	Peripheral blood	Positive	154			29	32	156		
8228-001_001800024	Low Risk	Y	Peripheral blood	Negative	159	138	137	28	19	137		Viral shedding day 99-138

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
8228-001_014700007	High Risk	Y	Peripheral blood	Negative	162	155	137	28	32	1189		Continued shedding through day 155
8228-001_010800007	High Risk	Y	Peripheral blood	Negative	167	160	137	33	33	10780		Continued shedding through day 160
8228-001_011700002	High Risk	Y	Peripheral blood	Negative	168			24	24	2076		
8228-001_000300009	Low Risk	Y	Peripheral blood	Positive	172							
8228-001_006400021	Low Risk	Y	Peripheral blood	Positive	174			55	54	137		

Table A1-3: Subjects Whose Death Occurred after Week 24 in Letermovir Arm (ITT)

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
8228-001_000300003	Low Risk	N	Bone marrow	Positive	349			101	102	397		
8228-001_000300012	Low Risk	Y	Peripheral blood	Negative	361							death on day 361
8228-001_000500001	Low Risk	Y	Peripheral blood	Negative	241							
8228-001_001200002	High Risk	Y	Peripheral blood	Positive	264							
8228-001_001700006	Low Risk	Y	Peripheral blood	Positive	249	225	281	104	108	39396		Continued shedding through day 225
8228-001_001800014	High Risk	Y	Peripheral blood	Negative	198							
8228-001_001800016	Low Risk	Y	Peripheral blood	Positive	225							
8228-001_001800020	Low Risk	Y	Peripheral blood	Negative	257	223	1797					No data after day 223
8228-001_001800021	Low Risk	Y	Peripheral blood	Positive	353							
8228-001_001800026	Low Risk	Y	Peripheral blood	Negative	194							
8228-001_001800027	High Risk	Y	Peripheral blood	Positive	201							
8228-001_001800048	High Risk	Y	Bone marrow	Positive	252			66	66	267		
8228-001_001900004	High Risk	Y	Bone marrow	Positive	300			58	58	387		
8228-001_002000008	Low Risk	Y	Peripheral blood	Positive	331							
8228-	Low Risk	Y	Bone	Positive	257			92	92	212		

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
001_003000011			marrow									
8228-001_004200007	High Risk	N	Peripheral blood	Positive	194	156	137	140	145	401		No data after day 156
8228-001_004200008	High Risk	Y	Peripheral blood	Positive	304							
8228-001_004400002	Low Risk	Y	Bone marrow	Positive	306							
8228-001_004500008	Low Risk	Y	Peripheral blood	Negative	197							
8228-001_006300002	High Risk	Y	Bone marrow	Positive	199							
8228-001_009100014	Low Risk	Y	Peripheral blood	Negative	387							
8228-001_009100020	Low Risk	Y	Peripheral blood	Negative	374						141	was shedding virus around time of HCMV disease (days 124-168); death on day 374
8228-001_010800023	Low Risk	Y	Peripheral blood	Positive	475							death on day 475
8228-001_011600016	High Risk	Y	Bone marrow	Positive	198							
8228-001_011600017	Low Risk	Y	Bone marrow	Positive	191							
8228-001_011600038	High Risk	Y	Bone marrow	Positive	198	168	263	126	127	137		Continued shedding through day 168 (2nd infection?)
8228-001_011700006	High Risk	N	Peripheral blood	Positive	296	239	189	46	46	25661		Continued shedding through day 239
8228-001_012200002	Low Risk	Y	Peripheral blood	Unknown	202							
8228-001_012300001	High Risk	Y	Peripheral blood	Positive	214							
8228-001_012300009	Low Risk	Y	Peripheral blood	Negative	185							
8228-001_012900016	Low Risk	Y	Peripheral blood	Unknown	321							
8228-001_013100009	Low Risk	Y	Peripheral blood	Positive	221							
8228-001_013100021	Low Risk	Y	Peripheral blood	Positive	231							
8228-001_013100023	Low Risk	N	Peripheral blood	Positive	236							
8228-001_014000002	Low Risk	Y	Peripheral blood	Positive	258							
8228-001_014000013	Low Risk	Y	Peripheral blood	Negative	192							
8228-001_014600002	Low Risk	Y	Peripheral blood	Positive	298							
8228-001_016100008	High Risk	Y	Peripheral blood	Negative	262			128	128	975		continued shedding through day 154

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Day of HCMV Disease	Notes
8228-001_016400012	Low Risk	Y	Bone marrow	Positive	297							
8228-001_017500007	Low Risk	N	Peripheral blood	Negative	295			130	130	3825		
8228-001_018500006	Low Risk	Y	Bone marrow	Positive	210							

Table A1-4: Subjects Whose Death Occurred after Week 24 in Placebo Arm (ITT)

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (copies/mL)	Day of HCMV Disease	Notes
8228-001_000300005	High Risk	Y	Peripheral blood	Positive	226	224	160	46	47	331		Continued shedding through day 224
8228-001_001300005	Low Risk	N	Peripheral blood	Nega ive	239	185	137	21	10	844		The viral shedding at late points may be blips
8228-001_001300006	Low Risk	Y	Peripheral blood	Positive	204							
8228-001_001800030	Low Risk	Y	Peripheral blood	Nega ive	231						204	
8228-001_001800038	Low Risk	Y	Peripheral blood	Positive	259							
8228-001_001900002	High Risk	Y	Bone marrow	Nega ive	238			34	34	1343		
8228-001_006400012	Low Risk	Y	Peripheral blood	Nega ive	330	262	5237	16	21	4564	312	No data after day 262, continued shedding through day 262
8228-001_006900003	Low Risk	Y	Peripheral blood	Positive	368			39	39	243		
8228-001_009100018	High Risk	N	Peripheral blood	Positive	452			24	19	15101		continued shedding through day 130, death on day 452
8228-001_010200006	Low Risk	Y	Peripheral blood	Nega ive	251	217	864	19	19	137		Second infection day 203
8228-001_011600037	Low Risk	Y	Bone marrow	Nega ive	183							
8228-001_012400002	Low Risk	Y	Bone marrow	Nega ive	193			36	40	2951		Shedding on days 141-169
8228-001_012900018	Low Risk	Y	Peripheral blood	Positive	210	165	840					No data after day 165, day 143 172 copies/mL
8228-001_014000012	Low Risk	Y	Peripheral blood	Nega ive	200			54	54	137		
8228-001_014600001	Low Risk	Y	Bone marrow	Nega ive	285			38	48	10298		
8228-001_014800005	High Risk	Y	Cord blood	Nega ive	192	153	1834	37	40	212		No data after day 153, continued shedding through day 153
8228-	Low Risk	N	Bone	Positive	293			19	17	137		

Subject ID	Risk Stratification	FAS Flag	Stem cell source	Donor HCMV Serostatus	Day of Death	Day of Measure Viral Load around Death	Viral Load (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (copies/mL)	Day of HCMV Disease	Notes
001_016300002			marrow									
8228-001_016400015	Low Risk	N	Peripheral blood	Positive	271							

Table A1-5: Summary of Local Laboratory pp65/PCR and Central Laboratory PCR Data (Study MK-8228-001)

Subject ID	Day of Death	Day of PET																
8228-001_000100001		89	Day	1	4	7	7	14	14	21	23	28	28	35	42	44	49	49
			Viral Load	TND	Negative	TND	Negative	TND	Negative	197	Negative	150	Negative	150	Negative	TND	TND	Negative
			Day	56	60	63	63	70	70	77	79	84	85	89	94	98	107	112
			Viral Load	150	Negative	150	Negative	TND	Negative	150	Negative	871	2081	27946	46000 copies/mL	7083	88000 copies/mL	2232
8228-001_000100005		40	Day	1	7	14	19	29	33	40	41	43	47	47	54	61	69	76
			Viral Load	TND	TND	TND	TND	150	150	600 copies/mL	150	150	150	negative	150	TND	TND	TND
			Day	82	89	103	112	117	127	131	145	159	205	271				
			Viral Load	TND	TND	150	positive	266	600 copies/mL	150	TND	TND	150	TND				
8228-001_000400002		36	Day	1	8	15	22	29	32	36	43	46	50	58	64	72	92	99
			Viral Load	TND	TND	150	190	668	1236 copies/mL	691	228	negative	457	150	150	150	150	TND
			Day	113	127	141	197	284	354									
			Viral Load	TND	TND	TND	TND	TND	TND									
8228-001_001200003		41	Day	1	8	14	19	26	33	40	41	47	54	61	68	75	82	89
			Viral Load	TND	TND	TND	TND	150	150	886	2867	4882	2672	150	TND	TND	TND	TND
			Day	103	117	131	145	159	201	215	271	284	323					
			Viral Load	TND	TND	TND	TND	TND	654 IU/mL	751 IU/mL	733	5152 IU/mL	TND					
8228-001_001300005	239	21	Day	1	6	7	10	10	17	20	20	27	27	34	34	41	44	48
			Viral Load	150	354 IU/mL	864	925	624 IU/mL	895 IU/mL	1081	2322 IU/mL	371	213 IU/mL	150	less than 137 IU/mL	150	Detected less than 137 IU/mL	TND
			Day	50	56	60	63	66	70	80	84	94	98	107	112	122	126	129
			Viral Load	Not Detected	TND	Detected less than 137 IU/mL	TND	Detected less than 137 IU/mL	150	Detected less than 137 IU/mL	TND	Not Detected	150	Detected less than 137 IU/mL	TND	336 IU/mL	241	364 IU/mL
			Day	143	153	185	185											
			Viral Load	150	detected lessthan 137	150	Not Detected											
8228-001_002000015			Day	1	7	14	18	21	27	27	40	40						
			Viral Load	TND	TND	755.7 IU/mL	290 IU/mL	582	1548	20460 IU/mL	5052	26180 IU/mL						
8228-001_003		18	Day	1	8	15	19	23	31	38	45	52	59	66	73	80	87	94
			Viral	TND	150	150	150	TND	TND	224	860	2941	1638	1033	217	150	TND	TND

Subject ID	Day of Death	Day of PET															
000017			Load														
			Day	101	115	129	141	149	156	156	169	239	270	344			
			Viral Load	150	150	150	TND	80 copies/mL	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	TND	TND			
8228-001_003 300002		10	Day	-27	1	8	8	15	22	29	36	43	50	57	64	71	78	92
			Viral Load	negative	150	353	403	150	150	150	150	150	150	TND	TND	TND	TND	TND
			Day	106	120	134	148	197	260	316								
			Viral Load	TND	TND	TND	TND	TND	TND	TND								
8228-001_003 300003			Day	1	8	15	22	29	36	43	50	57	65	71	78	99	113	127
			Viral Load	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	328
			Day	135	141	155	187	221	261	281	316							
			Viral Load	1293	400	150	3126 copies/mL	TND	TND	1075	TND							
8228-001_003 400003	154	29	Day	1	8	15	22	29	29	32	36	43	50	57	57	64	68	75
			Viral Load	150	TND	TND	150	168	150	171	150	TND	150	TND	1483 copies/mL	TND	TND	TND
			Day	85	92	99	113	127										
			Viral Load	TND	TND	TND	TND	TND										
8228-001_004 200011		42	Day	1	8	15	19	26	32	42	42	48	54	61	68	75	83	92
			Viral Load	TND	TND	TND	TND	TND	150	150	150	150	150	150	150	150	150	150
			Day	97	112	125	139	155	160	167	172	218	273	286	328			
			Viral Load	1282	150	TND	TND	TND	307 copies/mL	150	479 copies/mL	TND	150	331 copies/mL	150			
8228-001_004 400004		9	Day	1	3	7	7	9	14	21	29							
			Viral Load	150	453 copies/mL	842	1905 copies/mL	1092	208	TND	TND							
8228-001_004 400005		24	Day	1	8	12	19	19	24	26	33	40	47	60	66	71	85	92
			Viral Load	TND	TND	TND	150	272 copies/mL	242	150	371	150	TND	TND	TND	TND	150	217 copies/mL
			Day	106	114	127	142	207	260	320								
			Viral Load	TND	TND	150	TND	TND	TND	TND								
8228-001_004 500004		23	Day	1	7	14	21	21	23	28	35	42	52	56	63	70	77	84
			Viral Load	TND	TND	150	150	Positive	325	150	523	150	150	TND	TND	TND	TND	TND
			Day	91	107	121	135	149	162	226	296	343						
			Viral Load	150	150	150	TND	TND	TND	TND	TND	TND						
8228-001_004 700001		88	Day	1	8	15	22	29	36	44	50	58	67	74	80	85	88	89
			Viral Load	TND	150	150	200	179	150	150	150	245	189	413	1014	777	2100 copies/mL	523
			Day	89	103	124	141	156	170	222	264	358						
			Viral Load	342	TND	TND	Negative	Negative	TND	TND	TND	TND						
8228-001_004		25	Day	1	8	16	23	25	25	35	42	49	56	63	72	81	95	109
			Viral	TND	TND	150	728	3958	16000	1333	1164	150	TND	TND	TND	TND	TND	TND

Subject ID	Day of Death	Day of PET															
700002			Load						copies/mL								
			Day	123	135	165	219	255	346								
			Viral Load	TND	TND	TND	TND	TND	TND								
8228-001_004 700006		10	Day	1	8	9	13	16	22	23	30	36	42	51	57	65	73	78
			Viral Load	150	7700 copies/mL	354	514	150	TND	0 copies/mL	150	TND	150	457	799	541	150	TND
			Day	85	94	107	122	136	153	168	204	267	344					
			Viral Load	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND					
8228-001_005 800006		11	Day	1	8	9	14	21	28	38	45	50	57	64	72	79	87	92
			Viral Load	150	150	Cytomegalovirus pp65 Antigen: 1	150	449	376	1051	300	235	TND	150	TND	TND	TND	TND
			Day	106	147	169	274	316										
			Viral Load	TND	TND	TND	TND	TND										
8228-001_005 900001		22	Day	-7	1	6	13	20	20	22	22	27	29	35	37	42	48	58
			Viral Load	95.0 copies/mL	TND	TND	150	1140	positive	1488	positive	1516	735	198	100 0 copies/mL	150	TND	TND
			Day	65	72	79	87	97	107	122	136	170	212	247	308			
			Viral Load	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND			
8228-001_005 900014		15	Day	1	6	10	15	27	35	41	48	56	66	76	104	106	113	125
			Viral Load	150	150	positive	2679	262	150	TND	TND	TND	150	TND	150	150	150	TND
			Day	139	146	154	209	255	332									
			Viral Load	TND	not detected	150	150	TND	TND									
8228-001_006 400001			Day	1	7	15	22	28	34	43	43	48	48	55	55	62	69	76
			Viral Load	TND	TND	TND	TND	TND	TND	TND	negative	TND	Cytomegalovirus pp65 Antigen: normal	TND	Cytomegalovirus pp65 Antigen: normal	TND	TND	TND
			Day	83	90	105	118	132	146	161	203	272	328					
			Viral Load	TND	TND	TND	150	432	150	TND	TND	TND	TND					
8228-001_006 400012	330	16	Day	-1	1	7	8	14	18	21	28	28	37	37	44	44	51	51
			Viral Load	negative	TND	Cytomegalovirus pp65 Antigen: negative	150	1245	Cytomegalovirus pp65 Antigen: 16 cells	4998	50083	Cytomegalovirus pp65 Antigen: 14 cells	1282	Cytomegalovirus pp65 Antigen: negative	377	Cytomegalovirus pp65 Antigen: negative	388	Cytomegalovirus pp65 Antigen: negative
			Day	58	58	64	64	71	71	78	78	85	85	92	92	102	102	121
			Viral Load	1627	Cytomegalovirus pp65 Antigen: 8 cells	4425	12500 copies/mL	11436	Cytomegalovirus pp65 Antigen: 2 cells	582	Cytomegalovirus pp65 Antigen: negative	150	Cytomegalovirus pp65 Antigen: negative	150	Cytomegalovirus pp65 Antigen: negative	1199	Cytomegalovirus pp65 Antigen: 62 cells	150
			Day	121	137	137	147	172	192	225	246	262	273					
			Viral Load	Cytomegalovirus pp65 Antigen: negative	1211	Cytomegalovirus pp65 Antigen: 26 cells	Cytomegalovirus pp65 Antigen: 278 cells	1042	Cytomegalovirus pp65 Antigen: 21 cells	352	Cytomegalovirus pp65 Antigen: 119 cells	5735	Cytomegalovirus pp65 Antigen: 176 cells					

Subject ID	Day of Death	Day of PET															
8228-001_006 400021	174	55	Day	-4	1	4	6	11	14	22	26	29	29	33	33	40	40	47
			Viral Load	Cytomegalovirus pp65 Antigen: positive	TND	Cytomegalovirus pp65 Antigen: negative	TND	Cytomegalovirus pp65 Antigen: negative	TND	TND	Cytomegalovirus pp65 Antigen: 3 cells	150	Cytomegalovirus pp65 Antigen: negative	150	Cytomegalovirus pp65 Antigen: 1 cells	150	Cytomegalovirus pp65 Antigen: negative	150
			Day	47	54	54	55	61	69	69	77	77	81	88	88	96	109	124
			Viral Load	Cytomegalovirus pp65 Antigen: negative	150	Cytomegalovirus pp65 Antigen: 8 cells	150	Cytomegalovirus pp65 Antigen: negative	TND	Cytomegalovirus pp65 Antigen: negative	TND	Cytomegalovirus pp65 Antigen: negative	TND	TND	Cytomegalovirus pp65 Antigen: negative	TND	TND	TND
			Day	144	151	165	172											
			Viral Load	TND	TND	TND	Cytomegalovirus pp65 Antigen: negative											
8228-001_006 400025		49	Day	1	7	14	21	28	34	40	47	47	49	61	68	75	82	89
			Viral Load	TND	TND	TND	TND	TND	150	150	3534	Cytomegalovirus pp65 Antigen: 8 cells	6637	12247	398	150	150	150
			Day	96	110	124	138	152	166	215	266							
			Viral Load	150	TND	TND	TND	268	TND	TND	TND							
8228-001_006 900003	368	39	Day	1	15	21	28	34	35	39	42	49	57	63	70	78	91	105
			Viral Load	TND	TND	150	150	375 copies/mL	232	267	209	TND	TND	TND	TND	TND	TND	TND
			Day	116	140	150	204	259										
			Viral Load	TND	TND	TND	TND	TND										
8228-001_006 900007		62	Day	1	6	13	20	27	34	41	48	55	55	62	69	69	76	76
			Viral Load	TND	150	150	150	TND	TND	TND	TND	296	296 copies/mL	1076	150	137IU/ml	150	negative
			Day	90	104	115	129	146	207	209	213	223	262	321				
			Viral Load	150	TND	TND	TND	TND	272	272 copies/mL	150	150	TND	TND				
8228-001_007 800003		17	Day	-22	1	7	15	17	21	23	24	28	36	42	49	56	63	73
			Viral Load	Negative	150	1066	13264	31847	4.2 log IU/mL	11020	4.11 log IU/mL	66364	2054	150	150	150	150	150
			Day	80	99													
			Viral Load	150	150													
8228-001_007 800004		131	Day	-27	-3	5	13	20	27	33	40	48	55	62	69	76	90	104
			Viral Load	negative	TND	TND	TND	150	TND	TND	TND	TND	TND	TND	TND	TND	TND	Not detected
			Day	118	126	131	153	195	251	313								
			Viral Load	1661	3.87 log IU/mL	21577	925	TND	TND	150								
8228-001_009 100020	374		Day	1	7	14	21	25	32	39	46	53	60	67	75	81	88	95
			Viral Load	TND	150	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND
			Day	109	123	131	138	145	155	167	214	273	280					
			Viral Load	TND	472	48000 copies/microL	54654	21190	607	150	150	150	Not detected					

Subject ID	Day of Death	Day of PET															
8228-001_010 200007		15	Day	1	6	12	15	21	26	33	41	47	57	62	72	77	82	89
			Viral Load	TND	150	1000 copies/mL	150	150	150	150	TND	150	150	150	273	934	516	949
			Day	110	120	140	140	148	162	225	274							
			Viral Load	150	150	879	818	250	TND	150	150							
8228-001_010 200008			Day	1	7	14	19	29	36	43	49	54	61	70	77	84	98	118
			Viral Load	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	150
			Day	133	144	144	146	162	203	259								
			Viral Load	150	150	1200 copies/mL	151	TND	TND	TND								
8228-001_010 800007	167	33	Day	1	8	15	19	26	26	33	33	40	40	47	47	55	61	62
			Viral Load	TND	TND	150	150	1376	9865 IU/mL	11803	81516 IU/mL	15073	31408 IU/mL	28749	44154 IU/mL	6437	1797 IU/mL	561
			Day	68	71	75	75	82	82	89	89	96	96	110	110	124	139	160
			Viral Load	338	600 IU/mL	183	600 IU/mL	1289	5720 IU/mL	18767	76148 IU/mL	87418	211565 IU/mL	437	2059 IU/mL	150	TND	150
8228-001_010 800015		29	Day	1	8	15	22	26	28	33	43	50	57	61	71	78	85	92
			Viral Load	TND	150	150	150	3317 IU/mL	150	150	150	150	385	666	411	641	150	TND
			Day	99	119	127	148	162	176	212	275	362						
			Viral Load	TND	150	150	TND	150	TND	TND	TND	TND						
8228-001_011 300004		15	Day	1	1	8	15	15	15	22	22	29	36	42	49	57	71	92
			Viral Load	150	POSITIVE	TND	734	675	675 copies/mL	1157	1157 copies/mL	150	TND	TND	TND	150	TND	TND
			Day	101	113	127	144	204	260									
			Viral Load	TND	TND	TND	TND	TND	TND									
8228-001_011 600002		12	Day	1	7	13	21	28	35	42	49	56	63	70	76	85	97	111
			Viral Load	TND	150	611	1475	233	150	150	150	178	798	150	150	TND	TND	TND
			Day	124	138	154	203	273	281	307								
			Viral Load	TND	150	150	TND	150	379 IU/mL	150								
8228-001_011 600011		11	Day	1	3	8	10	11	15	16	21	28	35	42	49	56	63	65
			Viral Load	TND	positive	289	732 copies/mL	150	150	Positive	TND	TND	TND	TND	TND	TND	150	positive
			Day	70	84	98	114	126	140	211	246	351						
			Viral Load	667	1638	150	150	TND	150	TND	TND	TND						
8228-001_011 600025		14	Day	1	8	14	17	21	24	28	31	35	38	42	48	50	56	57
			Viral Load	TND	150	662	POSITIVE	3171	POSITIVE	9293	POSITIVE	723	POSITIVE	327	positive	189	positive	157
			Day	63	63	70	70	77	79	91	133	147	224	268	310			
			Viral Load	150	positive	TND	positive	TND	positive	TND	TND	TND	TND	TND	TND			
8228-001_011 600033		15	Day	1	8	14	15	17	21	28	29	32	36	42	43	46	50	57
			Viral Load	TND	150	943	941	Positive	271	Positive	6153	Positive	150	Positive	150	Positive	TND	TND
			Day	66	70	77	85	108	136	141	164	203	269					
			Viral Load	TND	TND	150	TND	150	TND	TND	TND	TND	TND					
8228-001_011	296	46	Day	1	8	22	31	39	44	44	46	49	51	58	58	65	71	74
			Viral	150	449	TND	150	969	6973	Cytomeg	28096	11386	Cytomeg	8466	Cytomeg	3757	7003	7942

Subject ID	Day of Death	Day of PET															
700006			Load							alovirus pp65 Antigen: positive			alovirus pp65 Antigen: positive		alovirus pp65 Antigen: negative		
			Day	78	86	93	100	114	128	143	184	239					
			Viral Load	Cytomeg alovirus pp65 Antigen: positive	2064	Cytomeg alovirus pp65 Antigen: negative	282	718	3878	11178	328	208					
8228-001_012 200003	163	108	Day	1	7	15	18	29	36	43	50	56	64	70	77	92	106	106
			Viral Load	150	150	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	150	4523	7994 IU/mL
			Day	108	119	126	137	137										
			Viral Load	6092	831	2096 IU/mL	221	438 IU/mL										
8228-001_014 000006		16	Day	1	8	10	15	16	22	30	37	46	49	53	64	71	79	87
			Viral Load	TND	TND	300 copies/mL	TND	150	TND	150	150	150	361	150	527	244	1430	150
			Day	94	106	120	140	155	169	217	263	319						
			Viral Load	TND	TND	150	150	367	150	TND	TND	TND						
8228-001_014 000009	84	54	Day	1	8	15	23	30	37	45	52	52	54	57	60	65	65	71
			Viral Load	TND	TND	TND	TND	TND	TND	150	150	900 copies/mL	402	458	1400 copies/mL	150	900 copies/mL	898
			Day	71	78	78												
			Viral Load	500 copies/mL	659	600 copies/mL												
8228-001_014 000011	131		Day	1	8	15	23	29	32	36	39	43	43	50	57	60	64	71
			Viral Load	TND	TND	150	TND	TND	400 copies/mL	150	50 copies/mL	150	50 copies/mL	TND	TND	100 copies/mL	150	150
			Day	78	87	92	99	101	106	113	123	127						
			Viral Load	150	150	150	100 copies/mL	TND	50 copies/mL	150	Negative	TND						
8228-001_014 000012	200	54	Day	1	8	15	24	30	36	43	52	52	54	58	58	66	73	73
			Viral Load	TND	TND	TND	TND	TND	TND	TND	172	1000 copies/mL	150	150	300 copies/mL	150	150	less than 50 copy/ml
			Day	80	87	87	92	96	100	114	128	142	156					
			Viral Load	TND	TND	less than 50 copy/ml	TND	LT 50 copy/ml	TND	TND	TND	TND	TND					
8228-001_016 100001		14	Day	-1	1	7	8	14	14	15	21	22	28	29	35	36	44	49
			Viral Load	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 1 cells	160	318	Cytomeg alovirus pp65 Antigen: 9 cells	151	Cytomeg alovirus pp65 Antigen: 40 cells	1973	Cytomeg alovirus pp65 Antigen: 28 cells	2934	Cytomeg alovirus pp65 Antigen: 7 cells	853	1667	Cytomeg alovirus pp65 Antigen: 52 cells
			Day	50	56	57	64	64	71	71	78	78	85	85	99	99	113	117
			Viral Load	195	Cytomeg alovirus pp65 Antigen: 6 cells	561	237	Cytomeg alovirus pp65 Antigen: 2 cells	150	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 1 cells	150	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells
			Day	127	127	141	141	155	155	211	260	329						
			Viral	TND	Cytomeg	TND	Cytomeg	TND	Cytomeg	TND	TND	TND						

Subject ID	Day of Death	Day of PET																
			Load		alovirus pp65 Antigen: 0 cells		alovirus pp65 Antigen: 0 cells		alovirus pp65 Antigen: 0 cells									
8228-001_016 100002		8	Day	-1	1	6	7	8	13									
			Viral Load	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 2 cells	244	174	Cytomeg alovirus pp65 Antigen: 6 cells									
8228-001_016 100005		46	Day	1	1	8	8	15	22	23	31	31	36	36	39	43	43	46
			Viral Load	TND	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	TND	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 1 cells	150	150	Cytomeg alovirus pp65 Antigen: 5 cells	150
			Day	50	53	53	64	64										
			Viral Load	Cytomeg alovirus pp65 Antigen: 11 cells	182	Cytomeg alovirus pp65 Antigen: 5 cells	150	Cytomeg alovirus pp65 Antigen: 1 cells										
8228-001_016 100008	262	128	Day	1	8	9	15	16	24	29	30	36	37	43	44	50	51	57
			Viral Load	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells
			Day	58	64	71	72	78	79	85	86	99	100	106	114	121	128	128
			Viral Load	TND	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 1 cells	781	1067
			Day	135	142	142	156											
			Viral Load	Cytomeg alovirus pp65 Antigen: 13 cells	1397	Cytomeg alovirus pp65 Antigen: 4 cells	TND											
8228-001_016 400003		23	Day	1	7	8	14	15	21	22	23	28	29	35	36	42	43	49
			Viral Load	150	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 1 cells	150	Cytomeg alovirus pp65 Antigen: 3 cells	150	150	Cytomeg alovirus pp65 Antigen: 3 cells	150	Cytomeg alovirus pp65 Antigen: 2 cells	150	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 0 cells
			Day	50	56	57	62	64	69	71	76	78	84	84	91	91	105	105
			Viral Load	150	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	TND	Cytomeg alovirus pp65 Antigen: 0 cells	158	Cytomeg alovirus pp65 Antigen: 0 cells	1300	Cytomeg alovirus pp65 Antigen: 14 cells
			Day	119	126	133	141	147	154	161	210	217	267	273	287	329		
			Viral Load	1102	Cytomeg alovirus pp65 Antigen: 4 cells	150	Cytomeg alovirus pp65 Antigen: 2 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 0 cells	150	Cytomeg alovirus pp65 Antigen: 0 cells	TND	Cytomeg alovirus pp65 Antigen: 1 cells	TND		
8228-001_016 400007		120	Day	1	6	8	13	15	20	22	27	28	34	35	41	48	51	55
			Viral Load	TND	Cytomeg alovirus	TND	Cytomeg alovirus	TND	Cytomeg alovirus	TND	Cytomeg alovirus	TND	Cytomeg alovirus	TND	Cytomeg alovirus	Cytomeg alovirus	TND	Cytomeg alovirus

Subject ID	Day of Death	Day of PET																
					pp65 Antigen: 0 cells		pp65 Antigen: 0 cells		pp65 Antigen: 0 cells		pp65 Antigen: 0 cells		pp65 Antigen: 0 cells		pp65 Antigen: 0 cells	pp65 Antigen: 0 cells		pp65 Antigen: 0 cells
			Day	58	64	65	69	72	76	79	83	86	90	93	103	106	110	117
			Viral Load	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 1 cells	Cytomegalovirus pp65 Antigen: 6 cells
			Day	120	131	138	145	152	159	194	198	198						
			Viral Load	3706	2639	Cytomegalovirus pp65 Antigen: 2 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells						
8228-001_016 400010		56	Day	1	8	15	19	22	26	29	33	36	40	43	47	47	54	54
			Viral Load	TND	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	459	1417	Cytomegalovirus pp65 Antigen: 1 cells	1209	Cytomegalovirus pp65 Antigen: 5 cells
			Day	56	61	61	68	75	78	82	82	89	95	103	110	117	123	131
			Viral Load	2057	150	Cytomegalovirus pp65 Antigen: 1 cells	206	150	Cytomegalovirus pp65 Antigen: 1 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND
			Day	138	145	153	158	161	221	277								
			Viral Load	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND								
8228-001_016 500001		121	Day	1	1	8	15	15	22	22	29	29	36	36	40	50	50	57
			Viral Load	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND
			Day	57	65	71	71	78	78	85	93	100	108	114	121	121	128	128
			Viral Load	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	150	1256	Cytomegalovirus pp65 Antigen: 8 cells	1260	Cytomegalovirus pp65 Antigen: 0.5 cells
			Day	141	149	156	156	214	277	325								
			Viral Load	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	TND								
8228-001_016 500005		29	Day	1	8	13	15	20	22	27	29	30	34	35	41	48	49	56
			Viral Load	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0.5 cells	150	150	Cytomegalovirus pp65 Antigen: 0 cells	150	150	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 0 cells
			Day	57	62	64	69	71	72	76	78	84						
			Viral Load	150	Cytomegalovirus pp65	150	Cytomegalovirus pp65	519	272	Cytomegalovirus pp65	150	Cytomegalovirus pp65						

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					Antigen: 0 cells		Antigen: 3 cells			Antigen: 0 cells		Antigen: 0 cells					
8228-001_016 500006		128	Day	1	8	13	15	20	22	27	28	34	41	43	50	50	55	55
			Viral Load	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells
			Day	62	62	69	69	76	76	83	83	90	90	104	104	125	125	128
			Viral Load	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	150	Cytomegalovirus pp65 Antigen: 2 cells	150
			Day	139	139	153	153	167	195	223	251	272	272					
			Viral Load	150	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells	TND	Cytomegalovirus pp65 Antigen: 0 cells					
8228-001_017 800002	326	127	Day	1	8	14	20	25	34	39	46	53	63	74	84	91	110	119
			Viral Load	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	TND	NEGATIVE
			Day	130	130	134	148	161	216	260	315							
			Viral Load	255	575 copies/mL	196	150	150	150	TND	TND							
8228-001_018 500001	49	26	Day	1	8	12	18	20	26	26	33							
			Viral Load	TND	TND	150	421	1327	15387	Positive	29667							
			Day	115	126	137	140	148	156	156	157	204	247	261	305	318	318	
			Viral Load	4800 copies/mL	150	Negative	150	Negative	5189	4800 copies/mL	12112	TND	Negative	TND	Negative	150	Negative	

Green: Central Laboratory results.
Yellow: Local Laboratory results.

Table A1-6: Subjects with Opportunistic Infections other than HCMV Infection (Letermovir Arm)

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_000100001		89	Y		N	Bilateral conjunctivitis, worsening, Epstein Barr Virus Viremia, Extended Spectrum Beta Lactamases (ESBLs) Escherichia coli Pneumonia, Pneumocystis jiroveci Pneumonia, Staphylococcus aureus Respiratory Tract Infection
8228-001_000100004			Y		N	Angular Cheilosis, Folliculitis, Bilateral Lower Extremities
8228-001_000300003	349	101	N		N	adenovirus pneumonitis, bronchitis, Corynebacterium pneumonia, Human herpesvirus 6 viremia
8228-001_000300004			Y		N	paronychial infection toe
8228-001_000300006			Y		N	upper respiratory tract infection due to coronavirus
8228-001_000300010			Y		N	viridans group streptococcal bacteremia
8228-001_000300011			Y		N	upper respiratory tract infection
8228-001_000300014			Y		Y	***acute gastroenteritis***
8228-001_000400008			Y		N	Rhinovirus Infection
8228-001_000400014		112	Y		N	Coronavirus
8228-001_000400017			Y		N	eye infec ion
8228-001_000400019		12	N		N	Human Rhinovirus Upper URTI Nasopharyngitis

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_000400024		120	Y		Y	***hemorrhagic cystitis due to BK virus***
8228-001_000500001	241		Y		Y	***Herpes Zoster (Shingles) Recurrence***
8228-001_001200002	264		Y		Y	Disseminated Fusarium infection, E coli urinary tract infection, ***S epidermidis bacteremia***
8228-001_001200004			Y		N	BK viremia, E. cloacae bacteremia
8228-001_001400001	30		Y		N	C. difficile enteritis
8228-001_001700006	249	104	Y		N	Carbapenem Resistant Enterobacteriaceae Klebsiella pneumoniae, Oral Candida Glabrata, Vancomycin Resistant ENTEROCOCCUS
8228-001_001700007			Y		N	Sinusitis
8228-001_001700008			Y		N	Upper Respiratory infection
8228-001_001700009	138		Y		N	BK Virus Infection hemorrhagic cystitis, Clostridium difficile ENTEROCOLITIS, CLOSTRIDIUM DIFFICILE POSITIVE DIARRHEA, HUMAN HERPES VIRUS-6 MENINGENCEPHALITIS, Mycobacterium Avium Infection, Vancomycin Resistant Enteroccus Faecium
8228-001_001700010			Y		Y	Shingles, ***TOXIN-MEDIATED GASTROENTERITIS***
8228-001_001700013	41		Y		Y	BK VIREMIA, PHARYNGITIS, RHINOVIRUS INFECTION, STAPHYLOCOCCUS EPIDERMIDIS INFECTION, ***STENOTROPHOMONAS Pneumonia***
8228-001_001800001			Y		N	BK viruria, Folliculitis, torso, Pneumonia
8228-001_001800002		125	Y		N	Oral candidiasis, Urinary tract infection
8228-001_001800007		159	Y		N	EBV reactivation, HHV-6 encephalitis, MRSA bacteremia, Oral thrush
8228-001_001800009	137		Y		N	Invasive Pulmonary Aspergillosis
8228-001_001800011	90		Y		N	Cellulitis, Upper Respiratory Infection
8228-001_001800013	17		N		Y	Candidemia (Candida dubliniensis), Enterococcus faecium bacteremia, ***Septic Shock***
8228-001_001800014	198		Y		N	Oral Thrush, Pneumonia
8228-001_001800019			Y		Y	***Rotavirus infection (previously submitted as Worsening diarrhea)***
8228-001_001800021	353		Y		N	Acute Otitis Media, Left ear, Right Lower extremity cellulitis
8228-001_001800026	194		Y		N	Fungal rash (Axillae)
8228-001_001800027	201		Y		N	Bronchopneumonia, Septic shock, upper respiratory tract infection
8228-001_001800029			Y		N	Upper respiratory tract infection
8228-001_001800034			Y		N	Folliculitis
8228-001_001800035			Y		N	Pneumonia
8228-001_001800037	121		Y		Y	***C. difficile associated diarrhea***
8228-001_001800041			Y		N	Oral candidiasis
8228-001_001800042			Y		N	Upper respiratory infection
8228-001_001800043			Y		N	Oral candidiasis
8228-001_001800045		125	N		N	BK virus cystitis
8228-001_001800048	252	66	Y		N	Acute disseminated candidiasis (C. parapsilosis), Candidemia
8228-001_001800050	101		Y		Y	coagulase-negative Staphylococcus Bacteremia, pneumonia, ***septic shock***
8228-001_001800051	115		Y		N	E. coli bacteremia, Facial Folliculitis, Respiratory infection, Rothia mucilaginosa bacteremia
8228-001_001800052			Y		Y	***aseptic meningitis***
8228-001_001900003	116		Y		N	abscess of right parotid mass, BK virus infection of bladder
8228-001_001900004	300	58	Y		N	pulmonary aspergillosis, RSV pneumonia, urinary tract infection, Urinary Tract Infection, VRE in stool
8228-001_001900005			Y		N	bacteremia, coag neg staph and Acinetobacter, C. difficile colitis, Gram positive cocci blood infection, rotavirus infec ion of bowel, yeast infection, vaginal
8228-001_002000003			Y		N	Cold Sore to Lip, Klebsiella pneumoniae Bacteremia, Rhinovirus, Staphylococcus aureus Bacteremia
8228-001_002000004			Y		N	Cold, Enterovirus, Rhinovirus
8228-001_002000005			Y		N	Bilateral Elbow Cellulitis
8228-001_002000007			Y		N	Rhinitis
8228-001_002000008	331		Y		N	Cold, Enterovirus, Rhinovirus
8228-001_002000009			Y		N	Klebsiella pneumoniae Urinary Tract Infec ion
8228-001_002000010			Y		N	Corynebacterium jeikeium Bacteremia
8228-001_002000013			Y		N	Haemophilus parainfluenzae Bacteremia, Perineal Abscess

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_002000016			Y		N	Folliculitis to Face, Folliculitis to Scalp
8228-001_002000018	46		Y		N	Bilateral Pneumonia, Clostridium difficile Colitis, Sepsis
8228-001_002000020			Y		N	Atypical Pneumonia to Right Lung, Pharyngitis
8228-001_003000003			Y		N	folliculitis, FOLLICULITIS, rhinitis
8228-001_003000004		36	N		N	folliculitis, sepsis
8228-001_003000005			Y		N	CYSTITIS, folliculitis
8228-001_003000006			Y		N	invasive pulmonary aspergillosis
8228-001_003000011	257	92	Y		N	Oral candidiasis
8228-001_003000017		18	Y		Y	Acute sinusitis, Conjunctivitis, Cystitis, Fever (Klebsiella Pn. infection), pneumonia, ***VZV retinitis***
8228-001_003300001			Y		N	CVC Infection, otitis left ear, otitis right ear
8228-001_003300003			Y	144	N	HHV6 REACTIVATION
8228-001_003300004			Y		N	HHV6 reactivation
8228-001_003300006			Y		N	catheter infection, cystitis, folliculitis, Human herpesvirus 6 infection reactivation
8228-001_003300009		128	Y		N	EBV reactivation
8228-001_003300011			Y		N	respiratory infection
8228-001_003300012			Y		N	cystitis
8228-001_003400001		147	Y		N	EBV INFECTION (viremia)
8228-001_003400004			Y		N	folliculitis
8228-001_003400005	116		Y		Y	folliculitis, infection HHV6(viremia), ***sepsis without focus***
8228-001_004100001			Y		N	HERPES ZOSTER IN NECK
8228-001_004100003			Y		N	common cold
8228-001_004100005	63		Y		Y	***Tract Urinary Infection***
8228-001_004200001			Y		N	COMMON COLD, FLU, HERPES SIMPLEX IN TONGUE, ORAL HERPES, PAROTIDITIS, RESPIRATORY INFECTION upper tract
8228-001_004200006			Y		N	boil in groin
8228-001_004200007	194	140	N		N	herpes zoster, Pneumonia, Respiratory infection
8228-001_004200008	304		Y		N	viremia (Parainfluenza 1): upper respiratory tract infection
8228-001_004200012			Y		N	Fever by Staphylococcus coagulase-negative
8228-001_004400002	306		Y		N	ocular conjunctivi is, Urinary tract infection
8228-001_004400004		9	N		Y	***Bacteremia by Streptococcus viridans***
8228-001_004500002			Y		N	Common cold
8228-001_004500003			Y		Y	***Cold***
8228-001_004500005			Y		N	Cold, Pneumonia
8228-001_004500007			Y		N	Cold
8228-001_004500008	197		Y		Y	Parainfluenza infection, ***Tonsillitis***
8228-001_004500013			Y		N	Sepsis
8228-001_004500014			Y		Y	***Rhinovirus infection***
8228-001_004700003			Y		N	Fungus in mouth
8228-001_004700006		10	N		Y	***pneumonia***
8228-001_004700008			Y		N	Fungal infection scrotum, Urinary Infection
8228-001_005300001		8	N		Y	***Urinary Tract Infection***
8228-001_005800002			Y		Y	Parotitis left, ***Soft tissue Infection (cellulitis)- left area port a cath implantation***
8228-001_005800004		144	Y		N	Pharyngitis
8228-001_005800005			Y		N	Cystitis
8228-001_005800007	104	25	Y		N	Bacteremia, Clostridium difficile Infection (Bacteremia), Pulmonary Infiltrates (proven fungal Infection-candida albicans), Renal Infiltrates (suspected fungal Infection-candida albicans), suspected Infection
8228-001_005800008			Y		N	Folliculitis
8228-001_005900002			N		N	thrush mouth
8228-001_005900003			Y		Y	***bronchitis***
8228-001_005900012			N		N	rhinitis

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_005900016			Y		N	thrush, urinary tract infection
8228-001_005900017			Y		N	common cold
8228-001_006100005			Y		N	Rhinitis
8228-001_006100006	146		Y		Y	bacterial Meningi is, Cheilitis angularis, ***pulmonary aspergillosis***
8228-001_006100008			Y		N	Pharyngitis
8228-001_006300002	199		Y		N	atypical pneumonia, atypical pneumonia, Bacteremia, Cystitis, gram posi ive staphylococcal bacteremia, Herpes simplex DNA test positive (viremia), HHV6 Reactivation
8228-001_006300003	16		Y		N	Aspergillus Pneumonia, Sepsis, Worsening Aspergillosis (Aspergillus AG posi ive in blood)
8228-001_006300006	147		Y		Y	conjunctivitis of the right eye, ***Sepsis***
8228-001_006400001			Y		N	Infection / RSV viremia, Urinary tract infection
8228-001_006400004			Y		N	EBV Reactiva ion process, Infection with Staphylococcus epidermis, Sore oral cavity/ Candidiasis intermittent
8228-001_006400009			Y		N	Oral candidiasis
8228-001_006400010			Y		N	Infection Candida / oral thrush
8228-001_006400014			Y		N	EBV Reactivation, Enterococcus in Urine, Pneumonia, Staphylococcus epidermis bacteremia, Urinary Tract Infection
8228-001_006400015			Y		N	Cold Symptoms
8228-001_006400016			Y		N	Cold Symptoms
8228-001_006400017		54	Y		N	Infection enterococcus faecuim, Urinary tract infection
8228-001_006400020			Y		Y	Diverticulitis, Sepsis, Tonsillitis, ***Urinary Tract Infection***
8228-001_006400023			Y		N	EBV Reactivation
8228-001_006900005		123	Y		N	acute pharyngitis, Herpes Zoster, maxillary sinusitis
8228-001_006900006			Y		N	Oral Candidiasis, Pharyngitis, upper respiratory infection, upper respiratory infection, UPPER RESPIRATORY INFECTION: FRONTAL SINUSITIS, Upper Respiratory infection, Urinary Infection
8228-001_006900007		62	Y		N	acute pharyngoamygdalitis, upper respiratory tract infection
8228-001_006900008			Y		N	oral candidiasis
8228-001_006900012			N		N	acute media o itis, left eye conjunc ivitis, right external otitis
8228-001_007100003			Y		N	acute otitis media
8228-001_007100006			Y	140	N	epididymitis, Esophageal candidiasis
8228-001_007200002			N		N	otitis media with effusion, Pharyngitis, Upper respiratory infection
8228-001_007300004			Y		N	herpes zoster in buttock, urinary tract infection
8228-001_007500007			Y		N	acute viral respiratory infection
8228-001_007800001			N		N	Common cold, Mild cold
8228-001_007800008	54		N		Y	EBV reactivation, ***Pneumonia***
8228-001_009100014	387		Y		N	Oral thrush, Paraflu
8228-001_009100016			Y		Y	***Paraflu type 1 viremia***
8228-001_009100020	374		Y	141	Y	Paraflu virus, peri-orbital cellulitis, ***Peri-orbital cellulitis***
8228-001_009300001			Y		N	coryzal symptoms
8228-001_010000001			N		N	urine infection: Bk and JC
8228-001_010000002	149		Y		Y	***soft tissue infection cellulitis: left inferior extremity***
8228-001_010000005		8	N		N	reactivation BK, reactivation JC
8228-001_010100005		101	Y		N	Gastrointestinal inf. susp.-Candida glabrata, Pneumonia
8228-001_010200003			Y		N	Bacterial pharyngitis (Escherichia coli ESBL, Klebsiella pneumoniae ESBL), Bronchitis, Tonsillitis
8228-001_010200008			Y		N	Pneumonia
8228-001_010800001			Y		N	common cold
8228-001_010800003		11	N		N	staphylococcus epidermidis sepsis
8228-001_010800006	116		N		N	bacteremia, herpes simplex infection mouth, invasive aspergillosis, lip infection
8228-001_010800012		137	Y		N	varicella zoster
8228-001_010800013		144	Y		N	bronchiolitis, Bronchitis: upper respiratory tract infection (Pseudomonas aeruginosa), common cold, lip infection (herpes simplex virus 1)
8228-001_010800015		29	Y		N	bronchitis, catheter related infection (staphylococcus epidermidis), urinary tract infection
8228-001_010800016		101	Y		N	rhinitis

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_010800019			Y		N	catheter related infection, pyuria
8228-001_010800021			Y		N	bronchiolitis
8228-001_010800023	475		Y		N	catheter related infection, folliculi is head, herpes simplex infection mouth
8228-001_010800027	69		Y		N	invasive pulmonary aspergillosis, Klebsiella pneumoniae sepsis, staphylococcus aureus sepsis
8228-001_010800028			Y		N	bacteremia (Streptococcus mitis)
8228-001_011000002			Y		Y	rhinitis, ***viral gastro-enteritis***
8228-001_011000004	171	146	Y		Y	acute sinusitis, clostridium difficile colitis, ***rhinovirus infection***
8228-001_011000005			Y		N	para-influenza type 1 infection
8228-001_011000008			Y	132	N	bronchioli is, polyomavirus infection, rhinovirus infection, upper respiratory infection
8228-001_011000009			Y		N	campylobacter bacteremia, possible pulmonary invasive aspergillosis
8228-001_011300001	60		Y		N	EBV reactivation
8228-001_011300002			Y		N	NASOPHARYNGITIS, URI, METAPNEUMOVIRUS POSITIVE
8228-001_011300003			Y		N	CANDIDA ALBICANS, INFLUENZA A, ORAL THRUSH, PNEUMONIA, SINUSITIS
8228-001_011600001	155		Y		Y	Coagulase negative staphylococcus aureus blood infection, ***Pneumonia***
8228-001_011600003			Y		Y	***Pneumonia***
8228-001_011600004	106		Y		N	Coagulase negative staphylococcus blood infection, Parainfluenza Pneumonia, Vancomycin resistant enterococci colitis
8228-001_011600009			Y		Y	***C Difficile colitis***
8228-001_011600011		11	Y		N	HHV6 viremia
8228-001_011600016	198		Y		Y	BK Cystitis, septic shock, thrush, ***worsening pneumonia***
8228-001_011600017	191		Y		N	human rhinovirus, Stomatococcus bacteremia
8228-001_011600018	72		Y		N	BK Hemorrhagic Cysti is, worsening enterococcus faecium pneumonia, worsening of aspergillosis fungemia
8228-001_011600020			Y		N	gram negative rod UTI
8228-001_011600023			Y		N	Klebsiella pneumoniae urinary tract infection, thrush
8228-001_011600027			Y		N	bacillus species blood infection, Staph Coag Neg bacteremia, staphy epidermidis bacteremia, thrush
8228-001_011600028			Y		Y	Right eye periorbital celluli is, sinusitis, thrush, ***urinary tract infection***
8228-001_011600030			Y		N	bacillus bacteremia, Klebsiella pneumoniae UTI
8228-001_011600031			Y		N	C diff stool infection, common cold, E Coli UTI, thrush
8228-001_011600032		50	Y		N	bacillus species bacteremia, Staph Coag Neg bacteremia
8228-001_011600034		32	N		N	coronavirus, HSV viremia, Human Rhinovirus, intermittent CMV Reactivation, Klebsiella pneumoniae UTI, nasopharyngitis
8228-001_011600035			Y		N	BK Viremia
8228-001_011600036			Y		N	nasopharyngitis
8228-001_011600038	198	126	Y		N	Coronavirus NL63 respiratory infection, HHV6 viremia
8228-001_011600039		91	Y		Y	bacteremia, bacterial upper respiratory tract infection, ***coronal virus pneumonia***
8228-001_011600041			Y		Y	***Gastroenteritis Norovirus***
8228-001_011600044			Y		N	cellulitis of left extremity, Corynebacterium bacteremia, enterococcus faecalis bacteremia, salmonella UTI, staphylococcus epidermidis bacteremia, thrush
8228-001_011600045			Y		N	coronavirus, human rhinovirus
8228-001_011600048		65	Y		N	BK Cystitis, coronavirus, urinary tract infection staphylococcal
8228-001_011700004	142		Y		Y	folliculitis- face, ***sepsis***
8228-001_011700005			Y		Y	candida albicans infection BAL-oral, ***pneumonia- unknown origin***
8228-001_011700006	296	46	N		N	pneumonia without causal agent, upper respiratory infection symptoms
8228-001_012200003	163	108	N		N	C. difficile infection, EBV Viremia
8228-001_012300001	214		Y		Y	Neutropenic Sepsis, ***Sepsis***
8228-001_012300003			Y		N	Diarrhea (viral gastroenteritis), urinary viral infection
8228-001_012300006			Y		N	thrush
8228-001_012300007	80		Y		Y	Acute uncomplicated diverticulitis, ***Sepsis***
8228-001_012300009	185		Y		N	HERPES ZOSTER, RIGHT INNER LEG, multifocal community-acquired pneumonia, thrush
8228-001_012600003	92		Y		N	Epidermidis bloodstream infection, Lung infection/ Pneumonia, Pulmonary infection -invasive pulmonary Aspergillus infection
8228-001_012600006			N	150	N	Enterocoli is infectious, Urinary tract infection (UTI)

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_012900005			Y		N	Bacteremia
8228-001_012900008		12	Y		N	Bacteremia
8228-001_012900011			Y		Y	***Fungal Infection (fungal infection in the lungs aspergilloma)***
8228-001_012900016	321		Y		Y	Cystitis, ***Sepsis of unknown etiology***
8228-001_012900017			Y		N	Sepsis
8228-001_013100001		105	N		N	bacteremia
8228-001_013100009	221		Y		Y	bacteremia, herpes in lip, ***Pneumonia***
8228-001_013100013			N		Y	bacteremia, ***sinusitis***
8228-001_013100015			Y		Y	bacteremia, Sinusitis, upper respiratory infection, ***viremia***
8228-001_013100016			Y		N	abscess in pilonidal sinus, bacteremia
8228-001_013100017	15		Y		N	bacteremia
8228-001_013100021	231		Y		Y	bacteremia, cranial involvement in mucormicosis, mucor mucosis, ***sinusitis***
8228-001_013100023	236		N		N	bacteremia (S. epidermidis)
8228-001_013100025			Y		N	bacteremia, Epstein-Barr virus infection, sinusitis
8228-001_013100028			Y		N	bacteremia
8228-001_013300001			Y		N	Localized Cutaneous Infection, Upper respiratory tract infec ion
8228-001_014000001		126	Y		Y	***Fungal infection of gut (Candida dubliniensis and Candida glabrata)***
8228-001_014000002	258		Y		N	Bronchitis, Ca heter site cellulitis of right, Respiratory infection, Susp. Aspergillosis lobus superior pulmonis sinistri
8228-001_014000005			Y		N	Candida albicans, Cystitis, Oral candidiasis, Urinary tract infection
8228-001_014000006		16	Y		N	Adenovirus viremia
8228-001_014000008		129	Y		Y	***Dental infection***
8228-001_014000010			Y		N	Fungal infection of mouth
8228-001_014000011	131		Y	37	N	Pneumonia, Polyoma virus, Rhinovirus
8228-001_014000013	192		Y		N	Unspecified infection, Urinary tract infection
8228-001_014000014			Y		Y	Fungal infection of mouth, Loose stools (Clostridium difficile positive), ***Unknown Pneumonia***
8228-001_014600003			Y		Y	***Candida albicans to esophageal candidiasis***
8228-001_014600004			Y		N	ringworm
8228-001_014600008			Y		N	bacteremia
8228-001_014700003		174	Y		N	otitis externa due to Aspergillus niger, possible genital herpes (or erosive rash lesions), Septicemia due to Pseudomonas aeruginosa, Septicemia due to Staphylococcus haemolyticus
8228-001_014700005			Y		N	nails infection, nasopharyngitis flu
8228-001_014700011	30		N		Y	EBV reactivation, ***Septic shock not microbiologically documented***
8228-001_014700012			Y		N	aspergillosis, EBV reactivation, Proteus mirabilis pneumonia
8228-001_014700013		6	N	99	N	E. coli urinary tract infec ion, EBV reactivation, Stenotrophomonas maltophilia bacteremia
8228-001_014700014			Y		N	EBV reactivation in blood, enterococcus fecalis urinary infection, Vaginal mycosis (not microbiologically documented)
8228-001_014800002			Y		N	EBV reactivation, ENT infection, labial oral herpes, nasal herpes, urinary infection
8228-001_014800006			Y		N	EBV reactiva ion, Frontal sinusitis
8228-001_016100003			Y		N	Human herpesvirus 6 infection
8228-001_016200002			Y		N	Gram positive bacteremia
8228-001_016200004			Y		N	Gram positive bacteremia
8228-001_016300003		128	Y		N	Pericoronitis of wisdom tooth, Sepsis
8228-001_016300004		156	Y		N	Pharyngitis
8228-001_016400001		143	Y		N	Parotitis
8228-001_016400004	18		Y		N	Sepsis
8228-001_016400007		120	Y		N	Pharyngitis, Sepsis
8228-001_016400009			Y		N	MRSE detect in pharyngitis
8228-001_016400012	297		Y		N	Adenoviral hemorrhagic cystitis
8228-001_016400013			Y		N	Catheter site infection, common cold, Urinary tract infec ion
8228-001_016400014			Y		N	common cold, Pneumonia

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_016500001		121	Y		N	Pneumonia
8228-001_016500004			Y		Y	***Invasive pulmonary asperillosia***
8228-001_016500006		128	Y		N	pneumonia
8228-001_017500002			Y		N	Epstein-Barr Virus Viremia
8228-001_017500006			Y		Y	BK Viremia, EBV, ***Urinary Tract Infection***
8228-001_017500007	295	130	N		Y	Bacteremia, ***Viral infection without focus***
8228-001_017800001			Y		N	Enterococcus faecium in faeces, labial herpes, RHINOCONJUNCTIVITIS
8228-001_017800002		127	Y		N	Subclavian abscess in area of venous catheter explant
8228-001_018500003			Y		Y	***BK infection***
8228-001_018600001			Y		N	C. difficile colitis, sinusitis, staphylococcus epidermidis bacteremia

Table A1-7: Subjects with Opportunistic Infections other than HCMV Infection (Placebo Arm)

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_000100002			Y		N	BK Viremia, Epstein Barr Virus Viremia, Genital Labial Yeast Infection, Hemorrhagic Cystitis, BK Virus Related
8228-001_000100005		40	Y		N	adenovirus, atypical pneumonia, coronavirus infection, Epstein-Barr Virus
8228-001_000300002	82	48	N		N	folliculitis, Human herpesvirus 6 viremia
8228-001_000300005	226	46	Y		Y	disseminated aspergillus, enterococcus faecalis bacteremia, Enterococcus faecalis bacteremia, enterococcus faecalis bacteriuria, enterococcus infection right thigh lesion, ***severe septic shock***
8228-001_000400004			Y		N	Respiratory Syncytial Virus Viremia
8228-001_000400015			Y		Y	***Upper Respiratory Syncytial Virus Infection pharyngitis***
8228-001_000400016			Y		N	upper respiratory tract infection
8228-001_000400023	60		Y		N	HHV6 Viremia, pneumonia
8228-001_001200003		41	Y	285	Y	***C difficile Colitis***
8228-001_001300002			Y		N	Human Rhinovirus/Enterovirus Viremia
8228-001_001300005	239	21	N		Y	bacterial pneumonia, human rhinovirus, ***upper respiratory infection***
8228-001_001300006	204		Y		N	URI - Coronavirus Type HKU1
8228-001_001400002			Y		N	perianal abscess
8228-001_001700003	21		Y		N	Septic shock due to vancomycin-resistant enterococcus, Staphylococcus haemolyticus infection (in blood)
8228-001_001700012	118		Y		N	Neutropenic pharyngitis
8228-001_001700014			Y		N	C. Difficile infection
8228-001_001800004	153	41	Y		N	BK cystitis, Thrush
8228-001_001800015	99		Y		Y	Bacteremia, Parainfluenza Respiratory Tract Infection, ***Septic Shock***
8228-001_001800022			Y		N	Upper respiratory tract infection, Upper thoracic region Folliculi is
8228-001_001800030	231		Y	204	N	c. difficile diarrhea, Gingivi is
8228-001_001800033			Y		N	Epstein-Barr virus viremia, Folliculitis
8228-001_001800040			Y		N	Gingivitis
8228-001_001800046			Y		N	Urinary tract infection
8228-001_001900002	238	34	Y		N	Haemophilus parainfluenza bacteremia
8228-001_001900007			Y		N	Staphylococcus Bacteremia
8228-001_002000002			Y		N	Left Chest Wall Cellulitis
8228-001_002000012	21		Y		N	Oral Thrush, Sepsis, Septic Shock, Worsening Left Lower Lobe Pneumonia
8228-001_002000014		26	Y		N	Boil to Right Thigh
8228-001_002000017			Y		N	Coagulase negative Staph species Bacteremia, Enterococcus species Urinary Tract Infection
8228-001_002000019	141	85	Y		Y	Clostridium ramosum Bacteremia, Enterococcus faecalis Bacteremia, Exacerba ion of Staphylococcus species coagulase negative Bacteremia, ***Worsening Staphylococcus aureus Pneumonia***
8228-001_003000002		22	Y		N	CYSTITIS, folliculitis, herpes labialis
8228-001_003000007			Y		Y	***sepsis***

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_003000008		23	Y		N	re-occurring CMV infection
8228-001_003000013		34	Y		N	acute sinusitis, Cystitis
8228-001_003000016	16		Y		N	pneumonia, sepsis
8228-001_003200005			Y		Y	labyrinthi is, ***septic shock***
8228-001_003300002		10	N		N	CISTYTIS, clostridium difficile infection (colitis), EBV REACTIVATION, flu syndrome, HHV6 REACTIVATION
8228-001_003300007			Y		N	catheter site infection, 'Human herpesvirus 6 reactivation' (viremia), invasive fungal infection
8228-001_003300010			Y		N	respiratory infection
8228-001_003400002			Y		N	conjunctivitis
8228-001_003400003	154	29	N		N	Pneumonia 'organism unknown.', TB mycobacteria infection(LUNG), widespread folliculitis
8228-001_004100002	39		Y	12	Y	***SEPTIC SHOCK***
8228-001_004100006			Y		N	ORAL HERPES
8228-001_004200005		30	Y		N	Pseudomembranous colitis (Clostridium difficile)
8228-001_004200011		42	Y		N	ASTROVIRUS INFECTION, fever by S. Aureus
8228-001_004400001			Y		N	common cold
8228-001_004500001			Y		N	Stomach flu
8228-001_004500009			Y		N	Dental root infection., Pneumonia, Pneumonia, Sepsis, Vaginal candidiasis
8228-001_004700001		88	Y		N	Fungal, Saccharomyces cerevisiae, infection mouth, Skin infection caused by central venous catheter
8228-001_004700002		25	Y		Y	***Gastroenteritis***
8228-001_005800003			Y		N	Sinusitis
8228-001_005800006		11	N		N	cronical Sinusitis, febrile Infection
8228-001_005800012		43	Y		N	Pharyngitis, polysinusitis, respiratory infection without infection focus (viremia), urinary tract infection
8228-001_005900011			Y		N	cold
8228-001_006300007			Y		N	Epstein-Barr virus reactivation (viremia), meningoencephalitis (EBV), Rhinitis, Staphylococcus hemolyticus Sepsis, Stomatitis (HSV1-PCR pos), urinary tract infection (enterococcus faecalis), worsening pulmonary aspergillosis, wound infection of BMA Puncture Location
8228-001_006400006			Y		N	Infection clostridium difficile
8228-001_006400008			Y		N	EBV reactivation, Infection Clostridium difficile, RSV viremia, staphylococcus epidermis bacteremia
8228-001_006400012	330	16	Y	312	N	Adenovirus infection, Bronchiolitis, Cold Symptoms, Infection E. coli, Pneumonia, Rhinitis
8228-001_006400013			Y		N	Cold Symptoms
8228-001_006400021	174	55	Y		N	Cold Symptoms
8228-001_006400025		49	Y		N	Panaritium right bunion
8228-001_006900003	368	39	Y		Y	acute pharyngitis, acute mucopurulent conjunctivitis, Acute Pharyngitis, Fever syndrome caused by acute pharyngitis, Maxillary sinusitis, Pneumonia, ***Probable Invasive fungal Infection by Aspergillosis***
8228-001_006900004			Y		N	cold, foot mycosis, pharyngitis
8228-001_007100001		12	N		N	pneumonia
8228-001_007500006	97		Y		N	EBV reactivation, Lung infection (aspergillus fumigatus), sepsis, Upper respiratory infec ion
8228-001_007800003		17	N		Y	***Viral Upper Respiratory Tract Infection***
8228-001_009100001		42	Y		N	Lower respiratory tract infection
8228-001_009100004		16	N		Y	Diarrhoea C. diff positive, ***Influenza A respiratory tract infection viremia***
8228-001_009100017		27	Y		N	Adenoviral Diarrhoea, Adenoviremia
8228-001_009100019		36	Y		N	Coryzal Symptoms, Paraflu 3 infection
8228-001_010100009			Y		N	Upper respiratory tract infection
8228-001_010200005		55	Y		N	Epstein-Barr Virus infection - viremia, Pharyngitis
8228-001_010200007		15	Y		N	Central nervous system Toxoplasmosis, EBV Infection, Infection of upper respiratory tract, Pharyngitis (bacterial Staphylococcus haemolyticus MRCNS, MSB), Upper respiratory tract infection
8228-001_010800007	167	33	Y		N	parotitis right, urinary tract infection
8228-001_010800010		36	Y		N	eye infection, staphylococcus epidermidis infection blood
8228-001_010800011		36	Y		Y	herpes genitalis, catheter infection, ***sinusitis***
8228-001_010800017	66	24	Y		N	catheter related infection (enterococcus faecium), urinary tract infection (Klebsiella pneumoniae, polyomavirus)
8228-001_010800018		53	Y		Y	***HHV-6 encephalitis***

Subject ID	Day of Death	Day of PET	FAS Flag	Day of HCMV Disease	AE Serious	Emergent Co-infection (excluding HCMV infection)
8228-001_010800020		57	Y		N	bronchitis, catheter related infection, erysipelas left leg, pyuria, urinary tract infection
8228-001_010800025			Y		N	lip infection
8228-001_011000007			N		N	E. coli sepsis, E.coli sepsis, herpes labialis, upper respiratory tract infection
8228-001_011300004		15	N		N	EPSTEIN BARR VIRUS REACTIVATION, PAPULOPUSTULAR RASH, PHARYNGITIS, PNEUMONIA, REPIRATORY SYNCYTIAL VIRUS, URI, HUMAN RHINOVIRUS, URI, RHINOVIRUS POSITIVE
8228-001_011600002		12	Y		N	BK cystitis, BK Viremia Infection, Enterococcal bacteremia, UTI: enterococcus, Vancomycin Resistant Enterococcus infection
8228-001_011600007		36	Y		N	Cellulitis, Thrush
8228-001_011600008			Y		N	BK viremia, gastroenteritis
8228-001_011600012			Y		N	Oral Thrush
8228-001_011600013			Y		N	bacteremia, nasopharyngitis
8228-001_011600021			Y		N	coronavirus respiratory infection
8228-001_011600025		14	Y		N	BK Viremia, coronavirus, enterococcus UTI, Gram negative UTI
8228-001_011600026		25	Y		N	BK Viremia, enterococcus faecium UTI, herpes simplex virus (nasal cavity), HHV6 Infection, nasal septal mucormycosis, oral thrush, parvovirus in bone marrow, Sinus bacterial colonization
8228-001_011600037	183		Y		N	coronavirus, E Coli bacteremia, thrush
8228-001_011600043			Y		N	coronavirus, staphylococcus coag neg bacteremia, urinary tract infection
8228-001_011700002	168	24	Y		Y	respiratory infection (upper and lower), ***sepsis***
8228-001_011700003			Y		N	Upper respiratory infection symptoms
8228-001_012200001			Y		N	HHV-6 Infection, Rhinovirus infection
8228-001_012300004			Y		N	BK virus, by PCR-urine
8228-001_012600002	118		Y		N	Streptococcus viridans blood stream
8228-001_012900013			Y		N	Bacteremia - Escherichia Infection
8228-001_012900014		13	Y		N	Bacteremia
8228-001_012900018	210		Y		N	Bacterial infection, Urinary infection
8228-001_013100011	28		Y		Y	sepsis, ***septicemia due basil Gram negative infection)***
8228-001_013100014			Y		Y	***Bacteremia (sepsis)***
8228-001_013100018			Y		N	bacteremia
8228-001_013100027		40	Y		N	E. coli infection in blood
8228-001_014000012	200	54	Y		Y	Lip herpes, ***Unknown Pneumonia***
8228-001_014200007	126	64	Y		Y	Bacterial Pneumonia-pulmonary infection, ***clostridium difficile infection***
8228-001_014600001	285	38	Y		N	coronavirus
8228-001_014600006		44	Y		N	gastroenteritis
8228-001_014700001			Y		N	Bronchiolitis, oral candidiasis, rhinitis, Rhinopharyngitis
8228-001_014700007	162	28	Y		N	EBV reac ivation, Staphylococcus epidermidis urinary tract infection, staphylococcus septicemia
8228-001_014700015	96		Y		Y	dental abscess, folliculitis, ***Pneumocystis pneumonia***
8228-001_014700017			Y		N	EBV reac ivation, not documented pulmonary infection
8228-001_014800005	192	37	Y		N	EBV reactivation, Oral candidosis
8228-001_016100001		14	N		N	cystitis (bladder)
8228-001_016300002	293	19	N		N	perionychia
8228-001_016400005		101	Y		N	Adenoviral hemorrhagic cystitis, Bacteria infection, Candida infection, Otitis media serous, Sepsis
8228-001_016400010		56	Y		N	Pharyngitis
8228-001_016500005		29	Y		N	Corynebacterium bacteremia
8228-001_018500002			Y		N	septic shock, Upper respiratory tract infection
8228-001_018600002		62	Y		N	bacteremia (vancomycin resistant enterococcus faecium), BK viremia, C difficile colitis, sinusitis

Table A1-8: Subjects with Detectable Baseline Viral Load in Letermovir Arm (non-FAS)

Subject ID	Viral Load at Baseline (copies/mL)	Risk Stratification	Outcome at Week 14	Outcome at Week 24	Day initiation of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Discontinue Reason
8228-001_000300003	150	Low Risk			101	102	397	Death on day 337
8228-001_000300007	150	Low Risk	Success	Success				
8228-001_000400019	150	High Risk	Failure	Failure	12	12	203	
8228-001_001300001	150	High Risk	Failure	Failure	9	9	325	
8228-001_001800013	150	High Risk	Failure	Failure				Death on day 10
8228-001_001800028	150	Low Risk	Success	Success				
8228-001_001800045	150	High Risk	Success	Failure	125	125	379	
8228-001_001900006	150	High Risk	Success	Success				
8228-001_003000004	150	High Risk	Failure	Failure	36	36	415	
8228-001_003200006	150	High Risk	Failure	Failure	9	8	218	
8228-001_004200007	150	High Risk	Success	Failure	140	145	401	Dea h on 185
8228-001_004400004	150	High Risk	Failure	Failure	9	9	997	
8228-001_004700006	150	Low Risk	Failure	Failure	10	9	323	
8228-001_005300001	150	High Risk	Failure	Failure	8	8	2726	
8228-001_005900002	150	Low Risk	Failure	Failure				
8228-001_005900012	150	Low Risk	*Success*	*Success*				
8228-001_006200001	150	Low Risk	*Success*	*Success*				
8228-001_006200005	150	Low Risk	Failure	Failure	14	14	615	
8228-001_006900009	150	Low Risk	Success	Success				
8228-001_006900012	150	High Risk	Success	Success				
8228-001_007100002	150	High Risk	Success	Success				
8228-001_007100005	150	Low Risk	Success	Success				
8228-001_007200002	150	Low Risk	Success	Success				
8228-001_007800001	150	Low Risk	Success	Success				
8228-001_007800008	265	Low Risk	Failure	Failure				Death on day 29

Subject ID	Viral Load at Baseline (copies/mL)	Risk Stratification	Outcome at Week 14	Outcome at Week 24	Day initiation of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)	Discontinue Reason
8228-001_010000001	150	High Risk	Failure	Failure				Withdrawal By Subject on day 7
8228-001_010000005	150	High Risk	Failure	Failure	8	8	529	
8228-001_010200004	150	Low Risk	Failure	Failure				Death on day 123
8228-001_010800003	150	Low Risk	Failure	Failure	11	11	306	
8228-001_010800006	150	Low Risk	Success	Failure				Death on day 110
8228-001_011600015	150	High Risk	Failure	Failure				Wi hdrawal By Sub ect on day 15
8228-001_011600034	150	Low Risk	Failure	Failure	32	32	277	
8228-001_011700006	150	High Risk	Failure	Failure	46	46	25661	Death on day 268
8228-001_012200003	150	Low Risk	Success	Failure	108	106	4131	Death on day 145
8228-001_012600006	150	Low Risk	Success	Failure				End-organ-disease on day 150
8228-001_012900015	314	Low Risk	Failure	Failure	4	4	658	
8228-001_013100001	150	Low Risk	Success	Failure	105	109	522	
8228-001_013100013	150	Low Risk	Success	Success				
8228-001_013100019	150	Low Risk	Failure	Failure	18	11	190	
8228-001_013100023	150	Low Risk	Success	Failure				Death on day 231
8228-001_014200001	150	Low Risk	Success	Success				
8228-001_014200002	716	Low Risk	Success	Success				
8228-001_014200006	150	High Risk	Success	Success				
8228-001_014700011	150	High Risk	Failure	Failure				Death on day 23
8228-001_014700013	157	High Risk	Failure	Failure	6	6	960	End-organ-disease on day 99
8228-001_017500004	150	Low Risk	Success	Success				
8228-001_017500007	150	Low Risk	Success	Failure	130	130	3825	Death on day 292
8228-001_018900006	150	Low Risk	Success	Success			0	

Table A1-9: Subjects with Detectable Baseline Viral Load in Placebo Arm (non-FAS)

Subject ID	Viral Load at Baseline (copies/mL)	Risk Stratification	Outcome at Week 14	Outcome at Week 24	Day initiation of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (copies/mL)	Discontinue Reason
8228-001_000300002	150	Low Risk	Failure	Failure	48	48	598	Death on day 81
8228-001_001300005	150	Low Risk	Failure	Failure	21	20	925	Death on day 212
8228-001_003300002	150	High Risk	Failure	Failure	10	8	403	
8228-001_003400003	150	High Risk	Failure	Failure	29	32	171	Death on day 147
8228-001_005800006	150	High Risk	Failure	Failure	11	8	150	
8228-001_005900014	150	Low Risk	Failure	Failure	15	15	2679	End-organ-disease on day 93
8228-001_007100001	213	High Risk	Failure	Failure	12	12	1624	
8228-001_007300001	189	Low Risk	Failure	Failure				Wi hdrawal By Sub ect on day 15
8228-001_007800003	150	Low Risk	Failure	Failure	17	17	31847	
8228-001_007800005	150	Low Risk	Failure	Failure				Wi hdrawal By Sub ect on day 11
8228-001_009100004	150	Low Risk	Failure	Failure	16	14	1225	
8228-001_009100018	150	High Risk	Failure	Failure	24	24	16534	
8228-001_010800004	150	Low Risk	Failure	Failure	15	15	12874	
8228-001_011000007	150	Low Risk	Success	Success				
8228-001_011300004	150	Low Risk	Failure	Failure	15	15	675	
8228-001_012900006	150	High Risk	Failure	Failure				Physician Decision on day 27
8228-001_014000015	150	Low Risk	Failure	Failure	12	12	150	
8228-001_016100001	150	High Risk	Failure	Failure	14	14	318	329
8228-001_016100002	150	High Risk	Failure	Failure	8	7	244	26
8228-001_016300002	150	Low Risk	Failure	Failure	19	17	150	Death on day 279
8228-001_016400003	150	Low Risk	Failure	Failure	23	23	150	
8228-001_016400015	253	Low Risk	Success	Success				

Table A1-10: Subjects who developed HCMV End-organ-disease (ITT)

Subject ID	Risk Stratification	FAS Flag	Treatment Arm	Stem cell source	Donor HCMV Serostatus	Day of HCMV End-organ-Disease	Day Measure Viral Load around time of End-organ-disease	Viral load around time of End-organ-disease (IU/mL)	Day of PET	Day Measure Viral Load around time of PET	Viral load around time of PET (IU/mL)
8228-001_001200003	High Risk	Y	Placebo	Peripheral blood	Positive	285	281	669	41	41	2618
8228-001_001800030	Low Risk	Y	Placebo	Peripheral blood	Negative	204	169	137			
8228-001_002000001	Low Risk	Y	Placebo	Bone marrow	Negative	159	164	1703			
8228-001_003300003	High Risk	Y	Letermovir	Bone marrow	Negative	144	135	1180			
8228-001_004100002	Low Risk	Y	Placebo	Peripheral blood	Positive	12	15	6995			
8228-001_004100008	Low Risk	Y	Letermovir	Peripheral blood	Positive	201	170	5456	150	150	5456
8228-001_005900014	Low Risk	N	Placebo	Peripheral blood	Negative	93	15	2309	15	15	2446
8228-001_006400012	Low Risk	Y	Placebo	Peripheral blood	Negative	312	276	5237	16	21	4564
8228-001_007100006	High Risk	Y	Letermovir	Peripheral blood	Positive	140	140	137			
8228-001_009100020	Low Risk	Y	Letermovir	Peripheral blood	Negative	141	138	49917			
8228-001_011000008	Low Risk	Y	Letermovir	Peripheral blood	Negative	132	126	5109			
8228-001_011000009	High Risk	Y	Letermovir	Peripheral blood	Negative	259	288	147			
8228-001_012600006	Low Risk	N	Letermovir	Peripheral blood	Negative	150	149	1422			
8228-001_014000007	Low Risk	Y	Placebo	Peripheral blood	Negative	65	150	137			
8228-001_014000011	Low Risk	Y	Letermovir	Peripheral blood	Negative	37	36	137			
8228-001_014700013	High Risk	N	Letermovir	Peripheral blood	Positive	99	83	TND	6	6	960
8228-001_016100006	High Risk	Y	Letermovir	Peripheral blood	Positive	256	238	137	4	4	137

8228-001_001800030: Death on day 222.
8228-001_004100002: Death on day 18.
8228-001_006400012: Death on day 316.
8228-001_009100020: Death on day 373.
8228-001_014000011: Death on day 129.

Table A1-11: Individual Resistance Data (Study P020)

Subject ID	Dose (mg/day)	Day of last dose	Sample Day	Viral load (DNA IU/mL)	Amino acid substitutions in pUL56	Reasons for Discontinuation
101006	60 mg	8	8	5116	L134V, Q228H	Alternative anti-HCMV treatment initiated
			63	986	V425A, G430V	
			63 (whole blood)	986	V425A, N586D	

103001	60 mg	23	15	209	-	Alternative anti-HCMV treatment initiated
			22	1043	none	
103002	60 mg	14	8	219	-	Alternative anti-HCMV treatment initiated
108003	60 mg	51	9	328	none	Alternative anti-HCMV treatment initiated
			43	110	-	
			50	650	V236M, V425A	
201007	60 mg	13	1	119	none	Alternative anti-HCMV treatment initiated
			7	35	none	
			15	584	none	
201011	60 mg	15	8	34	V425A	Alternative anti-HCMV treatment initiated
			18	284	D414N, V425A	
201016	60 mg	3	1	414	none	Alternative anti-HCMV treatment initiated
			4	1093	V425A	
103006	120 mg	14	8	892	none	Alternative anti-HCMV treatment initiated
			13	1221	none	
			20	513	none	
103007	120 mg	7	1	46	-	Alternative anti-HCMV treatment initiated
			10	646	-	
			10 (whole blood)	646	V425A, N586D	
108011	120 mg	8	1	109	none	Alternative anti-HCMV treatment initiated
			7	2048	none	
			7 (whole blood)	2048	V425A, A464T, E495Q, N586D	
109001	120 mg	13	1	62	-	Alternative anti-HCMV treatment initiated
			8	5256	S227I	
109002	120 mg	19	8	4900	-	Alternative anti-HCMV treatment initiated
			15	2496	none	
			15 (whole blood)	2496	V425A, N586D	
			20	449	none	
206004	120 mg	35	15	321	none	Alternative anti-HCMV treatment initiated
			21	1846	none	
			28	2278	none	
			35	2190	R410G	
			36	1050	D414N	
105006	240 mg	6	1	11902	none	Discontinued due to adverse event
110002	240 mg	8	1	34	-	Alternative anti-HCMV treatment initiated
			9	41	-	

Table A1-12: Individual Resistance Data (Study MK-8228-001, Letermovir Arm)

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
8228-001_000100001		T037A, K095E	Initiation of PET based on	EBV, influenza B	85

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
			documented CMV viremia	virus, rhinovirus	
8228-001_000100001		T037A, K095E	Initiation of PET based on documented CMV viremia	EBV, influenza B virus, rhinovirus	89
8228-001_000100001		T037A, K095E	Initiation of PET based on documented CMV viremia	EBV, influenza B virus, rhinovirus	157
8228-001_001800002	I535V	T037A, K095E	Initiation of PET based on documented CMV viremia		125
8228-001_001800007	S255L		Initiation of PET based on documented CMV viremia	HHV6	160
8228-001_001800045	S445N		Initiation of PET based on documented CMV viremia	BK Cystitis	126
8228-001_001800048	S445DEL, N446DEL, S447DEL, E485G, T775I	D665E	Death		66
8228-001_003000017			Initiation of PET based on documented CMV viremia		19
8228-001_003200006			Initiation of PET based on documented CMV viremia		9
8228-001_003300003	P800L	T037A, K095E, I531V	CMV end-organ disease	BK virus, JC virus, HHV6	135
8228-001_003300003	P800L, P846L	I531V	CMV end-organ disease	BK virus, JC virus, HHV6	281
8228-001_003400001	P800L	D094N	Initiation of PET based on documented CMV viremia	EBV	147
8228-001_004200010			Initiation of PET based on documented CMV viremia		42
8228-001_004400004	A366	T037A, K095E	Initiation of PET based on documented CMV viremia		9
8228-001_004700006	C493S		Initiation of PET based on documented CMV viremia		13
8228-001_005800004	S445G	T037A, K095E	Initiation of PET based on documented CMV viremia		145
8228-001_006400001	L658S	T037A, K095E, P176S, M406V		RSV	132
8228-001_006400009					8
8228-001_006900007	V236M, S445DEL, N446DEL, S447DEL, E485G	D665E	Initiation of PET based on documented CMV viremia		62
8228-001_007800004			Initiation of PET based on documented CMV viremia		131
8228-001_009100020		R816W	CMV end-organ disease		145
8228-001_010100005			Initiation of PET based on documented CMV viremia		127
8228-001_010200008	S705F, V778A	A532T, D665E			146
8228-001_010800005	N446DEL/S, A464T	T037A, K095E	Initiation of PET based on documented CMV viremia	HSV-1, polyoma virus	147
8228-001_010800012	N446DEL/S, A464T	T037A, K095E	Initiation of PET based on	polyoma virus JC,	131

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
			documented CMV viremia	VZV, BK virus	
8228-001_010800013		T037A, D094N, K095E	Initiation of PET based on documented CMV viremia	HSV-1	144
8228-001_010800015			Initiation of PET based on documented CMV viremia		28
8228-001_010800016	A425V/A	T037A, D094N, K095E, L522P	Initiation of PET based on documented CMV viremia	herpes simplex	95
8228-001_011600011			Initiation of PET based on documented CMV viremia	HHV6	15
8228-001_011700006	C325W		Death		46
8228-001_012200003		D665E	Death		108
8228-001_012600006	I313V, S445N		Death		162
8228-001_014000006			Death		16
8228-001_014000011			Death		36
8228-001_014700003	S445N, S749N	N074S, D309G	Death	adenovirus, BK virus	175
8228-001_014700013	Y575C	D309D/V	Death	coronavirus	6
8228-001_016100005	N446DEL/S, A464T	D094N, A633V	Death		46
8228-001_016100008	A164V, N446DEL, A464T		Death		128
8228-001_016400007	Y575Y	D094N	Death		120
8228-001_016500001	N446DEL/S, A464T, A779V	A633V	Death		121
8228-001_016500006	E157G, N446DEL/S, A464T, A779V		Death		128
8228-001_017500007	S445N		Death		130
8228-001_017800002			Death		134

Table A1-13: Individual Resistance Data (Study MK-8228-001, Placebo Arm)

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
8228-001_000100005	S749N		Death	coronavirus, parainfluenza 3, rhinovirus	40
8228-001_000100005	S749N, T821A	T097A	Death	coronavirus, parainfluenza 3, rhinovirus	41
8228-001_000100005	S749N, L849P		Death	coronavirus, parainfluenza 3, rhinovirus	131
8228-001_000400002	V584A	A145T	Death		36
8228-001_001200003	T189M, S765T ,P800L		Death		41
8228-001_001300005	M442T, S445N, T452I, S454N, T545N, S749N, V778A, R827H, Q842E		Death	Parainfluenza Virus Type 4, rhinovirus	10

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
8228-001_001800004	S445N	H047Y	Death	BK virus	33
8228-001_001800005		D094N, Y240C, F348L, A633V	Death		162
8228-001_001800018	H653L		Death		64
8228-001_001800024			Death		33
8228-001_001900002	E497G, P800L	T037A, K095E	Death		34
8228-001_002000015			Death		40
8228-001_003000013	T189M, P800L	T037A, K095E	Death		36
8228-001_003300002	V793V, P800L	T037A, K095E, L558I	Death	EBV, BK virus, HHV6	10
8228-001_003400003	P800L	T097M, R442H	Death		29
8228-001_004200005	M442T, T452I, S454N, V778A, A828V		Death		112
8228-001_004200011	N004D	H312R, T431A	Death	metapneumovirus, bocavirus	42
8228-001_004400005	S133N, P800L	L500P	Death		24
8228-001_004500004	N138K	T037A	Death		23
8228-001_004700001		T037A, K095E	Death		89
8228-001_004700002	N004D, T189M, P800L	I298V	Death		28
8228-001_005800006			Death		14
8228-001_005800011	T627S	T037A, K095E	Death		30
8228-001_005900001	S718P	N536S	Death		22
8228-001_005900008	S445N, S749N	T037A, K095E	Death		33
8228-001_005900014		T037A, K095E	Death		15
8228-001_006400012		T037A, K095E	Death		21
8228-001_006400021	I535V	Q191R	Death		55
8228-001_006400025	E161G, M442T, S445N, T452I, S454N, S749N, V778A	D094N	Death		49
8228-001_006900003	Y643N		Death		39
8228-001_007100001	N446DEL, A464T, Q577R, A779V	D094N	Death		12
8228-001_007800003		T037A, K095E	Death		17
8228-001_009100001	A061A/V, Q062Q/P, P800L	T037A, K095E	Death		42
8228-001_009100004	R246S	T037A	Death	influenza A virus	17
8228-001_010200005	R246C	T037A, K095E, A512T	Death	EBV	55
8228-001_010200005	L742DEL	L441S, A512T	Death	EBV	120
8228-001_010200006			Death		19
8228-001_010200006			Death		54
8228-001_010200006			Death		203
8228-001_010200007			Death		77

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
8228-001_010200007		F168L, Q184R	Initiation of PET based on documented CMV viremia		140
8228-001_010800004			Initiation of PET based on documented CMV viremia	EBV, norovirus, adenovirus	15
8228-001_010800007	S445N, S749N	D094N	Death	polyoma virus	33
8228-001_010800010	A180V, S445N, P800L	T037A, K095E	Death		36
8228-001_010800011	L070P, V778A	T037A, K095E	Death		36
8228-001_010800017	UL56S445N, UL56D586N/D	T037A, K095K/E, I187P	Death	polyoma virus JC	24
8228-001_010800018		T037A	Death	adeno virus	53
8228-001_010800020	UL56P800L	T037A, K095E	Death		57
8228-001_010800024			Death		57
8228-001_011300004	UL56S445N		Death	EBV, rhinovirus, RSV	15
8228-001_011600002				BK Virus, coronavirus,	13
8228-001_011600007	UL56I140V	V362A	Initiation of PET based on documented CMV viremia		36
8228-001_011600025	UL56S749N		Initiation of PET based on documented CMV viremia	BK Virus, coronavirus	14
8228-001_011600033	UL56S749N	R484Q	Initiation of PET based on documented CMV viremia		15
8228-001_011700002	UL56S445N	T037A, K095E	Death		24
8228-001_012400002	UL56H727L/P, UL56S749N	T037A, K095E	Death		40
8228-001_014000009	UL56H268R, UL56P606P/L, UL56Q692Q/H, UL56R745R/L		Death		54
8228-001_014000012	UL56S445N, UL56S749N	D094N, D149G	Death		54
8228-001_014200007	UL56D480N, UL56V778A	D094N	Death		64
8228-001_014600001	UL56S445N	T037A, K095E, Q191H, E502K	Death	coronavirus	48
8228-001_014600006	UL56V778A	A242T, I531V	Initiation of PET based on documented CMV viremia		42
8228-001_014700007		T037A, K095E, M658V	Death	EBV	28
8228-001_016100001	UL56N446DEL, UL56A464T, UL56A779V	S613G	Initiation of PET based on documented CMV viremia		14
8228-001_016100002	UL56N446DEL/S, UL56A464T, UL56P767L	A633V	Initiation of PET based on documented CMV viremia		8
8228-001_016400003	UL56A164V, UL56N446DEL/S, UL56A464T	F348L, A633V	Initiation of PET based on documented CMV viremia		23
8228-001_016400010	UL56N446DEL, UL56A464T, UL56E485K, UL56L704P	I417V, A633V	Initiation of PET based on documented CMV viremia		56
8228-001_016500005	UL56V132A, UL56N446DEL, UL56A464T, UL56D580G, UL56L600P, UL56M677V, UL56A779V	I164V, A633V	Initiation of PET based on documented CMV viremia		72

Subject ID	Amino acid substitutions in pUL56	Amino acid substitutions in pUL89	Reasons for Discontinuation	Other viruses	Sample Day
8228-001_018500001	UL56S133N, UL56P800L		Initiation of PET based on documented CMV viremia		20

Table A1-14: Changes to Immunosuppression (Study MK-8228-001, Letermovir Arm)

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
8228-001_000100001	89			Drug	tacrolimus	tacrolimus	tacrolimus	prednisone	methylprednisolone sodium succinate	methylprednisolone sodium succinate	tacrolimus	triamcinolone acetonide	prednisone	tacrolimus
				Dose	1.1 mg	1 mg	0 5 mg	80 mg	100 mg	160 mg	0.7 mg	1 APPLICATION	70 mg	4 mg
				Day	-17	-1	4	5	6	7	7	17	34	34
				Drug	prednisone	methylprednisolone sodium succinate	prednisone							
				Dose	160 mg	100 mg	60 mg							
				Day	35	61	65							
8228-001_001800002	125			Drug	tacrolimus	fludrocortisone acetate	prednisone							
				Dose	.	.	.							
				Day	-11	10	52							
8228-001_001800007	159			Drug	tacrolimus	methylprednisolone	tacrolimus	triamcinolone acetonide	hydrocortisone	methylprednisolone	tacrolimus	tacrolimus	methylprednisolone	tacrolimus
				Dose	6 mg	90 mg	3 5 mg	2 APPLICATION	1 APPLICATION	200 mg	4 mg	2 67 mg	100 mg	6 mg
				Day	-1	4	6	7	7	7	7	8	9	11
				Drug	prednisone	prednisone	prednisone	tacrolimus	methylprednisolone	tacrolimus	tacrolimus	prednisone	prednisone	prednisone
				Dose	125 mg	100 mg	80 mg	5 mg	170 mg	3 mg	6 mg	100 mg	200 mg	160 mg
				Day	11	12	13	13	14	14	15	17	18	20
				Drug	tacrolimus	tacrolimus	methylprednisolone	tacrolimus	tacrolimus	tacrolimus	tacrolimus	prednisone		
				Dose	8 mg	10 mg	170 mg	6 mg	5 mg	4 mg	.	.		
				Day	22	24	25	29	31	32	37	47		
8228-001_001800048	66		252	Drug	mycophenolic acid	tacrolimus	prednisone							
				Dose	.	.	.							
				Day	-2	-2	31							
8228-001_003000017	18			Drug	mycophenolate mofetil	cyclosporine	methylprednisolone sodium succinate	methylprednisolone sodium succinate	cyclosporine	methylprednisolone sodium succinate	cyclosporine	methylprednisolone sodium succinate	methylprednisolone sodium succinate	cyclosporine
				Dose	2000 mg	140 mg	80 mg	100 mg	160 mg	20 mg	180 mg	90 mg	90 mg	180 mg
				Day	-4	-1	1	2	2	5	6	9	9	13
				Drug	methylprednisolone sodium succinate	cyclosporine	cyclosporine	cyclosporine	methylprednisolone sodium succinate	cyclosporine	methylprednisolone sodium succinate	cyclosporine	cyclosporine	cyclosporine
				Dose	70 mg	70 mg	90 mg	120 mg	60 mg	130 mg	50 mg	80 mg	90 mg	90 mg
				Day	16	16	17	20	21	23	24	25	27	30
8228-001_003300003		144		Drug	sirolimus	mycophenolic acid	hydrocortisone							
				Dose	.	.	250 mg							
				Day	-7	-7	65							
8228-001_003400001	147			Drug	hydrocortisone sodium succinate	hydrocortisone								
				Dose	.	.								

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
				Day	-357	6								
8228-001_005 800004	144			Drug	cyclosporine	methylprednisolone	methylprednisolone	cyclosporine						
				Dose	.	40 mg	16 mg	.						
				Day	-10	7	10	14						
8228-001_006 400001				Drug	mycophenolate mofetil	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine
				Dose	2 g	125 mg	150 mg	125 mg	175 mg	150 mg	200 mg	300 mg	150 mg	100 mg
				Day	-12	0	0	1	1	2	2	4	5	6
				Drug	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	prednisolone	prednisolone	prednisolone
				Dose	125 mg	125 mg	100 mg	200 mg	100 mg	75 mg	200 mg	50 mg	25 mg	15 mg
				Day	6	7	7	8	9	9	10	11	12	15
				Drug	cyclosporine	cyclosporine	prednisolone	cyclosporine	prednisolone	cyclosporine	cyclosporine	prednisolone	prednisolone	cyclosporine
				Dose	125 mg	100 mg	10 mg	250 mg	7 5 mg	125 mg	100 mg	5 mg	2 5 mg	250 mg
				Day	15	15	18	19	23	27	27	28	33	34
				Drug	cyclosporine	prednisolone	mycophenolate mofetil	cyclosporine	cyclosporine	cyclosporine	cyclosporine	mycophenolate mofetil	cyclosporine	
				Dose	200 mg	2.5 mg	1500 mg	125 mg	100 mg	100 mg	75 mg	1000 mg	200 mg	
				Day	37	38	42	54	54	62	62	72	89	
8228-001_006 400009				Drug	cyclosporine	mycophenolate mofetil								
				Dose	.	.								
				Day	-4	-1								
8228-001_006 900007	62			Drug	cyclosporine	cyclosporine								
				Dose	300 mg	300 mg								
				Day	-1	18								
8228-001_007 800004	131			Drug	tacrolimus	tacrolimus	tacrolimus							
				Dose	4 mg	1 mg	1 5 mg							
				Day	-366	21	21							
8228-001_009 100020		141	374	Drug	prednisolone	cyclosporine								
				Dose	5 mg	150 mg								
				Day	-187	-3								
8228-001_010 100005	101			Drug	cyclosporine	cyclosporine	cyclosporine	cyclosporine	methylprednisolone	cyclosporine	cyclosporine	cyclosporine	methylprednisolone	hydrocortisone
				Dose	175 mg	150 mg	125 mg	100 mg	70 mg	150 mg	150 mg	200 mg	140 mg	200 mg
				Day	-2	28	35	48	54	55	58	64	68	68
				Drug	cyclosporine	mycophenolate mofetil	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	cyclosporine	methylprednisolone	cyclosporine	cyclosporine
				Dose	250 mg	2000 mg	120 mg	100 mg	80 mg	70 mg	200 mg	50 mg	100 mg	200 mg
				Day	71	72	75	77	79	81	85	85	86	86
				Drug	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	cyclosporine	methylprednisolone	tacrolimus	methylprednisolone	
				Dose	40 mg	30 mg	20 mg	16 mg	8 mg	250 mg	4 mg	2 mg	4 mg	
				Day	87	90	91	93	95	96	97	99	132	
8228-001_010 800005	147			Drug	cyclosporine	mycophenolate mofetil	cyclosporine	cyclosporine	cyclosporine	cyclosporine	methylprednisolone	cyclosporine	methylprednisolone	cyclosporine
				Dose	.	3500 mg	.	250 mg	200 mg	150 mg	32 mg	100 mg	24 mg	150 mg
				Day	-4	-1	13	22	24	32	38	38	53	59
				Drug	methylprednisolone	methylprednisolone	methylprednisolone	hydrocortisone sodium succinate						
				Dose	16 mg	8 mg	4 mg	200 mg						
				Day	60	74	88	108						
8228-001_010 800012	137			Drug	hydrocortisone sodium succinate	cyclosporine	mycophenolate mofetil	mycophenolate mofetil	mycophenolate mofetil	mycophenolate mofetil	cyclosporine	hydrocortisone sodium succinate	tacrolimus	methylprednisolone sodium succinate
				Dose	.	.	.	.	2 g	3 g	250 mg	100 mg	3 mg	60 mg
				Day	-8	-4	-2	9	13	14	16	24	37	40

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
				Drug	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	leflunomide	methylprednisolone	leflunomide	methylprednisolone	methylprednisolone
				Dose	48 mg	32 mg	16 mg	12 mg	16 mg	100 mg	12 mg	20 mg	8 mg	32 mg
				Day	43	46	53	57	58	59	59	62	63	81
8228-001_010800013	144			Drug	cyclosporine	mycophenolate mofetil	cyclosporine	cyclosporine	methylprednisolone sodium succinate	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone
				Dose	300 mg	3500 mg	250 mg	250 mg	80 mg	32 mg	16 mg	12 mg	8 mg	4 mg
				Day	-9	-7	7	7	50	54	63	74	81	94
8228-001_010800015	29			Drug	methylprednisolone	cyclosporine	mycophenolate mofetil	mycophenolate mofetil	mycophenolate mofetil	mycophenolate mofetil	cyclosporine			
				Dose	.	.	.	.	.	3750 mg	175 mg			
				Day	-8	-4	-2	-1	17	21	27			
8228-001_010800016	101			Drug	cyclosporine	mycophenolate mofetil	cyclosporine	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone
				Dose	.	3 g	250 mg	.	24 mg	16 mg	8 mg	16 mg	32 mg	16 mg
				Day	-6	-4	9	20	31	38	45	57	69	71
8228-001_011600011	11			Drug	tacrolimus	mycophenolate mofetil	hydrocortisone sodium succinate	hydrocortisone sodium succinate	dexamethasone sodium phosphate					
				Dose	3 mg	1000 mg	25 mg	100 mg	10 mg					
				Day	-12	-7	3	8	22					
8228-001_014000006	16			Drug	dexamethasone sodium phosphate	tacrolimus	mycophenolate mofetil	dexamethasone sodium phosphate						
				Dose	.	3 mg	2000 mg	10 mg						
				Day	-12	-6	-6	9						
8228-001_014000011		37	131	Drug	dexamethasone sodium phosphate	mycophenolic acid	everolimus	everolimus	methylprednisolone sodium succinate	methylprednisolone	everolimus	methylprednisolone	methylprednisolone	tacrolimus
				Dose	10 mg	2000 mg	1 5 mg	2 mg	160 mg	72 mg	1 mg	48 mg	32 mg	1 APPLICATION
				Day	-11	-5	-5	6	7	12	14	14	15	21
				Drug	everolimus	methylprednisolone	methylprednisolone sodium succinate	everolimus	methylprednisolone sodium succinate	methylprednisolone sodium succinate	methylprednisolone sodium succinate	everolimus	everolimus	methylprednisolone sodium succinate
				Dose	0.75 mg	64 mg	180 mg	1 mg	240 mg	1000 mg	240 mg	1.5 mg	2 mg	160 mg
				Day	22	22	26	28	28	30	32	37	42	42
				Drug	mycophenolate mofetil									
				Dose	1000 mg									
				Day	44									
8228-001_016100005	46			Drug	cyclosporine	mycophenolate mofetil	cyclosporine							
				Dose	.	.	.							
				Day	-15	-13	16							
8228-001_016100008	128		262	Drug	tacrolimus	methylprednisolone	tacrolimus	methylprednisolone sodium succinate	tacrolimus	methylprednisolone sodium succinate	hydrocortisone sodium succinate	prednisolone	prednisolone	prednisolone
				Dose	.	.	.	.	.	.	.	.	.	.
				Day	-14	-12	24	29	32	38	39	39	50	55
8228-001_016400007	120			Drug	tacrolimus	hydrocortisone sodium succinate	hydrocortisone sodium succinate	betamethasone butyrate propionate	methylprednisolone sodium succinate	tacrolimus	hydrocortisone sodium succinate	tacrolimus	methylprednisolone	hydrocortisone sodium succinate
				Dose	.	.	.	.	.	.	.	.	.	.
				Day	-10	-2	2	20	21	30	32	45	45	82
				Drug	methylprednisolone	betamethasone butyrate propionate								

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
				Dose	.	.								
				Day	83	94								
8228-001_016500001	121			Drug	hydrocortisone	cyclosporine	prednisolone							
				Dose	.	.	.							
				Day	-13	-13	54							
8228-001_016500006	128			Drug	cyclosporine	hydrocortisone sodium succinate	cyclosporine							
				Dose	.	.	.							
				Day	-8	2	25							
8228-001_017800002	127			Drug	tacrolimus	tacrolimus								
				Dose	.	3 mg								
				Day	-17	96								

Table A1-15: Changes to Immunosuppression (Study MK-8228-001, Placebo Arm)

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
8228-001_000100005	40			Drug	tacrolimus	dexamethasone sodium phosphate	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus			
				Dose	2 5 mg	4 mg	4 mg	6 mg	2 5 mg	2 mg	1 5 mg			
				Day	-8	2	8	11	17	17	41			
8228-001_000400002	36			Drug	sirolimus	tacrolimus								
				Dose	4 mg	6 mg								
				Day	-32	-14								
8228-001_001200003	41	285		Drug	tacrolimus	tacrolimus	hydrocortisone	triamcinolone	prednisone	prednisone	prednisone	prednisone	prednisone	prednisone
				Dose	2 mg	1 mg	1 APPLICATION	2 APPLICATION	40 mg	30 mg	.	.	.	.
				Day	-1	2	14	16	27	31	32	33	34	36
				Drug	prednisone	prednisone								
				Dose	.	.								
				Day	45	47								
8228-001_001800004	41		153	Drug	tacrolimus	methylprednisolone sodium succinate	prednisone							
				Dose	2 mg	.	80 mg							
				Day	-12	10	11							
8228-001_001800005	162			Drug	tacrolimus	prednisolone acetate	dexamethasone							
				Dose	.	.	.							
				Day	-13	88	88							
8228-001_001800018	62		152	Drug	prednisone	tacrolimus	sirolimus	prednisone	prednisone					
				Dose	20 mg	2 mg	.	20 mg	40 mg					
				Day	-270	-22	-19	73	74					
8228-001_001800024	28		159	Drug	sirolimus	tacrolimus								
				Dose	2 mg	3 mg								
				Day	-20	-18								
8228-001_001900002	34		238	Drug	mycophenolate mofetil	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	
				Dose	1 g	5 mg	1 5 mg	5 mg	2 mg	4 mg	3 mg	4 mg	5 mg	
				Day	-6	-6	-3	0	5	6	10	13	16	
8228-001_002000015				Drug	cyclosporine	cyclosporine	methylprednisolone sodium succinate							
				Dose	450 mg	400 mg	120 mg							
				Day	-11	10	13							

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
8228-001_003000013	34			Drug	cyclosporine	mycophenolate mofetil	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	dexamethasone sodium phosphate	dexamethasone sodium phosphate
				Dose	90 mg	1000 mg	110 mg	140 mg	50 mg	60 mg	80 mg	90 mg	4 mg	8 mg
				Day	-2	-1	0	4	8	10	11	14	20	21
				Drug	dexamethasone sodium phosphate	dexamethasone sodium phosphate	methylprednisolone	dexamethasone sodium phosphate	cyclosporine	dexamethasone	cyclosporine	dexamethasone sodium phosphate	cyclosporine	methylprednisolone
				Dose	8 mg	4 mg	24 mg	4 mg	50 mg	2 mg	60 mg	4 mg	100 mg	16 mg
				Day	21	23	24	25	26	26	28	29	31	31
				Drug	cyclosporine	cyclosporine	dexamethasone sodium phosphate	methylprednisolone	dexamethasone sodium phosphate	cyclosporine	cyclosporine	methylprednisolone		
				Dose	120 mg	300 mg	2 mg	12 mg	2 mg	100 mg	200 mg	12 mg		
				Day	32	34	35	38	39	41	43	45		
8228-001_004200005	30			Drug	cyclosporine	mycophenolate mofetil	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine			
				Dose	180 mg	.	200 mg	250 mg	225 mg	175 mg	125 mg			
				Day	-3	-3	3	6	15	20	41			
8228-001_004200011	42			Drug	sirolimus	tacrolimus	tacrolimus	mycophenolate mofetil	methylprednisolone	mycophenolate mofetil	sirolimus	sirolimus		
				Dose	0.4 mg	0.6 mg	2 mg	3 g	120 mg	3 g	0.4 mg	0.5 mL		
				Day	-7	-6	11	24	40	40	41	44		
8228-001_004500004	23			Drug	cyclosporine	prednisolone								
				Dose	.	.								
				Day	-7	17								
8228-001_004700001	88			Drug	cyclosporine	prednisolone								
				Dose	75 mg	20 mg								
				Day	-6	42								
8228-001_004700002	25			Drug	cyclosporine	prednisolone								
				Dose	300 mg	15 mg								
				Day	-2	8								
8228-001_005800011	30			Drug	cyclosporine	cyclosporine	methylprednisolone							
				Dose	.	.	.							
				Day	-11	9	16							
8228-001_005900001	22			Drug	cyclosporine	prednisolone sodium succinate	prednisolone sodium succinate	prednisolone						
				Dose	250 mg	100 mg	100 mg	25 mg						
				Day	-23	4	9	20						
8228-001_005900008	33			Drug	cyclosporine	prednisolone sodium succinate								
				Dose	.	.								
				Day	-22	-1								
8228-001_006400012	16	312	330	Drug	tacrolimus	mycophenolate mofetil	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	prednisolone	tacrolimus
				Dose	2 mg	.	1 5 mg	2 mg	4 mg	2 mg	1 5 mg	4 mg	.	2 mg
				Day	-44	-15	-1	-1	1	2	2	5	8	10
				Drug	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus				
				Dose	1 5 mg	1.5 mg	1 mg	1.5 mg	1 mg	3 mg				
				Day	10	11	11	20	20	27				
8228-001_006400021	55		174	Drug	mycophenolate mofetil	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine
				Dose	.	250 mg	200 mg	200 mg	100 mg	150 mg	100 mg	100 mg	75 mg	200 mg
				Day	-2	-1	-1	0	0	1	1	2	2	8
				Drug	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine
				Dose	75 mg	100 mg	150 mg	200 mg	100 mg	100 mg	200 mg	150 mg	100 mg	75 mg

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
				Day	9	9	10	11	14	14	15	17	18	21
				Drug	cyclosporine	cyclosporine	prednisolone	cyclosporine	cyclosporine					
				Dose	50 mg	150 mg	.	75 mg	50 mg					
				Day	21	27	46	59	59					
8228-001_006400025	49			Drug	dexamethasone	tacrolimus	mycophenolate mofetil	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus
				Dose	.	2.016 mg	.	2 mg	1 5 mg	1.5 mg	2 mg	2 mg	2 5 mg	2.5 mg
				Day	0	2	2	4	4	5	5	7	7	8
				Drug	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	prednisone	tacrolimus	tacrolimus	tacrolimus
				Dose	2.016 mg	1.5 mg	3 mg	1.5 mg	2 mg	3 mg	.	4 mg	2 mg	2.5 mg
				Day	8	11	12	16	16	18	19	19	20	20
				Drug	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus	tacrolimus
				Dose	2 5 mg	3 mg	3 mg	2.5 mg	3 mg	1 mg	2 5 mg	1.5 mg	2 5 mg	4 mg
				Day	21	21	22	22	25	25	26	27	27	29
				Drug	tacrolimus	tacrolimus								
				Dose	3 mg	2 mg								
				Day	32	39								
8228-001_006900003	39		368	Drug	hydrocortisone	prednisone	prednisone	prednisone						
				Dose	250 mg	50 mg	40 mg	10 mg						
				Day	11	13	16	23						
8228-001_010200006	19		251	Drug	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	methylprednisolone hemisuccinate	dexamethasone	cyclosporine	cyclosporine	cyclosporine
				Dose	300 mg	250 mg	50 mg	175 mg	100 mg	160 mg	3 APPLICATION	125 mg	175 mg	75 mg
				Day	-10	6	6	7	7	8	8	8	8	9
				Drug	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	cyclosporine	mycophenolate mofetil	methylprednisolone	
				Dose	250 mg	25 mg	125 mg	100 mg	200 mg	225 mg	250 mg	2000 mg	32 mg	
				Day	9	10	10	11	12	13	16	20	24	
8228-001_010200007	15			Drug	cyclosporine	hydrocortisone								
				Dose	100 mg	.								
				Day	-8	-1								
8228-001_010800007	33		167	Drug	cyclosporine	mycophenolate mofetil	cyclosporine	cyclosporine	methylprednisolone	tacrolimus				
				Dose	.	3000 mg	.	150 mg	32 mg	5 mg				
				Day	-4	-2	12	17	35	40				
8228-001_010800010	36			Drug	cyclosporine	mycophenolate mofetil	hydrocortisone sodium succinate	cyclosporine						
				Dose	.	.	.	50 mg						
				Day	-7	-4	2	10						
8228-001_010800011	36			Drug	cyclosporine	cyclosporine	cyclosporine	tacrolimus	methylprednisolone	methylprednisolone	tacrolimus			
				Dose	.	400 mg	350 mg	4 mg	16 mg	8 mg	4 mL			
				Day	-3	20	22	33	42	46	46			
8228-001_010800017	24		66	Drug	dexamethasone sodium phosphate	tacrolimus	mycophenolate mofetil	tacrolimus						
				Dose	.	.	.	2 mg						
				Day	-68	1	1	18						
8228-001_010800018	53			Drug	cyclosporine	mycophenolate mofetil	hydrocortisone sodium succinate	methylprednisolone	cyclosporine	methylprednisolone	tacrolimus	methylprednisolone		
				Dose	180 mg	3750 mg	100 mg	64 mg	300 mg	32 mg	8 mg	24 mg		
				Day	-8	-5	5	10	10	14	24	28		
8228-001_010800020	57			Drug	cyclosporine	mycophenolate mofetil	methylprednisolone sodium succinate	cyclosporine	methylprednisolone sodium succinate	methylprednisolone	methylprednisolone	methylprednisolone	methylprednisolone	cyclosporine

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
				Dose	264 mg	4 g	40 mg	450 mg	40 mg	32 mg	24 mg	16 mg	16 mg	300 mg
				Day	-5	-2	-1	13	14	18	22	26	55	59
				Drug	methylprednisolone									
				Dose	8 mg									
				Day	65									
8228-001_010800024	57			Drug	cyclosporine	mycophenolate mofetil	tacrolimus	tacrolimus						
				Dose	200 mg	.	.	.						
				Day	-5	-3	7	11						
8228-001_011600002	12			Drug	hydrocortisone sodium succinate	tacrolimus	triamcinolone acetonide	tacrolimus	hydrocortisone sodium succinate					
				Dose	600 mg	1.2 mg	1 APPLICATION	6 mg	50 mg					
				Day	-30	-24	-2	1	14					
8228-001_011600007	36			Drug	tacrolimus	methylprednisolone sodium succinate								
				Dose	11 mg	375 mg								
				Day	-10	38								
8228-001_011600025	14			Drug	mycophenolate mofetil hydrochloride	tacrolimus	methylprednisolone sodium succinate	mycophenolate mofetil	tacrolimus	prednisone	mycophenolate mofetil			
				Dose	3000 mg	2.1 mg	130 mg	3000 mg	4 CAPSULE	100 mg	3000 mg			
				Day	-19	-15	-2	2	2	3	5			
8228-001_011600033	15			Drug	tacrolimus	tacrolimus	tacrolimus	hydrocortisone sodium succinate	fludrocortisone acetate					
				Dose	50 mg	2 mg	4 mg	25 mg	0.1 mg					
				Day	-17	-16	2	7	7					
8228-001_012400002	36		193	Drug	tacrolimus	hydrocortisone	hydrocortisone	hydrocortisone	hydrocortisone	tacrolimus	methylprednisolone	prednisone	prednisone	
				Dose	1 mg	.	200 mg	100 mg	100 mg	0.5 mg	125 mg	80 mg	60 mg	
				Day	-4	0	13	14	20	24	25	27	38	
8228-001_014000009	54		84	Drug	dexamethasone sodium phosphate	everolimus	mycophenolate mofetil	everolimus	mycophenolate mofetil	methylprednisolone sodium succinate	methylprednisolone	methylprednisolone	methylprednisolone	everolimus
				Dose	.	2 mg	2000 mg	1 mg	2000 mg	120 mg	48 mg	48 mg	32 mg	1 mg
				Day	-11	-5	-5	50	50	50	53	58	63	63
8228-001_014000012	54		200	Drug	dexamethasone	tacrolimus	mycophenolic acid	everolimus	tacrolimus	everolimus	everolimus			
				Dose	.	.	1000 mg	.	2 mg	0.75 mg	0 5 mg			
				Day	-10	-5	-5	22	24	35	42			
8228-001_014200007	64		126	Drug	cyclosporine	methylprednisolone								
				Dose	100 mg	40 mg								
				Day	-31	42								
8228-001_014600001	38		285	Drug	cyclosporine	cyclosporine	methylprednisolone sodium succinate	cyclosporine	prednisone	cyclosporine	prednisone	prednisone		
				Dose	230 mg	160 mg	160 mg	130 mg	160 mg	260 mg	80 mg	60 mg		
				Day	-3	8	11	18	19	20	26	29		
8228-001_014600006	44			Drug	cyclosporine	methylprednisolone sodium succinate	cyclosporine							
				Dose	.	.	.							
				Day	-3	22	27							
8228-	28		162	Drug	cyclosporine	mycophenolat	methylprednis	prednisolone	mycophenolat	methylprednis	cyclosporine	methylprednis	cyclosporine	methylprednis

Subject ID	Day of PET	Day of HCMV Disease	Day of Death											
001_014 700007						e mofetil	olone		e mofetil	olone		olone		olone
				Dose	.	.	.	.	2000 mg	.	80 mg	65 mg	150 mg	50 mg
				Day	-10	-8	17	21	23	24	33	33	37	40
8228-001_016 400010	56			Drug	hydrocortisone sodium succinate	tacrolimus	methylprednisolone sodium succinate	methylprednisolone	tacrolimus					
				Dose	.	.	.	.	.					
				Day	-30	-6	23	38	59					
8228-001_016 500005	29			Drug	betamethasone sodium phosphate	cyclosporine								
				Dose	.	.								
				Day	-13	-7								
8228-001_018 500001	26		102	Drug	mycophenolate mofetil	cyclosporine	methylprednisolone	cyclosporine	cyclosporine	dexamethasone	cyclosporine	methylprednisolone sodium succinate		
				Dose	.	200 mg	40 mg	400 mg	300 mg	8 mg	150 mg	250 mg		
				Day	-12	-5	-2	2	3	13	14	35		

Table A1-16: Summary of Local Laboratory pp65/PCR and Central Laboratory PCR Concordance Data (Study MK-8228-020)

Site	Local Laboratory pp65 and Central Laboratory PCR	Local and Central Laboratory PCR
101	63.6% LL-/CL+	5.6% LL-/CL+ and 5.6% LL+/CL-
102	-	1% LL-/CL+
103	-	19.5% LL-/CL+ and 4.2% LL+/CL-
104	-	5.5% LL-/CL+
105	14.3% LL-/CL+	-
107	15.8% LL-/CL+ and 23.1% LL+/CL-	-
108	7.2% LL-/CL+ and 21.2% LL+/CL-	8.7% LL-/CL+ and 45.5% LL+/CL-
109	66.7% LL-/CL+	-
110	-	6.3% LL-/CL+ and 33.3% LL+/CL-
201	5.1% LL-/CL+ and 23.1% LL+/CL-	-
202	-	10.9% LL-/CL+
203	-	-
206	-	2.3% LL-/CL+ and 13.3% LL+/CL-
209	-	12.5% LL-/CL+
211	-	-
213	6.3% LL-/CL+	-
214	-	5.0% LL-/CL+
215	-	-
216	-	9.1% LL-/CL+

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/s/

TAKASHI E KOMATSU
08/08/2017

ANAMARIS M COLBERG POLEY
08/08/2017

JULIAN J O REAR
08/08/2017

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW: Eric F. Donaldson, Ph.D.
NDA#: 209939 **SDN 000 DATE REVIEWED:** 07/07/2017

NDA#: 209939 and 209940 **Serial #: 000**

Reviewer's Name: Eric F. Donaldson, Ph.D.

Sponsor's Name and Address:

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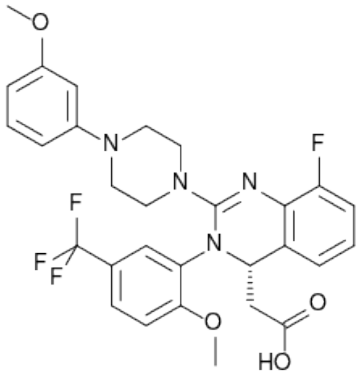
Amendments:

SDN	Date Submitted	Date Received	Date Assigned
003	March 8, 2017	March 8, 2017	March 10, 2017
006	March 24, 2017	March 24, 2017	March 27, 2017
009	April 24, 2017	April 24, 2017	April 24, 2017

Related/Supporting Documents: IND 104706, IND 118361, NDA 209940, DMF (b) (4)

Chemical Name: (S)-{8-fluoro-2-[4-(3-methoxyphenyl)-1-piperazinyl]-3-[2-methoxy-5-(trifluoromethyl)-phenyl]-3,4-dihydro-4-quinazolinyl} acetic acid

Structure:



LETERMOVIR

Molecular formula: $C_{29}H_{28}F_4N_4O_4$

Molecular weight: 572.56

Drug category: Antiviral

Indication(s): Prophylaxis of cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT)

Dosage Form(s): 480 mg administered once daily orally or as an intravenous (IV) infusion over 1 hour through 100 days post-transplant

Route(s) of Administration: Tablet: 240 mg; 480 mg or Injection: 240 mg/12 mL or 480 mg/24 mL in a single-dose vial for intravenous infusion

Recommended Dosage: 480 mg administered once daily

Dispensed: Rx _X_ OTC ___ (Discipline relevant)

Abbreviations: AraT, All Randomized and Treated; DAVP, Division of Antiviral Products; (b) (4) FAS, full analysis set; GAP, Genotyping Analysis Population; GV, genotypic variant; HCMV, human cytomegalovirus (human herpesvirus 5), HSCT, hematopoietic stem cell transplant; LLOQ, lower limit of quantification; LOD, limit of detection; NGS, next-generation DNA sequencing; PET, pre-emptive therapy; PO, dose orally; QC, quality control; QD, dose once daily; R+, HCMV-seropositive recipients RSV, respiratory syncytial virus; SOT, solid organ transplant; UL, unique long;

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EXECUTIVE SUMMARY

Merck Sharp & Dohme Corp. (Merck, M), submitted this original NDA for letermovir (240 mg/12 mL or 480 mg/24 mL) for prophylaxis of human cytomegalovirus (HCMV) infection or disease in adult HCMV-seropositive recipients (R+) of an allogeneic hematopoietic stem cell transplant (HSCT). This Clinical Virology review focused on the independent assessment of next generation sequencing (NGS) data generated during resistance assessments of virologic failure samples and provided in support of the NDA. Overall, assessment of the NGS data by the Division of Antiviral Products (DAVP) indicated that the data and analyses provided by the sponsor and performed by (b) (4) were acceptable and this NDA is approvable with respect to this aspect of the Clinical Virology review. For overall conclusions for Clinical Virology, please see the primary Clinical Virology review of Dr. Takashi Komatsu, Ph.D., RAC (NDA 209939 SDN 000).

Letermovir (MK-8228) is an HCMV terminase complex inhibitor. The HCMV terminase complex is made up of at least two viral proteins, pUL56 and pUL89, and the main function of the terminase complex is to cleave HCMV concatemeric genomes into single units of functional HCMV monomers. Letermovir inhibits the HCMV terminase activity, preventing the formation of mature genomes that can be packaged to form infectious virions. The cell culture antiviral activity of letermovir was determined in multiple cell lines. The median EC_{50} values of letermovir against clinical HCMV isolates were 0.0019 µM for subtype gB1 (range 0.0001-0.0058 µM, N=29), 0.002 µM for subtype gB2 (range 0.0007-0.0061 µM, N=27), 0.0023 µM for subtype gB3 (range 0.0015-0.0034 µM, N=11), and 0.0029 µM for subtype gB4 (range 0.0026-0.0032 µM, N=3). The EC_{50} values were 3.5 to 5.6 nM when human fibroblasts from different origins (foreskin, normal and embryonic lung, and dermis) were infected with CMV AD169 and incubated with letermovir. HCMV mutants with reduced susceptibility to letermovir were selected in cell culture. The substitutions mapped to pUL56 and occur at amino acid residues between 231 and 369 (V231A, V231L, V236L, V236M, E237D, L241P, T244K, T244R, L257I, F261C, F261L, F261S, Y321C, C325F, C325R, M329T, R369G, R369M, R369S). EC_{50} values for virus expressing these substitutions were 13 to 5,870-fold higher than those observed for the wild-type reference virus. One resistance-associated substitution in pUL89 was selected for in cell culture using letermovir. In the selection experiment A345S was identified, which was described by the sponsor as a site of interstrain variation. A second pUL89 substitution was selected by an analog of letermovir, BAY 66-6047, which selected for pUL89 substitution Q256E. The substitutions of interest for pUL89 were Q256E and A345S.

Phase 3 clinical trial P001 (MK-8228-001) was a randomized, placebo-controlled, multi-site, double-blind, trial that evaluated the efficacy and safety of letermovir compared to placebo in the prevention of clinically significant HCMV infection in adult (≥18 years of age) HCMV-seropositive allogeneic hematopoietic stem cell transplant (HSCT) recipients. The primary objective of the study was to evaluate the efficacy of letermovir in the prevention of clinically significant HCMV infection through Week 24 post-transplant following administration of letermovir or placebo. The sponsor reported that a lower proportion of subjects in the letermovir group (37.5%) developed clinically significant HCMV infection compared to the placebo group (60.6%) through Week 24 post-transplant in the Full Analysis Set population and the letermovir group exhibited an increased time to onset of clinically significant HCMV infection through Week 24 post-transplant. In addition, the sponsor concluded that all-cause mortality was substantially lower in the letermovir group compared to placebo through Week 24 post-transplant. Please see the review of Senior Clinical Virology Reviewer, Dr. Takashi Komatsu, Ph.D., RAC for specific information regarding the conclusions for this clinical trial (Clinical Virology review of NDA 209939 SDN 000).

In order to identify HCMV variants potentially associated with resistance to letermovir (compared to a reference strain) in subjects failing HCMV prophylaxis in study P001 (MK-8228-001), genotypic analyses were conducted on subjects who had clinically significant HCMV infection through Week 24 post-transplant with documented HCMV DNaemia who had a plasma sample available for analysis. Total DNA (including HCMV DNA) was isolated from plasma, and the UL56 and UL89 protein coding regions were amplified and subjected to DNA sequence analysis using NGS Illumina MiSeq

technology. NGS analysis of the entire UL56 and UL89 coding regions was performed on samples obtained from 22 letermovir-treated subjects in the FAS population who experienced prophylaxis failure and for whom samples were available for analysis. The sponsor reported that one subject had a letermovir-resistant genotypic variant at pUL56 amino acid position V236M. In addition, samples from 54 subjects from the Placebo arm were subjected to NGS analysis and these were used as the comparator to identify substitutions that only occurred on treatment with letermovir.

The goal of the independent assessment of NGS data was to confirm the results reported by the sponsor, to determine which known resistance-associated substitutions were present in the virus of subjects who failed treatment, and to determine if additional substitutions could potentially be associated with treatment failure. The independent analysis focused on the amino acid positions of interest identified in the two HCMV proteins that have been associated with resistance to letermovir based on cell culture resistance selection experiments. For pUL56, substitutions at positions 231, 236, 237, 241, 244, 257, 261, 321, 325, 329, and 369 are considered to be resistance-associated. For pUL89, a substitution at position 345 was selected in cell culture using letermovir, but the sponsor concluded that this was an interstrain variation not associated with letermovir resistance. However, this conclusion is limited by the failure of the sponsor to phenotype this substitution. In addition, substitutions at pUL89 position 256 were selected in cell culture with a close analog of letermovir (see NDA 209939 SDN 000).

Overall, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few minor exceptions that could be rectified using a third NGS analysis pipeline. pUL56 substitutions E237G, V236M, and C325W were detected in the virus of subjects from the letermovir arm who failed treatment while on letermovir. These substitutions occurred at known cell culture resistance positions and therefore, are considered resistance-associated substitutions. In addition, pUL56 E485G substitution and 445-SNS-447 deletion occurred at high frequency (>70%) in 2 of 8 (25%) of subjects who failed treatment while on letermovir. Because this deletion and substitution occurred at highly conserved pUL56 amino acid positions, these are also considered resistance-associated. These substitutions should be included in the resistance section of the label.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability:

This application is approvable based on the analysis of NGS data. There was good agreement with the results presented by the sponsor and independently determined by DAVP. However, interpretation of the results varied, and will likely result in labeling changes.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, If Approvable:

(b) (4)

BACKGROUND AND SUMMARY

The HCMV terminase complex is made up of at least two viral proteins, pUL56 and pUL89, and the main function of the terminase complex is to cleave HCMV concatemeric genomes into single units of functional HCMV monomers. Letermovir inhibits the HCMV terminase activity, preventing the formation of mature genomes that can be packaged to form infectious virions. The cell culture antiviral activity of letermovir was determined in multiple cell lines. The median EC_{50} values of letermovir against clinical HCMV isolates were 0.0019 µM for subtype gB1 (range 0.0001-0.0058 µM, N=29), 0.002 µM for subtype gB2 (range 0.0007-0.0061 µM, N=27), 0.0023 µM for subtype gB3 (range 0.0015-0.0034 µM, N=11), and 0.0029 µM for subtype gB4 (range 0.0026-0.0032 µM, N=3). The EC_{50} values were 3.5 to 5.6 nM when human fibroblasts from different origins (foreskin, normal and embryonic lung, and dermis) were infected with CMV AD169 and incubated with letermovir.

HCMV mutants with reduced susceptibility to letermovir were selected in cell culture. The substitutions mapped to pUL56 and occur at amino acid residues between 231 and 369 (V231A, V231L, V236L, V236M, E237D, L241P, T244K, T244R, L257I, F261C, F261L, F261S, Y321C, C325F, C325R, M329T, R369G, R369M, R369S). EC_{50} values for these substitutions were 13 to 5,870-fold higher than those observed for the wild-type reference virus. One resistance-associated substitution in pUL89 was selected for using letermovir. In the selection experiment A345S was identified, which was described by the sponsor as a site of interstrain variation. A second pUL89 substitution was selected by a close analog of letermovir known as BAY 66-6047, which selected for pUL89 substitution Q256E. Based on these studies, two substitutions of interest have been identified for this viral protein, although the sponsor did not report if these substitutions confer cross-resistance to letermovir or determine the phenotypes of these substitutions in relation to letermovir susceptibility.

Phase 3 clinical trial P001 (MK-8228-001) was a randomized, placebo-controlled, multi-site, double-blind, trial that evaluated the efficacy and safety of letermovir compared to placebo in the prevention of clinically significant HCMV infection in adult (≥18 years of age) HCMV-seropositive allogeneic hematopoietic stem cell transplant (HSCT) recipients. The primary objective of the study was to evaluate the efficacy of letermovir in the prevention of clinically significant HCMV infection through Week 24 post-transplant following administration of letermovir or placebo. The sponsor reported that a lower proportion of subjects in the letermovir group (37.5%) developed clinically significant HCMV infection compared to the placebo group (60.6%) through Week 24 post-transplant in the Full Analysis Set population and the letermovir group exhibited an increased time to onset of clinically significant HCMV infection through Week 24 post-transplant. In addition, the sponsor concluded that all-cause mortality was substantially lower in the letermovir group compared to placebo through Week 24 post-transplant. Next generation sequencing of the HCMV UL56 and UL89 genes was performed for 85 subjects, 54 in the placebo group and 31 subjects who were on treatment with letermovir at the time of failure (Table 1).

Table 1. Subjects for whom NGS data were submitted (DAVP Analysis). TF, treatment failure; TS, treatment success.

# Subjects	Arm	Outcome	# fastq files
54	Placebo	All TF	120
31	Letermovir	29 TF/ 2 TS	66
1	Unknown	unknown	2

The NGS data provided by the sponsor included: 1) frequency tables showing amino acid variation that occurred at each position of the two viral proteins pUL56 and pUL89 for the virus from each subject that was successfully sequenced using Illumina MiSeq technology; 2) raw nucleotide sequence data in fastq format for all samples that were deep sequenced; and 3) summary resistance data for the study.

Given that next generation sequencing is an emerging technology with no current standards for analysis, the division requested raw data in fastq format so that an independent analysis could be

performed on the NGS data. The sponsor's summary NGS data were compared to the results generated by DAVP following these criteria:

1. The sponsor's frequency tables were used to generate a summary and do a direct comparison of the results reported by the sponsor;
2. Frequency tables were generated by DAVP using an independent mapping of reads to a reference for each sample and using an optimized NGS analysis pipeline in the High-Performance Integrative Virtual Environment (HIVE). A second optimized NGS analysis pipeline using CLC Genomics Workbench was used to assess variants for which there was disagreement (novel variants or major disagreements in frequency) between the sponsor's results and the HIVE pipeline. The results of the independent assessment were compared with those reported by the sponsor and those generated using the sponsor's frequency table; and
3. The conclusions from the NGS data were compared to the results reported by the sponsor using Sanger population sequence analysis when applicable.

Rationale for Requesting and Analyzing NGS Data

In general, the FDA does not analyze raw nucleotide sequence data in conjunction with new drug applications (NDAs). However, because of the complexity and size of NGS data, the lack of data standards and standardized analyses pathways, and the large number of tools available for different operations in an analysis pathway the Division of Antiviral Products has determined that an independent analysis of NGS data should be performed for new drug applications to ensure that resistance pathways for new drugs and new combinations are carefully characterized and defined in the label. DAVP provides an NGS template entitled, ***Submitting Next Generation Sequencing Data to the Division of Antiviral Products*** to sponsors who intend to use NGS technology in support of new drug approvals.

NGS Data Analysis Pipeline

DAVP used the HIVE, in collaboration with CBER, to perform the majority of the independent assessment of the NGS data for this NDA. DAVP virologists, in collaboration with the HIVE team, have developed specific tools that allow DAVP to batch rename files to meet nomenclature rules for the analysis pipeline, to assess quality control on all sequence files, to convert variant call files (VCF) to frequency tables at the amino acid level, and to generate tables comparing results submitted by the sponsor and those generated by HIVE or CLC Genomics (Figure 1).

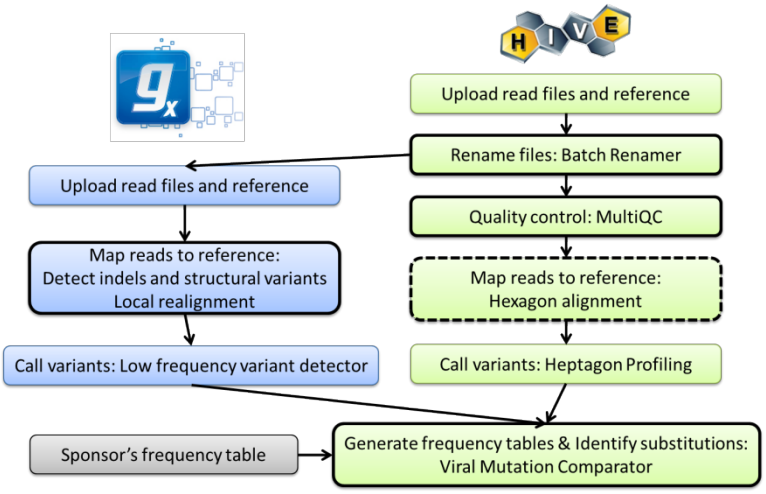

Figure 1. Overview of the NGS analysis pipeline using HIVE and/or CLC Genomics Workbench (DAVP Analysis).

The initial analysis of NGS data was generated using HIVE. A second optimized NGS analysis pipeline using CLC Genomics Workbench was used to assess variants for which there was disagreement (novel variants or major disagreements in frequency of 10% or greater) between the sponsor's results and the HIVE pipeline (Figure 1).

NGS Analysis Parameters and Overview of Data Analysis

DAVP used an optimized HIVE analysis pipeline for the primary NGS analyses and an optimized CLC Genomics workbench pipeline for the secondary NGS analyses (assessing disagreements). The workflow in HIVE was as follows: 1) sequence reads were mapped to the reference sequence using Hexagon aligner tool, 2) variants were called at the amino acid level using the Heptagon profiling tool, and 3) frequency tables were generated using the Viral Mutation Comparator tool.

The parameters used in HIVE were the following:

1. Mapping using Hexagon aligner
    a. Match benefit: 5
    b. Mismatch penalty: -4
    c. Mismatch continuation penalty: -6
    d. Gap continuation cost: -4
    e. Gap opening cost: -12
    f. Local alignment
2. Heptagon profiling
    a. Minimum coverage was set to 100

Briefly, the workflow in CLC Genomics workbench was as follows: 1) trimmed reads were mapped to the reference sequence, 2) structural variants were detected based on the information derived from unaligned ends and locally realigned to the reference sequence, 3) consensus sequences were generated, 4) variants were called at the amino acid level, exported in Excel, and later imported into HIVE for analysis using HIVE's Viral Mutation Comparator tool and to generate frequency tables.

The parameters used in CLC Genomics were the following:

1. Mapping using Hexagon aligner
    a. Match score: 1
    b. Mismatch cost: 2
    c. Insertion cost: 3
    d. Deletion cost: 3
    e. Length fraction of read required for mapping: 0.5
    f. Similarity fraction: 0.8
    g. Local alignment
2. InDels and Structural Variants
    a. p-value threshold of unaligned end breakpoints: 0.0001
    b. maximum number of mismatches: 3
    c. minimum number of reads to filter variants: 2
3. Local realignment
    a. multi-pass realignment: 2
    b. maximum guidance-variant length (defined by InDels and Structural Variants): 100
4. Low frequency variant detection
    a. Minimum frequency: 1%
    b. Minimum coverage was set to 100
    c. Frequency was set to 1%
    d. Significance: 1%

Each step of the analysis process is briefly described below.

1. **Processing fastq files**. Data were submitted to the FDA on a portable hard drive, which included fastq files for each subject's samples that were sequenced using paired end chemistry and the Illumina MiSeq sequencing platform. The nucleotide sequences were uploaded via the HIVE interface and assessed for quality control by looking at the following parameters: position statistics, where the mean phred score is calculated; read length, the relative base population of A, C, G, T for each sample; and average base quality for each file. Outlier files were flagged and evaluated more closely.
2. **Preparing sequence reads and reference sequences prior to mapping**. The fastq files were imported into HIVE or CLC and the Merlin strain of HCMV (NC_006273.2) was used as the reference sequence. The UL56 and UL89 genes were annotated separately and uploaded into HIVE for use as independent reference sequences, whereas for CLC both genes were annotated in the Merlin genome and the entire genome was used as the reference sequence. The individual reads from each fastq file were subjected to trimming using the default parameters for CLC Genomics Workbench (when used).
3. **Mapping reads to the appropriate reference sequence.** The reads from each fastq file were aligned to the appropriate reference sequence to generate a mapping for each sample. The mapping contained the target of interest (the UL56 and/or UL89 gene sequences) and was used to identify variants that differed from the reference sequence. In general, the mappings were assessed to determine the depth of coverage at each nucleotide position and to evaluate read directionality (ratio of forward to reverse reads) to identify regions of bias.

NGS Analysis Pipeline Output

4. **Generating frequency tables of amino acid substitutions.** From the read mappings, variants were called and variant tables were generated for each nucleotide sequence run using the built in variant caller in HIVE (Simonyan et al., 2017) and the low frequency variant caller in CLC Genomics workbench (only used in cases of disagreement between HIVE and the sponsor). The variant call tables were converted into amino acid frequency tables using the HIVE Viral Mutation Comparator tool. Below are descriptions for the variant callers and the frequency table:
    a. **HIVE Variant Detector (HVD)** – calls variants from a read mapping using the Heptagon Sequence Profiler tool (Simonyan et al., 2017). Following alignment, the reference-based variant profile is computed by mapping nucleotides of short read sequences and counting occurrences of distinct bases at every genomic position on the reference. This variant-calling procedure produces consensus, per-base, forward/reverse, and total coverage maps for all reference segments of the specified reference genomes/sets. The frequencies of each variant call are then computed relative to the reference genome or relative to the accumulated consensus genome, depending on user specifications. Amino acids (AA) are called based on the contribution of every read and its alignment in relation to an annotated open reading frame (ORF). AA substitution calls are made from codon translations of individual reads, based on mapping to annotated open reading frames. This is to contrast the variant calling from a consensus based AA calling procedure.
    b. **Low Frequency Variant Detection (LFVD)** – according to the CLC Genomics Workbench Manual: *"a statistical test is performed at each site to determine if the nucleotides observed in the reads at that site could be due simply to sequencing errors, or if they are significantly better explained by there being one (or more) alleles than the reference present in the sample at some unknown frequency. If the latter is the case, a variant corresponding to the significant allele will be called, with estimated frequency".*
    c. **Frequency tables** – Tables were generated using the HIVE Viral Mutation Comparator tool. This table contains information for each position of each gene and each subject for which variation from the reference occurs. The frequency table contains the following columns: unique subject identifier (USUBJID), treatment regimen (ARM), visit (VISIT), the amino acid position within the gene of interest (AAPOS), total coverage at the

nucleotide position (TCOV), the amino acid found in the reference sequence (AAREF), the amino acid substitution (AASUB), the coverage at the nucleotide level for the variant (VCOV), and the frequency by which the variant was detected (AAFREQ). Frequency tables are generated by:

i. The variant tables were combined by arm (letermovir or placebo)
ii. The variant tables were filtered to remove synonymous substitutions
iii. The variant tables were reformatted to be directly comparable to the frequency tables submitted by the sponsor
iv. Any amino acid substitution ≥1% was presented in the table, although the sponsor's analysis was performed with a ≥5% cutoff.

Resistance Analysis Definitions

5. **Generating resistance analysis tables.** The HIVE Viral Comparator tool and Excel macros were used to convert the frequency tables into resistance analysis tables, allowing the resistance tables to be populated using different frequency thresholds. For example, the frequency tables generated from CLC Genomics Workbench output or submitted by the sponsor contained all variants with a frequency greater than or equal to 1%, and this tool allowed resistance analysis tables to be generated showing variants at different levels of sensitivity (5%, 15%, 25%, etc.) as defined by the user.
6. **Conducting independent resistance analysis.** The frequency tables and resistance analysis tables were then analyzed to identify substitutions that occurred above a defined frequency threshold of 5%, using the following criteria:
    a. **Resistance criteria** – resistance-associated substitutions were defined by several criteria listed below. In general, substitutions that occurred at highly conserved amino acid positions in the virus of subjects in the letermovir, but not the placebo arm were of interest, particularly if these occurred at known resistance positions, occurred in more than one subject, or occurred while the subject was still on letermovir treatment.
        i. Amino acid positions conserved at ≥96% identity (based on conservation values provided by the sponsor)
        ii. Substitution occurs in <5% of subjects in the placebo arm
        iii. Amino acid substitution frequency ≥5% in a subject in the letermovir arm; looking for patterns above 2%
        iv. Substitutions occurred in n≥2 subjects
        v. Detected at known positions at any frequency
        vi. Resistance detected prior to D100 (while on drug)
        vii. Novel, unreported substitutions at a polymorphic position
        viii. Identify amino acid changes that occurred as the result of two or more mutations in a codon
    b. In addition, known resistance-associated substitutions that have been identified in pUL56 and pUL89 were analyzed at the 1% frequency cutoff, given that amino acid replacements at these sites may represent viral populations that are of low fitness and therefore may rapidly be overgrown once treatment stops. For pUL56, substitutions at positions 231, 236, 237, 241, 244, 257, 261, 321, 325, 329, and 369 are considered to be resistance-associated. For pUL89, positions 256 and 345 are considered to be resistance-associated.

NGS Data Comparison

7. **Comparing results to those submitted by the sponsor.** The remainder of this review provides details on how the NGS data submitted by the sponsor were independently evaluated using the above described NGS analysis pipeline. In general, the NGS data analysis was performed using data generated in this pipeline and provided by the sponsor, and the results were compared as follows:

a. Frequency and resistance analysis tables were compared directly and major differences were noted
b. Amino acid substitutions were identified by at least two and up to three algorithms, including the sponsor's algorithm (M) and HVD and LFVD (used by DAVP) and major differences between algorithms were reported
c. Novel resistance-associated amino acid substitutions reported by different NGS analysis approaches were compared and major differences were reported
d. NGS analysis results were compared to results obtained and reported by the sponsor using Sanger population sequencing when applicable
e. Novel resistance-associated substitutions identified by the independent analysis were noted and discussed with the review team for potential labeling/post-marketing actions

CLINICAL STUDIES

The sponsor submitted NGS data for one phase 3 clinical trial to support the NDA for letermovir. This portion of the clinical virology NDA review focused exclusively on the independent analysis of NGS data from treatment failures from the phase 3 clinical trial P001 (MK-8228-001). The primary review of the phase 3 and phase 2 clinical trials and overall conclusions drawn from phase 2 and phase 3 resistance data can be found in the review of Senior Clinical Virology Reviewer Takashi Komatsu, Ph.D., RAC (NDA 209939 SDN 000).

Phase 3 clinical trial P001 (MK-8228-001) was a randomized, placebo-controlled, multi-site, double-blind, trial that evaluated the efficacy and safety of letermovir compared to placebo in the prevention of clinically significant HCMV infection in adult (≥18 years of age) HCMV-seropositive allogeneic hematopoietic stem cell transplant (HSCT) recipients. The primary objective of the study was to evaluate the efficacy of letermovir in the prevention of clinically significant HCMV infection through Week 24 post-transplant following administration of letermovir or placebo. The sponsor reported that a lower proportion of subjects in the letermovir group (37.5%) developed clinically significant HCMV infection compared to the placebo group (60.6%) through Week 24 post-transplant in the Full Analysis Set population and the letermovir group exhibited an increased time to onset of clinically significant HCMV infection through Week 24 post-transplant (Table 2). In addition, the sponsor concluded that all-cause mortality was substantially lower in the letermovir group compared to placebo through Week 24 post-transplant (Figure 2).

Table 2. Summary of the efficacy analyses for primary and secondary endpoints (FAS population) (Table , page 17, P001v1 Synopsis).

	Letermovir (N=325)		Placebo (N=170)		Difference†	
	n	%	n	%	Difference(95% CI)	p-value
Primary Endpoint						
Clinically significant CMV infection through Week 24 post-transplant‡	122	37.5	103	60.6	-23.5 (-32.5, -14.6)	<0.0001
Secondary Endpoints						
Clinically significant CMV infection through Week 14 post-transplant‡	62	19.1	85	50.0	-31.3 (-39.9, -22.6)	<0.0001
CMV End-organ Disease through Week 14 post-transplant§	1	0.4	2	1.4	-1.0 (-3.5, 1.5)	0.2258
CMV End-organ Disease through Week 24 post-transplant§	5	2.0	3	2.4	-0.4 (-4.0, 3.2)	0.4056
Initiation of PET for documented CMV viremia through Week 24 post-transplant‡	119	36.6	101	59.4	-23.3 (-32.3, -14.3)	<0.0001
Initiation of PET for documented CMV viremia through Week 14 post-transplant‡	61	18.8	84	49.4	-31.0 (-39.6, -22.4)	<0.0001

† 95% CIs and p-value for the treatment differences in percent response were calculated using stratum-adjusted Mantel-Haenszel method with the difference weighted by the harmonic mean of sample size per arm for each stratum (high or low risk). A 1-sided p-value ≤0.0249 was used for declaring statistical significance for primary analysis of the primary endpoint. Nominal one-sided p-values (not adjusted for multiplicity) are provided for other analyses as a measure of the strength of the relationship between treatment and response.
‡ Approach to handling missing values: Non-Completer=Failure (NC=F) approach. With NC=F approach, failure was defined as all subjects who developed clinically significant CMV infection or prematurely discontinued from the study or had a missing outcome through Week 24/Week 14 post-transplant visit window.
§ Approach to handling missing values: Data-as-Observed (DAO) approach. With DAO approach, any subject with missing value for a particular endpoint was excluded from the analysis.
N = Number of subjects in analysis population.
n = Number of subjects with outcome.

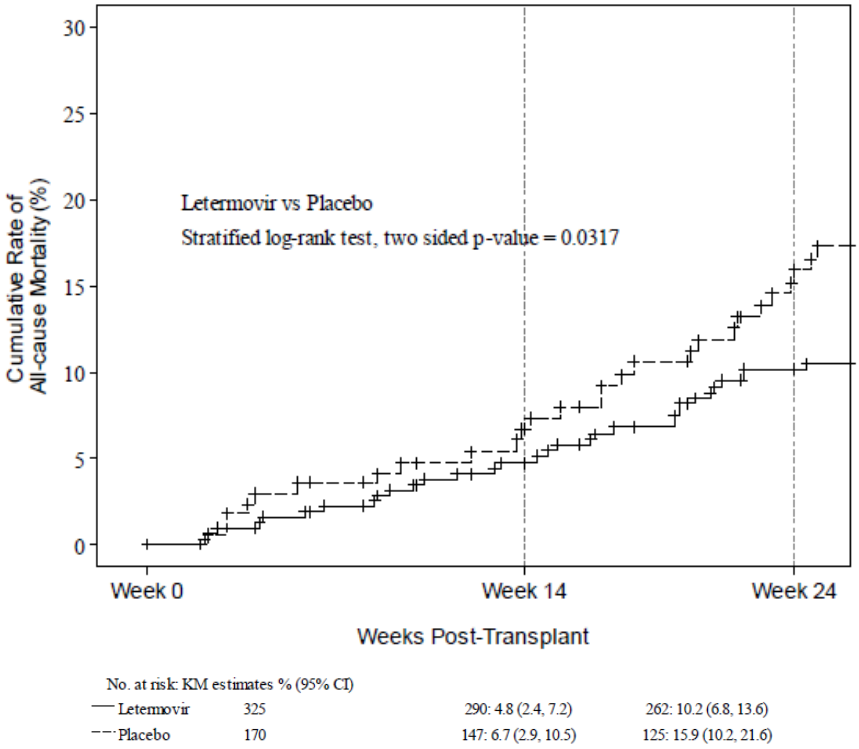

Figure 2. P001: Kaplan-Meier plot of time to all-cause mortality through Week 24 post-transplant (FAS population) (Figure , page 18, P001v1 Synopsis).

Resistance Analysis Population

Among 565 subjects in study P001 (MK-8228-001) who were randomized and treated, the sponsor reported that 167 met the primary endpoint of clinically significant HCMV infection through Week 24 post-transplant with documented HCMV DNaemia. Of these, the sponsor reported that 116 subjects had at least one plasma sample available for CMV UL56/UL89 genotypic analysis. According to the sponsor, the results for 2 subjects were invalid and there was insufficient plasma for repeat testing. The remaining 114 subjects were defined as the All Randomized and Treated (ARaT) Genotyping Analysis Population (GAP), and a subset of these (84 subjects) were defined as the FAS GAP. The sponsor stated that subjects in the FAS GAP were the primary population used to identify resistance-associated substitutions in pUL56 and pUL89. The 84 subjects in the FAS GAP included 34 subjects who received at least one dose of letermovir and 50 subjects who received placebo. Of note, NGS data were provided for 85 subjects from both groups.

Importantly, the sponsor initially used [redacted] (b) (4) to conduct the resistance analysis for their Phase 3 study, P001 (MK-8228-001); however, the genotyping assay used by [redacted] (b) (4) was not adequate for a prophylaxis study. Of 56 subject samples tested by [redacted] (b) (4) only 0/56 and 2/56 complete amino acid sequences were generated for the pUL56 and pUL89 coding regions, respectively. It is important to note that several subject plasma samples had low HCMV DNA copy numbers due to the study design which recommended that pre-emptive therapy be initiated in high risk subjects when the HCMV DNA level was ≥137 IU/mL and in low risk subjects when the HCMV DNA level was ≥274 IU/mL. The low viral load in these samples made it impossible to perform genotypic analysis of UL56 and UL89 with the [redacted] (b) (4) assay that has a limit of detection (LOD) of at least 1,875 copies/mL. Given these issues, the sponsor switched to the [redacted] (b) (4) for conducting the genotypic analysis using next generation sequencing. However, by the time of the switch, there were no remaining plasma samples available for several subjects and therefore, genotypic analysis could not be performed. Overall, there were 40/73 (22 with [redacted] (b) (4) and 62/85 (42 with [redacted] (b) (4) subjects with genotypic data.

Resistance Analysis Methodologies Used by the Sponsor

For this Phase 3 study (MK-8228-001), plasma samples were collected for monitoring HCMV DNAemia using the FDA approved Roche COBAS® AmpliPrep/COBAS TaqMan® (CAP/CTM) assay tested by the central laboratory. The lower limit of quantification (LLOQ) for this assay was 137 IU/ml which is ~150 copies/mL (using a conversion factor of 1.1 copies/IU as per the assay package insert). To perform HCMV UL56/UL89 genotypic analysis, total DNA was isolated from plasma, and the UL56 and UL89 protein coding regions were amplified and subjected to DNA sequence analysis. Two different methods were used to perform HCMV UL56 and UL89 DNA sequence analysis.

(b) (4) The HCMV UL56 and UL89 genes were amplified by PCR using five and ten HCMV-specific primer sets, respectively. PCR products were analyzed by fluorescent dye terminator dideoxy sequencing on an ABI 3730xl capillary DNA Sequencer. The DNA sequences were base-called and assembled into contigs, and the deduced UL56 or UL89 amino acid sequences were aligned with the appropriate reference sequence from the HCMV AD169 isolate (obtained from American Type Culture Collection, Manassas, Virginia, United States). All of the UL56 and UL89 amplicons had a measured LOD value of at least 1,875 copies/mL.

(b) (4) The genes encoding pUL56 and pUL89 were amplified in 3 different nested PCR assays. The amplicons generated by the nested PCR were analyzed by NGS using the Illumina MiSeq system. QC analysis and variant calling compared to a reference sequence was performed using the 'Athena' pipeline, which is custom software developed by (b) (4) specifically for the analysis of amplicon-based deep sequencing data. The reference sequences used were from the HCMV Merlin strain (GenBank accession number: HCMV_Merlin_NC006273_rev2). The LOD value was 108 copies/mL for UL56, 37 copies/mL for UL89A (Exon 1), and 92 copies/mL for UL89B (Exon 2). For the sponsor's analyses, differences detected at a frequency of ≥5% at a given position were identified as HCMV genotypic variants.

For a complete overview of the NGS analysis process used by (b) (4) please see Appendix 1.

The Sponsor's Resistance Analyses

For the resistance analyses, any nucleotide difference in the coding sequence of HCMV pUL56 or pUL89 that resulted in an amino acid substitution relative to the reference sequence was defined as a genotypic variant; however, DAVP prefers the term 'substitution' and so that terminology was used instead of the sponsors. Each of the substitutions were categorized further using the following criteria:

1. **Characterized** – defined as substitutions that occurred at sites identified in cell culture selection experiments that led to decreased susceptibility to letermovir; substitutions found in this category for UL56 were further categorized as susceptible or resistant to letermovir. The sponsor reported that no substitutions in UL89 that confer resistance to letermovir have been published to date.
2. **Not characterized** – defined as substitutions that include: 1) common polymorphisms in either pUL56 or pUL89 whose impact on letermovir susceptibility is unknown; and 2) observed changes in the pUL56 or pUL89 that have not been reported or seen previously.

The sponsor noted that substitutions detected in either pUL56 or pUL89 that have not been characterized will undergo phenotypic analysis to determine if the substitution has an impact on susceptibility to letermovir. The phenotypic analyses are not completed, and will be the subject of a separate report.

Clinical Trial P001 (MK-8228-001) Sponsor's Resistance Results

For clinical trial P001 (MK-8228-001), 114 subjects were defined as the ARaT GAP, and a subset of these (84 subjects) were defined as the FAS GAP. The sponsor stated that subjects in the FAS GAP

were the primary population used to identify resistance-associated substitutions in pUL56 and pUL89. The 84 subjects in the FAS GAP included 34 subjects who received at least one dose of letermovir and 50 subjects who received placebo.

The sponsor analyzed all of the genotypic variants detected in subjects in the ARaT GAP who received letermovir and had DNA sequence available (Table 3). The sponsor stated that Table 3 also includes identification of all FAS GAP substitutions, as well as substitutions in subjects who had detectable HCMV DNaemia on the day letermovir dosing was initiated. According to the sponsor, previously characterized letermovir-susceptible substitutions were not included in (Table 3). The sponsor noted that among the 6 subjects who received letermovir and had detectable HCMV DNaemia on the day dosing was initiated, UL56 substitutions in the "hot spot" were identified in the sample collected at prophylaxis failure in 2 subjects; one (I313V, not previously described or characterized) has no reported association with letermovir resistance, while the second (C325W) was a novel substitution at a residue where letermovir-resistant substitutions (C325F and C325R) have been previously identified. In addition, the sponsor reported that there were no other observed UL56 or UL89 substitutions identified at prophylaxis failure in the 6 ARaT GAP subjects who had detectable HCMV DNaemia on the day letermovir dosing was initiated.

Table 3. HCMV pUL56 and pUL89 substitutions for individual subjects in the ARaT GAP who received letermovir (Table 8, page 15, Virology Report Genotypic Analysis).

	UL56 GVs [Observed (Not Previously Described or Characterized), Common Non-Characterized, and Previously-Characterized Resistant] †														
Subject ID	E157	A164	V236	S255	I313	C325	S445	N446	I535	Y575	T775	A779	R816	R826	UL89 GVs‡	Total GVs
100008*						W										1
100074								S								1
100077								S								1
100078															L522P	1
100165															I531V	1
100278#															#	0
100283															D94N	1
100348		V														1
100351										C					D94N	2
100391								S				V			A633V	3
100399*															#	0
100402							SNS445^				I				Q625STOP, D665E	4
101625			M				SNS445^								D665E	3
101640															N74S, D309G	2
101749							G								S102F	2
101760															#	0
101765																0
101773*					V										#	1
101858															E95K	1
101880									V							1
101890*																0
101909															D94N	1
101957				L												1
101969															#	0
102029								S							D94N, A633V	3
102078#															D665E	1
102090	G							S				V		L	#	4
102193													W			1

† E157G, A164V, I313V, C325W, S255L, S445G, SNS445, I535V, Y575C, T775I, R816W, R826L are observed (not previously described or characterized) UL56 GVs. N446S, A779V are common, non-characterized UL56 GVs. V236M is a previously-characterized, letermovir-resistant GV.
‡ D94N, E95K, I531V, A633V, D665E are common, non-characterized UL89 GVs; N74S, S102F, D309G, L522P, Q625STOP are observed (not previously described or characterized) UL89 GVs.
§ Subjects with detectable CMV viremia on Day 1
^ Codons for S445-N446-S447 are deleted
# Subjects 100278, 101760, 101773, 101969, and 102090 were missing UL89 AA 1-674; Subject 100399 was missing UL89 AA 1-296.
GAP = Genotyping Analysis Population; GV = Genotypic Variant.
Note: Differences detected at a frequency of ≥5% of the total sequence data at a given position indicate the presence of a CMV genotypic variant (GV).

Clinical Trial P001 (MK-8228-001) Resistance Conclusions from the Sponsor

The sponsor concluded that the overall incidence of HCMV UL56 and UL89 substitutions observed in their analysis was similar in subjects who received either letermovir or placebo, met the primary endpoint, and had a plasma sample available for HCMV UL56/UL89 genotypic analysis. Their overall analyses reported that 1 of 22 (5%) letermovir subjects in the FAS GAP who had NGS results had a UL56 substitutions known to confer resistance to letermovir, and 7 of the 22 subjects (32%) had a single substitution in either pUL56 or pUL89 that had not been previously described or characterized for susceptibility to letermovir. In addition, the sponsor reported that 4 letermovir subjects (18%) had multiple observed substitutions (not previously described or characterized) in pUL56 and/or pUL89. In the subpopulation of letermovir subjects from the GAP who had HCMV DNaemia when letermovir

dosing was initiated, the sponsor stated that no subjects had letermovir-resistance-associated pUL56 substitutions in the sample collected at prophylaxis failure, but 2 of 6 subjects had novel substitutions in the "hot spot" of pUL56. The sponsor stated that phenotypic analysis will be performed to determine which uncharacterized substitutions, if any, affect HCMV susceptibility to letermovir in cell-culture models of infection.

Clinical Trial P001 (MK-8228-001) DAVP Analysis

The NGS analysis approach used by DAVP was different from the one employed by the sponsor, but generally provided the same overall results. DAVP used two NGS analysis pipelines to analyze the 190 fastq files provided by the sponsor (188 files for subjects and 2 controls)(Table 4). Briefly, the sequence files were segregated by protein (pUL56 and pUL89) and by arm, such that subjects who received letermovir were analyzed as one group and subjects in the placebo arm were analyzed as a different group. The sequence read files for each subject and timepoint (some subjects had samples available from multiple timepoints) were aligned to the UL56 and UL89 genes of the Merlin HCMV reference sequence and the alignments were then analyzed to determine nucleotide changes that resulted in amino acid differences. Frequency tables were generated which captured the variation detected at all amino acid positions of both proteins, and then the amino acid substitutions that were detected in the letermovir arm were compared to the amino acid substitutions detected in the placebo arm to determine which sites might be polymorphic and which might be associated with the development of resistance to letermovir.

Table 4. Subjects for whom NGS data were provided for the resistance analysis (DAVP Analysis). Yellow rows indicate subjects who failed on treatment with letermovir.

8228-001-000100001	101765	D85, D89, D157	6	Letermovir	8228-001-010800004	101695	D15	2	Placebo
8228-001-000100005	101767	D40, D41, D131	6	Placebo	8228-001-010800005	100074	D147	2	Letermovir
8228-001-000400002	101778	D36	2	Placebo	8228-001-010800007	100075	D33	2	Placebo
8228-001-001200003	100135	D41	2	Placebo	8228-001-010800010	100076	D36	2	Placebo
8228-001-001300005	102119	D10	2	Placebo	8228-001-010800011	101698	D36	2	Placebo
8228-001-001800002	101880	D125	2	Letermovir	8228-001-010800012	100077	D131	2	Letermovir
8228-001-001800004	101882	D33	2	Placebo	8228-001-010800013	101909	D144	2	Letermovir
8228-001-001800005	101883	D162	2	Placebo	8228-001-010800016	100078	D95	2	Letermovir
8228-001-001800007	101957	D160	2	Letermovir	8228-001-010800017	100307	D24	2	Placebo
8228-001-001800018	102011	D64	2	Placebo	8228-001-010800018	101912	D53	2	Placebo
8228-001-001800045	100399	D126	2	Letermovir	8228-001-010800020	101914	D57	2	Placebo
8228-001-001800048	100402	D66	2	Letermovir	8228-001-010800024	102067	D57	2	Placebo
8228-001-001900002	100109	D34	2	Placebo	8228-001-011300004	101725	D15	2	Placebo
8228-001-002000015	101948	D40	2	Placebo	8228-001-011600002	100043	D13	2	Placebo
8228-001-003300002	100164	D10	2	Placebo	8228-001-011600007	101667	D36	2	Placebo
8228-001-003300003	100165	D135, D281	4	Letermovir	8228-001-011600025	100451	D14	2	Placebo
8228-001-003400001	100283	D147	2	Letermovir	8228-001-011600033	102217	D15	2	Placebo
8228-001-003400003	100285	D29	2	Placebo	8228-001-011700006	100008	D46	2	Letermovir
8228-001-004200005	100142	D112	2	Placebo	8228-001-012200003	102078	D108	2	Letermovir
8228-001-004200010	101760	D42	2	Letermovir	8228-001-012400002	101867	D40	2	Placebo
8228-001-004200011	101761	D42	2	Placebo	8228-001-012600006	101773	D162	2	Letermovir
8228-001-004400004	100278	D9	2	Letermovir	8228-001-013100003	**NA**	**Dxx**	2	**Unknown**
8228-001-004400005	101916	D24	2	Placebo	8228-001-014000009	101806	D54	2	Placebo
8228-001-004500004	101672	D23	2	Placebo	8228-001-014000012	102025	D54	2	Placebo
8228-001-004700002	101634	D28	2	Placebo	8228-001-014200007	102003	D64	2	Placebo
8228-001-005800004	101749	D145	2	Letermovir	8228-001-014600001	101651	D48	2	Placebo
8228-001-005900001	101657	D22	2	Placebo	8228-001-014600006	101655	D42	2	Placebo
8228-001-005900008	101874	D33	2	Placebo	8228-001-014700003	101640	D175	2	Letermovir
8228-001-005900014	102018	D15	2	Placebo	8228-001-014700007	100019	D28	2	Placebo
8228-001-006400001	101717	D132	2	Letermovir	8228-001-016100001	100343	D14	2	Placebo
8228-001-006400012	102005	D21	2	Placebo	8228-001-016100002	100344	D8	2	Placebo
8228-001-006400021	102103	D55	2	Placebo	8228-001-016100005	102029	D46	2	Letermovir
8228-001-006400025	102106	D49	2	Placebo	8228-001-016100008	100348	D128	2	Letermovir
8228-001-006900007	101625	D62	2	Letermovir	8228-001-016400003	102036	D23	2	Placebo
8228-001-007100001	100361	D12	2	Placebo	8228-001-016400007	100351	D120	2	Letermovir
8228-001-007800003	101857	D17	2	Placebo	8228-001-016400010	102039	D56	2	Placebo
8228-001-007800004	101858	D131	2	Letermovir	8228-001-016500001	100391	D121	2	Letermovir
8228-001-009100001	101741	D42	2	Placebo	8228-001-016500005	100393	D72	2	Placebo
8228-001-009100020	102193	D145	2	Letermovir	8228-001-016500006	102090	D128	2	Letermovir
8228-001-010200005	101689	D55, D120	4	Placebo	8228-001-017500007	101890	D130	2	Letermovir
8228-001-010200006	101690	D19, D54, D203	6	Placebo	8228-001-017800002	101969	D134	2	Letermovir
8228-001-010200007	100067	D77, D140	4	Placebo	8228-001-018500001	102197	D20	2	Placebo
8228-001-010200008	101691	D146	2	Letermovir					

For this analysis, we used the resistance criteria presented in the Resistance Analysis Definitions section above. In general, we considered substitutions that occurred at highly conserved amino acid positions (≥96% identity based on conservation values provided by the sponsor) that did not occur or

rarely occurred in the placebo arm (<5% of subjects) to be of interest, particularly if these substitutions: 1) occurred in more than one subject, 2) occurred at a frequency >2%, 3) were detected at known amino acid positions previously associated with resistance, 4) occurred as the result of multiple changes to the codon, 5) occurred as novel amino acids at a known polymorphic site, and 6) were only present in subjects who failed while on letermovir treatment.

For the most part, the DAVP analysis of NGS data from clinical trial P001 (MK-8228-001) was consistent with the results reported by the sponsor (Table 5 and Table 8). Most of the differences between the two analyses occurred at frequencies below 2.5%. However, there were several positions of disagreement between the results provided by the sponsor and those reported by HIVE (Tables 5 and 8, red lines), although these differences occurred at positions of low frequency (<5%) or at positions that were associated with deletions in the coding sequence. Samples with discrepancies were reanalyzed using the CLC Genomics workbench, and in all cases, there was agreement between the results detected by the CLC and those reported by the sponsor (Table 5 and 8).

Table 5. Amino acid substitutions in pUL56 that meet the DAVP resistance definition (DAVP Analysis).

USUBJID	SUBJID	AAPOS	VISIT	SUBS2	AAFREQ	Conserved	PBO	RESISTANCE	AGREEMENT
8228-001-001800002	101880	535	D125	I535V	0.05	100	2	RESIST	H,M
8228-001-001800007	101957	26	D160	L26P	0.04	100	0	RESIST	H M
		255	D160	S255L	1	100	0	RESIST	H,M
8228-001-001800048	100402	445-447	D66	SNS Deletion	0.7	95.7, 95.7, 98.9	0*	RESIST	M,C
		485	D66	E485G	0.99	99 5	4	RESIST	H,M
		775	D66	T775I	1	99 5	0	RESIST	H M
8228-001-003300003	100165	846	D281	P846L	0.11	100	0	RESIST	H,M
8228-001-004200010	101760	237	D42	E237G	0.04	100	0	RESIST	H M
		826	D42	R826L	0.03	100	0	RESIST	H,M
8228-001-004400004	100278	213	D9	Q213R	0 0234	100	0	RESIST	M,C
8228-001-006400001	101717	103	D132	A103V	0.04	100	0	RESIST	H M
		658	D132	L658S	0.15	100	0	RESIST	H,M
8228-001-006900007	101625	48	D62	I48M	0.04	100	0	RESIST	H,M
		236	D62	V236M	0.99	100	2	RESIST	H,M
		445-447	D62	SNS Deletion	0.7	95.7, 95.7, 98.9	0*	RESIST	M,C
		485	D62	E485G	0.99	99 5	4	RESIST	H,M
8228-001-009100020	102193	816	D145	R816W	0.99	100	0	RESIST	H,M
8228-001-010200008	101691	276	D146	E276G	0 0204	100	4	RESIST	M,C
		705	D146	S705F	0.09	100	2	RESIST	H,M
8228-001-010800012	100077	706	D131	V706A	0.04	100	0	RESIST	H,M
8228-001-010800013	101909	269	D144	S269G	0.05	100	0	RESIST	H,M
8228-001-011700006	100008	325	D46	C325W	0.99	100	0	RESIST	H,M
		378	D46	S378N	0.024	100	0	RESIST	M C
8228-001-012200003	102078	3	D108	M3V	0.03	100	0	RESIST	H,M
		148	D108	N148D	0 0227	100	0	RESIST	M,C
8228-001-012600006	101773	313	D162	I313V	0.18	100	0	RESIST	H M
		750	D162	L750P	0.03	100	0	RESIST	H,M
8228-001-016100008	100348	164	D128	A164V	0.99	100	2	RESIST	H,M
8228-001-016400007	100351	575	D120	Y575C	0.99	100	0	RESIST	H M
8228-001-016500006	102090	3	D128	M3I	0.03	100	0	RESIST	H,M
		41	D128	F41L	0.03	100	0	RESIST	H,M
		141	D128	E141*	0.03	100	2	RESIST	H M
		157	D128	E157G	0.07	100	0	RESIST	H,M
		182	D128	Q182K	0.03	100	0	RESIST	H,M
		542	D128	E542*	0.03	100	0	RESIST	H,M
		779	D128	A779V	1	99 5	2	RESIST	H,M
		826	D128	R826L	0.05	100	0	RESIST	H M
8228-001-017500007	101890	757	D130	Y757H	0.03	100	0	RESIST	H,M
8228-001-017800002	101969	641	D134	M641T	0.04	100	0	RESIST	H M
		667	D134	Y667H	0 0251	100	0	RESIST	M,C

USUBJID, unique subject ID; **SUBJID**, subject ID; **AAPOS**, amino acid position; **VISIT**, study day that sample was taken; SUBS2, substitutions at a frequency ≥2% that met resistance criteria; **AAFREQ**, the NGS frequency of the substitution; **Conserved**, percent identity at the amino acid position; **PBO**, percent occurrence in the placebo arm; **RESISTANCE**, RESIST = met DAVP resistance criteria; **AGREEMENT**, show algorithms that were in agreement with the variant call at that position; M, Merck; H, HIVE; C, CLC Genomics workbench; **Red**, substitutions detected by the sponsor but not HIVE that were assessed by CLC and determined to be in agreement; **yellow**, positions that required two nucleotide changes to the codon. *, deletion not detected in the PBO group, but variation was noted at the positions that were deleted.

For pUL56, there were 36 amino acid positions that met DAVP resistance criteria with 4 substitutions occurring in 2 or more subjects and 32 occurring in one subject (Table 6). Of note, substitutions previously associated with resistance were detected in the virus of three subjects, including V236M

(0.99 frequency) in subject 8228-001-006900007, C325W (0.99 frequency) in subject 8228-001-011700006, and E237G (0.04 frequency) in subject 8228-001-004200010 (Table 6). One substitution that was detected in the virus of two subjects required two changes to the codon to result in an E (GAA) to G (GGG) change at position 485, which occurred in the virus of subjects 8228-001-006900007 and 8228-001-001800048.

Table 6. List of pUL56 substitutions from the letermovir arm meeting the DAVP resistance criteria (DAVP analysis).

AAPOS	No. Subj	SUBS	RATIO
3	2	M3I/M3V	1-1
445-447	2	SNS Deletion	2
485	2	E485G	2
826	2	R826L	2
26	1	L26P	1
41	1	F41L	1
48	1	I48M	1
103	1	A103V	1
141	1	E141*	1
148	1	N148D	1
157	1	E157G	1
164	1	A164V	1
182	1	Q182K	1
213	1	Q213R	1
236	1	V236M	1
237	1	E237G	1
255	1	S255L	1
269	1	S269G	1
276	1	E276G	1
313	1	I313V	1
325	1	C325W	1
378	1	S378N	1
535	1	I535V	1
542	1	E542*	1
575	1	Y575C	1
641	1	M641T	1
658	1	L658S	1
667	1	Y667H	1
705	1	S705F	1
706	1	V706A	1
750	1	L750P	1
757	1	Y757H	1
775	1	T775I	1
779	1	A779V	1
816	1	R816W	1
846	1	P846L	1

Orange, substitutions detected at previously identified sites of potential resistance; yellow, substitutions which required two changes to the codon.

Substitutions that were detected in the virus of subjects who were on treatment at the time of letermovir failure are shown in Table 7.

Table 7. pUL56 substitutions meeting the DAVP resistance criteria that occurred in subjects while on treatment with letermovir (DAVP analysis). Orange, known resistance site; red, detected in 2 subjects.

USUBJID	SUBJID	Timepoints	Resistance Conclusion
8228-001-000100001	**101765**	D85, D89, D157	No substitutions met Resistance criteria
8228-001-001800048	**100402**	D66	445-447 SNS deletion, E485G, and T775I
8228-001-004200010	**101760**	D42	E237G and R826L
8228-001-004400004	**100278**	D9	Q213R
8228-001-006900007	**101625**	D62	I48M, V236M, 445-447 SNS deletion, and E485G
8228-001-010800016	**100078**	D95	No substitutions detected >1%
8228-001-011700006	**100008**	D46	C325W and S378N
8228-001-016100005	**102029**	D46	No substitutions met Resistance criteria

Two substitutions E485G and a 445-SNS-447 deletion were detected in the virus of two subjects, including subjects 8228-001-006900007 and 8228-001-001800048 (Table 7). Of note, E485G has been described in an independent study as a pUL56 polymorphism that does not have an impact on letermovir susceptibility (Champier et al., 2008; Lishka et al., 2016); however, given that this substitution at a conserved amino acid position emerged in the virus from two subjects who failed treatment while on letermovir, it is considered a resistance-associated site. The 445-SNS-447 deletion has been observed in pUL56 for another terminase complex inhibitor (Champier, et al., 2008), but its impact on letermovir susceptibility is unknown.

For the pUL89 analyses, there was also good agreement between DAVP and the results reported by the sponsor. Differences between HIVE and the results reported by the sponsor occurred at frequencies below 2.5% (Table 8). However, there were several positions of disagreement between the results provided by the sponsor and those reported by HIVE (Table 8, red lines), although these differences occurred at positions of low frequency (<5%) or at positions that were associated with deletions in the coding sequence. Samples with discrepancies were reanalyzed using the CLC Genomics workbench, and in all cases, there was agreement between the results detected by the CLC and those reported by the sponsor (Table 8).

Table 8. Amino acid substitutions in pUL89 that meet the DAVP resistance definition (DAVP Analysis).

USUBJID	SUBJID	AAPOS	VISIT	SUBS2	AAFREQ	Conserved	PBO	RESISTANCE	AGREEMENT
8228-001-000100001	101765	323	D157	L323P	0.0216	100	4	RESIST	M,C
8228-001-001800002	101880	146	D125	V146I	0.03	100	0	RESIST	H,M
8228-001-001800007	101957	243	D160	H243R	0.04	100	0	RESIST	H,M
		373	D160	S373G	0.04	99.5	2	RESIST	H,M
		531	D160	I531T	0.04	96.8	4	RESIST	H,M
		656	D160	V656A	0.0222	100	0	RESIST	M,C
8228-001-001800048	100402	246	D66	H246R	0.03	100	0	RESIST	H,M
		625	D66	Q625*	0.16	100	4	RESIST	H,M
8228-001-003300003	100165	132	D281	T132A	0.03	100	2	RESIST	H,M
		458	D281	L458P	0.04	100	0	RESIST	H,M
		531	D135	I531V	1	96.8	4	RESIST	H,M
		531	D281	I531V	1	96.8	4	RESIST	H,M
8228-001-005800004	101749	102	D145	S102F	0.1	100	2	RESIST	H,M
8228-001-006400001	101717	124	D132	F124L	0.04	100	4	RESIST	H,M
		176	D132	P176S	0.12	100	0	RESIST	H,M
		406	D132	M406V	0.09	100	0	RESIST	H,M
		572	D132	I572V	0.0249	100	2	RESIST	M,C
8228-001-010200008	101691	532	D146	A532T	0.07	100	0	RESIST	H,M
8228-001-010800016	100078	522	D95	L522P	0.07	100	0	RESIST	H,M
		637	D95	T637A	0.0231	99.5		RESIST	M,C
8228-001-014700003	101640	41	D175	K41E	0.0206	100	0	RESIST	M,C
		74	D175	N74S	0.06	100	0	RESIST	H,M
		309	D175	D309G	0.05	100	2	RESIST	H,M
		426	D175	N426D	0.0236	100	2	RESIST	M,C
		521	D175	S521G	0.0239	100	0	RESIST	M,C
8228-001-016100005	102029	331	D46	T331A	0.0207	100	0	RESIST	M,C

USUBJID, unique subject ID; **SUBJID**, subject ID; **AAPOS**, amino acid position; **VISIT**, study day that sample was taken; SUBS2, substitutions at a frequency ≥2% that met resistance criteria; **AAFREQ**, the NGS frequency of the substitution; **Conserved**, percent identity at the amino acid position; **PBO**, percent occurrence in the placebo arm; **RESISTANCE**, RESIST = met DAVP resistance criteria; **AGREEMENT**, show algorithms that were in agreement with the variant call at that position; M, Merck; H, HIVE; C, CLC Genomics workbench; **Red**, substitutions detected by the sponsor but not HIVE that were assessed by CLC and determined to be in agreement; **yellow**, positions that required two nucleotide changes to the codon. *, deletion not detected in the PBO group, but variation was noted at the positions that were deleted.

For pUL89, there were 24 amino acid positions that met DAVP resistance criteria with one substitution occurring in 2 or more subjects and 23 occurring in one subject (Table 9). Of note, substitutions previously associated with resistance (at positions 256 and 345) were not detected in the virus of any subjects in the letermovir arm or the placebo arm. Two different substitutions (I531V and I531T) were

detected at amino acid position 531 in the virus of two subjects, including I531T (0.04 frequency) in subject 8228-001-001800007 and I531V (1.0 frequency) in subject 8228-001-003300003. Of note, this substitution was also observed in the placebo group at a frequency of ~4%.

Table 9. List of pUL89 substitutions from the letermovir arm meeting the DAVP resistance criteria (DAVP analysis).

AAPOS	No. Subj	SUBS	RATIO
531	2	I531V/I531T	1-1
41	1	K41E	1
74	1	N74S	1
102	1	S102F	1
124	1	F124L	1
132	1	T132A	1
146	1	V146I	1
176	1	P176S	1
243	1	H243R	1
246	1	H246R	1
309	1	D309G	1
323	1	L323P	1
373	1	S373G	1
406	1	M406V	1
331	1	T331A	1
426	1	N426D	1
458	1	L458P	1
521	1	S521G	1
522	1	L522P	1
532	1	A532T	1
572	1	I572V	1
625	1	Q625*	1
637	1	T637A	1
656	1	V656A	1

Substitutions in UL89 that were detected in the virus of subjects who were on treatment at the time of letermovir failure are shown in Table 10. None of these substitutions occurred at positions previously associated with resistance to letermovir and no substitutions occurred in more than one subject.

Table 10. pUL89 substitutions meeting the DAVP resistance criteria that occurred in subjects while on treatment with letermovir (DAVP analysis).

USUBJID	SUBJID	Timepoints	Resistance Conclusion
8228-001-000100001	101765	D85, D89, D157	L323P
8228-001-001800048	100402	D66	H246R and Q625*
8228-001-004200010	101760	D42	No substitutions detected >1%
8228-001-004400004	100278	D9	No substitutions detected >1%
8228-001-006900007	101625	D62	No substitutions met Resistance criteria
8228-001-010800016	100078	D95	L522P and T637A
8228-001-011700006	100008	D46	No substitutions detected >1%
8228-001-016100005	102029	D46	T331A

The overall resistance picture is summarized in Table 11. pUL56 substitutions E237G, V236M, and C325W were detected in the virus of subjects from the letermovir arm who failed treatment while on letermovir. These substitutions occurred at known cell culture resistance positions and therefore, are considered resistance-associated substitutions. In addition, the pUL56 445-SNS-447 deletion and

E485G substitution, occurred at high frequency (>70%) in 2 of 8 (25%) of subjects who failed treatment while on letermovir, and these two substitutions are also considered resistance-associated. In addition, there were several other substitutions that occurred in the virus of one subject who failed treatment while on letermovir or was detected in 2 subjects (either both were off-treatment failures or one was from both categories, i.e., one off-treatment failure and one on-treatment failure). No substitutions were detected at previously identified resistance-associated sites in subject who failed treatment after letermovir dosing had been completed.

Table 11. Summary of substitutions meeting the DAVP resistance criteria that occurred in subjects while on treatment with letermovir (DAVP analysis). Orange, known resistance site; red, detected in 2 subjects.

USUBJID	SUBJID	Timepoints	UL56	UL89
8228-001-000100001	101765	D85, D89, D157	No substitutions met Resistance criteria	L323P
8228-001-001800048	100402	D66	445-447 SNS deletion, E485G, and T775I	H246R and Q625*
8228-001-004200010	101760	D42	E237G and R826L	No substitutions detected >1%
8228-001-004400004	100278	D9	Q213R	No substitutions detected >1%
8228-001-006900007	101625	D62	I48M, V236M, 445-447 SNS deletion, and E485G	No substitutions met Resistance criteria
8228-001-010800016	100078	D95	No substitutions detected >1%	L522P and T637A
8228-001-011700006	100008	D46	C325W and S378N	No substitutions detected >1%
8228-001-016100005	102029	D46	No substitutions met Resistance criteria	T331A

Of note, substitutions at previously identified resistance-associated sites occurred in 3/8 (37.5%) of the subjects that failed while on letermovir (Table 11), and no two subjects had substitutions at the same positions. Moreover, no substitutions were detected in the virus of subjects who failed off-treatment at previously identified resistance-associated sites. These observations indicate that: 1) letermovir likely has a low barrier to resistance; 2) there may be multiple low frequency resistance pathways instead of a one major pathway to resistance; and 3) longer term treatment with letermovir may be an option for patients who had their HCMV DNAemia controlled by letermovir at the end of 100 days.

Clinical Trial P001 (MK-8228-001) DAVP Conclusions

In general, there was agreement between the variants detected by the sponsor and those detected by DAVP; however, there was disagreement in how these data were interpreted. pUL56 substitutions V236M, E237G, and C325W were detected in the virus of subjects from the letermovir arm who failed treatment while on letermovir. These substitutions occurred at known cell culture resistance positions and therefore, are considered resistance-associated substitutions. Of note, V236M was selected in cell culture and also was observed as a resistance-associated substitution that emerged in their Phase 2 study (see of NDA 209939 SDN 000). In addition, the pUL56 445-SNS-447 deletion and E485G substitution, occurred at high frequency (>70%) in 2 of 8 (25%) of subjects who failed treatment while on letermovir, and these two substitutions are also considered resistance-associated. These substitutions should be included in the resistance section of the label. Several other substitutions were detected in pUL56 and pUL89 and these substitutions should be assessed to determine the impact on susceptibility to letermovir in a phenotypic assay (see Post Marketing Recommendations below). In general, there are three conclusions that can be drawn from the independent assessment of resistance: 1) letermovir likely has a low barrier to resistance; 2) there may be multiple low frequency resistance pathways instead of a one major pathway to resistance; and 3) longer term treatment with letermovir may be an option for patients who had their HCMV DNAemia controlled by letermovir at the end of 100 days.

OVERALL CONCLUSIONS

DAVP performed an independent analysis of the NGS data submitted for one phase 3 clinical trial and compared the results to those reported by the sponsor. In general, there was good agreement between these results. However, the different analysis pipelines used much different default filtering and mapping criteria and this was apparent when comparing frequency table values such as Total Coverage, Variant Coverage, and Amino Acid Frequency. However, despite these differences, the general trends observed were very similar and there was generally good agreement at frequencies greater than 2%.

The goal of the independent assessment of NGS data was to confirm the results reported by the sponsor, to determine which known resistance-associated substitutions were present in the virus of subjects who failed treatment, and to determine if additional substitutions could be potentially be associated with treatment failure. The independent analysis focused on the amino acid positions of interest identified in the two HCMV proteins that have been associated with resistance to letermovir based on cell culture resistance selection experiments. For pUL56, substitutions at positions 231, 236, 237, 241, 244, 257, 261, 321, 325, 329, and 369 are considered to be resistance-associated. For pUL89, positions 256 and 345 were determined to be resistance-associated in cell culture based experiments with a close analog of letermovir (see NDA 209939 SDN 000).

Overall, the NGS analyses results reported by the sponsor were in agreement with the results generated by DAVP, with a few minor exceptions that could be rectified using a third NGS analysis pipeline. pUL56 substitutions E237G, V236M, and C325W were detected in the virus of subjects from the letermovir arm who failed treatment while on letermovir. These substitutions occurred at known cell culture resistance positions and therefore, are considered resistance-associated substitutions. In addition, pUL56 substitutions 445-SNS-447 deletion and E485G, occurred at high frequency (>70%) in 2 of 8 (25%) of subjects who failed treatment while on letermovir, and these two substitutions are also considered resistance-associated. These substitutions should be included in the resistance section of the label.

Several other substitutions were detected in pUL56 and pUL89 and these substitutions should be assessed to determine the impact on susceptibility to letermovir in a phenotypic assay (see Post Marketing Recommendations below). In general, there are three conclusions that can be drawn from the independent assessment of resistance: 1) letermovir likely has a low barrier to resistance; 2) there may be multiple low frequency resistance pathways instead of a one major pathway to resistance; and 3) longer term treatment with letermovir may be an option for patients who had their HCMV DNAemia controlled by letermovir at the end of 100 days.

For complete labeling details, please see the review of NDA 209939 SDN 000 by Senior Clinical Virology Reviewer Takashi Komatsu, Ph.D., RAC.

POST MARKETING RECOMMENDATIONS

(b) (4)

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[redacted]

ADMINISTRATIVE

Reviewer's Signature(s)

Eric F. Donaldson
Eric F. Donaldson, Ph.D.
Clinical Virology Reviewer

Concurrence(s)

_____Date: _____
HFD-530/Clin Micro TL/J O'Rear

cc:
HFD-530/NDA
HFD-530/Division File
HFD-530/RPM/Tyson

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/s/

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07/20/2017

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07/20/2017