NCI Protocol #: Not applicable

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Sponsor Study #: Merck MISP 57433

TITLE: A Phase 2 Study of letermovir treatment for patients experiencing refractory or resistant cytomegalovirus infection or disease with concurrent organ dysfunction

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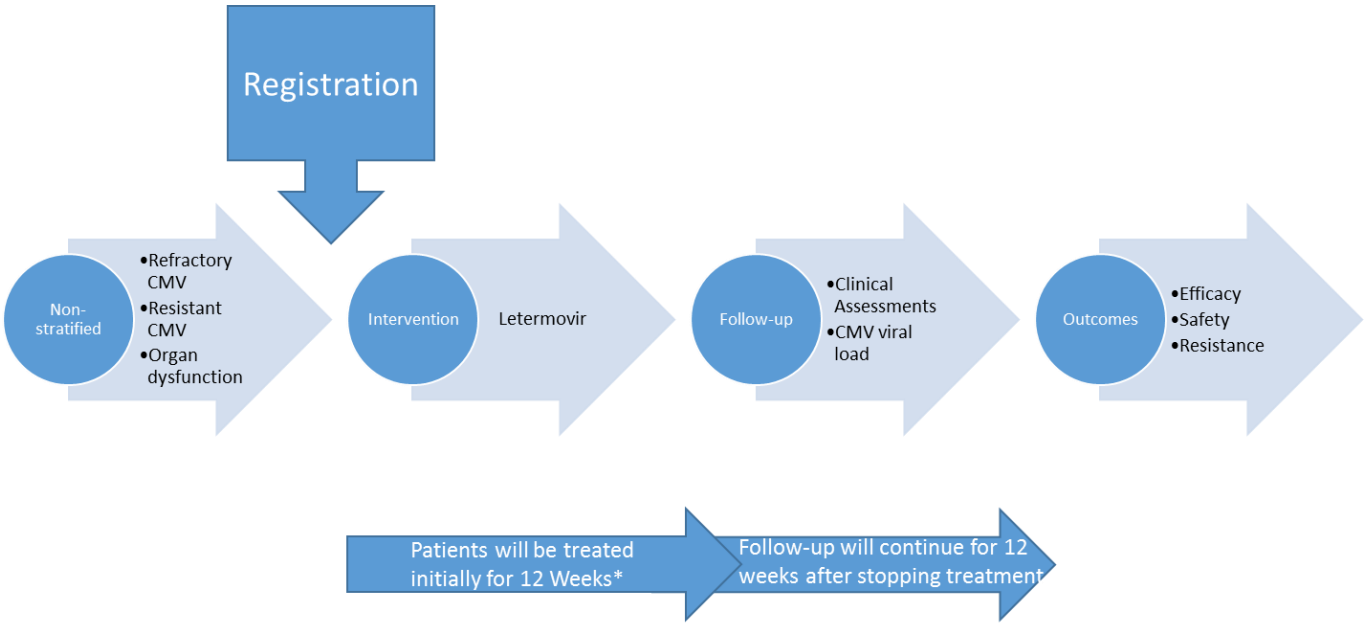
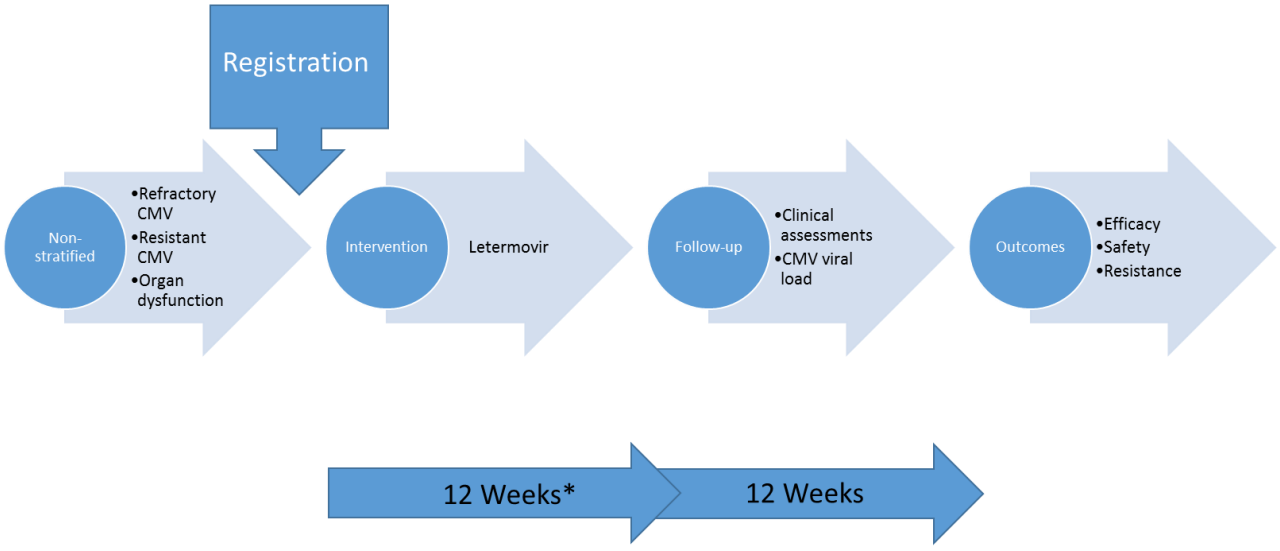
SCHEMA



*Patients may be treated for an additional 12 weeks of letermovir for a total of 24 weeks on treatment



*Patients may be treated for an additional 12 weeks of letermovir for a total of 24 weeks on treatment.

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1. OBJECTIVES

1.1 Study Design

This is an investigator-initiated, single-arm, open-label trial of letermovir treatment for patients with documented cytomegalovirus (CMV) infection or CMV disease that is refractory or resistant to FDA-approved treatments and/or who have baseline organ dysfunction (myelosuppression or renal dysfunction) for which treatment with available antiviral agents would likely cause further dysfunction and increased risk of dialysis or death. We plan to enroll up to 32 immunocompromised patients including hematopoietic-cell (HCT) and solid-organ (SOT) transplant recipients who meet eligibility criteria (see section 3).

CMV infection is defined as the detection of CMV nucleic acids by polymerase chain reaction (PCR) or other molecular methods in blood or plasma in immunocompromised patients at risk of CMV disease. CMV disease is defined as end-organ involvement with concurrent organ-specific clinical symptoms caused by CMV and usually demonstrated by involved tissue biopsies. The criteria of Ljungman and colleagues (CID 2017) will be used to classify CMV disease.

Patients will receive letermovir treatment for up to 12 weeks with optional additional 12 weeks of treatment for secondary prophylaxis if clinically indicated.

Clinical assessments and CMV viral load (VL) will be measured at each study visit. Samples will also be collected for CMV genotyping and pharmacokinetic evaluations.

While on treatment, patients will be assessed weekly from Week 1 through Week 6, every other week from Week 8 through Week 12, and if necessary, once per month at Weeks 16, 20, and 24.

Upon treatment discontinuation, patients will be followed for 12 weeks (with study visits at 1, 4, 8, and 12 weeks after discontinuation).

The study calendar (Section 10) provides detail of study visits and procedures.

1.2 Primary Objectives

To evaluate the clinical efficacy, antiviral activity, and safety of letermovir treatment for patients with refractory or resistant CMV infection or CMV disease who have toxicities or baseline organ dysfunction for which treatment with available CMV treatments are either ineffective or will likely worsen organ dysfunction.

1.3 Secondary Objectives

- To describe kinetics of viral clearance and potential emergence of letermovir-resistant CMV virus in patients treated in this setting.
- Overall survival

- CMV progression-free survival: time from study enrollment to CMV progression or death whichever occurs first
- Characterize pharmacokinetics of letermovir in adolescent patients

1.4 Exploratory Objectives

- To longitudinally describe CMV-specific CD4 and CD8 T-cell levels in patients with CMV infection or disease in this trial

2. BACKGROUND

2.1 Study Disease(s)

CMV remains the most common viral infection in transplantation and in other patients who are immunocompromised.[1] Rates of CMV infection and disease vary depending on baseline CMV-specific immunity and the overall state of immunosuppression.[2]

The clinical effects of CMV can be divided into direct and indirect effects. Direct effects attributed to CMV include CMV syndrome and CMV end-organ disease, while indirect effects of CMV include an increased risk of opportunistic infections and metabolic derangements.[3]

CMV disease (consisting of CMV end-organ disease and CMV syndrome) is associated with increased morbidity, mortality, as well as poor outcomes following SOT and HCT.[4,5]

Current strategies to minimize the impact of CMV in transplant recipients include CMV antiviral prophylaxis during the initial period post-transplantation and preemptive antiviral treatment by surveillance of CMV infection by systematic monitoring of CMV nucleic acids in blood or plasma and antiviral administration for patients in whom CMV viremia is detected above prespecified thresholds.[6] Both strategies have been effective in reducing the burden of CMV disease, but infections continue to occur after prophylaxis is discontinued and available antivirals to treat CMV frequently cause organ toxicity (myelosuppression with ganciclovir, renal toxicity with foscarnet and cidofovir), and resistance to these antivirals develops with certain frequency in transplant recipients, making imperative the need to develop and test new antivirals for treatment of CMV infection and CMV disease.

The FDA approved letermovir in November 2017 for CMV prophylaxis in allogeneic HCT patients who are CMV seropositive (who have evidence of CMV infection prior to HCT),[7] but there is minimal experience on its use to treat active infection.[8,9] This trial is a proof-of-concept study to address the feasibility of using letermovir for treatment of active CMV infection. Letermovir use for treatment of active CMV infection and its use in SOT recipients and other immunocompromised hosts is considered off-label use.

2.2 IND Agent

Letermovir is an anti-CMV agent that recently received FDA approval for CMV prophylaxis in CMV seropositive, allogeneic hematopoietic-cell transplantation (HCT) recipients.[7] Letermovir inhibits the viral terminase complex (UL51/UL56/UL89),[10–12] an enzyme that plays an important role in cleavage of concatenated viral DNA into individual genomes that are subsequently inserted into CMV procapsids to generate infectious CMV virions. Letermovir has demonstrated potent, selective, and reversible inhibition of CMV activity in preclinical studies *in vitro* and efficacy against the virus *in vivo*.[11,13]

Letermovir has been shown to be generally well tolerated in 28 Phase 1 studies, two Phase 2 studies, and a pivotal Phase 3 study in HCT recipients. In the latter study, CMV seropositive allogeneic-HCT recipients received letermovir or placebo from the early post-transplant period (within 4 weeks post-transplant) through Week 14 post-transplant (Day +100) and were followed for an additional 34 weeks.[7] In this study, letermovir demonstrated statistical significance in the prevention of clinically significant CMV infection compared to placebo, defined as onset of CMV end-organ disease or initiation of anti-CMV pre-emptive therapy based on documented CMV DNAemia as measured by the central laboratory. Letermovir prophylaxis also resulted in statistically significant lower all-cause mortality relative to placebo through Week 24 post-transplant.

2.3 Rationale

Although letermovir prophylaxis will likely decrease the overall burden of CMV in HCT recipients, treatment of active CMV infection and CMV disease remains an area of unmet clinical need as we lack effective non-toxic options for treatment. Current CMV antiviral treatment relies on ganciclovir or valganciclovir, which is frequently myelosuppressive. Foscarnet is prescribed in patients who develop ganciclovir resistance or experience significant myelosuppression from ganciclovir, but it is associated with significant renal toxicities.[14] Cidofovir is uncommonly used for treatment of CMV infections, is not as effective as ganciclovir or foscarnet, and is nephrotoxic.[15]

Given letermovir's outstanding safety profile, especially its lack of myelosuppression or nephrotoxicity, and the efficacy results of the phase 3 prophylaxis trial, many clinicians will be inclined to start using letermovir for treatment of active CMV infections and CMV disease even though the drug lacks approval for this indication. There are very limited data regarding letermovir's activity during active CMV infection. Stoelben and colleagues reported a phase 2 trial where 40mg or 80mg twice-daily of letermovir were effective in preemptively treating CMV viremia in renal transplant recipients (67% were receiving concomitant cyclosporine).[9] Kaul and colleagues reported that a patient with multidrug-resistant CMV disease was successfully treated with letermovir 240mg/day after failing multiple previous treatments.[8] The phase 3 study evaluating letermovir prophylaxis in allogeneic-HCT recipients included 70 patients who had detectable CMV DNA at the time of randomization. Although not included in the main analysis of the trial, the 48 patients who received letermovir with detectable CMV at baseline were less likely than 22 patients on placebo to develop progressive infection requiring

preemptive treatment.[7]

We believe that it is important to formally test letermovir's ability to treat active CMV infection and CMV disease. We propose that treatment be studied initially in a proof-of-concept, open-label trial in patients who lack effective therapeutic options and in those with baseline organ dysfunction where currently available treatments would likely lead to poor clinical outcomes such irreversible kidney toxicity or severe myelosuppression. These patients would thus most likely benefit from letermovir treatment. The data generated will inform future development programs for CMV treatment with letermovir and will likely help patients in this situation.

Given the safety profile and relatively wide therapeutic index of letermovir to date,[7] there is equipoise at this time to consider letermovir use for patients 12 years of age or older, who weigh 30 or more kilograms, who are experiencing CMV infections or disease that meet the eligibility criteria for the trial, for whom available treatment options are likely to be unsuccessful or lead to greater toxicity and morbidity.

2.4 Correlative Studies Background

Not applicable

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Participants in the study must meet the following eligibility criteria:

- Age ≥12 years
- Weight ≥30 kg
- Transplant recipient (HCT, SOT) or other immunocompromised patients who require antiviral treatment for CMV.
- Documented CMV disease or persistent CMV infection (CMV virus load above 500 IU/mL on consecutive measurements, at least one day apart).
- CMV infection is refractory to treatment (defined as ≥14 days of standard CMV treatment without clinical improvement for CMV disease, or failure to achieve >1 log reduction in CMV VL after ≥14 days of standard treatment for CMV infection)[16,17]
- Current CMV infection has documented genotypic resistance to ganciclovir or foscarnet. Please see Appendix E for the full list of amino acid substitutions that qualify as drug-resistant mutations.
- For patients with any prior CMV infection episode that broke through letermovir prophylaxis, but not during the current CMV infection, documentation of letermovir susceptibility testing should demonstrate absence of letermovir mutations known to confer resistance to letermovir.
- Severe myelosuppression (ANC <1000/μL, Hemoglobin <8g/dL, or Platelets <25,000/μL)[17] or renal dysfunction (estimated creatinine clearance <60 mL/min by

MDRD in adults or < 60 ml/min/1.73 m^2 by bedside Schwartz equation in < 18 years-old) at baseline or which develops during antiviral treatment.
  - Patients who develop severe myelosuppression or renal dysfunction during antiviral treatment as defined above are eligible without having to meet the refractoriness/antiviral resistance criterion. See Table below for further details on eligibility.
  - Combinations of genotypic antiviral resistance and organ dysfunction that lead to eligibility are presented in the following table.
- The effects of letermovir on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and 3 months after completion of letermovir administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 3 months after completion of letermovir administration.
  - Patients must have a negative serum or urine pregnancy test
- Able to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

- History of allergic reactions attributed to compounds of similar chemical or biologic composition to letermovir.
- Known history of cirrhosis with Child-Pugh Class C hepatic insufficiency at screening
- Acute liver injury at baseline meeting Hy's law.
- Current CMV infection broke through letermovir prophylaxis.
- Patients with life expectancy of less than a week. Determination of life expectancy will be discussed with the patient's primary treatment physician.
- Have known human immunodeficiency virus (HIV) infection

Refractory	Ganciclovir Resistant	Foscarnet Resistant	Myelosuppression Criteria*	Renal dysfunction*	Eligible?
≥2 weeks of anti-CMV treatment and <1 log decline in CMV VL from start of treatment	Genotypic resistance based on reference lab results	Genotypic resistance based on reference lab results	ANC <1000/μL, or Hemoglobin <8g/dL, or Platelets <25,000/μL	GFR <60 mL/min by MDRD or < 60 ml/min/1.73 m2 by bedside Schwartz equation if < 18 years-old	
Yes	Yes or No	Yes or No	Yes or No	Yes or No	**Yes**
No	Yes	No	No	Yes	**Yes**
No	No	Yes	Yes	No	**Yes**
No	Yes	Yes	No	No	**Yes**
No	No	No	Yes	Yes	**Yes**
No	No	No	Yes	No	No
No	Yes	No	No	No	No
No	No	No	No	Yes	No

No	No	Yes	No	No	No
No	Yes	No	Yes	No	No
No	No	Yes	No	Yes	No
No	No	No	No	No	No

*Either at the onset of treatment or arising during treatment with other anti-viral therapy.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION AND RANDOMIZATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered. Protocol therapy will begin within 24 hours of registration.

The study will be available to eligible patients seven days a week. If after hours, all standard procedures set forth by Dana-Farber Cancer Institute's Office of Data Quality will be followed.

4.1 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.2 General Guidelines for Other Investigative Sites

Not applicable

4.3 Registration Process for Other Investigative Sites

Not Applicable

5. TREATMENT PLAN

5.1 Treatment Regimen

Letermovir will be administered daily for up to 12 weeks, with the optional additional 12 weeks of treatment for secondary prophylaxis if clinically indicated. Treatment will be administered on an inpatient or outpatient basis. Reported adverse events and potential risks are described in Section 7. No dose modifications will be permitted. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's CMV infection or disease.

Dosing for letermovir will be 480 mg PO/IV daily for patients not receiving concomitant cyclosporine. The dose is decreased to 240 mg PO/IV daily for patients receiving concomitant cyclosporine.[18]

Given that the half-life of letermovir is ≤24 hours, a second dose on day 1 of treatment (~12 hours apart) will be administered as a loading dose to patients who are ≥40 kg, to reach steady state drug levels on day 1. For patients who are 30 to 40 kg, no loading dose will be administered.

Pediatric patients will receive the same daily dose, but will not receive the loading dose. Because of the weight-based inclusion requirement for study enrollment, the adult dose will be acceptable for the pediatric population.

Merck conducted additional PK modeling and simulations which suggest that the letermovir exposure at the end of day 1 would be similar to exposures achieved at day 3-4 if a second dose of 480 mg is administered 12 hours after the first dose and that the exposure with 2 doses of letermovir 480 mg 12 hours apart is within the safety margin for participants who are ≥ 40 kg. Given that this protocol is enrolling patients with active CMV infection (i.e. not being administered for prophylaxis which is the approved indication) and it is desirable to reach steady state exposure as soon as possible to avoid the emergence of viral resistance, it is reasonable to test a loading dose in the context of treatment of active infection in this study. For participants who are between 30-39 kg, no loading dose is necessary given the exposures are predicted to be higher already due to lighter weight.

Letermovir will be provided PO or IV as needed.

If dosed with the oral formulation while outpatient, the participant will be requested to maintain a medication diary of each dose of medication. (See Appendix C) The medication diary will be reviewed during each study visit.

5.2 Agent Administration

As applicable, describe in detail the following parameters for each agent used as part of the protocol therapy:

Administration – Letermovir 480 mg PO/IV daily, with administration per the FDA's package insert with the addition of a second, loading dose on day 1 of treatment for patients ≥40 kg.

Dosing – 480 mg daily in patients without concomitant cyclosporine therapy and 240 mg in patients with concomitant cyclosporine therapy. The drug should be taken at approximately the same time every day +/- 2 hours and can be taken without regard for food intake. Pediatric patients should not receive the loading dose. Otherwise, pediatric patients are given the same dose.

Drug, Tubing and Filtration – Letermovir injection is compatible with the following IV bags and infusion set materials. PREVYMIS injection is not recommended for use with polyurethane-containing IV administration set tubing.

IV Bags Materials:
Polyvinyl chloride (PVC), ethylene vinyl acetate (EVA) and polyolefin (polypropylene and polyethylene)

Infusion Sets Materials:
PVC, polyethylene (PE), polybutadiene (PBD), silicone rubber (SR), styrene–butadiene copolymer
(SBC), styrene-butadiene-styrene copolymer (SBS), polystyrene (PS)

Plasticizers:
Diethylhexyl phthalate (DEHP), tris (2-ethylhexyl) trimellitate (TOTM), benzyl butyl phthalate (BBP)
Catheters:
Radiopaque polyurethane

Hydration – No pre-treatment, treatment or post-treatment hydration is required or recommended.

Special Equipment – No special infusion pump or overfills will be required.

Observation period – No specific observation period is required.

Protocol specific procedures – No specific protocol procedures are required.

Infusion Reactions – Infusion reactions were not observed in the phase III trial. Patients are not required to stay after infusion for monitoring, but institutional procedures should be followed.

Oral Agents – Letermovir therapy can be taken without regard to food intake. The pill may not be crushed. If a dose is missed or vomited, the pill should be taken again and document this in the drug diary (see Appendix C). If a dose is missed or forgotten for any reason, it should be taken when possible. A minimum of 8 hours is suggested between doses when the dose is remembered. Otherwise, the missed dose should be skipped and the next dose should be taken at its scheduled time.

5.3 General Concomitant Medication and Supportive Care Guidelines

Patients who are receiving concomitant cyclosporine will receive 240 mg of letermovir per day owing to a drug–drug interaction that is mediated by the organic anion transporters OATP1B1 and OATP1B3.

Because letermovir is a weak-to-moderate inhibitor of cytochrome P-450 3A (CYP3A) and a weak-to-moderate inducer of CYP2C9 and CYP2C19 enzymes (encoding cytochrome P-450, family 2, subfamily C, polypeptides 9 and 19, respectively), the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Overall PI should be alerted if the participant is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

Appendix D presents guidelines for identifying medications/substances that could potentially interact with letermovir.

Given the lack of pediatric experience for the use of letermovir, the study will hold enrollment for additional pediatric patients (<18 years-old) after 3 pediatric patients have been enrolled to assess their letermovir pharmacokinetic profiles and assure letermovir exposures are adequate from an efficacy and safety perspective. Treatment dosing may be adjusted depending on results of these analysis. For example, if letermovir blood levels are below the efficacy targets as determined in the adult phase III trial, the dose will be increased to achieve efficacy targets, and enrollment will resume with this new dose after obtaining permission form the IRB and FDA.

With an estimated accrual rate of 5 patients per month and a total duration of follow-up of 6 months from enrollment, we expected to finish the study within 1 year from trial start.

5.4 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance.

Patient-level treatment response milestones:
Patients will be required to achieve minimum virologic and clinical response milestones for treatment to continue.

The CMV virological response milestones[16] for patients without CMV disease are defined as:

1) Any decrease in CMV DNA from baseline (week 0), measured on week 3
2) A ≥2 log decreased from baseline, or an undetectable CMV DNA, on week 6.

The clinical response milestones for patients with CMV disease are defined as:

1) Stabilization of clinical disease by week 3 (i.e., no worsening signs or symptoms compared to week 1) as assessed by the site investigator and
2) Improvement or resolution of clinical disease by week 6 as judged by investigator (i.e., improvement in signs and symptoms of affected organs [resolution of diarrhea, pneumonia, hepatitis, retinitis, etc.])

Patients who do not meet the response milestones should discontinue letermovir treatment. If there are no other treatments available to a particular patient (because of known multidrug antiviral resistance), patients with stable CMV disease or CMV viremia that has not reached the virologic milestones could continue treatment.

In the absence of treatment interruptions due to adverse event(s), treatment may continue for 12 weeks of treatment, followed by an additional 12 weeks of secondary prophylaxis, or until one of the following criteria applies:

Patient-level safety events that require holding or stopping letermovir

- Drug-induced liver injury (DILI) meeting Hy's law criteria without alternative explanation. Patients with confirmed liver GVHD, for example, will be allowed to continue treatment, or if patients have abnormal LFTs due to CMV hepatitis.
- Any CTCAE grade 4 adverse event(s) with no alternative explanation whether attributable to drug or not
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant is removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

Study-level stopping rules

The study-level stopping rules are detailed in section 13.3. If the number of responses is not met after the first stage or safety is compromised, then the trial will be placed on hold and available data analyzed to decide whether to terminate the study early or consider a higher dose of letermovir for treatment.

Given the lack of pediatric experience for the use of letermovir, the study will hold enrollment for additional pediatric patients (<18 years-old) after 3 pediatric patients have been enrolled to assess their letermovir pharmacokinetic profiles and assure letermovir exposures are adequate from an efficacy and safety perspective.

5.5 Duration of Follow Up

While on treatment, patients will be assessed weekly from Week 1 through Week 6, every other week from Week 8 through Week 12, and if necessary, once per month at Weeks 16, 20, and 24.

Upon treatment discontinuation, patients will be followed for 12 weeks (at 1, 4, 8, and 12 weeks after discontinuation).

Clinical assessments and CMV VL will be measured at each visit. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

5.6 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

For patients who discontinue from active study participation we will request the possibility of passive follow up for survival and CMV events by review of their medical record.

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

6. DOSING DELAYS/DOSE MODIFICATIONS

Interruptions of the trial regimen for up to a total of 7 days during the treatment period are allowed.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

The safety of letermovir was evaluated in one Phase 3 randomized, double-blind, placebo-controlled trial in which 565 allogeneic-HCT recipients were randomized and treated with letermovir (N=373) or placebo (N=192) through Week 14 post-transplant. Adverse events were those reported while subjects were on study medication or within two weeks of study medication completion/discontinuation.

Cardiac Adverse Events: The cardiac adverse event rate (regardless of investigator-assessed causality) was higher in subjects receiving letermovir (13%) compared to subjects receiving placebo (6%). The most common cardiac adverse events were tachycardia (reported in 4% of letermovir subjects and in 2% of placebo subjects) and atrial fibrillation (reported in 3% of letermovir subjects and in 1% of placebo subjects).

Common Adverse Events The rate of adverse events occurring in at least 10% of subjects in the letermovir group and at a frequency at least 2% greater than placebo are outlined in Table 1.

Table 1: All Grade Adverse Events Reported in ≥ 10% of letermovir-Treated HCT Recipients at a Frequency at least 2% Greater than Placebo Adverse Events	**Letermovir (N=373)**	**Placebo (N=192)**
nausea	27%	23%
diarrhea	26%	24%
vomiting	19%	14%
peripheral edema	14%	9%
cough	14%	10%
headache	14%	9%
fatigue	13%	11%
abdominal pain	12%	9%

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Adverse Event Reporting

7.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

7.3.2 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.3 DF/HCC Adverse Event Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

7.3.4 Protocol-Specific Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the Overall PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form).

CTCAE SOC	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
Gastrointestinal disorders	nausea	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported
Gastrointestinal disorders	diarrhea	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication,	Only Grade IV and above AEs should be reported

				or underlying disease	
Gastrointestinal disorders	vomiting	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported
General disorders	peripheral edema	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported
Respiratory, thoracic and mediastinal disorders	cough	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported
Nervous system disorders	headache	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported
General disorders	fatigue	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported
Gastrointesitnal disorders	abdominal pain	Grade III and below	Either requiring hospitalization or prolongation of hospitalization	Either letermovir, other concomitant medication, or underlying disease	Only Grade IV and above AEs should be reported

7.4 Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

The PI will also report any events that merit communication with the DFCI IRB via email concurrently (within 1 business day) to Merck.

7.5 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

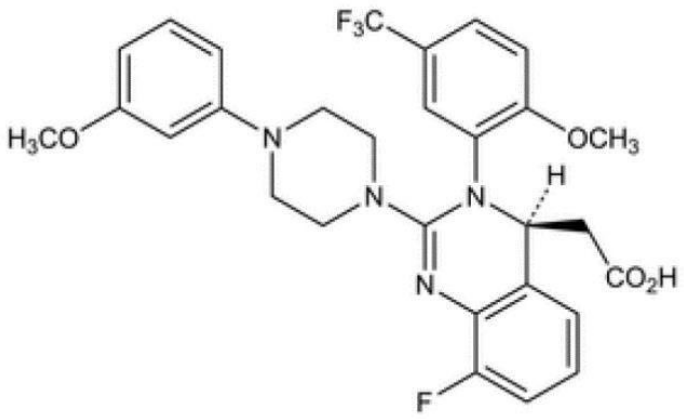
A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 Letermovir

8.1.1 Description

Letermovir has a molecular formula of C29H28F4N4O4 and a molecular weight of 572.55. The chemical name for letermovir is (4*S*)-2-{8-Fluoro-2-[4-(3-methoxyphenyl)piperazin-1-yl]-3-[2-methoxy-5(trifluoromethyl)phenyl]-3,4-dihydroquinazolin-4-yl}acetic acid. Letermovir is very slightly soluble in water.

The chemical structure of letermovir is:



The pharmacokinetic properties of letermovir are displayed below.

Pharmacokinetics in HSCT Recipients	
Treatment Regimen	Steady-state median (90% prediction interval) AUC (ng•hr/mL) of PREVYMIS
480 mg oral once daily, no cyclosporine	34,400 (16,900, 73,700)
480 mg IV once daily, no cyclosporine	100,000 (65,300, 148,000)
240 mg oral once daily, with cyclosporine	60,800 (28,700, 122,000)
240 mg IV once daily, with cyclosporine	70,300 (46,200, 106,000)
Pharmacokinetics in Healthy Subjects	

Treatment Regimen 480 mg oral once daily	Steady-state geometric mean AUC and Cmax of Cmax: 13,000 ng/mL AUC: 71,500 ng•hr/mL
Dose proportionality	Greater than proportional following single and multiple oral or IV doses of PREVYMIS 240 mg and 480 mg
Accumulation ratio†	Cmax: 1.03 AUC: 1.22
Time to steady-state	9-10 days
Absorption	
Bioavailability	Healthy subjects administered PREVYMIS without cyclosporine: 94% at an oral dose range of 240 mg to 480 mg HSCT recipients administered PREVYMIS without cyclosporine: 35% with 480 mg oral once daily HSCT recipients administered PREVYMIS with cyclosporine: 85% with 240 mg oral once daily
Median Tmax (hr)	45 min to 2.25 hr
Effect of food (relative to fasting) ‡	AUC: 99.63% [84.27% - 117.80%] Cmax: 129.82% [104.35% -161.50%]
Distribution	
Mean steady-state volume of distribution	45.5 L following IV administration in HSCT recipients
% *In vitro* bound to human plasma proteins	99% across the concentration range of 0.2 to 50 mg/L
In vitro blood-to plasma ratio	0.56 across the concentration range of 0.1 to 10 mg/L
Metabolism	
In vitro metabolism	UGT1A1/1A3 (minor)
Drug-related component in plasma	97% unchanged parent No major metabolites detected in plasma
Elimination	
Route of elimination	Hepatic uptake (OATP1B1/3)
Mean terminal $t_{1/2}$ (hr)	12 hrs after dosing of PREVYMIS 480 mg IV once daily
% of dose excreted in feces§	93%

% of dose excreted in urine§	<2%
% of unchanged drug excreted in feces§	70%

* Values were obtained in studies of healthy subjects unless otherwise indicated.

† Based on geometric mean data.

‡ Values refer to geometric mean ratio [fed/fasted] percentage and 90% confidence interval back transformed from linear mixed-effects model performed on natural log-transformed values. The meal administered was a standard high fat and high calorie meal (33 grams protein, 65 grams carbohydrates, 58 grams fat; 920 total calories).

§Single oral administration of radiolabeled letermovir in mass balance study.

8.1.2 Form

Letermovir is available as 240 mg and 480 mg tablets. Letermovir tablets contain either 240 mg or 480 mg of letermovir and the following inactive ingredients: colloidal silicon dioxide, croscarmellose sodium, magnesium stearate, microcrystalline cellulose, povidone 25, and film-coated with a coating material containing the following inactive ingredients: hypromellose 2910, iron oxide red (only for 480 mg tablets), iron oxide yellow, lactose monohydrate, titanium dioxide, and triacetin, Carnauba wax is added as a polishing agent.

Letermovir is also available as 240 mg and 480 mg injection for intravenous infusion. Letermovir injection is a clear, preservative-free sterile solution in single-dose vials of either 240 mg or 480 mg per vial. Each 1 mL of solution contains 20 mg letermovir, hydroxypropyl betadex (150 mg), sodium chloride (3.1 mg), sodium hydroxide (1.2 mg), and Water for Injection, USP. The amount of sodium hydroxide may be adjusted to achieve a pH of approximately 7.5.

8.1.3 Storage and Stability

Letermovir tablets can be stored at room temperature at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). Once mixed, the diluted solution should be stored for no longer than 24 hours at 2-8ºC.

8.1.4 Compatibility

See above description in section 8.1.2.

8.1.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the drug in a self-contained and protective environment.

8.1.6 Availability

Letermovir will be distributed to investigators via the DFCI central pharmacy. DFCI will be

maintaining the central drug supply and will distribute as patients are consented, registered, and enrolled in the trial.

8.1.7 Preparation

See above description in section 8.1.2.

8.1.8 Administration

The Letermovir IV formulation will be diluted in either 0.9% sodium chloride solution or 5% dextrose for a total volume of 250 mL, and the duration of infusion will be 60 minutes ±15 minutes. No filter is needed for administration.

8.1.9 Ordering

Pharmacy will email Merck Scientific Leadership and Research Manager (Boski Patel) when drug supplies are needed. Letermovir will be supplied from investigational supply and will be provided by Merck, free of charge, according to the internal protocol [Merck MISP 57433].

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form.

8.1.11 Destruction and Return

Unused supplies of the agent will be destroyed at respective site institutions if their standard operating procedure allows it. If not, unused drug supply will be returned to the DFCI central pharmacy and destroyed per standard operating procedures at DFCI.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies: N/A

9.2 Laboratory Correlative Studies:

An additional whole blood sample will be collected for monthly measurements of CMV-specific CD4 and CD8 T-cell levels using the Viracor CMV T Cell Immunity Panel (https://www.viracor-eurofins.com/test-menu/30360-cmv-t-cell-immunity-panel/). This panel measures the relative strength of T cell responses to CMV specific antigens. It evaluates and reports the activity of CD4 and CD8 T cell responses independently. Effective T cell immunity against CMV is a factor in controlling CMV viral latency and a prospective characterization of T cell responses has not been performed in this patient population.

9.3 Special Studies:

9.3.1 CMV Resistance variants

9.3.1.1 Outcome Measure: The presence of CMV isolates that are resistant to letermovir

9.3.1.2 Method of Assessment: Deep sequencing will be done on plasma DNA samples collected at the time of study assessments. Per usual practice, BWH lab keeps additional aliquots of samples collected for CMV testing, which is done at clinic visits as part of standard of care. Patients who have recurrent viremia while on letermovir treatment will have these aliquots tested for resistance variants. No additional specimens will be collected.
Resistance to letermovir will be assessed by genotypic analysis of the CMV terminase complex genes UL 51, UL56 and UL89. Samples will be analyzed by PCR amplification and next-generation DNA sequencing at DDL Diagnostic Laboratory B.V. (Rijswijk, The Netherlands). These samples will be submitted through Merck.

9.3.1.3 Timing of Assessment: At the end of the study

9.3.1.4 Method of Data Recording: Electronically captured data

9.3.1.5 Timing of Data Recording: After genetic assays are complete

10. STUDY CALENDAR

Patients will receive letermovir treatment for up to 12 weeks with the option of an additional 12 weeks of treatment for secondary prophylaxis if clinically indicated.

While on treatment, patients will be assessed weekly from Week 1 through Week 6, every other week from Week 8 through Week 12, and if necessary, once per month on Week 16, 20, and 24.

Upon treatment discontinuation, patients will be followed for 12 weeks (at 1, 4, 8, and 12 weeks after discontinuation).

Clinical assessments and CMV VL will be measured at each visit. (See section **10.1** for details). Genotypic evaluations will be done retrospectively based from samples collected.

Pre-study evaluations are to be conducted within 3 days prior to start of protocol therapy. Baseline evaluations are to be conducted within 1 day prior to start of protocol therapy. Assessments must be performed prior to administration of any study agent. Study assessments will be performed weekly of the protocol-specified date, unless otherwise noted. Weekly windows will be Monday through Friday independent of the day of the week letermovir is started on Week 1.

Visit schedule while on therapy[a] (Adult ≥ 18 years-old)

	Pre-Study[b,c]	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 8	Wk 10	Wk 12	Wk 16	Wk 20	Wk 24
Letermovir administration (diary review)		X	X	X	*	*	*	*	*	*	*	*	*	*
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X												X
Physical exam (as indicated)	X													
Funduscopic exam[d]	X													
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X													
Weight	X					X			X		X	X	X	X
Performance status (Karnofsky score)[e]	X					X			X		X	X	X	X
CBC w/diff	X	X[c]	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[f]	X	X[c]	X	X	X	X	X	X	X	X	X	X	X	X
CMV viral load	X	X[c]	X	X	X	X	X	X	X	X	X	X	X	X
CMV genotype[g]	X													
CMV immunity panel		X				X			X		X	X	X	X
PK sample (predose, if on drug)				X		X		X	X	X	X			
EKG (as indicated)	X		X											
Adverse event evaluation		X												X
B-HCG	X[h]					X[h]			X[h]		X[h]	X[b,h]		

A: *Letermovir*: Dose as assigned; once daily for 12 to 24 weeks. Refer to section 5.1 for detailed administration regimen
* If patient meets treatment continuation criteria. See sections 5.1 and 5.4.
a: Study visits will be completed in outpatient clinic once patient is discharged.
b: Pre-study evaluations are to be conducted within 3 days prior to start of protocol therapy
c: If pre-study visit is completed on the same day as treatment initiation (week 0), the pre-study laboratory investigations need not be repeated.
d: Patients with CMV retinitis at baseline will have funduscopic exams at each study visit.
e: See appendix A.
f: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
g: If not previously done for the current CMV infection. Can be repeated if letermovir resistance is clinically suspected. Letermovir genotyping and deep sequencing will be done in a reference laboratory at the end of the study.
h: Serum or urine pregnancy test (in women of childbearing potential).

Visit schedule while on therapy[a] (Pediatric 12 to ≤ 18 years-old)

	Pre-Study[b,c]	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 8	Wk 10	Wk 12	Wk 16	Wk 20	Wk 24
Letermovir administration (diary review)	X	X	X	X	*	*	*	*	*	*	*	*	*	*
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X	X												X
Physical exam (as indicated)	X													
Funduscopic exam[d]	X													
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X													
Weight	X					X			X		X	X	X	X
Performance status (Lansky score if < 16 years-old, Karnofsky score if ≥ 16 years-old)[e]	X					X			X		X	X	X	X
CBC w/diff	X	X[c]	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[f]	X	X[c]	X	X	X	X	X	X	X	X	X	X	X	X
CMV viral load	X	X[c]	X	X	X	X	X	X	X	X	X	X	X	X
CMV genotype[g]	X													
CMV immunity panel		X				X			X		X	X	X	X
Routine PK sample (predose, if on drug)				X		X		X	X	X	X			
Intensive PK samples[h]			X or X											
EKG (as indicated)	X		X											
Adverse event evaluation		X												X
B-HCG	X[i]					X[i]			X[i]		X[i]	X[i]		

A: *Letermovir*: Dose as assigned; once daily for 12 to 24 weeks. Refer to section 5.1 for detailed administration regimen
* If patient meets treatment continuation criteria. See sections 5.1 and 5.4.
a: Study visits will be completed in outpatient clinic once patient is discharged.
b: Pre-study evaluations are to be conducted within 3 days prior to start of protocol therapy
c: If pre-study visit is completed on the same day as treatment initiation (week 0), the pre-study laboratory investigations need not be repeated.
d: Patients with CMV retinitis at baseline will have funduscopic exams at each study visit.
e: See appendix A and B.
f: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
g: If not previously done for the current CMV infection. Can be repeated if letermovir resistance is clinically suspected. Letermovir genotyping and deep sequencing will be done in a reference laboratory at the end of the study
h: Pediatric patients (<18 years old) will undergo intensive PK sampling on Week 1 or Week 2 of treatment with time points of 0, 1, 2.5, 8 and 24 hours[7] for better understanding of PK/PD given the current lack of pediatric data on letermovir.
i: Serum or urine pregnancy test (in females of childbearing potential).

Visit schedule[a] upon drug discontinuation (all ages)

	End of Treatment[b]	Post-treatment Week 1	Post-treatment Week 4	Post-treatment Week 8	Post-treatment Week 12
Medical history					
Physical exam	X	X	X	X	X
Funduscopic exam	X				
Vital signs	X	X	X	X	X
Weight	X	X	X	X	X
Concurrent meds	X				X
Performance status (Lansky score if < 16 years-old, Karnofsky score if ≥ 16 years-old)[c]	X	X	X	X	X
CBC w/diff	X	X	X	X	X
Serum chemistry[d]	X	X	X	X	X
CMV viral load	X	X	X	X	X
CMV genotype[e]					
CMV immunity panel	X		X		X
EKG (as indicated)					
Adverse event monitoring[f]	X				X

a: Study visits will be completed in outpatient clinic once patient is discharged.
b: Visit to be conducted upon study treatment discontinuation
c: See appendix A and B.
d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
e: If not previously done for the current CMV infection. Can be repeated if letermovir resistance is clinically suspected. Letermovir genotyping and deep sequencing will be done in a reference laboratory at the end of the study
f: Off-study evaluation. Note: for IND/IDE trials, follow up visits or other contact are required in order to identify SAEs during the 30 days following the end of study treatment.

10.1 CMV Viral Load Assessment

Plasma CMV testing for study purposes will be obtained at each study visit. Testing will be done at Viracor Eurofins who will serve as the central laboratory for the trial. Quantitative testing of CMV in plasma will be done using the COBAS Amplicor/COBAS Taqman CMV test (https://www.viracor-eurofins.com/test-menu/5500-cytomegalovirus-cmv-quantitative-pcr/). Specific timepoints can be found in the study calendar in section **10.0**.

CMV virus load testing for clinical care will be at the discretion of the treating clinicians. Local laboratory CMV results will be captured in the study database. Please see Appendix F for assay details.

11. MEASUREMENT OF EFFECT

Each patient will be required to achieve a minimum virological and clinical response milestones for their treatment on study to continue.

The virological response milestones are defined as:

1) Any decrease in CMV DNA from baseline (Week 0), measured on week 3 and
2) A ≥2 log decrease in CMV DNA from baseline, or an undetectable CMV DNA, measured on week 6.

For patients with clinical CMV disease, the clinical response milestones are defined as:

1) Stabilization of clinical disease by week 3 (i.e. no worsening signs or symptoms compared to week 1) as assessed by the site investigator and
2) Improvement or resolution of clinical disease by week 6 (i.e., improvement in signs and symptoms of affected organs [resolution of diarrhea, pneumonia, hepatitis, retinitis, etc.])
3) Patients with clinical CMV disease should also meet virological response milestones outlined above to support the continuation of letermovir therapy. Patients who enter the study based solely on documented CMV disease by histopathology in the absence of quantifiable CMV virus load should remain nonquantifiable (less than 137 IU/mL).
4) Patients who are enrolled with CMV retinitis will be considered failures if they require additional intravitreal therapy after study week 3. Intravitreal therapy before this time will be at the discretion of the treating ophthalmologist.

A complete virological response is defined as two consecutive CMV DNA levels <137 IU/mL in the central laboratory.

CMV recurrence is defined as plasma CMV DNA ≥137 IU/mL in ≥2 consecutive samples in the central laboratory in a patient who had previously achieved plasma CMV DNA <137 IU/mL.

Patients will be considered to have experienced virological failure if CMV virus load is unchanged or higher from baseline when measured on week 3. if the decline in CMV DNA is <2 log on week 6, or if the patient experiences CMV recurrence while on letermovir treatment.

CMV progression-free survival will be assessed from time from study enrollment to CMV progression or death whichever occurs first.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

Data collection will be performed using InForm, which is an approved software which complies with Section 21 CFR, Part 11 requirements needed to use electronic data for supporting a New Drug Application. Case Report Forms will be developed by DF/HCC Clinical Trials Research Informatics Office (CTRIO), and the data will be maintained by the Office of Data Quality (ODQ).

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to ODQ in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Collaborative Agreements Language: N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design and Objectives

This is a single-arm, two-stage Phase II study of letermovir to treat refractory or resistant CMV infection or disease in patients who underwent hematopoietic cell or solid organ transplantation. The primary objective of the study is to formally test letermovir's ability to treat active CMV infection and CMV disease in patients who lack effective therapeutic options and in those with baseline organ dysfunction where currently available treatments would likely lead to poor clinical outcomes such as irreversible kidney toxicity or severe myelosuppression. These patients would thus most likely benefit from letermovir treatment. The data generated will inform future development programs for CMV treatment with letermovir and will likely help patients in this

situation.

13.2 Endpoints

13.2.1 Primary Endpoint

The primary endpoint is complete virological response assessed on week 6 of treatment for patients with CMV infection or clinical response with concurrent virological response assessed by week 6 for patients with CMV disease. (See Section 11 for definitions).

13.2.2 Secondary Endpoints

- Proportion of patients with a clinically meaningful treatment response to letermovir treatment, defined as a virological response and a concomitant clinical response in patients with CMV disease by Week 6 of treatment.
- Proportion of patients with a clinically meaningful treatment response to letermovir treatment, defined as a virological response and a concomitant clinical response in patients with CMV disease by Week 3 of treatment.
- Proportion of patients with complete virological and clinical response at study week 12 independent of duration of letermovir treatment.
- Proportion of patients with complete virological and clinical response at study week 24 independent of duration of letermovir treatment.
- Frequency and time to recurrent CMV viremia in patients who have virological response to letermovir treatment
- Overall survival
- CMV progression-free survival
- Kinetics of viral clearance and potential emergence of letermovir-resistant CMV virus in patients treated in this setting.

13.3 Sample Size/Power Calculation

We plan to enroll 32 evaluable patients. Based on a recent phase 2 trial of maribavir in the comparable patient population,[16] the proposed treatment will be regarded as efficacious in treating refractory and resistant CMV if the success rate is 60% or higher and inefficacious if the success rate is 35% or lower. Here, response refers to complete virological response for CMV infection and clinical response with concurrent virological response for CMV disease (primary endpoint). With the sample size 32, there will be 91% power to detect a 25% difference in response rate.

The study is a two-stage design with an early stopping rule if the proposed treatment shows no efficacy after the first stage. In the first stage, 12 evaluable patients will be accrued. Of these 12, if 4 or fewer patients respond, then the study will be terminated early for lack of efficacy. If, however, 5 or more patients respond, then additional 20 patients will be accrued. After the second stage, if 15 or more patients in a total of 32 respond, the proposed treatment will be considered efficacious. Conversely, if 14 or fewer patients respond, the proposed treatment will

be regarded as inefficacious and the treatment will not be considered further. If 15 out of 32 patients respond, the 95% confidence interval for the response rate is (30%, 68%). This confidence interval is calculated based on the Atkinson and Brown method (Biometric, 1985).

With this design, the probability of concluding the proposed treatment is efficacious is 0.91 if the true but unknown response rate is 60% and 0.095 if the true rate is 35%. The probability of early stopping is 0.58 if the rate is 35% and 0.057 if the rate is 60%. This decision rule is calculated using an exact binomial distribution. Patients will be considered unevaluable for the primary endpoint if they are removed from the study due to death that is unrelated to the proposed treatment, withdrawal of consent or lost to follow up prior to the response assessment. These patients will be replaced. Table 1 below presents the operating characteristics of this design.

Table 1. Operating Characteristics

	True but unknown response rate					
	0.35	0.4	0.45	0.5	0.55	0.6
Prob. of stopping early (<=4 in 12)	0.583	0.438	0.304	0.194	0.112	0.057
Overall prob. of accepting the treatment	0.095	0.231	0.427	0.636	0.806	0.913

13.4 Monitoring Treatment Related Toxicity

Continuous monitoring on treatment related toxicity will be conducted for all patients who receive any amount of the study drug. Data safety and monitoring will be performed per Dana-Farber/Harvard Cancer Center Data and Safety Monitoring Committee (DF/HCC DSMC) guidelines. The stopping guidelines serve as a trigger for consultation with the DF/HCC DSMC for additional review, and are not regarded as formal stopping rules that would mandate automatic closure of study enrollment.

The treatment related toxicities would include the following events that occurring on therapy.

- Drug-induced liver injury (DILI) meeting Hy's law criteria without alternative explanation.
- Any CTCAE grade 4 adverse event(s) potentially related to letermovir, with no alternative explanation.

In the previous studies of letermovir[16], letermovir was well tolerated. Based on this information, we project the treatment related toxicity rate <=15%. In this study, we will monitor DILI and grade 4 or higher treatment related adverse events in parallel. That is, in the first 10 patients, if 2 or more patients experience DILI and/or if 3 or more patients experience grade 4 or higher treatment related adverse events, then the accrual will be halted and the DF/HCC DSMC will be consulted. With this design, the probability of halting accrual is 0.15 if the true but unknown rate of DILI (p1) is 5% and the true but unknown rate of grade 4 or higher treatment related adverse events (p2) is 10%; the probability of halting accrual is 0.63 if these rates are 15% and 20%, respectively. Table 2 shows probabilities of halting accrual under various levels of p1 and

p2.

Table 2. Probability of Halting Accrual

True but unknown rate of DILI (p1)	True but unknown rate of grade ≥4 treatment related AE (p2)					
		0.05	0.1	0.15	0.2	0.25
	0.05	0.097	0.150	0.250	0.381	0.520
	0.1	0.272	0.316	0.396	0.501	0.613
	0.15	0.462	0.494	0.554	0.631	0.714
	0.2	0.629	0.651	0.692	0.745	0.802
	0.25	0.759	0.773	0.800	0.835	0.872

The probability in this table is calculated based on the bivariate binomial distribution assuming that two events are independent. The independence assumption is made since the dependence of these two events is unknown. It is possible that two events might be slightly positively dependent. If that is the case, the probability of halting accrual will be slightly larger than the one presented in this table. If the accrual continues after the first 10 patients, the same rule will apply to the next 10 patients.

13.5 Accrual

Based on the current practice, the projected accrual rate will be 3-4 patients per month. Conservatively extrapolating this projection, we anticipate that the accrual will complete approximately in one year.

Accrual Targets								
Ethnic Category	**Sex/Gender**							
	Females			**Males**			**Total**	
Hispanic or Latino	3		+	3		=	6	
Not Hispanic or Latino	13		+	13		=	26	
Ethnic Category: Total of all subjects	16	(A1)	+	16	(B1)	=	32	(C1)
Racial Category								
American Indian or Alaskan Native	0		+	0		=	0	
Asian	1		+	1		=	2	
Black or African American	3		+	3		=	6	
Native Hawaiian or other Pacific Islander	0		+	0		=	0	
White	12		+	12		=	24	
Racial Category: Total of all subjects	16	(A2)	+	16	(B2)	=	32	(C2)
	(A1 = A2)			(B1 = B2)			(C1 = C2)	

13.6 Analysis of Secondary Endpoints

Secondary endpoints include assessment of overall response rate, duration of response, CMV progression-free survival and overall survival. Due to the exploratory nature, the analysis for secondary endpoints will largely be descriptive. Response rate and duration of response will be summarized descriptively and the Kaplan Meier method will be used to estimate CMV progression-free survival and overall survival. A similar descriptive analysis will be performed for laboratory correlative studies of Kinetics of viral clearance. If feasible, association between clinical response and laboratory endpoints will be explored.

14. PUBLICATION PLAN

The results will be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. A full report of the outcomes will be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B LANKSY PERFORMANCE STATUS CRITERIA FOR PATIENTS AGE < 16

Rating	Description
100	Full active, normal
90	Minor restrictions with strenuous physical activity
80	Active, but gets tired more quickly
70	Both greater restriction of, and less time spent in, active play
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Lying around much of the day, but gets dressed; no active play; participates in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	Stuck in bed; needs help even for quiet play
20	Often sleeping; play is entirely limited to very passive activities
10	Does not play nor get out of bed
0	Unresponsive

APPENDIX C STUDY PATIENT DRUG DIARY

General Instructions for oral tablet:

- Can be taken without regard to food intake.
- Do NOT crush pill to ingest.
- Take the pill at approximately the same time every day (within 2 hours).
- If a dose is missed or vomited, the pill should be taken again. If this occurs, document this in the drug diary.
- If a dose is missed or forgotten for any reason, it should be taken when possible. If not remembered, the missed dose should be skipped and the next dose should be taken at its scheduled time.
- Bring your Daily Dosing Diary to every visit with your doctor.

Daily Subject Diary

Study:
Subject Number:_____

Date	Dose Taken (Y/N) Please circle	Number of pills taken	Time of Dose
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		

	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		
	Y / N		

APPENDIX D POTENTIAL DRUG INTERACTIONS WITH LETERMOVIR

Potential for other drugs to affect letermovir

Letermovir is a substrate of organic anion-transporting polypeptide 1B1/3 (OATP1B1/3) transporters. Co-administration of letermovir with drugs that are inhibitors of OATP1B1/3 transporters may result in increases in letermovir plasma concentrations (Table).

Potential for letermovir to affect other drugs

Co-administration of letermovir with midazolam results in increased midazolam plasma concentrations, indicating that letermovir is a moderate inhibitor of CYP3A. Co-administration of letermovir with drugs that are CYP3A substrates may result in clinically relevant increases in the plasma concentrations of co-administered CYP3A substrates (Table).

Letermovir is an inhibitor of OATP1B1/3 transporters. Co-administration of letermovir with drugs that are substrates of OATP1B1/3 transporters may result in a clinically relevant increase in plasma concentrations of co-administered OATP1B1/3 substrates (Table).

The magnitude of CYP3A-and OATP1B1/3-mediated drug interactions on co-administered drugs may be different when letermovir is co-administered with cyclosporine. See the prescribing information for cyclosporine for information on drug interactions with cyclosporine.

The following Table summarizes drug-drug interactions and precautions related to letermovir administration.[18]

Concomitant Drug Class and/or Clearance Pathway: Drug Name	**Effect on Concentration†**	**Clinical Comments**
Anti-arrhythmic agents		
amiodarone	↑ amiodarone	Close clinical monitoring for adverse events related to amiodarone is recommended during co-administration. Frequently monitor amiodarone concentrations when amiodarone is co-administered with letermovir.
Anticoagulants		
warfarin	↓ warfarin	When letermovir is co-administered with warfarin, frequently monitor International Normalized Ratio (INR)§.
Anticonvulsants		
phenytoin	↓ phenytoin	When letermovir is co-administered with phenytoin, frequently monitor phenytoin concentrations§.
Antidiabetic agents		
Examples: glyburide, repaglinide, rosiglitazone	↑ glyburide ↑ repaglinide ↑ rosiglitazone	When letermovir is co-administered with glyburide, repaglinide, or rosiglitazone, frequently monitor glucose concentrations§. When letermovir is co-administered with cyclosporine, use of repaglinide is not recommended.
Antifungals		

voriconazole‡	↓ voriconazole	If concomitant administration of voriconazole is necessary, closely monitor for reduced effectiveness of voriconazole§.
Antimycobacterial		
rifampin	↓ letermovir	Co-administration of letermovir and rifampin is not recommended.
Antipsychotics		
pimozide	↑ pimozide	Co-administration is contraindicated due to risk of QT prolongation and torsades de pointes
Ergot Alkaloids		
ergotamine, dihydroergotamine	↑ ergotamine, dihydroergotamine	Co-administration is contraindicated due to risk of ergotism
HMG-CoA Reductase Inhibitors		
atorvastatin‡	↑ atorvastatin	When letermovir is co-administered with atorvastatin, do not exceed an atorvastatin dosage of 20 mg daily§. Closely monitor patients for myopathy and rhabdomyolysis. When letermovir is co-administered with cyclosporine, use of atorvastatin is not recommended.
pitavastatin, simvastatin	↑ HMG-CoA reductase inhibitors	Co-administration of letermovir and pitavastatin or simvastatin is not recommended. When letermovir is co-administered with cyclosporine, use of either pitavastatin or simvastatin is contraindicated due to significantly increased pitavastatin or simvastatin concentrations and risk of myopathy or rhabdomyolysis
fluvastatin, lovastatin, pravastatin, rosuvastatin	↑ HMG-CoA reductase inhibitors	When letermovir is co-administered with these statins, a statin dosage reduction may be necessary§. Closely monitor patients for myopathy and rhabdomyolysis. When letermovir is co-administered with cyclosporine, use of lovastatin is not recommended. When letermovir is co-administered with cyclosporine, refer to the statin prescribing information for specific statin dosing recommendations.
Immunosuppressants		
cyclosporine‡	↑ cyclosporine ↑ letermovir	Decrease the dosage of letermovir to 240 mg once daily Frequently monitor cyclosporine whole blood concentrations during treatment and after discontinuation of letermovir and adjust the dose of cyclosporine accordingly§.
sirolimus‡	↑ sirolimus	When letermovir is co-administered with sirolimus, frequently monitor sirolimus whole blood concentrations during treatment and after discontinuation of letermovir and adjust the dose of sirolimus accordingly§.

		When letermovir is co-administered with cyclosporine and sirolimus, refer to the sirolimus prescribing information for specific sirolimus dosing recommendations§.
tacrolimus‡	↑ tacrolimus	Frequently monitor tacrolimus whole blood concentrations during treatment and after discontinuation of letermovir and adjust the dose of tacrolimus accordingly§.
Proton pump inhibitors		
omeprazole	↓omeprazole	Clinical monitoring and dose adjustment may be needed.
pantoprazole	↓ pantoprazole	Clinical monitoring and dose adjustment may be needed.
CYP3A Substrates		
Examples: alfentanil, fentanyl, midazolam, and quinidine	↑ CYP3A substrate	When letermovir is co-administered with a CYP3A substrate, refer to the prescribing information for dosing of the CYP3A substrate with a moderate CYP3A inhibitor§. When PREVYMIS is co-administered with cyclosporine, the combined effect on CYP3A substrates may be similar to a strong CYP3A inhibitor. Refer to the prescribing information for dosing of the CYP3A substrate with a strong CYP3A inhibitor§. CYP3A substrates pimozide and ergot alkaloids are contraindicated
* This table is not all inclusive. † ↓ =decrease, ↑=increase ‡ These interactions have been studied. § Refer to the respective prescribing information.		

Drugs without Clinically Significant Interactions with letermovir

No clinically significant interactions were observed in clinical drug-drug interaction studies of letermovir and acyclovir, digoxin, mycophenolate mofetil, posaconazole, ethinyl estradiol, and levonorgestrel.

APPENDIX E CMV GENOTYPE MUTATIONS THAT CONFER ANTIVIRAL RESISTANCE TO GANCICLOVIR, FOSCARNET, OR CIDOFOVIR

Source: Viracor Eurofins Revision 6 (10/2018)

Reportable Mutations - UL54 (DNA Polymerase)[1]

Viral Phenotype Confirmed by Marker Transfer[2]				
Mutation	**Cidofovir**	**Foscarnet**	**Ganciclovir**	**REFERENCE**
D301N	R	S	R	19,33,56
E303D[4]	R	S	R	93
E303G[4]	R	S	R	93
N408D	R	S	R	9,12,23,31,33,35,56,72
N408K	R	S	R	13, 74
N408S	R	S	R	84, 88
N410K	R	S	R	19, 23
F412C	R	S	R	2,9,20,23,33,35,65,72
F412L	R	S	R	74
F412S	R	S	R	25,74,111
F412V	R	S	R	9,23,72
D413A	R	S	R	69,73
D413E	R	S	R	19,23,31,33,52,56,72,73
D413N	R	S	R	89
D413Y[4]	R	S	R	79
P488R	R	S	R	79,111
N495K	S	R	R	73, 76,103
K500N	R	S	R	79,111
L501I	R	S	R	2,9,12,23,33,64,72
T503I	R	S	R	12,19,33,35,72,73
K513N	R	S	R	23,33,35,56,72
K513E	R	S	R	9,23,33,34,35,72
K513R	R	S	R	35, 72, 89
D515E	S	S	R	90
D515Y	S	R	R	91
L516R	R	S	R	19, 33
I521T	R	S	R	66, 68, 72
P522A	R	S	R	9,19,25,33,37,68,72
P522S	R	S	R	68, 72
del524	R	S	R	88
V526L	R	S	R	82
C539G	R	S	R	89
C539R	R	S	R	79,111

[1] Most UL54 mutations also have UL97 mutations.
[2] R and S denote resistant and sensitive, respectively. ND indicates Not Determined.
[3] Low-grade or variable resistance.
[4] Also confers resistance to CMX001.

Reportable Mutations - UL54 (DNA Polymerase)[1]

Viral Phenotype Confirmed by Marker Transfer[2]				
Mutation	**Cidofovir**	**Foscarnet**	**Ganciclovir**	**REFERENCE**
D542E[4]	R	S	S	81
A543P	R	S	R	108
L545S	R	S	R	9,12,33,72
L545W	R	S	R	74,111
T552N	S	R	R	79,103,111
Q578H	R	R	R	24, 74,111
Q578L	S	R	S	83
S585A	S	R	S	79,111
D588E	S	R	S	9,12,23,35,72
D588N	S	R	R	12,23,27,33, 74
F595I	S	R	S	79,111
T700A	S	R	S	5,9,18,33,47,72
V715A	S	R	S	92
V715M	S	R	S	5,9,18,25,33,37,47,72
I726T	S	S	R	83
E756K	R or S[3]	R	R	12,19,23,33,36,74,82,110
E756D	S	R	S	12,19,31,33
E756Q	S	R	S	12,18,33,37
L773V	R	R	R	24,89
L776M	S	R	R	67,73
V781I	S	R	R[3]	9,23,74
V787A	S	R	R	91
V787L	S	R	R	18,37,48,73
L802M	S	R	R or S[3]	9,12,18,20,23,27,33,35,65,73
K805Q	R	S	S	9,18,28,35,72
A809V	S	R	R	18,19,25,28,33,47,72,103
V812L[4]	R	R	R	12,18,20,27,28,33,72,73,78,93
T813S	R	R	R	28,73
T821I	S	R	R	9,18,28,33,35,72,73
P829S	S	S	R	79,111
A834P	R	R	R	13,73
T838A	S	R	S	27,28,73
G841A	R	R	R	28,72,73
G841S	S	R	R	83
V946L	S	R	S	79,111

L957F	ND	ND	R	79,111
del981 to 982	R	R	R	11,19,33,63,72,73,108
A987G	R	S	R	1,9,22,23,25,43,72
S290R	S	R	S	103
N495K+Q783R	S	R	R	103
E951D	S	R	R	103

Reportable Mutations - UL54 (DNA Polymerase)[1]

Viral Phenotype found in Clinical Isolates (Unconfirmed by Marker Transfer)[2]				
Mutation	**Cidofovir**	**Foscarnet**	**Ganciclovir**	**REFERENCE**
M393R	R	R	R	20, 72
M393K	R	R	R	20, 72
T419M	S	R	S	24
L501F	R	S	R	23,25,31,56,72
Y(I)722V	R	S	R	35, 72
H729Y	S	R	S	74
Y751H	R	S	R	35, 72
V787I	S	R	S	33

[1] Most UL54 mutations also have UL97 mutations.
[2] R and S denote resistant and sensitive, respectively. ND indicates Not Determined.
[3] Low-grade or variable resistance.
[4] Also confers resistance to CMX001.

Reportable Mutations - UL56 (Terminase)

Viral Phenotype Confirmed by Marker Transfer[1]		
Mutation	**Letermovir**	**REFERENCE**
S229F	R	101
V231A	R	99
V231L	R	95,99,101,108
N232Y	R	102
V236A	R	108
V236L	R	99
V236M	R	94,95,101,110
E237D	R	99,102
L241P	R	95,99,100,101
T244K	R	99,101
L254F	R	101
L257F	R	101
L257I	R	99,101
K258E	R	102
F261L	R	99,101,102
Y321C	R	99
C325F	R	99
C325W	R	108
C325R	R	99
C325Y	R	95,99
M329T	R	99,102
A365S	R	108
N368D	R	101
R369G	R	95
R369M	R	95,101
R369S	R	95,100
E237D, T244K, F261L	R	99
V236L, L257I	R	99
V236M, L257I, M329T	R	99
S229F, L254F, L257I	R	101

[1] R denotes resistance.

Reportable Mutations - UL97 (Phosphotransferase)

Viral Phenotype Confirmed by Marker Transfer[1]		
Mutation	**Ganciclovir**	**REFERENCE**
F342S	R	105,106,107
V356G	R	106,107
L405P	R	75
M460I	R	3,5,6,7,8,10,12,23,25,33,109
M460T	R	75
M460V	R	6,7,8,10,11,17,24,33,43,50,109
V466G	R	73,77
C518Y	R	84,85
H520Q	R	6,7,10,25,33,43,109
del590 to 593	R	12,72
del591 to 594	R	8,10,33,34,35,72,104
del591 to 607	R	51,72
C592G	R[2]	6,8,10,11,20,23,27,33,51,103,104,109
A594E	R	73,75
A594G	R	84,86
A594P	R	6,25,59,72
A594T	R	10,25,33,36,50,51,54
A594V	R	5,6,8,10,11,17,25,27,33,50,104,109
L595F	R	10,33,41,72
L595S	R	5,6,8,10,11,12,17,23,25,33,109
L595W	R	6,8,10,25,33,51,72
del595	R	4,10,17,72,104
del595 to 603	R	14,33,48,72,104
E596G	R	10,20,33,35,36,51,72
E596Y	R	90
del597 to 599	R	92,104
G598S	R	33,58,72
K599T	R	60,72
del600	R	10,33,36,51,72,104
del601 to 603	R	69,104
C603R	R	73,77,109
C603W	R	6,8,10,20,23,25,33,35,72,109
C607F	R	8,50,51,72
C607Y	R	10,25,33,34,35,39,51,72
I610T	R	90
A613V	R	84, 87
A591V	R	104
del596	R	104
del597 to 598	R	104
del599	R	104
del600 to 601	R	104

del601	R	104
del601 to 602	R	104

[1] R denotes resistance. [2] Alone confers modest resistance. When found in conjunction with UL54 del 981 to 982, there is a much higher level of resistance.

Viral Phenotype found in Clinical Isolates (Unconfirmed by Marker Transfer)[1]		
Mutation	**Ganciclovir**	**REFERENCE**
M460L	R	49, 72
A590T	R	10,49,72
del590 to 600	R	17, 72
del590 to 603	R	56, 72
A591D	R	10,49,72
C592F	R	72
del594 to 601	R	unpublished clinical isolate
L595T	R	10,35,72
N597I	R	10,49,72
del597 to 603	R	25
G598V	R	10,49,72
K599M	R	10,49,72
C603Y	R	6,10,49,72
A606D	R	10,49,72

[1] R denotes resistance.
[2] Alone confers modest resistance. When found in conjunction with UL54 del 981 to 982, there is a much higher level of resistance.

APPENDIX F DETAILS OF LOCAL CMV TESTING FOR PARTICIPATING HOSPITALS

Boston Children's Hospital uses a locally developed assay with their own primers and probes. The assay uses whole blood. The sample is extracted with Qiagen Qiacube extraction kit and the PCR is done in a Cepheid Smart Cycler. The lower limit of quantification is 500 copies/mL.

Brigham and Women's Hospital uses the COBAS Amplicor/COBAS Taqman CMV test developed by Roche Molecular Systems using plasma. The Lower limit of quantification is 137 IU/mL.

Massachusetts General Hospital uses the COBAS Amplicor/COBAS Taqman CMV test developed by Roche Molecular Systems using plasma. The Lower limit of quantification is 137 IU/mL.