EVALUATION OF THE TOLERABILITY AND CLINICAL EFFECTIVENESS OF LETERMOVIR IN HEART TRANSPLANT RECIPIENTS

NCT04904614

Date: 2/17/2025

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Sponsor: Merck and Company

STATEMENT OF COMPLIANCE

:

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following [use applicable regulations depending on study location and sponsor requirements; samples follow]:

- *U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)*
- *ICH GCP E6*
- *Completion of Human Subjects Protection Training*

http://www.fda.gov/cder/guidance/959fnl.pdf
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html
http://cme.cancer.gov/c01/

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:*

Signed: _____ Date: _____

Name

Title

** The protocol should be signed by the local investigator who is responsible for the study implementation at his/her specific site; i.e., if Investigational New Drug study, the individual who signs the Form FDA 1572.*

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1.1 Study objectives

We propose to study letermovir prophylaxis for cytomegalovirus (CMV) prevention in heart transplant recipients to establish tolerability and clinical effectiveness in a cohort of patients followed prospectively.

2.1 Specific Aims

1. To establish that letermovir can be well tolerated in cardiac transplant recipients and be associated with a lower rate of neutropenia in this population compared to historic controls in our well defined prior cardiac transplant cohort

2. To assess the rates of CMV infection and disease in cardiac transplant recipients who receive letermovir prophylaxis.

3. To compare this prospective cohort to our historic controls of 274 heart transplant recipients in the data base established through the Merck IISP program (accepted at ISHLT in April 2020) for all clinical outcomes including CMV infection, CMV disease, opportunistic infections, graft rejection and death

2.1.1 Clinical hypotheses

1. The clinical hypothesis is that letermovir will be well tolerated and less marrow toxic than valganciclovir or ganciclovir in a cohort of heart transplant recipients. We know that about 30% of heart transplants develop neutropenia in the first year post transplant and that valganciclovir use is one of the associated risk factors. Knowing that letermovir is a less marrow toxic agent we hypothesize it will be better tolerated than valganciclovir or ganciclovir in this population.

2. We also hypothesize that letermovir will be clinically effective in preventing CMV disease in this population.

2.2 Background and Rationale

There is a need for alternative medications to prevent cytomegalovirus in organ transplantation (1). The current regimen of valganciclovir prophylaxis is often

associated with neutropenia, as well as the development of late onset CMV infection and disease, especially in the high risk subset of CMV donor positive to CMV recipient negative mismatched transplants (2). Letermovir has been licensed for prevention of CMV in stem cell transplantation (3). It is being studied in renal transplant recipients currently (4). We have had some experience in using letermovir to treat or provide secondary prophylaxis among heart transplant recipients who have had prolonged courses of CMV viremia, as an alternative to the toxicity which was associated with valganciclovir use. In addition, we have seen frequent relapse following cessation of secondary prophylaxis with valganciclovir as well as development of resistance.

2.3 Preliminary Data

In a cohort of heart transplant recipients we found that 30% of 274 patients developed neutropenia at a median of 142 days post transplant. Half required GCSF. In a multivariate analysis of causes of neutropenia in heart transplant recipients, being at high risk for CMV infection (D+/R-), lower baseline WBC at time of transplant, prior CMV infection and having a VAD pre heart transplant were all independently related to the development of neutropenia (5). We also have data on all clinical outcomes, including rates of CMV disease, rates of rejection, 1year mortality and the development of opportunistic infections to which we can compare the prospectively followed letermovir patients.

2.4 Rationale for Letermovir

Letermovir (LET) belongs to a new class of anti-CMV agents with a novel mechanism of action with:

(1) Significant anti-CMV activity in in vitro and in vivo pre-clinical studies;

(2) A favorable clinical safety profile demonstrated in Phase 1 and 2 studies, as well as in the Phase 3 P001 study in hematopoietic stem cell transplant (HSCT) recipients;

(3) Clinical efficacy as demonstrated in a study in HSCT recipients (3); and

(4) Activity against viral isolates resistant to marketed anti-CMV agents, also demonstrated in a case of multi-organ disease due to multi-resistant CMV (6). The activity of these agents map to the UL54 or UL97 genes, while LET activity maps to the UL56 (terminase) gene (7).

Letermovir inhibits the viral terminase complex (UL51/UL56/UL89), an enzyme that plays an important role in cleavage of concatenated viral DNA into individual unit-length genomes that are subsequently inserted into CMV procapsids to generate

infectious CMV virions (7). Letermovir has demonstrated potent, selective, and reversible inhibition of CMV activity in preclinical studies in vitro and efficacy against the virus in vivo (7,8).

Letermovir has been shown to be generally well tolerated in 28 Phase 1 studies, 2 Phase 2 studies, and a pivotal Phase 3 study, P001, in HSCT recipients. In P001, in which CMV seropositive allogeneic HSCT recipients received letermovir or placebo from the early post-transplant period (within 4 weeks post-transplant) through Week 14 post-transplant and were followed for an additional 34 weeks, Letermovir was superior to placebo in the prevention of clinically significant CMV infection (defined as onset of CMV end-organ disease OR initiation of anti-CMV preemptive therapy based on documented CMV DNAemia as measured by the central laboratory) and the clinical condition of the participant through Week 24 post-transplant (3). Letermovir prophylaxis also resulted in lower all-cause mortality relative to placebo through Week 24 post-transplant and Week 48 post-transplant in HSCT recipients.

Based on its mechanism of action that is distinct from other available anti-CMV agents, patients who are given Letermovir for prophylaxis and experience CMV infection or disease, i.e., a clinical and/or virological CMV “breakthrough” event, are still expected to retain available treatment options using existing anti-CMV medications.

2.5 The Environment

The Advanced Heart Failure Program, Tufts Medical Center

The Tufts Medical Center Heart Transplantation Program placed within the top 10 nationally both for volumes and outcomes in 2018. In 2019 there were 43 heart transplants. Our 1 year survival has been 94.7% with a 3-year survival of 94.1% and a 5-year survival 86.3% (from Jan 2020 SRTR data). We are the only hospital in Boston with a consistent 5 tier rating for 1year outcomes. The Tufts Advanced Heart Failure (HF) Program is located on the main campus of Tufts Medical Center and receives approximately 120 new referrals annually for advanced HF therapy evaluations – left ventricular assist device (LVAD) implantations and heart transplantations. The program also has a robust Mechanical Circulatory Support Program, accredited by the Joint Commission, with 30-40 durable LVAD implantations annually. We implant the FDA-approved HeartMate II®, HeartMate III® and HeartWare® LVADs and participate in the INTERMACS registry as well as several industry device trials such as the APOGEE study.

The Advanced HF Program research program also participates in a range of industry, federal and investigator-initiated trials. Tufts Medical Center has been a site in National Institutes of Health (NIH)-funded Heart Failure Network trials and participates in drug and device trials for patients with both HF with reduced ejection fraction and HF with preserved ejection fraction.

Division of Geographic Medicine and Infectious Diseases

Dr. Snydman has been conducting studies of CMV prophylaxis for 40 years, both in solid organ and stem cell transplantation. He has also been doing data base analyses of CMV infection and disease for over 35 years, as well as cost effectiveness analyses, and cohort studies. Dr. Snydman has experience and expertise in writing IND, and NDA reports as well as performing investigator initiated IND's, both for drugs, biologics, and probiotics.

The transplant ID program is fully integrated into the cardiac transplant program and follows about 100 heart transplants in the heart ID program lead by Dr. Helen Boucher, the Chief of the Division. Dr. Jennifer Chow has been involved in transplant ID studies for over a decade, and has experience in clinical trial cohort management, study design and data analysis in this population. She is an attending in the heartID program.

We propose to study letermovir prophylaxis in heart transplant recipients to establish tolerability and comparable efficacy at CMV prevention in a cohort of patients followed prospectively and compared to a well defined historic control group.

3.1 Study Design

We propose to enroll 100 heart transplant recipients over two years in order to have 35 heart transplant recipients to receive letermovir prophylaxis in an open label trial to prevent CMV infection and disease. This number is based on an estimate of about 40-45 transplants per year, of whom 30 (75%) would be eligible for CMV prophylaxis based on CMV donor and recipient status (we would not enroll CMV D-/R- transplant patients). There are competing clinical trials with immunosuppressive modalities so we anticipate enrolling about 15 patients per year who will consent and are able to be followed for the one year follow up.

Eligibility would be limited to heart transplant recipients who are not CMV D-/R- who are not enrolled in competing clinical trials and who are willing and able to participate in the trial. Written informed consent will be required.

3.1.1 Inclusion criteria

1. Adults between 18-70 will be eligible for participation

2. Written informed consent and able to participate with follow up

3. Heart transplant recipients who are not CMV donor negative and CMV recipient negative (CMV -/-)

4. Not enrolled in competing clinical trials

3.1.2 Exclusion Criteria

1. Dual heart and kidney transplant recipients
2. Patients who do not survive 72 hours post transplant
3. HIV infection
4. Patients with creatinine clearance less than 10 ml per min at time of enrollment
5. Hypersensitivity to letermovir
6. On CVVH or renal dialysis at the time of enrollment
7. Received a previous solid organ transplant or HSCT.
8. Has Child-Pugh Class C severe hepatic insufficiency at screening.
9. Has both moderate hepatic insufficiency AND moderate-to-severe renal insufficiency at screening.

   Note: Moderate hepatic insufficiency is defined as Child-Pugh Class B; moderate-to-severe renal insufficiency is defined as CrCl <50 mL/min, as calculated by the Cockcroft-Gault equation (as above), respectively.

10. Has a history of malignancy ≤5 years prior to signing informed consent except for adequately treated basal cell or squamous cell skin cancer or in situ cervical cancer or carcinoma in situ; or is under evaluation for other active or suspected malignancy.
11. Is pregnant or expecting to conceive, is breastfeeding, or plans to breastfeed from the time of consent through at least 90 days following cessation of study therapy.
12. Is expecting to donate eggs or sperm starting from the time of consent through at least 90 days following cessation of study therapy.
13. Has a history or current evidence of any condition, therapy, lab abnormality, or other circumstance that might confound the results of the study, interfere with the participant's participation for the full duration of the study, or put the participant at undue risk, as judged by the investigator, such that it is not in the best interest of the participant to participate in this study.
13. Has exclusionary laboratory value at screening, as listed in Table 1.

Table 1 Laboratory Exclusion Criteria

Laboratory Assessment	Exclusionary Value
Hemoglobin	<8 g/dL
Platelets	<25,000 cells/μL
Absolute neutrophil count	<1,000 cells/μL
Total bilirubin	>2.5 × ULN
ALT	>5 × ULN
AST	>5 × ULN
ALT = alanine aminotransferase; AST = aspartate aminotransferase; CMV = cytomegalovirus; IgG = immunoglobulin G; ULN = upper limit of normal	

14.

15. Is currently participating or has participated in a study with an unapproved investigational compound or device within 28 days, or 5× half-life of the investigational compound (excluding monoclonal antibodies), whichever is longer, of initial dosing on this study. Participants previously treated with an investigational monoclonal antibody will be eligible to participate after a 150-day washout period.

    Note: Investigational regimens involving combinations of approved agents are not permitted. Other non-interventional or other observational studies are allowed.

16. Has previously participated in this study or any other study involving LET.

17. Has previously participated or is currently participating in any study involving administration of a CMV vaccine or another CMV investigational agent, or is planning to participate in a study of a CMV vaccine or another CMV investigational agent during the course of this study.

No restriction will be made for CMV disease prior to transplant and up to 7 days of CMV prophylaxis with other medications will be allowed prior to enrollment.

3.2 Analysis Plan

The Principal Investigator and co-investigators will be responsible for maintaining the integrity of the data and analyzing it. We have considerable experience in this regard.

We will compare the historical cohort to the prospectively followed cohort. We will examine the following outcomes:

3.2.1 Primary Endpoint:

1. Proportion of patients with neutropenia at 12 months in the letermovir arm compared to historic controls.

3.2.2 Secondary Endpoints:

2. Rate of CMV infection at 1 year in the letermovir arm compared to the historic controls.
3. Rate of rejection at 1 year in the letermovir arm compared to historic controls
4. Rate of opportunistic infections or other infectious outcomes in the letermovir arm compared to historic controls.
5. Tolerability and compliance of patients taking letermovir
6. Use of GCSF in both groups.
7. Measures of CMV specific immunity in the letermovir group at cessation of prophylaxis.

3.2.3 Power Analyses

The power analysis will be based on the rate of neutropenia occurring within one year.

We know our baseline rate of neutropenia is 30% at 12 months (based on our cohort analysis of 274 heart transplant recipients) among ganciclovir/valganciclovir recipients (historic controls). In the stem cell transplant trial, the rate of neutropenia was 3.8 % and leukopenia 2.9% among patients on letermovir. If we assume a rate of neutropenia of 5% in the letermovir arm, we have a 90% power to demonstrate a difference between the letermovir group and our historic controls ($p<.05$).Even assuming an upper boundary of 10% for the rate of neutropenia in the letermovir arm, 30 patients will give us 82% power (alpha of $< .05$) to show a significant difference between our historical cohort and the letermovir treated patients for development of neutropenia.

3.3 Definitions

CMV disease will be defined according to the criteria of the CMV consensus conference definitions (9,10) and used in other studies. Briefly these definitions will be:

CMV infection: evidence of CMV replication regardless of symptoms; “defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen” (9,10).

CMV disease: evidence of CMV infection with attributable symptoms. CMV disease can be further categorized as a viral syndrome (i.e. fever, malaise, leukopenia, and/or thrombocytopenia), or as tissue invasive ("end organ") disease (9,10).

Blood stream infection: Positive blood culture. Bacteremia caused by common skin contaminants will be considered significant if the same organism is isolated from two blood cultures in the presence of clinical signs of infection and/or an intravascular device (11).

Invasive fungal infection: Identification of fungal or yeast species by cultures or histological examination from a normally sterile site (12). Solitary sputum, urine, or Foley catheter cultures were not counted as IFI events. Pulmonary and intracranial *Nocardia* infections will also be included in this category given that *Nocardia* is an opportunistic pathogen causing similar clinical disease to fungal organisms.

Neutropenia will be defined as an absolute neutrophil count of < 1000 per mm3(5)

Rejection

Rejection will be defined as the composite of a first episode of biopsy-proven cellular rejection requiring admission for intravenous corticosteroids or other parenteral therapies, antibody mediated rejection requiring admission for intravenous corticosteroids, plasmapheresis, or other parenteral therapies, or any rejection episode causing hemodynamic compromise, graft loss, re-transplantation, or death. Data on type of treatment for rejection will be captured for all hospitalizations and outpatient visits.

4.0 Study Procedures

Once enrolled giving written informed consent, patients who are not CMV D+/R- would receive 3 months of letermovir prophylaxis at a dose of 480 mg orally or intravenously once per day starting within day 7 of transplantation. If patients are receiving cyclosporine, the letermovir dose would be reduced to 240 mg per day. If the cyclosporine is discontinued, the letermovir dose would be increased back to 480 mg per day. Patients will receive HSV prophylaxis with famciclovir 500 mg orally twice a day as per our usual protocol (90 days). The standard duration of letermovir prophylaxis for all but the group at high risk of CMV disease would be 3 months, the high risk (D+/R-) group would receive 6 months of prophylaxis which is our standard protocol with valganciclovir.

We will collect demographic data including age, gender, underlying cardiac disease, use of ventricular assist device, other co-morbidities, labs including WBC at time of transplant, and infections at time of transplant well as donor information.

Patients will be followed prospectively with data collection obtained at the time of each clinic visit. We routinely follow patients daily while hospitalized then weekly for

about a month post-transplant, then every other week for two months and then monthly thereafter. Routine clinical management labs (including electrolytes, BUN, creatinine, CDC and differential, LFT's, immunosuppressive drug levels) are obtained at the time of each visit. We will assess compliance through pill counts at monthly visits. At each visit the tolerability of letermovir will be assessed as well using a standardized questionnaire for tolerability. Follow up phone calls will also be employed when necessary.

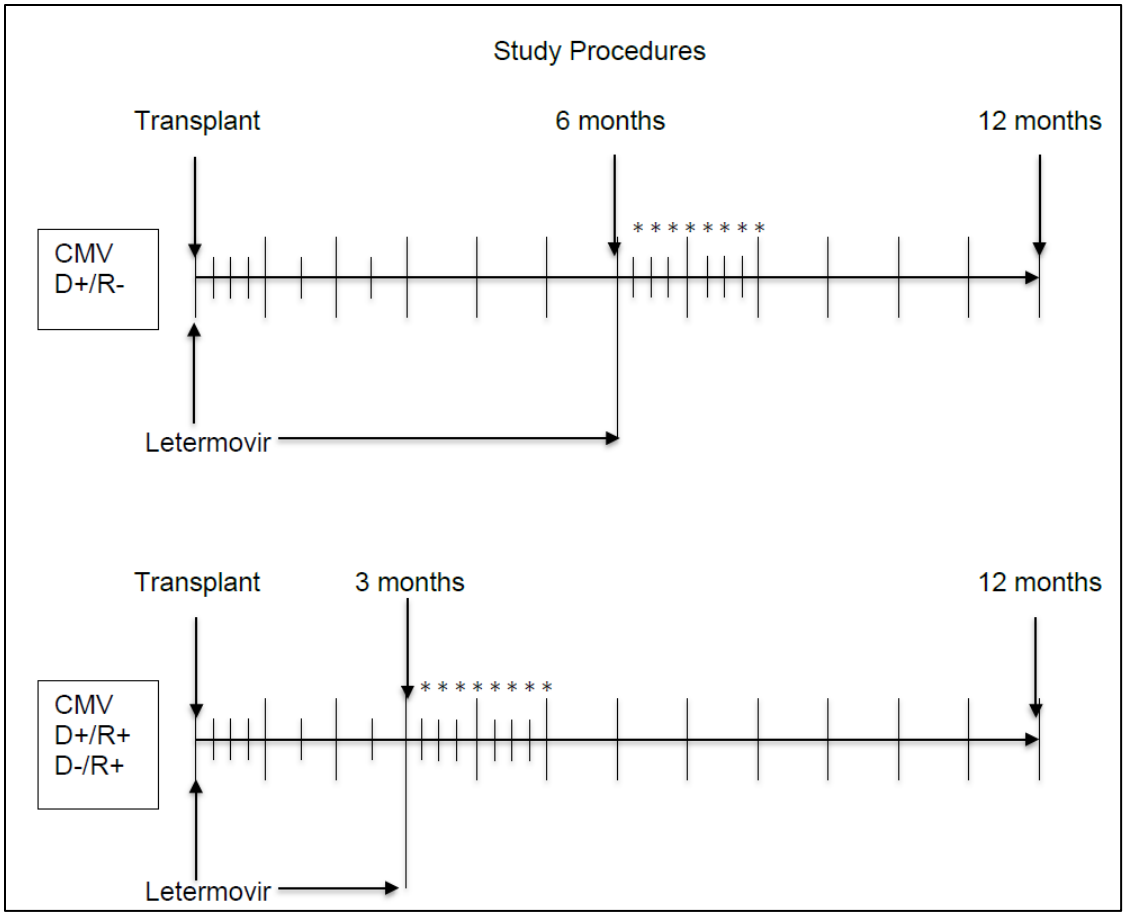
Cardiac catheterization is done routinely (every week for first 4 weeks, then every other week for two months, then monthly for the first year) to assess rejection, and at any sign of concern for rejection. Patients will be followed as part of the study protocol for one year post transplant in order to capture late onset CMV infection and disease as well as other infectious outcomes including opportunistic infections, graft rejection, treatment for rejection, or death.

With respect to CMV infection surveillance, we do not monitor patients for viral load during prophylaxis unless there is a suspicion of breakthrough viremia. Once patients discontinue prophylaxis, we do perform routine surveillance for CMV weekly for two months, then with any sign or symptom that might be consistent with CMV.

At cessation of letermovir prophylaxis (+/- 2 weeks) we will obtain a sample of measurement of CMV specific T cell immunity.

A schematic diagram and list of procedures is shown in Figure 1.

Figure 1. Schematic Diagram of Procedures



Each vertical line represents an outpatient follow up appointment after discharge. At each appointment, the following will be done:

- Exam
- AE assessment, and Compliance assessment (pill counts)
- Lytes, BUN, creatinine
- CBC with diff
- LFTs
- Cyclosporine or Tacrolimus levels
- * CMV viral load routinely measured post prophylaxis
- If clinical signs or symptoms compatible with CMV are suspected, CMV viral load will be performed

Table 1. Exams, labs, and assessments performed during the study

	At the time of transplant (day 0)	Post OHT weekly, week 1-4	Post OHT week 6	Post OHT Week 8	Post OHT week 12	Post OHT Month 4	Post OHT Month 5	Post OHT Month 6	Post OHT Monthly, month 7-12
Informed Consent	X								
Tacrolimus and/or sirolimus troughs		X	X	X	X	X	X	X	X
CBC with differential	X	X	X	X	X	X	X	X	X
Chem 7, LFTs (AST, ALT, AP, tot bilirubin)	X	X	X	X	X	X	X	X	X
CMV IgG					X*			X**	
CMV Quantiferon					X			X	
Baseline physical exam	X								
Visit for AE assessment and physical exam		X	X	X	X	X	X	X	X
Letermovir R+	X	X	X	X	X				
Letermovir D+/R-	X	X	X	X	X	X	X	X	
Compliance assessments (pill counts)		X	X	X	X	X	X	X	

* For CMV R+
**For CMV D+/R-

Table 2. CMV assessments at time of LET prophylaxis cessation

	Any Time of LET Prophylaxis cessation	**Weekly for 8 weeks**
CBC with differential	X	
CMV DNA		X
CMV IgG	X	
CMV Quantiferon	X	

4.1 Dosing of Letermovir (LET) Prophylaxis

From day of enrollment through either week 12 if CMV recipient is seropositive or Week 24 if the recipient is CMV seronegative (with CMV seropositive donor), participants will receive:

- LET 480 mg once daily (QD) given orally (either as one 480 mg tablet or two 240 mg tablets, based on participant's swallowing capability or, if the participant is receiving concomitant cyclosporin A (CsA), LET 240 mg QD given orally.

- Famciclovir 500 mg (FAM) given orally every 12 hours for prophylaxis against herpes simplex virus (HSV) and varicella zoster virus (VZV) as per our usual institutional protocol

- If participants in the LET treatment arm are unable to tolerate swallowing and/or have a condition (e.g., vomiting, diarrhea, or a malabsorptive condition) that may interfere with the absorption of the oral formulation then such participants can receive an IV formulation of LET. Participants on concomitant CsA will receive 240 mg IV LET QD, while participants not on will receive either 480 mg IV LET

4.2 Dose Modification (Escalation/Titration/Other)

Both oral (tablet) and IV formulations of LET will be available.

The IV formulation of LET contains the excipient hydroxypropyl betadex. Cyclodextrins can cause nephrotoxic effects in animals at systemic exposure; however, there is currently no evidence of these effects in humans. Hydroxypropyl

betadex amounts of approximately 250 mg/kg/day for 21 days were found to be safe in humans older than 2 years (13). Given this tolerability information in humans and the amount of cyclodextrin (3600 mg) contained in the highest dose (480 mg) of the IV formulation of LET administered in P001, nephrotoxic effects due to cyclodextrin were not expected in the trial population of adult HSCT recipients. Data from P001 suggest that the use of the cyclodextrin-containing IV formulation in this trial was not associated with renal toxicity and that dosing with the IV formulation is justified when it is necessary (3).

Based on the above, the use of IV LET is permitted in participants with renal insufficiency, provided CrCl is >10 mL/min. The IV formulation should only be used when participants are either unable to swallow or have a condition that may interfere with the absorption of the oral formulation at or after Day 1. Participants on IV LET should be switched to the oral formulation (ie, at the next planned dose) as soon as they are able to swallow and/or the condition that warranted the use of the IV formulation has resolved.

4.3 Dietary Restrictions for Taking Letermovir

Study therapy should be taken with food. Participants must avoid consumption of grapefruit, Seville oranges or their respective juices, and other quinine-containing drinks or food during the study from 2 weeks prior to study treatment administration until 72 hours after the final administration of study treatment

5.1 Treatment Compliance

The investigator/study coordinator will train the participant in the use of the Study Medication Diary. The participant will be instructed to record the number of tablets or capsules of study therapy taken during the study therapy period. At visits when used/unused study therapy are returned, site personnel will verify the accuracy of the dosing diary by comparing entries with amounts of returned study therapy. If a discrepancy is noted, the investigator/study coordinator must discuss the discrepancy with the participant, and the detailed explanation will be documented in the participant's study record.

If oral medication is administered by clinical personnel during any hospitalization or comparable inpatient setting (including but not limited to skilled nursing facility or rehabilitation facility) in which non-study and study medications are administered by clinical personnel), the site personnel will be responsible for documenting the adequacy of dosing adherence. We will document the compliance in the study case report form.

When administering IV formulation of study medication, the volume and the duration of infusion will be documented. The investigator/study coordinator will be responsible for transferring the appropriate information to the case report form.

Study therapy may be interrupted for any reason for a time period of ≤7 consecutive days (including suspected CMV Disease). Interruption from the protocol specified

treatment for a time period of ≤7 consecutive days due to an AE followed by re-starting of protocol specified treatment upon resolution of the AE is permitted.

5.2 Concomitant Therapy

Medications/therapies listed in this section pertain to co-administration with LET. When used, these agents should be administered in a manner consistent with the local product circular for these agents (if available for LET) including the complete list of prohibited medications (ie, those that are contraindicated or not recommended.

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the time periods specified by this protocol.

It is important for investigators to review each medication (prescription and non-prescription) the participant is taking before starting the study and at each study visit.

- At each visit, participants should be questioned about any new drug they are taking.

- To minimize the risk of adverse drug interactions, every effort should be made to limit the number of concomitant drugs to those that are truly essential.

Listed below are specific restrictions for concomitant therapy during the course of the trial.

- It should be noted that the magnitude of CYP3A- and OATP1B1/3-mediated drug interactions on co-administered drugs may be different when LET is co-administered with CsA (please also consult current prescribing information for CsA and for co-administered medication for drug interactions with CsA).

The following medications/therapies are <u>prohibited</u> during the dosing period and for 14 days after the dosing period:

- Certain HMG-CoA reductase inhibitors (statins): When LET is co-administered with CsA, the magnitude of the increase in statin plasma concentrations is expected to be greater than with LET alone.
    - Simvastatin or pitavastatin with LET or when LET is co-administered with CsA.
    - Atorvastatin or lovastatin when LET is co-administered with CsA.
        - Note: see below for co-administration of LET with atorvastatin, fluvastatin, lovastatin, rosuvastatin, or pravastatin.

- Fixed dose combination products containing statins are not allowed because the dosage of statins should be adjusted when LET is co-administered.

- Strong inducers, such as rifampin, phenytoin, carbamazepine, St John's wort (Hypericum perforatum), rifabutin and phenobarbital

- Moderate inducers, such as nafcillin, thioridazine, modafinil and bosentan

- Cytochrome P450 3A (CYP3A) substrates with narrow therapeutic range (NTR) that can lead to SAEs, including but not limited to:

    - Pimozide: Concomitant administration of LET may result in increased concentrations of pimozide due to inhibition of CYP3A by LET, which may lead to QT prolongation and torsade de pointes.

    - Ergot alkaloids: Concomitant administration of LET may result in increased concentration of ergot alkaloids (ergotamine and dihydroergotamine) due to inhibition of CYP3A by LET, which may lead to ergotism.

- Repaglinide when LET is co-administered with CsA (Note: see below for co-administration of LET with repaglinide).

- Everolimus when LET is co-administered with CsA (Note: see below for co-administration of LET with everolimus).

- The following medications/therapies are prohibited for the prevention/treatment of CMV while participants are on study therapy (except for cases of suspected CMV Disease). ACV, valacyclovir, and famciclovir may be used at thresholds lower than specified below (for a subset of these medications for which such thresholds are specified):

    - Foscarnet (see above)

    - Cidofovir (see above)

    - ACV (at doses >3200 mg PO per day or >25 mg/kg IV per day)

    - Valacyclovir (at doses >3000 mg or ≤500 mg PO per day, and not for HSV/VZV prophylaxis; see below)

    - Famciclovir (at doses >1500 mg

- Imipenem-cilastatin

- Investigational Agents: Unapproved investigational agents or investigational regimens involving combinations of *approved* agents (eg, immunosuppressive agents) are not permitted.

- Herbal Supplements: Herbal supplements are not permitted.

The following medications/therapies may require dose adjustment or closer monitoring during the dosing period and for 14 days after the dosing period:

- Inhibitors of organic anion-transporting polypeptide 1B1/3 (OATP1B1/3) transporters:
    - CsA: Co-administration of LET with CsA, a potent OATP1B1/3 inhibitor, increases the concentrations of LET. If LET is co-administered with CsA, the recommended dose of LET is 240 mg once daily.
- Substrates of organic anion-transporting polypeptide 1B1/3 (OATP1B1/3) and/or CYP3A:
    - Statins:
        - Atorvastatin: The dose of atorvastatin should not exceed a daily dose of 20 mg. Atorvastatin is prohibited when co-administered with LET and CsA (see above).
        - Fluvastatin, lovastatin, rosuvastatin, or pravastatin: The dose of fluvastatin, lovastatin, rosuvastatin, or pravastatin may need to be adjusted when co-administered with LET. Lovastatin is prohibited when co-administered with LET and CsA (see above). Monitoring for statin-associated adverse reactions (eg, myalgias, rhabdomyolysis) is recommended during co-administration.
    - Glyburide, a substrate of OATP1B1/3: Frequent monitoring of glucose concentrations is recommended during co administration of glyburide with LET.
- Substrates of CYP2C8 (repaglinide, rosiglitazone):
    - Repaglinide, rosiglitazone: LET is an in vitro inhibitor of CYP2C8. Co-administration of LET with CYP2C8 substrates (eg, repaglinide, rosiglitazone) may increase the plasma concentrations of CYP2C8 substrates. Frequent monitoring of glucose concentrations is recommended during co-administration of repaglinide and rosiglitazone and LET.
- CYP3A substrates:
    - Co-administration of LET with drugs that are CYP3A substrates may result in clinically relevant increases in the plasma concentrations of co-administered CYP3A substrates (examples: alfentanil, fentanyl, midazolam, quinidine). Therefore, frequent monitoring for adverse reactions related to these agents is recommended during co-

administration. When LET is co-administered with CsA, the combined effect on CYP3A substrates may be similar to a strong CYP3A inhibitor. Refer to the prescribing information for CsA and for co-administered medication for dosing of the CYP3A substrate with a strong CYP3A inhibitor.

- Substrates of CYP3A with NTR (examples given below; please also consult current prescribing information for monitoring and dosing these products with inhibitors of CYP3A); dose adjustment of CYP3A substrates with NTR may be needed.

    - CsA: Co-administration of LET with CsA increases CsA concentrations. Frequent monitoring of CsA whole blood concentrations should be performed during and at discontinuation of LET and the dose of CsA adjusted accordingly.

    - Sirolimus: Co-administration of LET with sirolimus increases concentrations of sirolimus. Frequent monitoring of sirolimus whole blood concentrations should be performed during and at discontinuation of LET and the dose of sirolimus adjusted accordingly.

      When LET is co-administered with CsA, refer to the sirolimus prescribing information for specific dosing recommendations for use of sirolimus with CsA.

    - Tacrolimus: Co-administration of LET with tacrolimus increases tacrolimus concentrations. Frequent monitoring of tacrolimus whole blood concentrations should be performed during and at discontinuation of LET and the dose of tacrolimus adjusted accordingly.

    - Everolimus: Co-administration of LET with everolimus may increase everolimus concentrations. Frequent monitoring of everolimus blood concentrations should be performed during and at discontinuation of LET and the dose of everolimus adjusted accordingly. The administration of everolimus when LET is co-administered with CsA is prohibited (see above).

        - Amiodarone: LET may increase the plasma concentrations of amiodarone (CYP3A and CYP2C8 substrates). Frequent monitoring for adverse reactions related amiodarone is recommended during co-administration. Frequent monitoring of amiodarone concentrations should be performed when co-administered with LET.

- Substrates of CYP2C9 and CYP2C19 (voriconazole, warfarin, omeprazole, and pantoprazole):

    - Voriconazole: Co-administration of LET with voriconazole decreases the plasma concentrations of voriconazole likely due to induction of CYP2C9 and/or 2C19. If concomitant administration is necessary, close monitoring for reduced effectiveness of voriconazole is recommended.

    - Warfarin: LET may decrease the plasma concentrations of CYP2C9 and/or CYP2C19 substrates (eg, warfarin). Frequent monitoring of international normalized ratio (INR) should be performed while warfarin is co-administered with LET.

    - Proton Pump Inhibitors, omeprazole and pantoprazole: LET may decrease the plasma concentrations of CYP2C19 substrates. Clinical monitoring and dose adjustment may be needed

6.0 CMV Disease Assessments and Management

All potential cases of CMV disease, as identified the study investigators will be assessed. Comparison will be made to our historical control cohort.

CMV infection is defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen.

CMV Definitions

CMV disease consists of the two following clinical definitions: 1) CMV end-organ disease; and 2) “probable CMV syndrome” (which will be termed “CMV syndrome” throughout this protocol).

- CMV end-organ disease will be further described by:

    - The specific type of end-organ disease (eg, pneumonia, gastrointestinal disease, or hepatitis); and

    - By categorization based on appropriate clinical signs/symptoms with detection/documentation of CMV:

        - Proven CMV end-organ disease

- Probable CMV end-organ disease.

Table 4. CMV assessment at time of treatment for CMV infection or disease

	At time of Initiation of Treatment for CMV Infection/Disease	Weekly During Treatment	At time of CMV Treatment Cessation	Weekly for 2 Months After Cessation of Treatment
CBC with Differential	X	X	X	
CMV DNA		X	X	X
CMV IgG	X		X	
CMV Quantiferon	X		X	

6.1 CMV Viral Resistance Testing

We do not routinely perform CMV resistance testing. Decisions to do so will be based on the judgement of the clinical investigators.

6.2 QuantiFERON-CMV Measurements

The development of CMV-specific T cell responses, which is the predominant adaptive immune response that confers protection against CMV (14-17) will be measured using the QuantiFERON-CMV assay within two weeks of the discontinuation of CMV prophylaxis or CMV treatment.

6.3 Rescue Medications & Supportive Care

In the event of CMV disease (suspected or confirmed by the study team) during the study therapy period (ie, prior to completion or early discontinuation of study therapy) or a clinical decision by the investigator to start CMV treatment due to any other reason. LET will be discontinued and the participant will be treated according to the local SOC.

In the event of HSV/VZV infection (as diagnosed by the investigator; herpes labialis, herpes zoster, and genital herpes) during the study therapy period (ie, prior to completion or early discontinuation of study therapy), will be treated according to the local SOC.

6.4 Treatment After the End of the Study

There is no study-specified treatment following the end of the study.

7.1 Safety Endpoints

The safety and tolerability of LET will be assessed by a clinical evaluation of AEs and inspection of other study parameters including vital signs, physical examination, and laboratory studies.

7.1.1 Leukopenia and Neutropenia

An evaluation of the safety and tolerability of LET, as assessed by review of the accumulated safety data, will be assessed as an endpoint in this study. For each episode of leukopenia or neutropenia that is reported as an AE during the treatment period, the corresponding laboratory values and normal ranges of total white blood cell (WBC) and absolute neutrophil count (ANC) will be collected (ie, local and central laboratory values).

Table 3. Assessment performed when ANC <1000

	Any Time ANC <1000	**Weekly Until ANC >1000 for 2 Consecutive Weeks**
CBC with differential	X	X
CMV DNA	X	

7.2 Method of Detecting AE, SAE and Other Reportable Safety Events

Care will be taken not to introduce bias when detecting AE and/or SAE and other reportable safety events. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

7.3 Follow-up of AE, SAE and Other Reportable Safety Event Information

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All AE, SAE and other reportable safety events including pregnancy and exposure during breastfeeding, ECI, Cancer and Overdose will be followed until resolution, stabilization, until the event is otherwise explained, or the participant is lost to follow-up. We will make every attempt to follow all non-serious AEs that occur.

7.4 Regulatory Reporting Requirements for SAE

- Prompt notification (within 24 hours) by the investigator to the sponsor of SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.

- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations, i.e., per ICH Topic E6 (R1) Guidelines for Good Clinical Practice.

- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAE) from the sponsor will file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8 Discontinuation/Withdrawal Criteria

8.1 Discontinuation of Study Treatment

Discontinuation of study treatment does not represent withdrawal from the study.

As certain data on clinical events beyond study treatment discontinuation may be important to the study, they will be collected through the participant's last scheduled follow-up, even if the participant has discontinued study treatment. Therefore, all participants who discontinue study treatment prior to completion of the protocol-specified treatment period will still continue to participate in the study.

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment.

- The participant develops confirmed or suspected CMV disease as determined by the investigator.

- The participant has a confirmed positive pregnancy test.

- An investigator feels it is in the best interest of the participant to discontinue.

- An elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) lab value that is greater than or equal to 3 × upper limit of normal (ULN) and an elevated total bilirubin lab value that is greater than or equal to 2 × ULN and, at the same time, an alkaline phosphatase lab value that is less than 2 × ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.

- The participant develops:

    - Both moderate hepatic insufficiency (Child-Pugh Class B) and moderate-to-severe renal insufficiency (defined as CrCl <50 mL/min as calculated by the Cockcroft-Gault equation),

      OR

    - Severe hepatic insufficiency (Child-Pugh Class C).

The participant **may** be discontinued from study therapy for any of the following reasons:

- Any AE/SAE assessed by the investigator as possibly or probably related to study therapy. The investigator may continue the participant in the study if it is deemed to be in the best interest of the participant to stay on study therapy.

- Failure to comply with the dosing, evaluations, or other requirements of the study.

- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or Sponsor, places the participant at unnecessary risk through continued participation in the study or does not allow the participant to adhere to the requirements of the protocol (e.g., if there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy may be required.

Discontinuation from study treatment is “permanent.” Once a participant is discontinued, he/she shall not be allowed to restart study treatment.

8.2 Withdrawal from the Study

A participant must be withdrawn from the study if the participant or participant’s legally acceptable representative withdraws consent from the study.

If a participant withdraws from the study, they will no longer receive study treatment or be followed at scheduled protocol visits.

8.3 Lost to Follow Up

If a participant fails to come to the clinic for one of their scheduled visits, and/or if the site is unable to contact the participant, the following procedures are to be performed:

- We will attempt to contact the participant and reschedule the missed visit. If the participant is contacted, the participant should be counseled on the importance of maintaining the protocol-specified visit schedule.

- We will make every effort to regain contact with the participant at each missed visit (e.g., phone calls and/or a certified letter to the participant's last known mailing address or locally equivalent methods). These contact attempts should be documented in the participant's medical record.

- Note: A participant is not considered lost to follow-up until the last scheduled visit for the individual participant.

8.3.1 Pregnancy and Exposure During Breastfeeding

Pregnancy in the first two years post transplant is highly discouraged and as part of the transplant program counseling against pregnancy is routinely done in the appropriate age groups. The immunosuppressive medications can be toxic to the fetus and pregnancy itself could put a strain on the heart.

Although pregnancy and infant exposure during breastfeeding are not considered adverse events, any pregnancy or infant exposure during breastfeeding in a participant (spontaneously reported to the investigator or their designee), including the pregnancy of a male participant's female partner, that occurs during the trial are reportable to the Sponsor.

All reported pregnancies willbe followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

8.3.2 Events of Clinical Interest (ECI)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor.

Events of clinical interest for this trial include:

1. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as

determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

8.4 Treatment of Overdose

In this study, an overdose is any dose higher than two times the prescribed dose specified. 7.2 (Dose Modification [Escalation/Titration/Other]). Overdose during the study will be a reportable safety event. .

8.4.1 Adverse Events Monitoring

Adverse event monitoring will include the collection of all AEs and SAEs from the time informed consent is signed through 14 days following the last dose of study treatment in all participants. Thereafter, any SAEs related to study medication will be collected through Week 52.

Clinical Safety Laboratory Assessments

8.4.2 Laboratory Safety Evaluations

The investigator will review the laboratory reports, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in study participant management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification), then the results must be recorded in the appropriate case report form.

For any laboratory tests with values considered clinically significantly abnormal during participation in the study or within 14 days after the last dose of study treatment, every attempt will be made to perform repeat assessments until the values return to normal or baseline or if a new baseline is established as determined by the investigator.

9.0 Literature Cited

1. Kotton CN, Kumar D, Caliendo AM, et al. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation. *Transplantation.* 2018;102(6):900-931.

2. Snydman DR. Epidemiology of infections after solid-organ transplantation. *Clin Infect Dis.* 2001;33 Suppl 1:S5-8.

3. Marty FM, Ljungman P, Chemaly RF, Maertens J, Dadwal SS, Duarte RF, et al. Letermovir Prophylaxis for Cytomegalovirus in Hematopoietic-Cell Transplantation. N Engl J Med. 2017; 377: 2433-2444. PMID: 29211658.

4. Letermovir versus valganciclovir to prevent human cytomegalovirus disease in kidney transplant recipients (MK8228-002). ClinicalTrials.gov.

5. Chow JLK, Ruthazer R, Boucher HW, Nierenberg N, Vest A, DeNofrio D, Snydman DR Factors associated with neutropenia post heart transplantation. Abstract accepted to ISHLT, April 2020 (not presented due to COVID 19)

6. Kaul DR, Stoelben S, Cober E, Ojo T, Sandusky E, Lischka P, et al. First report of successful treatment of multidrug-resistant cytomegalovirus disease with the novel anti-CMV compound AIC246. Am J Transplant 2011;11:1079-84.

7. Goldner T, Hewlett G, Ettischer N, Ruebsamen-Schaeff H, Zimmerman H, Lischka P. The novel anticytomegalovirus compound AIC246 (letermovir) inhibits human cytomegalovirus replication through a specific antiviral mechanism that involves the viral terminase. J Virol 2011;85(20):10884-93.

8. Lischka P, Hewlett G, Wunberg T, Baumeister J, Paulsen D, Goldner T, et al. In vitro and in vivo activities of the novel anticytomegalovirus compound AIC246. Antimicrob Agents Chemother. 2010 Mar;54(3):1290-7

9. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. *Clin Infect Dis.* 2017;64(1):87-91.

10. Gardiner BJ, Nierenberg NE, Chow JK, Ruthazer R, Kent DM, Snydman DR. Absolute Lymphocyte Count: A Predictor of Recurrent Cytomegalovirus Disease in Solid Organ Transplant Recipients. *Clin Infect Dis.* 2018;67(9):1395-1402.

11. Arbo MD, Snydman DR. Influence of blood culture results on antibiotic choice in the treatment of bacteremia. *Arch Intern Med.* 1994;154(23):2641-2645.

12. George MJ, Snydman DR, Werner BG, et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. *Am J Med.* 1997;103(2):106-113.

13. European Medicines Agency. Committee for Human Medicinal Products (CHMP): background review for cyclodextrins used as excipients

(EMA/CHMP/333892/2013) [Internet]. London: European Medicines Agency; 2014. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Report/2014/12/WC500177936.pdf.

14. Fernandez-Ruiz M, Kumar D, Humar A. Clinical immune-monitoring strategies for predicting infection risk in solid organ transplantation. Clin Transl Immunology. 2014 Feb 28;3(2):e12.

15. Abate D, Saldan A, Mengoli C, Fiscon M, Silvestre C, Fallico L, et al. Comparison of cytomegalovirus (CMV) enzyme-linked immunosorbent spot and CMV quantiferon gamma interferon-releasing assays in assessing risk of CMV infection in kidney transplant recipients. J Clin Microbiol. 2013 Aug;51(8):2501-7.

16. Cantisan S, Lara R, Montejo M, Redel J, Rodriguez-Benot A, Gutierrez-Aroca J, et al. Pretransplant interferon-γ secretion by CMV-specific CD8+ T cells informs the risk of CMV replication after transplantation. Am J Transplant. 2013 Mar;13(3):738-45.

17. Manuel O, Husain S, Kumar D, Zayas C, Mawhorter S, Levi ME, et al. Assessment of cytomegalovirus-specific cell-mediated immunity for the prediction of cytomegalovirus disease in high-risk solid-organ transplant recipients: a multicenter cohort study. Clin Infect Dis 2013;56(6):817-24.