MSK PROTOCOL COVER SHEET

An Open-label, Single-arm Study of Letermovir (LTV) for Prevention of Recurrent CMV Infection in High-risk Hematopoietic Cell Transplant (HCT) Recipients

Genovefa Papanicolaou, Medicine/Infectious Disease

Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, New York 10065

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is an open-label single arm trial of letermovir (LTV, Prevymis) for prevention of recurrent CMV infection in allogeneic hematopoietic cell transplantation (HCT) recipients with history of CMV infection. The study is being conducted at MSKCC and at the University of Minnesota.

Thirty-six HCT recipients who are ;?:12 years of age, had clinically significant CMV infection treated with CMV antivirals and are at high risk for recurrent CMV infection, defined as receiving a transplant from an HLA mismatched donor (including cord blood), acute or chronic graft versus host disease (GVHD) requiring either topical and/or systemic steroid treatment within 14 days prior to enrollment, or T cell-depleted (CD34+-selected) allograft, will be eligible to participate in the study.

All patients will receive LTV for prevention of recurrent CMV infection (secondary prophylaxis). Secondary prophylaxis will start after completion of antiviral therapy for CMV infection. LTV 480 mg (240mg daily for patients on cyclosporin A) will be administered daily until the patients meet the primary endpoint of clinically significant CMV infection or for maximum duration of 14 weeks. All patients will be followed for 24 weeks from Day 1 of LTV (Figure 1).

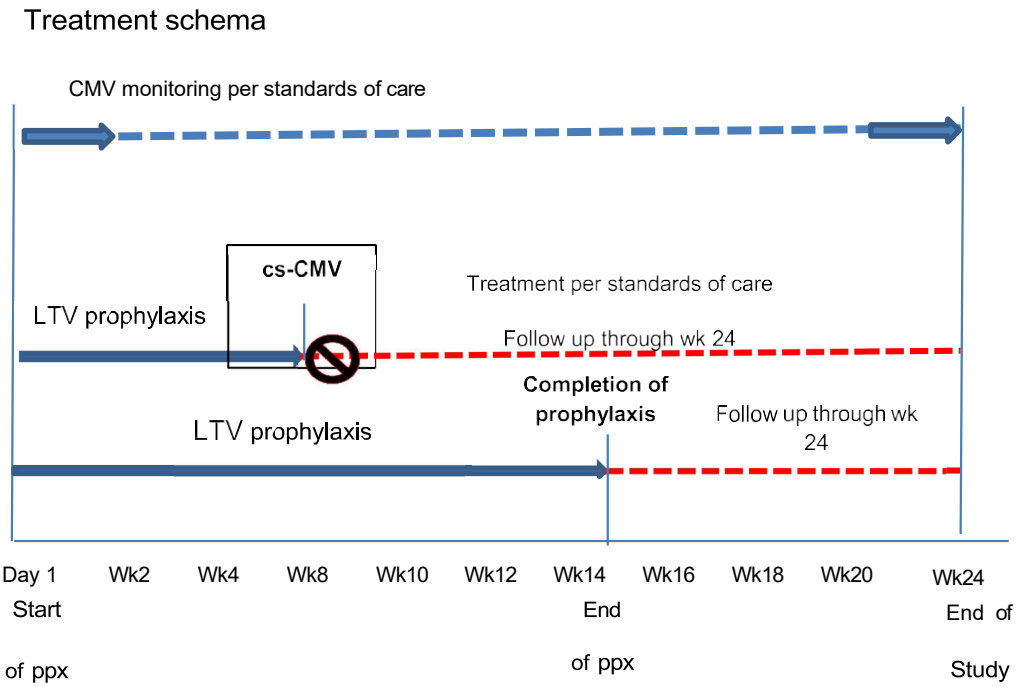
The primary endpoint will be discontinuation of LTV prior to week 14 due to recurrence of clinically significant CMV infection. Secondary endpoints include recurrence of clinically significant CMV infection during the 24-week follow-up period.

During the study patients will undergo routine surveillance for CMV by PCR of CMV DNA per standards of care at the enrolling institution. Patients that develop clinically significant CMV infection will discontinue LTV and will be treated according to the institutional standard of care.

Clinically significant CMV infection is defined as either CMV viremia requiring preemptive therapy with CMV antivirals [(val)ganciclovir or foscarnet] or CMV end organ disease (EOD). CMV EOD is defined as pneumonia, gastrointestinal disease with macroscopic mucosal lesions, hepatitis, neurological disease, cystitis, myocarditis, or pancreatitis in which CMV is detected in the affected tissue by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization; or retinitis with typical ophthalmological signs of CMV infection.

Patients with either an undetectable or a low CMV viral load (defined as <300 IU/ml) will be enrolled; this must be documented on at least two consecutive measurements at least 48 hours apart with most recent value :::; 7 days prior to Day 1. Patients with detectable CMV viral load may continue CMV antiviral therapy for 3 days concomitantly with LTV to allow LTV to reach steady state. Patients who meet the primary endpoint will discontinue letermovir and will be treated with preemptive therapy per standards of care at each Institution. Whenever possible, CMV should be sent for letermovir resistance testing prior to initiating preemptive therapy. A schema of the study design is shown below:

Figure 1.



Observational cohort

The objective for the observational cohort is to assess CMV specific immune responses at Day 100 post HCT in patients who received letermovir primary prophylaxis and did not have cs-CMV infection by Day 100.

The observational cohort consists of 50 patients that will be accrued at MSKCC only.

We will collect fresh mononuclear cells (PBMC) at D100 (day 90-120). Patients who do not have CMV specific responses on Day 100 will have repeat testing at D180 (5-7 months) post HCT. CMV immune responses will be measured by the CMV immunity T cell panel assay (Viracor-Eurofins).

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary objective:

To evaluate the efficacy of LTV in preventing CMV infection among patients receiving allogeneic hematopoietic cell transplant with history of CMV infection and who are at high risk of recurrent CMV infection, as assessed by the rate of breakthrough clinically significant

CMV infection by week 14. Patients who receive 3 doses of LTV will be included in the analyses.

Secondary objectives:

- The cumulative incidence of clinically significant CMV infection at 14 weeks will be estimated separately for patients who have undetectable CMV and detectable CMV at baseline.
- To estimate the extended efficacy of LTV by the cumulative incidence of clinically significant CMV infection through week 24.
- To assess the efficacy of LTV via additional endpoints:
    - Time to onset of clinically significant CMV infection among patients treated with LTV
    - Number of days free of CMV viremia, CMV EOD, CMV antivirals, alive, and out of hospital through week 14.
    - Utilization of CMV antivirals (type and duration) through week 24.
    - Number and reason(s) for readmissions and hospital length of stay (LOS)through week 24.
    - Invasive procedures (endoscopy, bronchoscopy, etc.) through week 24.
    - All-cause and non-relapse mortality at week 24.
- To determine the proportion of patients who develop LTV resistance (determined using CMV isolates from patients with CMV viremia during the study).
  To assess the occurrence of SAEs and toxicity leading to LTV discontinuation.
- To assess CMV-CMI at D +100 in a secondary observational cohort of HCT recipients that received LTV prophylaxis and did not have clinically significant infection. The percentage of patients with positive CMI will be estimated.

3.0 BACKGROUND AND RATIONALE

Cytomegalovirus (CMV) remains the most common clinically significant infection after allogeneic hematopoietic cell transplantation (HCT), occurring in up to 80% of CMV-seropositive HCT recipients (Ljungman Pet al. 2010).Following HCT, early CMV reactivation correlates with higher non-relapse mortality, with risk ratios ranging from 1.4 to 1.95, depending on the underlying hematologic disease and type of transplantation (Teira Pet al. 2016; Ramanathan M et al. 2016).Though preemptive therapy with CMV antivirals is effective in preventing CMV end organ disease (EOD), patients that do not develop CMV-specific immunity following CMV infection are at risk for recurrence of CMV infection after discontinuation of preemptive therapy. To prevent recurrent CMV infection, secondary prophylaxis is routinely used in these patients, which currently consists of a reduced (maintenance) dose of the same antivirals used for treatment of CMV [(val)ganciclovir or foscarnet]. Even at reduced doses, prolonged use of (val)ganciclovir or foscarnet is associated with myelosuppression or nephrotoxicity, respectively (Emery et al. 2013). Interruptions or dose reductions of anti-CMV antivirals due to toxicity often lead to sub-therapeutic drug concentrations, CMV resistance and potentially CMV EOD (Chou 2015). Thus, improved strategies for secondary prophylaxis are needed.

Letermovir (LTV) is a new, highly potent, CMV-specific terminase inhibitor that has shown in a phase 3 study, to be effective in preventing CMV in adult CMV-seropositive HCT recipients when administered in the first 100 days post-HCT (primary prophylaxis) (Marty FM et al. 2018).

In this multicenter, double-blind, placebo-controlled trial (P001, NCT02137772) in adult CMV-seropositive recipients of an allogeneic HCT, subjects were randomized (2:1) to receive either LTV at a dose of 480 mg once daily adjusted to 240 mg when co-administered with cyclosporine, or placebo. Randomization was stratified by investigational site and risk level for CMV reactivation at the time of study entry. Study drug was initiated after HCT (at any time from Day Oto Day 28 post-transplant) and continued through Week 14 post-transplant. Study drug was administered either orally or intravenously; the latter at investigators' discretion in subjects who were unable to take oral therapy. Subjects were monitored for CMV DNA weekly until post-transplant week 14 and then bi-weekly until post-transplant week 24 and treated with standard-of-care CMV pre-emptive therapy if CMV viremia was considered clinically significant. Subjects were followed through week 48 post-transplant.

The efficacy population consisted of 325 subjects who received LTV (including 91 subjects who received at least one IV dose) and 170 who received placebo (including 41 subjects who received at least one IV dose). The median time to starting study drug was 8 days after transplantation. Thirty-four percent (34%) of subjects were engrafted at baseline. The median age was 55 years (range:18 to 76 years); 57% were male; 84% were white; 9% were Asian; 2% were Black or African American; and 7% were Hispanic or Latino. At baseline, 30% of all subjects had one or more of the following factors associated with increased risk for CMV reactivation (high risk stratum): human leukocyte antigen (HLA)-related donor with at least one mismatch at one of the following three HLA-gene loci: HLA-A, -B or -DR; haploidentical donor; unrelated donor with at least one mismatch at one of the following four HLA-gene loci: HLA-A, -B, -C and -DRB1; use of umbilical cord blood as stem cell source; use of ex vivo T-cell-depleted grafts; Grade 2 or greater graft versus host disease (GVHD) requiring systemic corticosteroids. The remaining 70% of subjects did not meet any of these high-risk stratum criteria and were therefore included in the low risk stratum. Additionally, 48% of subjects received a myeloablative regimen (cyclosporine or tacrolimus). The most common primary reasons for transplant were acute myeloid leukemia (38%), myelodysplastic syndrome (16%), and lymphoma (12%).

The primary efficacy endpoint of Trial P001 was the incidence of clinically significant CMV infection through week 24 post-transplant (prophylaxis failure). Clinically significant CMV infection was defined as the occurrence of either CMV end-organ disease or initiation of anti-CMV pre-emptive therapy based on documented CMV viremia (using the Roche COBAS® AmpliPrep/COBAS TaqMan® assay, the lower limit of quantitation is 137 IU/ml, which is approximately 150 copies/ml). The protocol-specified guidance for CMV DNA thresholds for the initiation of pre-emptive therapy during the treatment period was 150 copies/ml or> 300 copies/ml for subjects in the high- and low-risk strata, respectively. From week 14 through week 24, the threshold was >300 copies/ml for both groups. The non-completer= failure approach was used, where subjects who discontinued LTV prior to week 24 post-transplant or had a missing outcome at week 24 post-transplant were counted as failures. The results of this study showed that patients who received LTV prophylaxis had significantly lower rates of clinically significant CMV infection through week 24 post-HCT [37.5%] compared with patients that received placebo [60.6%] ($p<0.001$). Efficacy results were similar between high- and low-risk strata for CMV reactivation.

In November 2017, LTV was approved by the FDA for primary CMV prophylaxis in HCT recipients.

Post-hoc analysis of Trial P001 demonstrated that among LTV-treated subjects, inclusion in the high-risk stratum for CMV reactivation at baseline, occurrence of GVHD, and steroid use at any time after randomization may be associated with the development of clinically significant CMV infection between week 14 and week 24 post-transplant. Approximately 10% of patients on LTV developed clinically significant CMV infection between weeks 14 and 24 post-HGT (after discontinuation of LTV). Of these patients, 47% had >1 baseline risk factor for CMV (HLA-mismatched donor, umbilical cord blood as the stem cell source, or T cell-depleted graft), and 81% had GVHD (Hodowanec A et al. 2018). Further, 19% required hospitalization for CMV, indicating that even post-prophylaxis CMV infections can be severe. Thus, even with widespread use of LTV as primary prophylaxis, the need for secondary prophylaxis remains, as certain groups of patients are at risk of developing CMV infection after discontinuing CMV prophylaxis.

Our hypothesis is that LTV will be effective as secondary prophylaxis in HGT recipients who complete treatment for clinically significant CMV infection and have risk factors for CMV recurrence. In addition to those listed above, risk factors for CMV recurrence include CMV-seronegative donor, lymphopenia, GVHD, and treatment with corticosteroids at doses > 0.5mg/kg (Boeckh M 2015).

While LTV is currently FDA-approved for treatment in patients >18 years, it is expected to be safe in and to benefit adolescent (ages 12 to 18 years) HGT recipients similarly to adults. Systemic exposure and clearance of drugs are generally similar in adolescent and adult patients after considering the effect of body size on pharmacokinetics (Momper JD 2013). LTV is transported by OATP1B1/3. In the literature, relative protein expression of OATP1B1 was highly variable among individuals, and no statistically significant difference was observed among studied age groups [Thomson, M. M., et al 2016] [Prasad, B., et al 2016]. For OATP1B3, protein expression was not significantly different between adults and children >1 year. By assuming weight distribution at each age according to CDC, the Phase 3 population PK model suggests that the adult doses of LTV will result in comparable exposures in ages 12 to <18 years as in adults.
Moreover, pediatric HGT recipients at risk for CMV recurrence currently have few options for prevention, given the side effects associated with prolonged use of (val)ganciclovir or foscarnet.

The long-term immunity against CMV relapse and end-organ disease requires functioning CMV-specific CDS+ and CD4+ T-cells (Hakki Met al 2003; Gabanti E et al, 2014). Low-level CMV replication is postulated to provide in vivo priming and expansion of CMV-specific T cell precursors (Li CR et al, 1994). Thus, effective CMV prophylaxis may hypothetically hinder CMV immune reconstitution through suppression of CMV replication. In a randomized study of ganciclovir prophylaxis after HGT, CMV-specific CD4+ proliferative and CDS+ cytotoxic responses at Day 100 post HGT were absent in a higher proportion of patients on ganciclovir compared with placebo. Other studies in HGT and SOT, suggest that ganciclovir prophylaxis may not completely suppress CMV replication and local CMV replication at the graft site (which may not lead to detectable CMV viremia) may be sufficient for triggering CMV-specific immune activation (La Rosa C et al, 2007; Hakki Met al, 2003). LTV blocks viral replication without inhibiting the synthesis of progeny CMV DNA or viral proteins. In fact, in vitro LTV exposure is associated with cytoplasmic accumulation of large amounts of sub-viral, noninfectious particles termed dense bodies (DB) (Cayatte C et al, 2013). It is unknown how LTV prophylaxis impacts CMV-specific immune activation. Because of the

unique mechanism of action of LTV leading to the release of noninfectious immunogenic DB, LTV prophylaxis may actually facilitate CMV immune reconstitution while providing effective prophylaxis against clinically significant CMV infection and end-organ disease.

Commercially available assays for functional assessment of CMV cell mediated immunity (CMV-CMI) include ELLISPOT assays measuring IFN-y as spot forming colony (SFC)/cells (T-Track ® CMV and T-SPOT.CMV ®), ELISA based assays measuring IFN- y (Quantiferon CMV®), methods to Quantify CMV-specific CD8+ T cells (Dextramer® CMV Kit (IVD)) and intracellular cytokine staining (CMV-immunity panel, VIRACOR®) (Yong MK, Curr Infect Dis Rep 2018). This CMV immunity panel is a flow cytometry assay developed to determine the percentages of CD4+ and CD8+ T cells that respond to stimulation with CMV antigens. Assessment of CMV specific response is based upon the cellular activation surface marker CD69 in conjunction with IFN-y, TNFa, and IL-2 cytokine production. Three CMV antigens are used to assess patient immunity; a whole viral lysate, a peptide pool of pp65, and a peptide pool of IE-1. The advantage of Intracellular cytokine staining assays is their ability to detect multiple cytokines and cell surface markers and differentiate T-cell phenotypes (Lileri, PLoS One. 2012; Whidmann T PLoS One. 2008; Lilieri DJ Infect Dis. 2009).

We propose to evaluate the efficacy of LTV as secondary prophylaxis in adult and adolescent HCT recipients at risk for recurrent CMV infection after successful preemptive treatment for CMV infection. Patients may or may not have received LTV as primary CMV prophylaxis. Additionally, we will evaluate the effects of LTV on CMV-specific immune reactivation at day 100 by using a flow cytometry based, CMV immunity panel on a cohort of patients treated with LTV.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is an, open-label, single-arm study of LTV for the prevention of recurrent CMV infection in HCT recipients with history of CMV infection. A total of 36 patients will be enrolled to the interventional cohort of the study.

Patients aged 12 years or older will be eligible for inclusion.

Patients enrolled on the study will receive oral LTV 480 mg daily (240 mg daily for patients receiving cyclosporine A). Patients that may have inadequate absorption due to vomiting, diarrhea, or a malabsorptive condition or are unable to receive oral medications due to difficulty swallowing will receive intravenous LTV at same doses.

The maximum duration of LTV administration will be 14 weeks. All patients will be followed for a total of 24 weeks from start of LTV. The total study duration will be 24 weeks. All patients will be monitored for CMV infection by their local quantitative RT-PCR of plasma (Roche) for the duration of the study (per Institutional standards of care). Patients will also be interviewed regarding symptoms of CMV EOD and will undergo routine laboratory evaluations post-HGT care (CBC, metabolic panel) (per standards of care). If symptoms or lab results suggest potential CMV EOD, appropriate

procedures for the affected organ will be performed (see 10.0 Evaluation During Treatment and 12.0 Criteria for Therapeutic Response for details). Any hospitalizations, invasive procedures undergone, and bacterial or fungal infections detected during participation in the study will be recorded.

Patients who develop clinically significant CMV infection while taking LTV will discontinue LTV and will be treated for CMV according to the standards of care at each Institution.

Observational cohort

CMV cell-mediated immunity (CMVI) will be assessed by the CMV inSIGHT™ (Viracor-Eurofins) at approximately 100 days post HCT. A 10 ml blood sample will be collected for CMV inSIGHT™. All efforts will be made to coordinate the collection for the CMV inSIGHT™ on the same day that immune phenotyping is performed per standards of care. Patients who at Day 100 have CMV inSIGHT™ below the reference range for either CD4 or CDS, will have a repeat sample collected at approximately Day 180 post HCT for repeat testing by CMV inSIGHT™. A test is evaluable, if it meets viability standards and adequate responses to positive control (staphylococcal endotoxin b). Patients with missing or non-evaluable tests will be replaced until 50 evaluable patients are accrued.

We propose to enroll 70 patients in order to accrue 50 evaluable patients.

4.2 Intervention

Open-label LTV (oral and intravenous) will be supplied by Merck.

Subjects will initially be administered the oral (tablet) formulation of study therapy provided they are able to swallow and do not have a condition that may interfere with gastrointestinal absorption. For subjects who cannot swallow and/or have a condition that may interfere with the absorption of the oral formulation, LTV can be given intravenously until patients are able to take oral LTV.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

Letermovir

5.1 Overview, Dosage and Administration

Letermovir (LTV, Prevymis, Merck) is a potent inhibitor of the CMV terminase complex, which cleaves viral deoxyribonucleic acid (DNA) into unit-length genome and packages it into procapsids. TV is approved for prophylaxis of cytomegalovirus (CMV) infection and disease in adult CMV-seropositive recipients of an allogeneic HCT. LTV has been found to be safe and generally well tolerated in 14 Phase 1-3 clinical trials.

The recommended dosage of LTV is 480 mg administered orally or intravenously (IV) once daily. Dosage of LTV should be decreased to 240 mg once daily when co-administered with cyclosporine.

LTV is formulated for oral administration as tablets, which should be ingested whole. LTV injection, which contains hydroxypropyl betadex, should be used only in patients unable to take oral therapy, and is administered via a peripheral catheter or central venous line at a constant rate over 1 h.

5.2 Contraindications

- LTV is contraindicated in patients receiving pimozide or ergot alkaloids:
  - Pimozide: Concomitant administration of LTV in patients receiving pimozide may result in increased concentrations of pimozide due to inhibition of cytochrome P450 3A (CYP3A) by LTV, which may lead to QT prolongation and torsades de pointes.
  - Ergot alkaloids: Concomitant administration of LTV in patients receiving ergot alkaloids may result in increased concentrations of ergot alkaloids (ergotamine and dihydroergotamine) due to inhibition of CYP3A by LTV, which may lead to ergotism.
- LTV is contraindicated with pitavastatin and simvastatin when co-administered with cyclosporine. Concomitant administration of LTV in combination with cyclosporine may result in significantly increased pitavastatin or simvastatin concentrations, which may lead to myopathy or rhabdomyolysis.

5.3 Safety of LTV in Adult CMV-seropositive Recipients [R+] of an Allogeneic HCT

The safety of LTV was evaluated in a Phase 3 randomized, double-blind, placebo-controlled trial (P001) in which 565 subjects were randomized and treated with LTV (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.

The cardiac adverse event rate (regardless of investigator-assessed causality) was higher in subjects receiving LTV (13%) compared with subjects receiving placebo (6%). The most common cardiac adverse events were tachycardia (4% of LTV subjects vs. 2% of placebo) and atrial fibrillation (3% of LTV subjects vs. 1% of placebo). Among those subjects who experienced one or more cardiac adverse events, 85% of LTV and 92% of placebo subjects had events reported as mild or moderate in severity. The rate of adverse events occurring in at least 10% of subjects in the LTV group and at frequency at least 2% greater than placebo is outlined in Table 1.

Table 1: All Adverse Events Reported in 10% of LTV-Treated HCT Recipients at a Frequency at least 2% Greater than Placebo in Trial P001

Adverse Event	LTV (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%

Overall, similar proportions of subjects in each group discontinued study medication due to an adverse event (13% of LTV subjects vs. 12% of placebo subjects). The most frequently reported adverse event that led to study drug discontinuation was nausea, occurring in 2% of LTV subjects and 1% of placebo subjects. Hypersensitivity reaction, with associated moderate dyspnea, occurred in one subject following the first infusion of IV LTV after switching from oral LTV, leading to treatment discontinuation.

Laboratory Abnormalities
Selected laboratory abnormalities reported during treatment or within 2 weeks of stopping treatment are presented in Table 2.

Table 2: Selected Laboratory Abnormalities Reported in Trial P001

	LTV n=373	**Placebo n=192**
Absolute neutrophil count (cells/µL)		
< 500	19%	19%
500- < 750	4%	7%
750 - < 1000	8%	9%
Hemoglobin (g/dL)		
< 6.5	2%	1%
6.5- < 8.0	14%	15%
8.0- < 9.5	41%	43%
Platelets (cells/µL)		
< 25000	27%	21%
25000 - < 50000	17%	18%
50000 - < 100000	20%	30%
Serum creatinine (mg/dL)		
> 2.5	2%	3%
> 1.5-2.5	17%	20%

The median time to engraftment (defined as absolute neutrophil count 500/mm^3 on 3 consecutive days after transplantation) was 19 days in the LTV group and 18 days in the placebo group.

5.4 Drug Interactions

Potential drug interactions for LTV are summarized in Appendix 1.

Potential for Other Drugs to Affect LTV

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

Potential for LTV to Affect Other Drugs
Co-administration of LTV with midazolam results in increased midazolam plasma concentrations, indicating that LTV is a moderate inhibitor of CYP3A. Co-administration of LTV with drugs that are CYP3A substrates may result in clinically relevant increases in the plasma concentrations of co-administered CYP3A substrates.
LTV is an inhibitor of OATP1B1/3 transporters. Co-administration of LTV 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.
The magnitude of CYP3A- and OATP1B1/3-mediated drug interactions on co-administered drugs may be different when LTV is co-administered with cyclosporine.

Drugs without Clinically Significant Interactions with LTV
No clinically significant interactions were observed in clinical drug-drug interaction studies of LTV and acyclovir, digoxin, mycophenolate mofetil, posaconazole, ethinyl estradiol, and levonorgestrel.

5.5 Use in Specific Populations

Renal Impairment
For patients with creatinine clearance (Cler) greater than 10 ml/min, no dosage adjustment of LTV is required based on renal impairment. There are insufficient data in patients with Cler 10 ml/min or less or in patients on dialysis to make LTV dosing recommendations. In patients with Cler less than 50 ml/min receiving LTV injection, accumulation of the intravenous vehicle, hydroxypropyl betadex, may occur. Serum creatinine levels should be closely monitored in these patients.

Pregnancy
Risk Summary: No adequate human data are available to establish whether LTV poses a risk to pregnancy outcomes. In a rat pre/post-natal development study, total litter loss was observed at maternal LTV exposures approximately 2 times higher than human exposure at the RHO.

Lactation
Risk Summary: It is not known whether LTV is present in human breast milk, affects human milk production, or has effects on the breastfed child. When administered to lactating rats, LTV was present in the milk of lactating rats as well as the blood of nursing pups.

Females and Males of Reproductive Potential
There are no data on the effect of LTV on human fertility. Decreased fertility due to testicular toxicity was observed in male rats.

5.6 Overdosage

There is no specific antidote for overdose with LTV. In case of overdose, it is recommended that the patient be monitored for adverse reactions and appropriate symptomatic treatment be instituted.
It is unknown whether dialysis will result in meaningful removal of LTV from systemic circulation.

5.7 Pharmacodynamics and Pharmacokinetics

The pharmacokinetic properties of LTV are displayed in Appendix 2.

Renal Impairment
LTV AUC was approximately 1.9- and 1.4- fold higher in subjects with moderate (eGFR greater than or equal to 30 to 59 ml/min/1.73m^2) and severe (eGFR less than 30 ml/min/1.73m^2) renal impairment, respectively, compared to healthy subjects.
Hydroxypropyl betadex present in the intravenous LTV formulation is mainly eliminated by glomerular filtration. Decreased elimination of hydroxypropyl betadex has been reported in the literature in patients with severe renal impairment.

Hepatic Impairment
LTV AUC was approximately 1.6-fold higher in subjects with moderate (Child-Pugh Class B [CP-B], score of 7-9) hepatic impairment compared with healthy subjects.

5.8 Efficacy in Adult CMV-seropositive Recipients of an Allogeneic Hematopoietic Stem Cell Transplant

The efficacy of LTV prophylaxis in transplant recipients at high risk for CMV reactivation was assessed in a multicenter, double-blind, placebo-controlled Phase 3 Trial (P001, NCT02137772) in adult CMV-seropositive recipients of an allogeneic HCT.
Efficacy results from Trial P001 are shown in Table 3.

Table 3: Trial P001 Efficacy Results in HCT Recipients (NC=F Approach, Full Analysis Population) Through Week 24

Parameter	Letermovir (n=325)	Placebo (n=170)
Proportion of subjects who failed prophylaxis	38%	61%
Reasons for failures*		
Clinically significant CMV infection by Week 24†	18%	42%
Initiation of preemptive therapy based on documented CMV Viremia	16%	40%
CMV end-organ disease	2%	2%
Discontinued from study before Week 24+	17%	16%
Missing outcome in Week 24 visit window	3%	3%
Stratum-adjusted treatment difference		

(LTV-Placebo) § Difference (95% CI)	-23.5 (-32.5, -14.6) ,r	

* Categories of failure are mutually exclusive and based on the hierarchy of categories in the order listed.
t Through week 14, 8% of subjects in the LTV group and 39% of subjects in the placebo group experienced clinically significant CMV infection.
+ Reasons for discontinuation included adverse event, death, lost to follow-up, physician decision, and withdrawal by subject.
§ 95% CI and p-value for the treatment differences in percent response were calculated using stratum-adjusted Mantel-Haenszel method with the difference weighted by the harmonic mean of sample size per arm for each stratum (high or low risk).
,r p-value <0.0001.
Note: Full analysis population includes randomized subjects who received at least one dose of study medication and excludes subjects with detectable CMV DNA at baseline.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

Patient must meet all the following criteria:

1. Age 12 years (any weight)
2. Have received allogeneic HCT
3. Have received preemptive therapy for clinically significant CMV infection post-HGT and have completed preemptive therapy no longer than 7 days prior to enrollment. Preemptive treatment includes ganciclovir, valganciclovir, foscarnet or cidofovir. Clinically significant CMV infection is defined as CMV viremia requiring preemptive therapy or CMV EOD. Patients who have received LTV prophylaxis prior to onset of clinically significant CMV infection are eligible as long as they also received preemptive therapy for clinically significant CMV infection prior to enrollment (see also exclusion criteria below)
4. Have one or more risk factors for recurrent CMV infection:
   a. Human leukocyte antigen (HLA)mismatch
      - HLA-related(sibling) donor with at least one mismatch at the HLA-A, -B or -DR gene loci,
      - Haploidentical donor
      - Unrelated donor with at least one mismatch at the HLA-A, -B, -C or-DRB1gene loci, or
      - Cord blood as stem cell source
   b. Acute or chronic GVHD requiring either topical steroids for gastrointestinal GVHD and/or systemic steroid treatment (1 mg/kg/day of prednisone or equivalent dose of another corticosteroid) within 14 days prior to enrollment
   c. T-cell-depleted allograft ex-vivo or in-vivo T-cell depleting agents including but not limited to ATG, alemtuzimab and post HCT cyclophosphamide.

5. For adult patients, able to provide written consent and complete the informed consent. For patients under 18 years, the patient's parent(s) or legal guardian(s)must provide informed consent and the patient must provide written assent to participation in the study.
6. Willing and able to comply with trial instructions and requirements
7. Male and female patients of childbearing potential must be willing to use a highly effective method of contraception for the course of the study. Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the patient.

Subject eligibility criteria for the observational cohort:

1. Age 18 years or older
2. First allogeneic peripheral blood or marrow HGT
3. LTV prophylaxis starting <30 days post HGT and given for at least 6 weeks

6.2 Subject Exclusion Criteria

1. Clinically significant CMV infection present at enrollment
2. Breakthrough CMV infection while on primary LTV prophylaxis (unless patient non-adherent or unable to adequately absorb letermovir) or documented resistance to LTV.
3. Glomerular filtration rate (GFR) :510 ml/min/1.73m^2(equivalent to creatinine clearance :510 ml/min)
4. Severe hepatic impairment
5. Routine use of high-dose acyclovir (doses of> 800 mg twice daily), valacyclovir (doses of> 500 mg twice daily), or famciclovir (doses> 500 mg/day) for varicella zoster virus (VZV)/herpes simplex virus prophylaxis; limited treatment courses at higher doses for VZV infections are permissible if treatment duration does not exceed 14 days total. Short courses of IV cidofovir for adenovirus (ADV) are permissible.
6. Suspected or known hypersensitivity to active or inactive ingredients of LTV formulations
7. Patients treated with a medication whose administration with LTV is contraindicated and whose discontinuation is not possible. Contraindicated medications include pimozide, ergot alkaloids and pitavastatin or simvastatin when co-administered with cyclosporine.
8. Imminent demise (expected survival< 6 weeks)
9. Documented positive result for human immunodeficiency virus antibody (HIV-Ab) or for hepatitis C virus antibody (HGV-Ab) with detectable HGV RNA, or hepatitis B surface antigen (HBsAg) at any time prior to HGT
10. Need for mechanical ventilation and/or vasopressor support at the time of enrollment
11. Pregnancy or breastfeeding
12. Plans to conceive or father children within the projected duration of the trial
13. History or current evidence of any condition, therapy, lab abnormality, or other circumstance that might confound the results of the study, interfere with the subject's participation for the full duration of the study, or would place the subject at undue risk as judged by the investigator, such that it is not in the best interest of the subject to participate in this study.
14. The following antivirals are allowed up to the listed dose limits:
    - Acyclovir up to 800 mg twice daily

- Valacyclovir up to 500 mg twice daily
- Famciclovir up to 500 mg/day for VZV/HSV prophylaxis; limited treatment courses at higher doses for VZV infections are permissible if treatment duration does not exceed 14 days total.
- Short courses of IV cidofovir for ADV (up to two doses)

Exclusion criteria for observational cohort:

1. Clinically significant CMV infection during the 100 days following HCT. Clinically significant CMV infection defined as either CMV viremia requiring preemptive therapy with CMV antivirals or CMV end organ disease (EOD)
2. Grade 3-4 GVHD
3. Cord blood as cell source for HCT
4. Treatment with systemic steroids (>0.5mg/kg for 2 weeks or longer) within 3 weeks prior to enrollment

7.0 RECRUITMENT PLAN

Eligible patients will be identified at the weekly BMT (Bone Marrow Transplantation) service meeting or referred by the BMT team or the infectious disease service. Eligibility will be confirmed by the investigator and the research team.

The principal investigator may also screen the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling in the study. During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at enrolling institution in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes. In most cases, the initial contact with the prospective subject will be conducted either by the treatment team, investigator or the research staff working in consultation with the treatment team.

The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened, and minimal PHI will be maintained as part of a screening log. For these reasons, we seek a (partial) limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) conversing with patients regarding possible

enrollment; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

The clinical trial will be listed on clinicaltrials.gov. All eligible patients will be approached for participation in the study. Patients will not be paid for participation in the study. All visits for the study will occur during visits for routine clinical care. No travel reimbursement will be provided for study visits. Patients are free to withdraw from the study without consequence at any time.

We expect to reach our accrual goal within 18 months.

8.0 PRETREATMENT EVALUATION

All evaluations will follow standards of care for HCT recipients at the enrolling institution. All aspects of the screening evaluation must be completed prior to entering the study:

- Two consecutive CMV tests must confirm low CMV viral load, or one CMV test must show undetectable CMV viral load providing the prior measurements showed a consistent decline in viral load. The last specimen must be collected::5 7 days prior to Day 1. Routine CMV testing will be performed at each enrolling Institution using a quantitative PCR assay in plasma (CobasAmpliprep/CobasTaqman (CAP/CTM) CMV, Roche Molecular Diagnostics, NJ, USA). The lower limit of quantification (LLOQ) and linear range (range) are 91 IU/ml and >137-9.1x10^6 IU/ml, respectively.
- Complete medical history will be taken, focusing on:
    - HCT variables
    - GVHD: grade, site(s), treatment
    - CMV infection: viremia, EOD, antivirals
    - Use of growth factors
    - Transfusions
    - Kidney and liver function
    - Pre-HCT infection with HIV, hepatitis C virus and hepatitis B virus
      Review of concomitant medications

For the observational cohort screening will include confirmation that no clinically significant CMV infection was present since HGT and at least one documented test showing T cell count of 2:100 cells/mcl taken no more than 1 week prior to enrollment.

9.0 TREATMENT/INTERVENTION PLAN

9.1 Study drugs

Treatment will be administered on an outpatient/inpatient basis. Patients will receive oral LTV 480mg daily (240mg daily for patients on cyclosporine). In patients with conditions affecting gastrointestinal absorption or who are unable to take oral medications, LTV will be administered Intravenously at the same doses until those conditions resolve. Patients

receiving oral medication will be administered a pill diary for drug compliance purposes. This will be administered and reconciled in clinic.

9.2 Duration of therapy and follow-up

The maximum duration of LTV administration will be 14 weeks. LTV will be discontinued for clinically significant CMV infection or toxicity. Up to 2 interruptions for a maximum combined interruption of 14 days are permitted during the study. Patients are allowed to restart LTV if toxicity is not deemed to be related to LTV.

All patients will be followed for a total of 24 weeks from start of LTV.

9.3 Concomitant medications

Only the following concomitant medications will be captured from the baseline visit until the end of study:

- CMV antiviral medications, including but not limited to:
  - Ganciclovir
  - Valganciclovir
  - Foscarnet
  - Cidofovir
- Immunosuppressants used for the treatment or prevention of GVHD, including but not limited to:
  - Tacrolimus
  - Mycophenolate mofetil (MMF)
  - Steroids

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Study Calendar

The schedule of visits and assessments for each visit is summarized in Table 4.

Table 4: Schedule of visits and assessments for each visit.

	Screening	Baseline	Treatment	Follow-up	End of study
	Day-30 to Day-1	Day 0	Weeks 2, 4, 6,8, 10, 14	Weeks 16 and 20	Week 24
Informed consent					
Medical history		+			
CMV monitoring[2]		+	+	+	+
CMV EOD assessment[4]			+	+	+
Documentation of LTV administration		+	+		
Laboratory evaluations[5]	+		+	+	+

Documentation of healthcare utilization[6]			+	+	+
Pill Diary Administration		+	+		
Pill Diary Reconciliation			+		
Documentation of invasive infections[7]and use of hematopoietic growth factors[8]		+	+	+	+
Collection of blood specimens for drug resistance testing[9]		IfCMV viremia detected	IfCMV viremia detected	IfCMV viremia detected	IfCMV viremia detected

Week refers to week of study, where the start of LTV administration is Day 0.

1. Screening medical history will assess eligibility as described in Section 8, Pretreatment evaluation. All clinically significant CMV infection(s) and/or disease prior to enrollment will be recorded; including dates, types and doses of antiCMV antivirals and all available CMV viral loads from the onset of clinically significant infection until enrollment. If probable/ definite CMV EOD is present, site, date of diagnosis and method of diagnosis will be captured.
2. CMV testing will be performed locally or University of Minessota. CMV will be monitored per local standard of care (typically weekly during treatment and at least every 2 weeks during follow up).
3. Two CMV tests of samples collected at least 48 h apart, with the last specimen collected :,; 7 days prior to Day 1.
4. CMV EOD will be diagnosed according to standard criteria (Ljungman Pet al. 2017). If patient symptoms and/or laboratory evaluations indicate potential EOD, appropriate diagnostic procedures will be performed as per standards of care at the enrolling institution (see 12.0 Criteria for therapeutic response for criteria to suspect each type of CMV EOD). Laboratory evaluations include complete blood count, basic metabolic profile, and hepatic function per local standards of care.
5. Laboratory evaluations include complete blood count, basic metabolic profile, and hepatic function per standards of care post HCT.
6. Health care utilization includes invasive procedures and hospital admissions. For hospital admissions, reason(s) (due to CMV versus other causes) and length of stay (LOS) will be recorded.
7. Bacterial, fungal or other viral infection:
   Bacterial infections include bloodstream infections, microbiologically or clinically documented bacterial Lower respiratory tract infection (LRTI) or organ/space infection. Fungal infections include bloodstream infections or invasive mold infections. Viral infections include LRTI by respiratory viruses or disseminated infection and/or end organ disease requiring therapeutic intervention and or hospitalization
8. Granulocyte/macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) with start and stop dates.
9. CMV resistance testing (Viracor, Eurofins) is advised for CMV viral load >500 IU/ml.

10.1.2 Study calendar for observational cohort: (D: days post HCT)

Procedure	D50-100 screening	D100 CMV CMI testing	D100-180 Follow up	D180 Repeat CMI testing
Eligibility screening	+			
Informed consent		+		
Assessment for non-clinically significant and clinically significant CMV infection			+	
Collection of blood specimens for CMI assessment		+		

Footnotes:

1. Repeat testing for CMV-CMI at D180 will only be done for patients with negative CMI on D100. We estimate approximately 40% cases to be retested on D180.

10.2 Required Laboratory Parameters for Treatment

Patients' laboratory evaluations must meet the following criteria for treatment to continue:

- Creatinine clearance (Cler) > 10 ml/min OR glomerular filtration rate (GFR) > 10 ml/min/1.73m^2

11.0 TOXICITIES/SIDE EFFECTS

11.1 Risks associated with letermovir

Only those events which are deemed to be at least possibly related to letermovir will be documented and attributed. The PI or Co-PI will attribute the relationship between the event and LTV. AEs will be graded per CTCAE v.5. In clinical trials conducted to date, LTV has not been associated with greater risk for adverse events, compared with placebo, when used in patients post-HSCT (see Section 5.3, Safety, Table 1). Similar proportions of subjects in

each group discontinued study medication due to an adverse event (13% of LTV subjects vs. 12% of placebo subjects).

11.2 Criteria for study drug discontinuation

Study drug will be discontinued if clinically indicated for treatment-emergent adverse events that are assessed by the physician investigator as possibly or probably related to study therapy.

11.3 Dose delays

Dosing interruptions (e.g. in the case of moderate, potentially treatment-related adverse effects such as nausea or vomiting) of up to 2 interruptions of maximum of 14 days in total during the 14-week treatment are allowed.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary outcome is the rate of breakthrough clinically significant CMV infection by week 14. Clinically significant CMV infection is defined as either CMV viremia requiring preemptive therapy with CMV antivirals [(val)ganciclovir or foscarnet or other anti-CMV antiviral] or CMV end organ disease (EOD). Patients will be monitored for CMV viremia weekly throughout treatment (Section 10, Evaluation during treatment). CMV EOD will be defined and scored according to the recommendations of the Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum (Ljungman Pet al. 2017; Appendix 3). The site(s) and date of diagnosis of CMV EOD will be recorded. CMV viral load threshold for PET initiation will be at the discretion of primary caregiver in a risk adaptive approach. In general. a single CMV viral load of >500 IU/ml or two consecutive viral loads >300 IU/ml with second value greater than the first is used as guidance for initiation of preemptive therapy.

13.0 CRITERIA FOR REMOVAL FROM STUDY

In accordance with the Declaration of Helsinki, ICH Good Clinical Practice Guidelines, and the US FDA Regulations, a patient has the right to withdraw from the study at any time for any reason without prejudice to his/her future medical care by the physician or at the institution. The Investigator also has the right to withdraw patients from the study (see below). A complete end-of-study evaluation will be made at the time of the patient's withdrawal with an explanation of why the patient is withdrawing, and an attempt should be made to perform a follow-up evaluation. Patients may be removed from the study by the treating clinician or Investigator for any of the following reasons:

- Significant noncompliance on the part of the patient
- Refusal of the patient to continue treatment or observations
- Unacceptable toxicity (AE at least probably related to LTV)
- Pregnancy
- Decision by the Investigator

14.0 BIOSTATISTICS

Primary Objective: This is a single-center open-label trial of LTV for prevention of recurrent CMV infection in patients undergoing HCT at MSK and participating sites. Patients who receive 3 doses of LTV are considered evaluable for the primary endpoint, which is the rate of clinically significant CMV infection through week 14. Patients who discontinue letermovir after receiving <3 doses for any reason will be replaced.

A total of 36 evaluable patients will be included in the primary endpoint analysis. If left untreated, the cumulative incidence of clinically significant CMV infection in this population is estimated to be 40% (Boeckh M et al. 2015). Therefore, LTV will be considered promising if the incidence decreases to 20%. Using these rates, a single stage exact design will be employed, and 36 evaluable patients will accrue. If at least 26 out of 36 patients are CMV-free at 14 weeks, LTV will be considered promising in this population. The type I and type II errors will be set at 0.10.

Patients who die or relapse while on prophylaxis remain evaluable for the primary endpoint as long as they receive at least 3 doses of LTV. To align with the cumulative incidence estimate, which treats death and relapse without CMV infection as competing risks, patients who die or relapse without CMV infection will be treated as "no CMV infection" for the primary analysis. Overall survival and non-relapsed mortality for the study population will be evaluated as secondary objectives.

The study does not include an interim analysis. Letermovir was approved by the FDA in November 2017. The drug has now been widely used at our Center on and off label including for secondary prophylaxis with encouraging results. This study will prospectively treat patients and estimate the cumulative incidence of CMV infection during letermovir secondary prophylaxis. If an interim analysis were included and accrual to this study were held, patients would instead receive the study drug off-protocol. Therefore, we feel it is a better approach to consecutively accrue 36 patients on this study, and not hold accrual for an interim analysis.

We will implement a stopping rule for futility. We will stop accrual in case 10 patients reach the primary outcome and develop breakthrough clinically significant CMV infection.

We anticipate accrual of approximately 2-3 patients per month and hence accrual should be completed in 18 months.

The secondary objectives of this study:

1. The cumulative incidence of clinically significant CMV infection at 14 weeks will be estimated separately for patients who have undetectable CMV and detectable CMV at baseline. Death and relapse are considered competing risks for this analysis.

2. The cumulative incidence of clinically significant CMV infection at 24 weeks will be estimated. Death and relapse are considered competing risks for this analysis.

3. Among patients who develop CMV viremia or EOD, the median time to onset of CMV viremia or EOD will be summarized

4. Descriptive statistics will be used to summarize the number of well days through week 14 (well days: defined as days free of CMV viremia, CMV EOD, CMV antivirals, alive, and out of hospital).

5. Summary statistics will be used to describe the type and duration of CMV antivirals during the study.

6. The number of hospital readmissions will be summarized through week 24. The reason for the readmission along with the duration of stay will also be summarized.

7. Rate and types of invasive procedures (endoscopy, bronchoscopy, etc.) during through week 24 will be summarized.

8. Overall survival at 24 weeks will be estimated using Kaplan-Meier methods. The cumulative incidence of non-relapse mortality rate will be estimated at week 24; relapse is considered a competing risk.
   The proportion of patients who develop LTV resistance will be estimated through week 24. The corresponding exact 95% confidence interval will also be reported.

For the observational cohort: Descriptive statistics of absolute lymphocyte count and lymphocyte subsets (CD4 and CD8) on 0100 will be reported. The proportion of patients who are positive CMI (+) on 0100 will be reported along with an 95% confidence interval. With 50 patients, the precision in which we can estimate the proportion of patients who are CMI positive is+/- 0.14. The odds ratio between CMI results on Day100 (positive/negative) and non-clinically significant CMV viremia (yes/no) in the study cohort will be estimated along with a 95% confidence interval. CMI at Day 100 will be associated with CMV infection after day 100 using cumulative incidence functions and Grays test. The incidence of CMV infection based on categorizes of absolute lymphocyte count and lymphocyte subsets (CD4 and CD8) will be similarly estimated and compared. P values lower than .05 will be considered statistically significant. To identify predictors of CMI responses on 0100, demographics baseline characteristics and presence of non-clinically significant CMV-viremia prior to 0100 will be examined in in multivariate models.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.2 Randomization

This is a single arm, open- label study. There is no randomization.

16.0 DATA MANAGEMENT ISSUES

This is a multi-institution study. All patients will be treated at Memorial Sloan Kettering Cancer Center and participating enrolling sites.

Laboratory data will be tabulated and summarized based on MSK normal ranges.

Data collected for this study will be entered in Medidata.

SAEs will be reported as described in section 17.2. AEs, including evidence of the relationship between the AE and study drug, will be documented in the patient's medical records **only if the event is considered at least possibly related to LTV.** If an event is reported as at least possibly related, the PI or Co-PI will review the patient's medical records and will grade the relationship between the event and LTV on a separate document. Any event that began before LTV therapy is initiated will be considered as medical history and will only be captured as an AE if the event worsens *after* LTV treatment is initiated and is deemed to be at least possible related to LTV. AEs will be recorded from LTV treatment initiation through 24 hours after the last dose.

Data will be transferred to Dr. Anat Stern at Rambam Medical Center. This will be a limited data set that do not include PHI identifiers. This is transferred under a Data Transfer Agreement between Memorial Sloan Kettering Cancer Center and Rambam Medical Center. Data will be provided deidentified only via the Secure File Transfer System. Data will be provided at the end of the study.

Viracor Eurofins will be performing analyses on samples collected from patients on the Observational Arm of the protocol following the institutional standard of care testing processes for CMV T Cell Immunity Panel. Only MSK will be collecting specimen and sending it to Viractor-Eurofins for analysis. Viracor Eurofins will receive PHI (name, DOB, MRN) as the test is approved for NYS; it is performed as institutional standard of care and results are available to the clinicians. Data will be provided when they result each sample throughout the study. The contact at Viracor Eurofins will be Tiffany Driver.

The University of Minnesota will be conducting the Therapeutic portion of the trial. Data will be collected and entered into the protocol's iMedidata database contemporaneous to the visits. The site PI of the University of Minnesota will be Dr. Jo-Anne Young.

16.1 Quality Assurance

Registration reports will be generated on a regular basis to monitor patient accrual and completeness of the registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Memorial Sloan Kettering Cancer Center (MSK) has established standard procedures for data safety monitoring of clinical research (see 16.2 Data and Safety Monitoring).

16.2 Data and Safety Monitoring

The data and safety monitoring (DSM) plans at MSKCC were approved by the National Cancer Institute in September 2001. The plans address the policies set forth by the NCI in the document entitled *Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials,* which can be found at http://cancertrials.nci.nih.gov/clinicaltrials. The DSM plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC DSM plans can be found on the MSKCC Intranet at: http://mskweb5.mskcc.org/intranet/html/70775.cfm.

There are several different mechanisms by which clinical trials are monitored for data, safety, and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research quality assurance) and departmental procedures for quality control, and there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the MSKCC Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

This protocol will be monitored by the MSK DSMB.

16.3 Regulatory Documentation

Prior to implementing this protocol at MSK, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MSK Institutional Review Board/Privacy Board (IRB/PB). There will be one protocol document and each participating site will utilize that document.

Participating sites that are conducting specimen analysis must submit the following documents to MSK before specimens can be shipped to the site:

- Participating Site 1572
- Conflict of Interest forms for Participating Site Investigators on the 1572

Participating sites that are conducting data and/or specimen analysis should submit this protocol to their IRB according to local guidelines. Copies of any site IRB correspondence should be forwarded to MSK.

17.0 PROTECTION OF HUMAN SUBJECTS

Consent process: Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to MSK IRB guidelines.
Risks: Risks associated with participation in this study will be explained to the patient as part of the ongoing consent discussion.

Benefits: LTV is effective in preventing CMV in HCT recipients. LTV, compared with other antivirals is associated with a lower frequency of adverse events including bone marrow suppression and nephrotoxicity.

Protocol Amendments and Study Termination: All protocol amendments will be reviewed and approved by MSK's Institutional Review Board before implementation.

Incentives: No incentives will be offered to patients/subjects for participation in this study. Participation is voluntary.

Costs: LTV will be provided by Merck. Participation in this study will incur no direct costs to patients for care.

Eligibility Exceptions: There will be no exceptions to the eligibility requirements for this protocol without the authorization of MSK Institutional Review Board.

Adverse Event Reporting Requirements: AEs will be graded per CTCAE v.5. The PI or Co-PI will attribute the relationship between the event and LTV. Only those events which are deemed to be at least possibly related to letermovir will be documented and attributed. Any event that began before LTV therapy is initiated will be considered as medical history and will only be captured as an AE if the event worsens after LTV treatment is initiated. AEs will be recorded from LTV treatment initiation through 1 day after the last dose.

Inclusion of Children in Research: LTV is currently FDA-approved for treatment in patients >18 years. Adolescent patients (ages 12 to 18 years) will be included in this study because LTV is expected to be safe and of potential benefit in HCT recipients of this age group. The protocol does not include children younger than 12 years old. **Inclusion of women and minorities:** Memorial Sloan Kettering Cancer Center has filed form HHS 441 (re: Civil Rights), form HHS 641 (handicapped Individuals), and form 639-A (re: Sex Discrimination). In selecting patients for this study, we have taken due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research. We expect that the study population will be fully representative of the range of patients seen at MSK without exclusion as to age, gender, or ethnic background.

Alternatives to the Planned Study: Alternative treatment options include receiving standard care and follow-up. If relevant, other investigational options will also be outlined.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential. Patient's names or any other personally identifying information will not be used.

17.1 Privacy

MSK's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health

information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with other qualified researchers.

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.
Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

For IND/IDE protocols:

The SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the IND Office

17.2.1 External SAE Reporting

Please refer to the MSK Multicenter Trial Addendum for multicenter SAE reporting. All required SAE reporting to the funders and/or drug suppliers will be completed by MSK only.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an !RB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study
2. The length of study and the likely follow-up required
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time
6. The consent indicates that individualized de identified information collected for the purpose of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with other qualified researchers.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research-specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Appendix 1: Potentially Significant Drug Interactions of LTV

Concomitant Drug Class and/or Clearance Pathway: Drug Name	**Effect on Concentrationt**	**Clinical Comments**
Anti-arrhythmic agents		
Amiodarone	j 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 LTV.
Anticoagulants		
Warfarin	t warfarin	When LTV is co-administered with warfarin, frequently monitor international normalized ratio **(INR)§.**
Anticonvulsants		
Phenytoin	t phenytoin	When LTV is co-administered with phenytoin, frequently monitor phenytoin concentrations§.
Antidiabetic agents		
Examples: glyburide, repaglinide, rosiglitazone	j glyburide j repaglinide j rosiglitazone	When LTV is co-administered with glyburide, repaglinide, or rosiglitazone, frequently monitor glucose concentrations§. When LTV is co-administered with cyclosporine, use of repaglinide is not recommended.
Antifungals		
Voriconazole+	t voriconazole	If concomitant administration of voriconazole is necessary, closely monitor for reduced effectiveness of voriconazole§.
Antimycobacterial		
rifampin	t letermovir	Co-administration of LTV and rifampin is not recommended
Antipsychotics		
Pimozide	j pimozide	Co-administration is contraindicated due to risk of QT prolongation and torsades de pointes
Ergot alkaloids		
Ergotamine, dihydroergotamine	j ergotamine, dihydroergotamine	Co-administration is contraindicated due to risk of ergotism
HMG-CoA Reductase Inhibitors		

Atorvastatin:f:	↓ atorvastatin	When LTV is co-administered with atorvastatin, do not exceed an atorvastatin dosage of 20 mg daily§. Closely monitor patients for myopathy and rhabdomyolysis. When LTV is co-administered with cyclosporine, use of atorvastatin is not recommended.
Pitavastatin, simvastatin	↓ HMG-CoA reductase inhibitors	Co-administration of LTV and pitavastatin or simvastatin is not recommended. When LTV 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 LTV is co-administered with these statins, a statin dosage reduction may be necessary§. Closely monitor patients for myopathy and rhabdomyolysis. When LTV is co-administered with cyclosporine, use of lovastatin is not recommended. When LTV is co-administered with cyclosporine, refer to the statin prescribing information for specific statin dosing recommendations.
Immunosuppressants		
Cyclosporine:t:	↓ cyclosporine ↓ letermovir	Decrease the dosage of LTV to 240 mg once daily. Frequently monitor cyclosporine whole blood concentrations during treatment and after discontinuation of LTV and adjust the dose of cyclosporine accordingly§.
Sirolimus:f:	↓ sirolimus	When LTV is co-administered with sirolimus, frequently monitor sirolimus whole blood concentrations during treatment and after discontinuation of LTV and adjust the dose of sirolimus accordingly§. When LTV is co-administered with cyclosporine and sirolimus, refer to the sirolimus prescribing information for specific sirolimus dosing recommendations§.
Tacrolimus:f:	↓ tacrolimus	Frequently monitor tacrolimus whole blood concentrations during treatment and after discontinuation of LTV and

		adjust the dose of tacrolimus accordingly§.
Proton pump inhibitors		
Omeprazole	Jomeprazole	Clinical monitoring and dose adjustment may be needed.
Pantoprazole	t pantoprazole	Clinical monitoring and dose adjustment may be needed.
CYP3A Substrates		
Examples: alfentanil, fentanyl, midazolam, and quinidine	t CYP3A substrate	When LTV is co-administered with a CYP3A substrate, refer to the prescribing information for dosing of the CYP3A substrate with a moderate CYP3A inhibitor§. When LTV 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. t t =decrease, t=increase + These interactions have been studied. § Refer to the respective prescribing information.		

Appendix 2: Absorption, Distribution, Metabolism, Elimination (ADME), and Pharmacokinetic Properties of LTV*

Pharmacokinetics in HCT Recipients		
Treatment regimen	Steady-state median AUC (ng•hr/ml)	90% prediction interval
480 mg oral 1x daily	34,400	16,900-73,700
480 mg IV 1x daily	100,000	65,300-148,000
240 mg oral 1x daily with cyclosporine	60,800	28,700-122,000
240 mg IV 1x daily with cyclosporine	70,300	46,200-106,000

Pharmacokinetics in Healthy Subjects				
Treatment regimen	Steady-state geometric mean AUC(ng•hr/ml)	Cmax (ng/ml)	Accumulation ratiot	Time to steady-state (days)
480 mg oral 1x daily	71,500	13,000	Cmax: 1.03 AUC: 1.22	9-10

Dose proportionality: Greater than proportional following single and multiple oral or IV doses of LTV 240 mg and 480 mg

Absorption		
Subjects	Treatment regimen	Bioavailability
Healthy subjects	Oral dose range of 240 mg to 480 mg	94%
HCT recipients	480 mg oral once daily	35%
HCT recipients	240 mg oral once daily with cyclosporine	85%
Median Tmax	45 min to 2.25 h	
Effect of food (relative to fasting) :t:	AUC: 99.63% (84.27% - 117.80%) Cmax: 129.82% (104.35% -161.50%)	

Distribution		
Indicator	*Value*	*Notes*
Mean steady-state volume of distribution	45.5 L	Following IV administration in HCT recipients
In vitro binding to human plasma proteins	99%	Concentration range: 0.2 to 50 mg/L
In vitro blood-to plasma ratio	0.56	Concentration range: 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)

Elimination	
Route of elimination	Hepatic uptake (OATP1B1/3)
Mean terminal t1/2	12 h after dosing of LTV 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.

t Based on geometric mean data.

:t: 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.

Appendix 3: Definitions of CMV end organ disease according to the Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum. Reproduced from Ljungman P, et al. Clin Infect Dis 2017.

CMV Pneumonia

Proven disease requires clinical symptoms and/or signs of pneumonia such as new infiltrates on imaging, hypoxia, tachypnea, and/or dyspnea combined with CMV documented in lung tissue by virus isolation, rapid culture, histopathology, immunohistochemistry, or DNA hybridization techniques.

Probable CMV pneumonia is defined as the detection of CMV by viral isolation, rapid culture of BAL fluid, or the quantitation of CMV DNA in BAL fluid combined with clinical symptoms and/or signs of pneumonia. A definite cut-off for CMV DNA load cannot be established at the present time. The cut-*off* is likely to vary between different patients and according to how the BAL procedure and processing are performed and the assay used for CMV DNA quantitation. Furthermore, CMV DNA levels may vary considerably between patients with varying degrees of severity of CMV pneumonia, which may impact the predictive values of any cut-off. It should be recognized that CMV shedding in the lower respiratory tract does occur and therefore a low CMV DNA load might well represent asymptomatic infection [7]. The likelihood for CMV pneumonia increases with increasing DNA viral load. In one study in HSCT patients, CMV viral load >200-500 IU/mL in BAL fluid was likely (with a positive predictive value of approximately 50% based on disease prevalence figures of approximately 10% among patients at risk for CMV pneumonia undergoing BAL testing) to represent pneumonia in HSCT recipients (M. Boeckh, unpublished data), while lower levels were likely indicating pulmonary shedding. Data from lung transplant patients suggest that the viral load in BAL fluid in patients with CMV pneumonia is approximately 1.5 log10 higher than the viral load in patients with detectable CMV DNA in BAL fluid without evidence of CMV pneumonia (a cut-off of 5500 IU/mL had a sensitivity of 91% and a specificity of 75%) (Lodding et al, abstract, IDWeek 2015). On the other hand, a negative CMV DNA test in the BAL fluid has a negative predictive value close to 100% and therefore excludes the possibility of CMV pneumonia. The use of quantitative PCR on biopsies is an evolving field. Presently, these findings could be defined as possible CMV pneumonia.

CMV Gastrointestinal Disease

Proven disease requires upper and/or lower gastrointestinal (GI) symptoms plus macroscopic mucosal lesions plus CMV documented in tissue by histopathology, virus isolation, rapid culture, immunohistochemistry, or DNA hybridization techniques. Studies should give information regarding the presence or absence of gut graft-vs-host disease (GVHD) in HSCT recipients.

Probable GI disease requires upper and/or lower GI symptoms and CMV documented in tissue but without the requirement for macroscopic mucosal lesions. Studies should give information regarding the presence or absence of gut GVHD in HSCT recipients.

CMV documented in blood by NAT (eg, PCR) or antigenemia or CMV documented by PCR from tissue biopsies is not sufficient for the diagnosis of CMV GI disease. The use of quantitative PCR on gut biopsies is an evolving field. Presently, these findings could be defined as possible GI disease.

CMV Hepatitis

Proven disease requires abnormal liver function tests plus CMV documented in tissue by histopathology, immunohistochemistry, virus isolation, rapid culture, or DNA hybridization techniques plus the absence of other documented cause of hepatitis.

Probable disease is not a recommended category for CMV hepatitis. Due to the risk for other confounders such as acute and chronic allograft rejection in liver transplant recipients or GVHD in HSCT recipients, as well as the common occurrence of drug-associated liver dysfunction, a probable CMV hepatitis category is not defined.

CMV Retinitis

Proven disease requires typical ophthalmological signs judged by an ophthalmologist experienced with the diagnosis of CMV retinitis. If the presentation is atypical or an experienced ophthalmologist is not available, it is recommended that the diagnosis be supported by CMV documented in vitreous fluid by NAT (such as PCR). A probable disease category should not be used.

CMV Encephalitis and Ventriculitis

Proven disease requires central nervous system (CNS) symptoms plus detection of CMV in CNS tissue by virus isolation, rapid culture, immunohistochemical analysis, in situ hybridization, or (preferably) quantitative PCR.

Probable disease requires CNS symptoms plus detection of CMV in CSF without visible contamination of blood ("bloody tap") plus abnormal imaging results or evidence of encephalitis on electroencephalography.

Nephritis

Proven disease is defined by the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization in a kidney allograft biopsy specimen obtained from a patient with renal dysfunction together with the identification of histologic features of CMV infection. The detection of CMV in urine by PCR or culture is not sufficient for the diagnosis of CMV nephritis as asymptomatic viral shedding in urine is common.

Cystitis

Proven disease is defined by the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization in a bladder biopsy specimen obtained from a patient with cystitis together with the identification of conventional histologic features of CMV infection. The detection of CMV in urine by PCR or culture is not sufficient for the diagnosis of CMV cystitis as asymptomatic viral shedding in urine is common.

Myocarditis

Proven disease is defined by the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization in a heart biopsy specimen obtained from a patient with myocarditis together with the identification of conventional histologic features of CMV infection.

Pancreatitis

Proven disease is defined as the detection of CMV by virus isolation, rapid culture, immunohistochemical analysis, or in situ hybridization in a pancreatic biopsy specimen obtained from a patient with pancreatitis together with the identification of conventional histologic features of CMV infection.

Other End-Organ Disease Categories

CMV can also cause disease in other organs, and the definitions of these additional disease categories include the presence of compatible symptoms and signs and documentation of CMV by biopsy by virus isolation, rapid culture, immunhistochemistry, or DNA hybridization in biopsy material.