Prophylaxis versus Preemptive Therapy for the Prevention of CMV in High-Risk R-D+ Liver Transplant Recipients

[CMV Antiviral Prevention Strategies

In D+R- Liver Transplants ("CAPSIL")]

DMID Protocol Number: 11-0073

DMID Funding Mechanism: Contract # HHSN272201100041C

IND status:

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Version Number: 4.0

January 21, 2016

STATEMENT OF COMPLIANCE

Each investigator must adhere to the protocol as detailed in this document. Each investigator will be responsible for enrolling only those study participants who have met protocol eligibility criteria. This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP) and the following applicable regulatory requirements:

U.S. Code of Federal Regulators applicable to clinical studies (45 CFR 46 and 21 CFR including parts 50 and 56 concerning informed consent and IRB regulations; and if under IND, 21 CFR 312)."

Completion of Human Subjects Protection Training
Refer to:

Http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html
Http://cme.cancer.gov/clinicaltrials/learning/humanparticipant-protections.asp

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 ICHE6 guidelines.

Site Investigator:

Signed: _____ Date: _____

Name
Title

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LIST OF ABREVIATIONS

AE	Adverse Event/Adverse Experience
ANC	Absolute Neutrophil Count
bid	Twice a day
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CMV	Cytomegalovirus
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CrCl	Creatinine Clearance
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EPC	End-Point Committee
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
HCT	Hematocrit
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HSV	Herpes Simplex Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IFN	Interferon
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
MedDRA ®	Medical Dictionary for Regulatory Activities

mg	Milligram
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PET	Preemptive Therapy
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
po	Per os (Latin for 'taken by mouth')
QA	Quality Assurance
QALY	Quality Adjusted Life Years
QC	Quality Control
Qd	One a day
RRT	Renal replacement therapy
SAE	Serious Adverse Event/Serious Adverse Experience
SOC	Standard of Care
SOT	Solid organ transplant
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
SPICE	Simplified Presentation of Incredibly Complex Evaluations
US	United States
WHO	World Health Organization

PROTOCOL SUMMARY

Title: Prophylaxis versus Preemptive Therapy for Prevention of CMV in High-Risk R-/D+ Liver Transplant Recipients ['CAPSIL' Study]

Population: CMV seronegative recipients (18 years of age or older) of a liver transplant from a CMV seropositive donor (R-/D+)

Phase: IV

Number of Clinical Sites: 6

Study Duration: 7 years

Subject Participation Duration: Until the closure of the study and not to exceed 7 years from enrollment.

Description of Agent or Intervention: Oral Valganciclovir hydrochloride: 2-[(2-amino-6-oxo-6, 9-dihydro-3H-purin-9-yl) methoxy]-3-hydroxypropyl (2S)-2-amino-3-methylbutanoate. Currently marketed as Valcyte ®.

Objectives:

Primary objective

The primary objective is to compare prophylaxis versus preemptive therapy using valganciclovir for the prevention of CMV disease in R-/D+ liver transplant recipients

Secondary objectives :To assess the two preventive strategies for:

- Clinical outcomes (major bacterial, fungal and non-CMV viral infections, rejection, graft loss and mortality)
- Hematologic toxicity (assessment of neutropenia and receipt of hematopoietic growth factor during study days 1-107)

Exploratory objectives :To assess the two preventive strategies for:

- The development of CMV-specific immunity following transplantation
- Cost effectiveness of the regimens

Study Outcome Measures

Primary outcome measure: The primary outcome measure is the incidence of CMV disease by 12 months post-transplant.

Secondary outcome measures (compared between 2 groups)

1. Clinical outcomes within 12 months post-transplant

- Incidence of late-onset CMV disease (CMV disease occurring after 100 days post-randomization)

- Incidence of major bacterial, fungal, and non-CMV viral infections
- Incidence of allograft rejection
- Incidence of graft loss (retransplantation)
- All-cause mortality

2. Hematologic toxicity (assessed until 107 days post-enrollment)
   - Incidence of neutropenia (ANC <1,000 and <500/μL)
   - Receipt of hematopoietic growth factor during study days 1-107)

Exploratory end-points (compared between 2 groups)

1. Clinical outcomes
   - Time to onset of CMV disease (disease free survival) through 12 months post-transplant
   - Incidence of graft loss (retransplantation) assessed until study closure
   - All-cause mortality assessed until study closure.
   - Incidence of post-transplant malignancies
   - Renal insufficiency requiring renal replacement therapy
   - Incidence of opportunistic infections through study closure

2. Hematopoietic growth factor (granulocyte colony stimulating factor) for neutropenia
   - Frequency of use
   - Number of dosages of growth factor required

Immunologic endpoints:

- CMV-specific immunity at 100 days, 6 and 12 months post-transplant
  a. CMV-specific T-cell responses
  b. CMV-specific neutralizing antibody responses

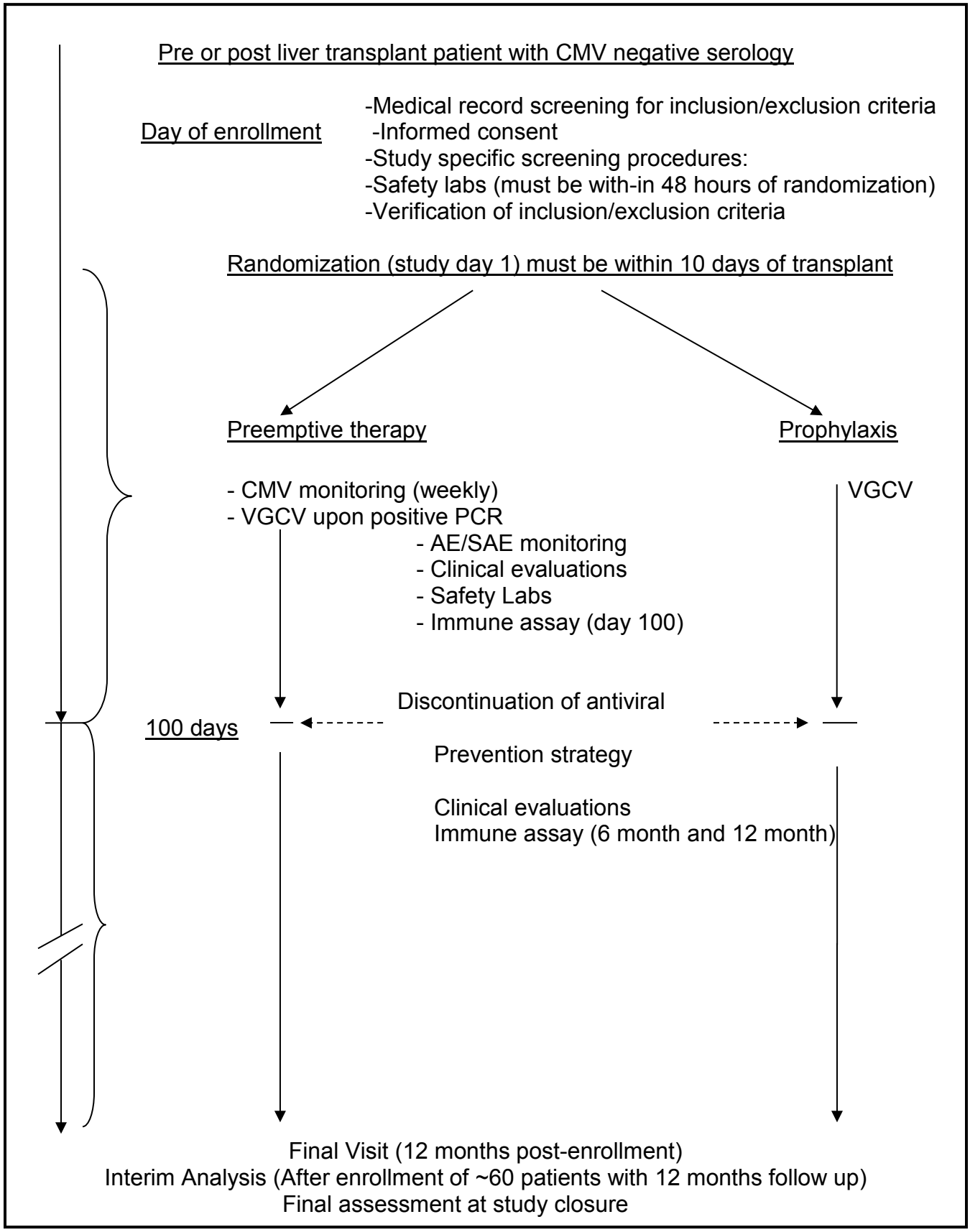
3. Cost effectiveness of the 2 approaches:
   - Total costs for each regimen
   - Cost utility analysis (cost per quality adjusted life-years)

Study Design: This is a prospective, randomized, multicenter trial of preemptive therapy vs. prophylaxis for prevention of CMV disease in R-D+ liver transplant patients. Patients meeting study criteria and who have provided informed consent will be randomized within 10 days of transplant to receive in an open label design, either antiviral prophylaxis with valganciclovir 900 mg orally once daily or preemptive therapy (weekly monitoring for CMV viremia by plasma PCR) for 100 days post- randomization with initiation of oral valganciclovir 900mg orally twice daily at onset of CMV viremia and continued until plasma PCR is negative on two consecutive weekly PCR tests). Valganciclovir dosages will be adjusted for renal dysfunction. Study participants will be followed during the intervention period (100 days post randomization) and until 12 months post-transplant for CMV disease, toxicity, and clinical outcomes (opportunistic infections, rejection, graft loss and mortality). All-cause mortality, rejection episodes, graft loss and retransplantation, opportunistic infections , need for dialysis, and post-transplant malignancies will be assessed at one year and until study closure (no longer than 7 years from enrollment

maximum). Additionally, the impact of the two CMV prevention strategies on CMV-specific cellular and humoral immune responses will be evaluated at 100 days after randomization, and 6 and 12 months post-transplant. A minimum of 176 subjects will be enrolled in the study. Allowing for over-enrollment for dropouts, withdrawals and deaths prior to 6 months, up to 205 subjects may be enrolled to achieve the target enrollment with one year follow up.

Estimated Time to Complete Enrollment: Approximately 3.5 years

Schematic of Study Design:



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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background

Cytomegalovirus (CMV) is a human herpes virus known to infect ~50-90% of the adults and is a major opportunistic pathogen in immunosuppressed populations such as transplant recipients. The term CMV infection indicates evidence of replicating virus as detected by laboratory testing in a blood sample (viremia). CMV disease on the other hand is defined by the presence of symptoms and signs attributable to this virus. CMV disease can manifest as viral syndrome with a flu-like illness or tissue invasive CMV disease and there are standardized criteria for their definitions in the literature (1, 2). Despite current preventive strategies, this virus continues to have a negative impact on outcomes in transplant recipients. The importance of preexisting immunity to CMV is evidenced by the substantially higher rates of infection and disease in subjects undergoing primary CMV infection (CMV naïve or seronegative recipients of CMV-seropositive allografts or R-/D+ recipients). In addition to overt disease, CMV-mediated immunomodulation can lead to indirect sequelae such as opportunistic infections, rejection, graft loss and reduced survival (3-5). Thus, strategies to optimally reduce the incidence and impact of CMV have the potential to significantly improve the outcome of transplantation.

2.2 Rationale

There are two major strategies for CMV prevention in clinical setting; prophylaxis and preemptive therapy(6). With the strategy of prophylaxis, antiviral drug is administered to all patients at any risk for CMV disease, usually for 100 days post-transplant. In preemptive therapy, antiviral drug is specifically targeted to patients deemed to be at highest risk for CMV disease, as determined by the detection of early viremia. Prompt initiation of antiviral therapy at the first detection of early viremia prevents progression to CMV disease (7-9). Each of the strategies has been studied individually and been shown to be effective for the prevention of CMV disease (compared to either placebo or no prevention strategy), but there is a striking paucity of direct comparative studies. The relative benefit of one strategy versus another has long been debated and there is strong support within the transplant community for a well-designed direct comparison of the two strategies for prevention of CMV disease in high-risk R-/D+ patients. This study will test the null hypothesis that the incidence of CMV disease in the prophylaxis intervention group is equal to the incidence in the preemptive therapy group. Defining the optimal strategy for the prevention of CMV is expected to have significant clinical benefit in the management of transplant recipients and to provide important insight into the immunologic mechanisms that are responsible for control of CMV in this setting.

Although the approach of preemptive therapy has been shown to be useful for prevention of CMV in transplant patients in previous studies, the totality of evidence supporting its use is more limited than for prophylaxis. As a result, prophylaxis with valganciclovir (given its high oral bioavailability and convenient once daily dosing) has emerged as the dominant strategy for the prevention of CMV in R-/D+ transplant patients (10, 11). However, late-onset CMV disease, defined as CMV disease occurring after 3 months post-transplant is now

recognized as a significant complication with the use of prophylaxis, particularly in R-/D+ patients (12-15). An evidence-based review showed that the incidence of CMV disease and specifically that of late-onset CMV disease in the valganciclovir era in these patients is substantially higher with prophylaxis than preemptive therapy (16). Overall, CMV disease has been documented in 2.6% of patients receiving preemptive therapy in the current era and in contrast to prophylaxis, it occurs largely within 100 days of transplantation (16). The frequency of CMV disease with prophylaxis and preemptive therapy based on studies in recent literature is summarized below. While, there are significant limitations of these data, including the fact that these are non-comparative studies and include heterogeneous transplant populations, there is a clear trend towards higher rates of CMV disease in patients who received prophylaxis compared to preemptive therapy.

CMV disease rates with antiviral prophylaxis for 3months

Study	Sample size	Type of transplant	Drug	R/D status	Follow up	CMV disease (%)
(9)	177	Liver	VGCV	R-/D+	12 mo.	19
(17)	29	Liver	VGCV	R-/D+	12 mo.	30
(12)	38	Liver	OGCV	R-/D+	12 mo.	26
(2)	163	Kidney	VGCV	R-/D+	12 mo.	36.8
(18)	158	Kidney-panc	VGCV	R-/D+	12 mo.	39
(19)	25	Kidney	VGCV	R-/D+	12 mo.	40
(13)	67	Liver	OGCV/VGCV	R-/D+	12 mo.	28
(14)	54	Liver	VGCV	R-/D+	19mo.	26
(15)	39	Kidney	OGCV	R-/D+	12 mo.	31

CMV disease rates with preemptive therapy

Study	Sample size	Type of transplant	Drug	R/D status	Follow up	CMV disease (%)
(20)	41	Liver	OGCV	R-/D+	4 mo.	0
(21)	22	Liver, Kidney	VGCV	R-/D+, R+	12 mo.	0
(22)	36	Liver	OGCV/ VGCV	R-/D+	20.4 pt/yr	0
(23)	42	Kidney, KP	VGCV	R-/D+	12 mo.	0
(24)	36	Liver	VGCV	R-/D+	62.8 pt/yr	0
(25)	11	Liver	VGCV	R-/D+	18 mo.	0
(26)	30	Liver	VGCV	All	12 mo.	0
(27)	36	Kidney	VGCV	R-/D+,R+	12mo.	5.5

In a recent evidence-based analysis of CMV prevention based on meta-analytic and multiple regression methodologies, 100 days of antiviral prophylaxis did not show superior efficacy for late-onset CMV disease and carried a significantly higher risk of neutropenia (28). An alternative approach that has been embraced by industry is to extend the duration of prophylaxis (2). Yet, prolonging the duration of prophylaxis is not entirely protective against late-onset CMV disease as evident by data showing CMV disease rate of 16% in the IMPACT study and 31-37% in other reports despite 6 months of prophylaxis (18, 19, 29). Ironically, it is plausible that shorter courses of antiviral agent implicit in preemptive therapy may be more effective for the long-term control of CMV since the lowest disease (30) rates reported with 6 months of prophylaxis are still substantially higher than those with preemptive therapy (2, 15).

There is compelling evidence to suggest that late-onset CMV disease is a significant contributor to poor outcomes after transplantation. CMV disease occurring after antiviral prophylaxis in liver transplant recipients was independently associated with higher overall mortality at 1 year (HR, 5.1, p=0.002) and even more strongly with bacterial and fungal infection-associated mortality (HR 11, p=0.002) (12). Furthermore, graft and patient survival in liver transplant recipients was significantly worse with late-onset compared to early-onset CMV(31). In R-/D+ kidney transplant recipients receiving prophylaxis, tissue-invasive disease was independently associated with allograft loss and mortality (32). Although not all studies have documented poor outcomes with late-onset disease, aforementioned data indeed suggest that late-onset CMV disease is not a benign occurrence.

Substantial evidence supports a role for CMV-specific immunity in the control of CMV in transplant patients (33-36) and there are several lines of evidence suggesting that a greater development of CMV-specific protective immune responses with preemptive therapy is the underlying mechanism for the relative protection against late-onset CMV disease with this approach. First, preemptive therapy, by its inherent design allows for controlled CMV replication to occur before an antiviral agent is administered. This antigen exposure is proposed to result in immune priming and hence enhancement of CMV-specific humoral and cellular immune responses that are critical for long-term control of the virus (37). In contrast, viral replication is almost completely suppressed with the use of antiviral prophylaxis (9) thereby preventing effective immune priming. Additionally, ganciclovir *per se* has an anti-proliferative effect on T cells (38). For example, at achievable serum concentrations, ganciclovir reduced T-cell proliferation by 50% and this effect was comparable to that of cyclosporine (38). There is however, precedence that delayed recovery of virus-specific host response is not unique with the receipt of prophylaxis for CMV, as this phenomenon has been observed with prolonged exposure to other nucleoside agents and for herpes viruses other than CMV (39, 40).

2.2.1 Proposed mechanism for protection against late-onset CMV with preemptive therapy compared to prophylaxis (preliminary observations)

To examine the hypothesis that preemptive therapy results in earlier and more robust CMV-specific immunity than prophylaxis, a prospective pilot study at the Universities of Washington and Pittsburgh was conducted in liver transplant patients. CMV-specific immune responses using multifunctional intracellular cytokine staining (ICS) were assessed at 3 months post-transplant in the following cohorts: preemptive therapy (R-/D+, n=16), prophylaxis (R-/D+, n=29), and an additional control group that received antiviral prophylaxis (R+, n=24). Although a precise quantitative immunologic correlate of protection against CMV disease in transplant patients has not been defined, virus-specific multifunctional cytokine-producing CD4 and CD8 T-cells have recently been shown to be functionally superior to mono-functional T-cells (41) and to be associated with clinical protection (42) in HIV-infected individuals. An association of multi-functionality with protection against CMV infection/disease has also been suggested in other studies (43, 44). There were a significantly higher proportion of patients with detectable monofunctional CMV-specific CD8 T-cells among the preemptive therapy group compared to prophylaxis; the proportion of responders in the preemptive therapy group was similar to the R+ positive control group (Table 1).

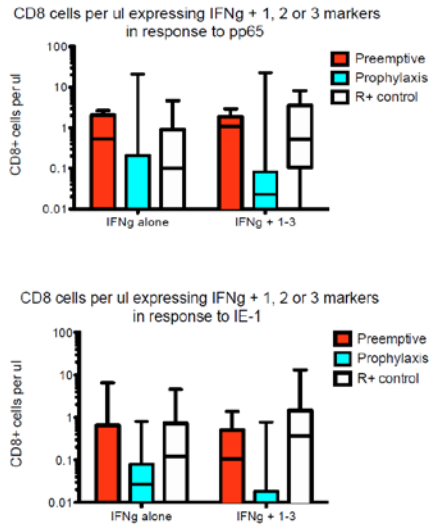
Table 1: Proportion of responders for CMV-specific CD8 lymphocytes producing IFN-γ according to the antiviral strategy

Assay	Stimulus	Preemptive (R-D+)	Prophylaxis (R-D+)	Control (R+)	P-value
CD8 IFN-γ	pp65 pepmix	8/15 (53%)	3/19 (16%)	7/20 (35%)	0.03
	SEB	14/15 (93%)	14/19 (93%)	20/20 (100%)	0.19

In addition, quantitative multifunctional intracellular cytokine analysis showed higher levels of mono- and polyfunctional CMV-specific IFN-γ-producing CD8 to both pp65 and IE-1 overlapping peptide pools in the preemptive therapy compared to prophylaxis group (Figure 1). Importantly, as for monofunctional CMV-specific CD8 T-cells, the levels among the preemptive therapy group were similar to those among the R+ positive control group.

These results suggest greater development of CMV-specific T-cell responses with preemptive therapy compared to prophylaxis. And similar to data from other studies summarized above, the incidence of late-onset of CMV disease in this cohort was significantly higher in the prophylaxis compared to preemptive therapy group (10/29 [34%] vs. 0/19 [0%]), respectively, p=0.0035). Multifunctional T cells were more common among subjects without CMV disease (Figure 2). However, since this was not a randomized controlled study, potential confounders (induction vs. no induction immunosuppression, intensity of maintenance immunosuppression etc.) might also explain, at least in part, these apparent differences, and thus there is a need to assess these parameters more definitively in the context of a randomized controlled trial.

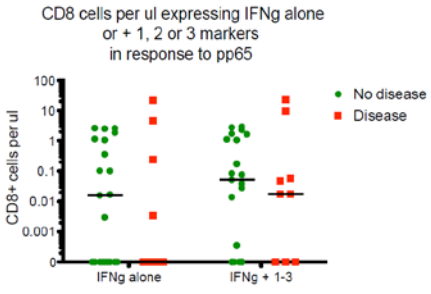
Figure 1: Mono- and multi-functional CMV-specific CD8 T-cells to pp65 and IE-1 according to antiviral strategy at 3 months after SOT



We will conduct immunologic testing for CMV-specific T-cell and neutralizing antibodies (UL128-131 epithelial/endothelial targets) in the study subjects. Previous studies have considered transplant patients with ≥ 0.4 CMV-specific CD4+ and CD8+ T-cells/μL as responders (45). Our study will assess differences in T-cell and antibody-mediated responses in the 2 groups and determine thresholds that confer protection against CMV disease.

Prophylaxis is considered to have a greater salutary effect on CMV-related indirect outcomes (46). However, this has not been incontrovertibly shown in liver transplant recipients. In a randomized, placebo-controlled trial, no difference in rejection, graft loss or mortality at 12 months was observed with the use of oral ganciclovir prophylaxis versus placebo (8). Studies comparing ganciclovir with other agents e.g., acyclovir have yielded similar results (47). A report of the Collaborative Transplant Study which is a large database from 435 transplant centers in 44 countries concluded that unlike other organ transplant recipients, CMV prophylaxis had no significant impact on the rate of acute rejection or graft survival in liver transplant recipients (48). There is also evidence that all outcomes with the use of preemptive therapy in liver transplant recipients are comparable to those in patients who never developed CMV infection (22).

Figure 2. Mono- and polyfunctional CMV-specific CD8 T cells at 3 months in patients with and without late CMV disease.



2.2.2 Valganciclovir use

The manufacturer's prescribing information states that valganciclovir is not indicated for use in liver transplant recipients. This is based on the results of the PV-16000 trial subgroup analysis in which liver transplant recipients receiving valganciclovir versus oral ganciclovir had a

higher rate of CMV disease. While not considered to be a statistical aberration (28), no published, proposed or biologically plausible explanation exists for these observations. Several lines of evidence discussed above suggest that greater development of CMV-specific protective immune responses is the underlying mechanism for the protection against CMV disease and that reconstitution of these responses is greater following preemptive therapy than prophylaxis. Additionally, ganciclovir per se has anti-proliferative effect on T-cells. Preemptive therapy appears to better facilitate the development of CMV-specific immune responses that ultimately allow for control of CMV and resultant protection from CMV disease. Thus, it is likely that the greater risk of CMV disease with valganciclovir in the PV-16000 trial was unrelated to any unique attribute of this drug, but rather to the approach or the manner in which it was employed i.e., as prolonged prophylaxis.

Valganciclovir is widely used as standard care for CMV prevention in liver transplant recipients. Furthermore, according to a survey of clinical practices in the US, valganciclovir is the most commonly used antiviral agent for prophylaxis in liver transplant recipients. International Consensus Guidelines of The Transplantation Society recommend either oral ganciclovir or valganciclovir for prophylaxis in R-/D+ liver transplant recipients (11). The guidelines of the Canadian Society of Transplantation's Consensus Workshop on CMV and the British Transplantation Society also recommend valganciclovir in liver transplant recipients (30, 49). In Canada and Europe, valganciclovir is approved for use in liver transplant recipients. Thus, use of valganciclovir in context of a clinical trial proposed herein is justifiable.

The other drug that was carefully considered but ultimately rejected was oral ganciclovir. The key argument against its use is its poor oral bioavailability and the consideration that the most active available anti-CMV agent should be used in the preemptive therapy arm since these subjects will have viremia. It is proposed that in a CMV-naïve host, an anti-CMV drug must be $\geq$ 93.3% effective to fully inhibit viral growth; the estimated efficacy of iv ganciclovir (5 mg/kg bid) for inhibition of viral growth is ~91.5% and that of oral ganciclovir is only~46.5% (50). Use of oral ganciclovir in the prophylaxis group was also rejected, the rationale being concerns for low bioavailability, large pill burden, and data suggesting that it is associated with a higher incidence of resistance compared to valganciclovir (51). Additionally, as of May 2009, oral ganciclovir is unavailable from the manufacturer (Ranbaxy) due to issues related to acquisition of raw materials.

The usual dosage of valganciclovir when used for prophylaxis is 900 mg orally daily. For preemptive therapy, a 900 mg bid dose is used (21, 24). The rationale for the dose in this group is that CMV infection (viremia) would already have occurred and therefore the purpose of antiviral therapy is to prevent the progression of asymptomatic infection to CMV disease. Indeed, preemptive therapy is often termed "early treatment" strategy for the aforementioned reason. Since the dose of valganciclovir that achieves blood levels equivalent to 5 mg/kg bid of iv ganciclovir (or the treatment dose for CMV) is 900 mg po bid (52), the drug dosage utilized for preemptive therapy will be 900 mg bid.

If a subject is unable to tolerate oral medications, intravenous ganciclovir may be substituted for oral valganciclovir because the study intends to compare the approach to prevention of CMV and not a particular formulation of ganciclovir. Since drug levels achievable with valganciclovir are similar to that with intravenous ganciclovir, brief substitution with the

latter agent is unlikely to confound any of the end-points and will prevent unnecessary discontinuation of the drug and exclusion of subjects.

The participating sites are currently using valganciclovir prophylaxis for 100 days after liver transplantation for CMV prevention as standard of care with drug costs charged to the patient or third-party payers as part of routine clinical care. In this study, one group of subjects will continue to receive standard of care (valganciclovir prophylaxis) and the other group will receive preemptive therapy with valganciclovir only upon evidence of CMV infection. For the group randomized to preemptive therapy, the drug use is estimated to be 43% less. No additional costs for valganciclovir use for study purposes will be incurred by the participating institution, subject or another party for participants randomized to either preemptive therapy or to prophylaxis.

Use of model for end-stage liver disease (MELD) scoring system as the basis for prioritizing organ allocation by the United Network for Organ Sharing in the current era has led to a substantially higher number of patients undergoing transplantation who have renal dysfunction or are requiring dialysis than time in the previous years (53,54). In order that our study is considered representative of liver transplantation practices at present and in order for our findings to be generalized to the transplant population undergoing in current clinical settings, it is important to include liver transplant subjects that require dialysis. Data in patients on dialysis have documented no pertinent issues that could influence the pharmacokinetics and bioavailability of valganciclovir in the study subjects (55). Furthermore, all study sites are currently using valganciclovir in dialyzed patients as standard of care. Receipt of dialysis has no effect on CMV disease rate in transplant recipients. However, given higher post-transplant mortality in patients receiving dialysis (56), subjects will be stratified by receipt of dialysis and sample size calculations will take into consideration the mortality within the first year.

We propose to conduct a randomized trial comparing prophylaxis versus preemptive therapy for the prevention of CMV disease in R-/D+ liver transplant recipients. The key goals of the study are to assess the efficacy of the 2 clinically used approaches for CMV prevention and not the efficacy of the drug per se. In this regard, this is a strategy trial based on the clinical practice of medicine using a drug that is currently the standard of care in these patients.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

2.3.1.1 Overview

The majority of transplant centers in the United States are currently routinely using valganciclovir or intravenous ganciclovir for prevention of CMV infection and disease in liver transplant recipients, either as primary antiviral prophylaxis or in a preemptive therapy strategy (10). Similarly, acyclovir at HSV suppressive doses is routinely used for the prevention of HSV reactivation for the first month post-transplant in patients who are not receiving CMV prophylaxis, and is a grade I recommendation in the American Society of Transplantation SOT ID Guidelines (53). The current study proposes to use these same antiviral drugs that are already in routine use in the majority of liver transplant centers. The present study will systematically compare two different strategies using the same antiviral drugs that are already

routinely used at US liver transplant centers. As a result, the potential risks of the antiviral medications for study subjects in the present study are deemed to not be different than in standard clinical practice. Similarly, since both CMV prevention strategies (prophylaxis and preemptive therapy) are currently used in clinical practice in US and European liver transplant centers, there is no anticipated increased risk to study participants of randomization to one or another strategy. The potential toxicities/side-effects of each of these agents are provided in the subsequent sections.

2.3.1.2 Ganciclovir

Valganciclovir when taken by mouth is rapidly converted in the body to its active form, ganciclovir. It is estimated that tens of thousands of persons have received either intravenous or oral formulation ganciclovir over the last 22 years since its initial approval. Based on its efficacy and general tolerability, ganciclovir is currently recommended as a first-line agent for prevention and treatment of CMV infection and disease in HIV, solid-organ transplant, and hematopoietic cell transplant (HCT) populations (49, 54). See the package insert for more information. Ganciclovir is generally well-tolerated. The most common adverse effects, which appear to be related to longer durations of exposure and use of concomitant drugs with similar toxicities, are various hematological adverse effects, most commonly leukopenia and neutropenia which are reversible after drug discontinuation. The potential toxicities of ganciclovir have been extensively studied *in vitro*, *in vivo*, and in placebo-controlled studies in humans. Based on animal and cell culture data, ganciclovir is considered a potential human carcinogen, teratogen, and mutagen. It is also considered likely to cause inhibition of spermatogenesis. No human data exist that estimate the actual risk of these effects. Thus, it is used judiciously and handled as a cytotoxic drug in the clinical setting.

2.3.1.2.1.1. Hematologic toxicity

Neutropenia is the principal toxicity of ganciclovir and valganciclovir. The incidence is highest in HCT recipients and HIV-infected individuals, followed by pediatric patients with congenital CMV disease and organ transplant recipients. Many studies have demonstrated the effect occurs late after drug administration (9,55, 56). In fact several studies in HCT recipients, the most susceptible population for this complication, show that the median time of onset is 5 weeks after start of drug administration. Neutropenia was documented in 8.2% of the patients who received 100 days of valganciclovir in the PV-16000 study however discontinuation of the drug was required in only 2% (9). Another recent randomized trial of valganciclovir vs. ganciclovir at treatment doses (900 mg twice daily and 5 mg/kg twice daily, respectively) for CMV disease in SOT recipients showed a neutropenia rate of 1.2% and 0%, respectively, at 21 days of treatment (57). Ganciclovir-related neutropenia is reversible (9, 55, 57). The time to recovery can be hastened by administration of G-CSF (54).

Most placebo-controlled randomized studies, including those in stem cell transplant patients, do not show a difference in the incidence of thrombocytopenia and platelet transfusion requirements (8, 9, 57-60). However, there are rare anecdotal reports of ganciclovir-related pancytopenia. One study of ganciclovir prophylaxis in HCT recipients reported delayed platelet engraftment (61). Overall, the potential to cause thrombocytopenia is considered low.

A trend towards anemia has been shown to occur in HIV-infected patients treated with valganciclovir. However, no strong evidence exists in transplant recipients and other patient populations, suggesting that the effect may be related to concomitant medications specific to the HIV setting. One recently completed phase III randomized trial of prolonged valganciclovir prophylaxis in HCT recipients, a population that would be considered at particularly high risk for this complication, did not show an increased rate of anemia or red blood cell transfusion requirements (62). Other recent randomized trials also did not show an increased risk of anemia (9, 63).

Key studies summarizing the incidence of neutropenia with valganciclovir use in organ transplant recipients are shown below.

Reference	Incidence of neutropenia (n/n)	Discontinuation of valganciclovir
(64)	4% (2/47)	2 (resolved in both)
(9)	8.2%	2%
(57)	1.2% (2/164)	Not known
(65)	4% (2/49)	0%
(66)	5.7% (4/70)	0%
(2)	15% (25/164)	<1%

2.3.1.2.2 Renal toxicity

Results from randomized trials do not support a role for ganciclovir or valganciclovir as causes of renal toxicity. None of the recently conducted randomized trials shows an increased risk or renal toxicity (62, 63), however, two earlier trials, one in heart transplant recipients with IV ganciclovir (67, 68) showed increased rates of renal insufficiency. While the potential to cause direct toxicity appears to be low, we will monitor renal function closely and adjust doses based on creatinine clearance according to the package insert.

2.3.1.2.3 Neurotoxicity

This is rarely observed and is not statistically significant between study arms of most randomized trial except one study in HCT recipients (63). This effect probably occurs only in a setting of concomitant drugs with neurotoxic potential and high blood levels of ganciclovir in the setting of renal insufficiency.

2.3.1.2.4 Carcinogenicity

Ganciclovir and valganciclovir are considered potential human carcinogens (see package insert). No studies have been performed to systematically assess this potential in humans. Although tens of thousands of transplant and HIV infected patients have been treated

with ganciclovir over the past ~20 years, no reports of an increased risk of cancer have been published. However, this does not rule out possible carcinogenic effect.

2.3.1.2.5 Teratogenicity

There are potential reports of ganciclovir-associated teratogenicity in laboratory animals but not in humans. Nevertheless, this drug is contraindicated in patients who are or are planning to become pregnant. For the purposes of this study, all subjects will be screened and excluded for pregnancy/possible pregnancy. For the three months following receipt of ganciclovir, abstinence or an effective method of birth control for both partners is recommended.

2.3.1.2.6 Summary of human toxicity data

Ganciclovir-related neutropenia occurs very uncommonly in persons without underlying bone marrow dysfunction and generally occurs at a median of 5 weeks after drug exposure. In patients without underlying bone marrow dysfunction, two recent trials showed very low rates of neutropenia after 3-4 weeks of ganciclovir at doses similar to those proposed in this protocol (2% within first 4 weeks with prophylaxis of 900 mg VGCV/day (9); 1.2% at day 21 with 900 mg valganciclovir twice daily; 0% at day 21 with 5 mg/kg ganciclovir twice daily (57). There is no convincing evidence that ganciclovir or valganciclovir cause thrombocytopenia.

Anemia has been observed in HIV-infected subjects, but there is no evidence that it is a problem in transplant patients or in the treatment of congenital disease.
There may be some risk of renal toxicity, however, this was not consistently observed across randomized trials. Other potential safety issues include teratogenicity and carcinogenicity.

Summary of ganciclovir and valganciclovir toxicities

Adverse effect	Human data	Documented in randomized trials
Neutropenia	Yes	Yes
Thrombocytopenia	Yes	No
Anemia	Yes	Some (HIV only)
Renal dysfunction	Yes	No (none in recent trials)
Gastrointestinal effects	Yes	Yes
Tumors	No	No
Birth Defects	No	No

Data in Table summarized from references 9, 55-63

2.3.2 Other risks

2.3.2.1 Blood draws

Bruising, soreness, anemia, or very rarely, infection, may occur as a result of venipuncture used to obtain blood from the participant's vein. The participant may feel lightheaded when the blood is drawn. Every attempt will be made to coordinate research blood draws with clinical blood draws to avoid extra needle sticks. The schedule of blood draws needed for the study are very similar to the schedule of blood draws that are considered standard of care post liver transplant. Only at time points at which blood is not being drawn for clinical purposes but needed for study purposes will a separate venipuncture be performed. Approximately 312 ml (~20 tablespoons) of blood will be collected specifically for study purposes over the 12 months of study participation in the preemptive therapy group and 172 ml (~11 tablespoons) in prophylaxis group. This total blood volume over the study period is well within accepted limits of blood draw.

2.3.2.2 Invasion of privacy

Personal Health information is to be collected about study participants during this study. There is a theoretical risk (as for any research study) that the participant's privacy may be breached. Study staff will take numerous precautions to protect participant privacy. Only authorized study personnel will access medical records and only specific clinical and laboratory information will be extracted from the medical record. Study codes (rather than subject identifiable information) will be used for all data collection documents. All study specimens will be de-identified and marked with only study codes before being sent to the central study laboratory. All study personnel have been appropriately trained regarding subject privacy and HIPAA regulations. Confidentiality of subject information will be strictly observed, and final publication of results will not include any subject identifiable information.

2.3.3 Potential benefits

There are no known direct benefits to study participants. All study participants will be followed more closely than is typically done during the course of routine clinical care. As a result, it is possible that some medical problems (specifically CMV disease) might be detected earlier for study participants than would have been the case if they had not been in the study.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary objective

The primary objective is to compare prophylaxis versus preemptive therapy using valganciclovir for the prevention of CMV disease in R-/D+ liver transplant recipients

3.1.2 Secondary objectives

To assess the two preventive strategies for:

- Clinical outcomes (major bacterial, fungal and non-CMV viral infections, rejection, graft loss and mortality) at one year post transplantation.
- Hematologic toxicity (assessment of neutropenia and receipt of hematopoietic growth factor during study days 1-107)

3.1.3 Exploratory objectives

To assess the two preventive strategies for:

- The development of CMV-specific immunity following transplantation
- Cost effectiveness of the regimens
- Differences in clinical outcomes (mortality, OI, malignancies, and the need for renal replacement therapy) at 1 to 7 years post transplantation.

3.2 Study Outcome Measures

3.2.1 Primary outcome measure

The primary outcome measure is the incidence of CMV disease by 12 months post-transplant.

3.2.2 Secondary outcome measures (compared between 2 groups)

1. Clinical outcomes within 12 months post-transplant
    - Incidence of late-onset CMV disease (CMV disease occurring after 100 days post-randomization)
    - Incidence of major bacterial, fungal, and non-CMV viral infections
    - Incidence of allograft rejection
    - Incidence of graft loss (retransplantation)

- All-cause mortality

2. Hematologic toxicity (assessed until 107 days post-enrollment)
   - Incidence of neutropenia (ANC <1,000 and <500/μL)
   - Hematopoietic growth factor (granulocyte colony stimulating factor) for neutropenia - (receipt of hematopoietic growth factor during study days 1-107)

3.2.3 Exploratory end-points (compared between 2 groups)

1. Clinical outcomes
   - Time to onset of CMV disease (disease free survival) through 12 months post-transplant
   - All-cause mortality, rejection episodes, graft loss and retransplantation, opportunistic infections, need for dialysis, and post-transplant malignancies will be assessed until study closure (7 years from enrollment maximum).

2. Immunologic endpoints:
   - CMV-specific immunity at 100 days, 6 and 12 months post-transplant
     a. CMV-specific T-cell responses
     b. CMV-specific neutralizing antibody responses

3. Cost effectiveness of the 2 approaches:
   - Total costs for each regimen
   - Cost utility analysis (cost per quality adjusted life-years)

4. STUDY DESIGN

This is a /prospective, randomized, multicenter trial of prophylaxis versus preemptive therapy for the prevention of CMV disease in R-/D+ liver transplant subjects. Subjects meeting study criteria and who have provided informed consent will be randomized within 10 days post-transplant to receive, in an open label design, either prophylaxis with valganciclovir 900 mg once daily for 100 days or preemptive therapy (weekly monitoring for CMV viremia by plasma PCR weekly for 100 days post-randomization with initiation of valganciclovir 900 mg twice daily at onset of CMV viremia and continued until plasma PCR is negative on two consecutive weekly PCR tests). All dosages will be adjusted for renal dysfunction. Subjects will be followed during the intervention period (100 days post randomization) and until 12 months post-transplant for CMV disease, toxicity, and clinical outcomes (opportunistic infections, rejection, graft loss, retransplantation and mortality).

All-cause mortality, rejection episodes, graft loss and retransplantation, opportunistic infections (bacterial, fungal), need for dialysis, and post-transplant malignancies will be assessed until study closure (7 years from enrollment maximum).

Additionally, the impact of the two CMV prevention strategies on CMV-specific cellular and humoral immune responses will be evaluated at 100 days after randomization, and 6 and 12 months post-transplant.

It is anticipated that ~20-22% of all liver transplant recipients will belong to the R-/D+ group and will qualify for the study. Approximate time to enrollment will be 3.5 years. A minimum of 176 subjects will be enrolled in the study. Allowing for over-enrollment to replace dropouts, up to 205 subjects may be enrolled to achieve the target enrollment of 176 (see section 5.4.3.3). There will be a single interim analysis for the primary endpoint of CMV disease as adjudicated by an independent End Points Committee. This analysis will be conducted after~ 60 subjects have been enrolled and followed for 1 year. Study closure will occur 2 years after the enrollment of the last subject.

5. STUDY POPULATION

Male and female liver transplant patients of any ethnicity are eligible for screening and enrollment.

5.1 Recruitment Strategies

Site investigators are all clinicians and also care providers for the liver transplant recipients at their respective institutions and therefore have direct access to the study population. The study population will be drawn from in hospital setting. Potential subjects will be identified by the site investigators and the study coordinators. Although the subjects may return to their hometown, which may be remote from the transplant center, close and longitudinal follow up of patients is a standard of care at all transplant centers.

5.2 Subject Inclusion Criteria

Subject must:

1. Be $\geq$ 18 years of age
2. Have negative CMV serology (confirmed within 6 months of transplant) and receive a liver from a donor with positive CMV serology (R-/D+)
3. Have received their first orthotopic liver transplant (the transplanted liver may be deceased donor or live donor graft) within 10 days prior.
4. Have absolute neutrophil count > 1000/ µL at randomization
5. If female, and not postmenopausal or surgically sterile, must have negative pregnancy test (serum or urine) within 48 hours prior to randomization and must also agree to use medically approved method of contraception. Acceptable methods include: barrier method, intrauterine device (hormonal or non-hormonal), oral hormonal contraceptives, abstinence for 100 days after randomization and 3 months after valganciclovir cessation. If male, and has not had a vasectomy, he must agree to practice barrier method of contraception for 100 days after randomization and 3 months after valganciclovir cessation
6. Subject or legally authorized representative has provided written informed consent.

5.3 Subject Exclusion Criteria

Subject must not:

1. Be currently enrolled in any interventional trial of an investigational therapeutic agent unless co-enrollment has been approved by study PIs and the DMID prior to enrollment

2. Have hypersensitivity to acyclovir, ganciclovir or valganciclovir
3. Be breast-feeding mother
4. Have known HIV infection (based on testing performed during the transplant evaluation process).
5. Be undergoing multi organ transplant or have undergone prior organ transplant
6. Have expected life expectancy of less than 72 hours.

5.4 Study Procedures

5.4.1 Screening

Potential subjects will be identified via assessment of medical record information using IRB-approved procedures. Since the investigating physician at all sites is both the caregiver and investigator, IRB-approved waiver [addressing Federal Policy criteria (45CFR 46.116(d)] for the screening portion of the consent will be obtained to access the medical record to identify potential research subjects. Medical records during screening will be reviewed for the limited purpose of determining potential eligibility of the subjects for the study. Eligible subjects will then be approached for informed consent. All subjects will provide an informed consent prior to enrollment in the study.

After appropriate informed consent has been obtained and before randomization of the subject, the following clinical laboratory tests as defined in the inclusion and exclusion criteria will be performed:

- In women of child bearing potential only: pregnancy test (serum or urine) - the results of this must be negative before proceeding. Pregnancy testing completed as SOC with-in 48 hours of randomization is acceptable.
- CMV serology (if not performed within 6 months of the transplant), absolute neutrophil count and serum creatinine level. These tests are performed as standard of care and are not study specific evaluations.

5.4.2 Randomization

Subjects will be randomized in 1:1 allocation to one of 2 study groups. All subjects that have met all criteria for study enrollment and have given written informed consent will be randomized via a web-based randomization system at the study web-portal.
The study team member randomizing the subject will get an immediate message providing group assignment. Randomization at each site will be stratified by: 1) the receipt of lymphocyte depleting induction and 2) dialysis at time of randomization. Randomization will occur as soon as it is feasible, but no later than 10 days after transplantation. Inadvertently randomized

subjects who do not meet the inclusion/exclusion criteria or those who are withdrawn prior to receiving the study drug will be replaced.

Subjects receiving lymphocyte depleting induction at the time of transplantation (even for those randomized to the preemptive therapy group) may receive up to 10 days of antiviral therapy with valganciclovir or IV ganciclovir after transplantation. Additionally, if the treating physician prescribes valganciclovir or IV ganciclovir for any length of time prior to randomization, it will not preclude subject enrollment. For subjects assigned to the prophylaxis group, the first dose of study drug should be given on the day of randomization regardless of lymphocyte induction. For subjects in the preemptive group that have received lymphocyte depleting induction, antiviral therapy with valganciclovir or IV ganciclovir will be given for ten days then stopped. Subjects that have not received lymphocyte depleting induction, but have received doses of valganciclovir or IV ganciclovir after transplantation prior to randomization, will have the pre-randomization treatment stopped and begin study treatment on randomization day based on their assigned group.

5.4.3 Discontinuation from Study and Withdrawal

5.4.3.1 Discontinuation from study

Under certain circumstances, an individual subject may be terminated from participation in this study. The site investigator must discuss potential subject discontinuation with the study PIs as soon as possible. Specific events that will result in discontinuation include:

- Site investigator decides to terminate participation for reasons of subject safety or to prevent compromising the scientific integrity of the study
- New scientific developments indicate that the treatment is not in the subject's best interest
- Subject was inappropriately enrolled based on inclusion/exclusion criteria
- Subject undergoes retransplantation after 30 days after randomization (please see section 6.2.5 for rationale)
- Study is terminated

For subjects who have received study product and discontinue from the study for any of the aforementioned reasons**,** every effort will be made to complete the safety specific evaluations.

5.4.3.2 Withdrawal

A subject may be withdrawn from study for the following reasons:

- Subject refuses further participation (consent withdrawn)
- Inappropriate enrollment (subject did not meet the inclusion criteria or met the exclusion criteria)

The date of last contact with the subject and the reason for withdrawal will be recorded in the CRF.

5.4.3.3 Handling of discontinuations and withdrawals

Should a subject discontinue prematurely, follow up assessments will be conducted per protocol. If the reason for removal of a subject from the study is an AE, specific event or test will be recorded on the CRF and follow up assessments will be continued. The exception to this would be if consent is fully withdrawn to any further collection of data. In the event that a subject discontinues participation after randomization but prior to receiving study drug or the subject was inappropriately enrolled, the subject will be replaced. No additional procedures or follow-up will be performed on subjects who discontinue prior to receiving study drug.

For subjects who withdraw consent from the study prior to receiving study product, there will be no further collection of data or study specific procedures. For a subject that has received study product then withdraws consent, the subject will not be required, but will be encouraged to complete safety specific evaluations.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Acquisition/Description

Valganciclovir is the standard of care for CMV prevention at all participating centers. Valganciclovir will be used on an open-label clinical basis and billed to the subject and/or insurer. The subjects may fill prescription at any pharmacy per standard clinical care at that institution. No medication will be provided specifically for study purposes.

Valcyte ® (valganciclovir HCl tablets) is available as 450 mg pink convex oval tablets with "VGC" on one side and "450" on the other side. Each tablet contains valganciclovir HCl equivalent to 450 mg valganciclovir. The dispensing of valganciclovir will be according to manufacturer recommendations and routine institutional practices at the transplant center.

It is possible that a generic formulation of valganciclovir might be approved and become available during the study period. Investigators may use either formulation of valganciclovir (brand vs. generic equivalent) for study subjects at the protocol-described doses according to institutional standards. Because of the uncertainty of if/when generic valganciclovir might become available; it will not be possible to specifically control for this variable in the pre-defined analytic plan. However, we will include post-hoc analyses to assess the potential impact of generic formulations of valganciclovir on the study safety and efficacy endpoints.

For this protocol, all references to “study drug” are referring to valganciclovir when prescribed per Section 6.2, and beginning with Study Day 1. “Study drug” includes dose adjustment when indicated and the use of ganciclovir when indicated per clinical care.

6.2 Valganciclovir Dosing and Administration

6.2.1 Dosage

Subjects assigned to the prophylaxis arm will receive valganciclovir at a dose of 900 mg po qd. Subjects assigned to the preemptive arm will receive valganciclovir 900 mg po bid upon detection of CMV viremia (at any level). Dose adjustments will be made for those subjects with renal dysfunction.

6.2.2 Drug administration

Drug administration will take place initially at the transplant hospital while the subject is an in-patient and will be administered by the clinical care team responsible for administering all other inpatient medications. If transferred or readmitted to a skilled nursing facility, rehabilitation facility, or outside hospital, the drug will be administered by the clinical care team responsible for administering all other medications at these facilities. If discharged home, the subject will self-administer the medication and compliance will be assessed as described in section 6.4.

6.2.2.1 Prophylaxis group

Subjects assigned to the prophylaxis arm will receive valganciclovir at a dose of 900 mg po qd (adjusted for renal dysfunction). Study drug will be given on the day of randomization (considered Day 1 of the study) and will be administered for 100 days.

For subjects randomized to the prophylaxis group that have been receiving prophylaxis therapy for CMV as part of their standard care prior to randomization, the prophylaxis dose will not be repeated on study day 1 if a dose has already been administered. However, if a dose was given as standard care, the dose will be counted as the 1st day of the 100 days of study treatment doses.

6.2.2.2. Preemptive therapy group

Day of randomization will be considered study Day 1 and all subsequent study days will start accordingly. Day 1 will begin immediately after randomization. In the event that a preemptive subject has already received a dose of valganciclovir as standard care prior to randomization on study Day 1: study Day 1 will still remain the day of randomization and the dose given prior to randomization will not be considered a protocol deviation. Subjects in the preemptive therapy group will undergo weekly testing of plasma using real-time CMV PCR for 100 days post randomization at the central site laboratory (see study manual for details of shipping and test result reporting). Valganciclovir 900 mg po bid (dose adjusted for renal function) will be employed upon detection of CMV viremia (at any level) and started within one week of initial positive PCR and continued until two consecutive tests performed one week apart are negative. Therapy will then be discontinued and weekly blood CMV PCR monitoring will continue until day 100 post randomization. Subjects with recurrent viremia (at any level) within 100 days post randomization will be treated with a repeat course of valganciclovir 900 mg po bid (dose adjusted for renal function) using similar criteria for discontinuation as for the initial episode of viremia. In the rare case in which viremia remains detectable at low levels or two negative PCRs cannot be achieved beyond 100 days post randomization, treatment with valganciclovir may be discontinued if the clinical team or the investigator deems that valganciclovir is no longer warranted.

All subjects assigned to the preemptive therapy group will receive acyclovir 400 mg po bid (adjusted for renal dysfunction) for at least 28 days after transplant for HSV prophylaxis as standard of care. Acyclovir will be discontinued during valganciclovir/iv ganciclovir preemptive

therapy within this period. This dose of acyclovir has no effect on CMV and is considered standard practice in the absence of routine use of valganciclovir post-transplant (53).

6.2.3 Conditions limiting valganciclovir administration

Subjects in either group who are unable to take valganciclovir orally at any time during the study period may receive IV ganciclovir until oral valganciclovir can be resumed. Subjects in either group may also be switched to IV ganciclovir if conditions that preclude use of oral valganciclovir develop such as inability to take oral medications or reduction in CrCl to < 10 ml/min or requirement for dialysis. These subjects may remain on IV ganciclovir until valganciclovir can be resumed. For handling of interruptions of valganciclovir due to neutropenia please see section 7.6.2.

6.2.4 Rejection episodes

Rejection episodes requiring lymphocyte depleting induction therapy are highly unusual in the current era. Nevertheless, subjects in either study group who receive lymphocyte depleting agent for the treatment of rejection at any time during the study period will receive valganciclovir 900 mg po qd or iv ganciclovir 5 mg/kg/d (if unable to take orally or use of oral valganciclovir is not possible) for up to one month. Valganciclovir and IV ganciclovir dosages will be adjusted for renal dysfunction. Subjects receiving other forms of augmented immunosuppression, including corticosteroids boluses or recycles will not receive concurrent antiviral agents.

6.2.5 Graft loss (retransplantation)

Subjects in either group undergoing retransplantation shall remain in the assigned study group. For subjects retransplanted within 30 days of randomization, the study drug (prophylaxis group) or surveillance monitoring for CMV PCR (preemptive therapy group) will be continued for 100 days from initial randomization. Subjects retransplanted after 30 days of randomization will be withdrawn from the study and receive standard care at that point since prolonged drug continuation can potentially impact the study outcomes, particularly the immunological end-points. The number of retransplantations after 30 days is expected to be low (less than 2 percent).

6.3 Dose Modifications for Renal Dysfunction

Dose modifications are recommended for subjects with renal impairment as outlined below.

Prophylaxis group valganciclovir dose modification	
CrCl (mL/min)	**Valganciclovir dose**
≥ 60	900mg once daily
40-59	450mg once daily
25-39	450mg every 2 days
10-24	450mg twice weekly
<10 (on hemodialysis)	Please see foot note below*
Preemptive therapy group valganciclovir dose modification	
CrCl (mL/min)	**Valganciclovir dose**
≥ 60	900mg twice daily
40-59	450mg twice daily
25-39	450mg once daily
10-24	450mg every 2 days
<10 (on hemodialysis)	Please see foot note below*

*Note: Manufacturer's instructions recommend IV ganciclovir 0.625mg/kg/ dose three times / week following hemodialysis in this setting. Some centers use valganciclovir 450 mg orally thrice weekly (after hemodialysis) instead of IV ganciclovir for patients on hemodialysis. Sites may follow standard medical practices at the transplant center for valganciclovir administration in hemodialyzed patients.

Recommended renal dosage adjustments for valganciclovir, ganciclovir, and acyclovir are standard medical practice and are recommended but not required for study. Renal dosage adjustments may be made by the study investigators and /or the clinical treating team when indicated. Switches between valganciclovir and ganciclovir are also standard medical practice and may be made by the study investigator and/or the clinical team when indicated. Please see Manual of Procedures for additional suggested renal dosage adjustments.

6.4 Medication Compliance

Compliance with valganciclovir will be assessed by the study coordinator using subject self-reporting 30, 60 days post randomization, and end of treatment. These assessments may occur up to 14 days after the specified visit day. Subjects will be asked about their recall of missed doses by questions asked in a nonjudgmental manner. Self-report correlates well with actual medication intake when a trusting subject-provider relationship exists as in transplant setting. Accuracy of the self-report can be maximized by (i) approaching the subject in a matter of fact and non-judgmental way, (ii) asking about the most recent days and missed doses, and (iii) using prompts to help recall. Pharmacy refill tracking was not deemed feasible for this study as the subjects may fill their antiviral medication from pharmacy other than the hospital pharmacy. Compliance to treatment regimen should be encouraged. However, it is expected that some patients will not be able to comply with all doses due to the chronicity of the condition

and underlying/concomitant illness. Therefore, compliance will not be reported as protocol deviations since overall compliance measures will be recorded for analysis.

6.5 Concomitant Medications

- Use of oral ganciclovir is prohibited during the study period.
- Intravenous ganciclovir may be used in subjects who are: unable to take oral medications; develop creatinine clearance less than 10ml/min or require hemodialysis; and during concomitant administration of lymphocyte depleting therapy (ATG, OKT3, alemtuzumab) as specified in protocol 5.4.2. For guidance for ganciclovir IV dose adjustment for subjects with renal impairment, please refer to Manual of Procedures (MOP).
- CMV prophylaxis with ganciclovir derivatives is adequate for herpes simplex virus (HSV) prevention. Guidelines recommend HSV prophylaxis with low-dose acyclovir within first month post-transplant in subjects who are not receiving other HSV-active antiviral therapy (53). Thus, subjects assigned to the preemptive therapy group will be allowed to receive low-dose acyclovir (400 mg po bid) for at least 28 days post-transplant as routine care. This dose of acyclovir has no effect on CMV. Acyclovir should be discontinued if preemptive therapy group subjects initiate valganciclovir. Acyclovir is not necessary in the prophylaxis group since valganciclovir has activity against HSV. For guidance for acyclovir dose adjustment for subjects with renal impairment, please refer to MOP.
- Use of CMV hyper immune globulin is prohibited except for the treatment of CMV disease as deemed necessary by the treating physician.
- Use of cidofovir and foscarnet is prohibited except for confirmed or suspected ganciclovir-resistant CMV disease.
- Immunosuppressant medications taken during study days 1-100 and any immunosuppression medications taken for rejection episodes during the 12 month period will be recorded. Immunosuppressive agent blood levels measured as standard of care during study days 1-100 will be recorded.
- Concomitant drugs with known hematologic toxicity or potential of drug interactions with valganciclovir that are taken during study days 1-100 will be recorded. These include: trimethoprim-sulfamethoxazole, dapsone, pentamidine, amphotericin B, abelcet, ambisome, probenecid, and imipenem.
- Use of acyclovir, valacyclovir or famciclovir is permitted without dose limit for the duration determined to be appropriate by the investigator for the prevention and or treatment of herpes simplex or herpes zoster

7 STUDY SCHEDULE

7.1 Screening

Site investigators are also clinicians and care providers for the liver transplant service at their respective institutions and therefore have direct access to the study population and medical records. Potential subjects will be identified via assessment of medical record information using IRB-approved procedures. Since the investigating physician at all sites is both the caregiver and investigator, IRB-approved waiver [addressing Federal Policy criteria (45CFR 46.116(d)] for the screening portion of the consent will be obtained to access the medical record to identify potential research subjects. Medical records during screening will be reviewed for the limited purpose of determining potential eligibility of the subjects for the study.

Initial screening will include subjects with known negative CMV serology that have not been approached for consent. If these patients are found to be ineligible prior to consent, they will be recorded on the electronic screening and randomization form noting reasons for exclusion. Data to be collected on screen failures that have not signed consent include only the reasons for ineligibility based on study inclusion and exclusion criteria. No research procedures will be performed on patients that have not given written informed consent. No identifiable information will be collected on patients that have not given written informed consent.

Screening may take place prior to transplant or post-transplant dependent on the enrolling site's preference. Either option is permissible for protocol adherence providing sites are using IRB-approved procedures.

7.2 Consent

Potentially eligible subjects or their surrogate, in accordance with the IRB approved procedures, may be approached after transplant or prior to transplant for consent if no exclusion criteria is met pending final determination of inclusion criteria eligibility. Consent may take place prior to transplant or post-transplant dependent on the enrolling site's preference. Either option is permissible for protocol adherence providing sites are using IRB-approved procedures. CMV seronegative subjects awaiting liver transplant may be consented prior to transplant. These subjects will become eligible for the study if they receive a CMV seropositive liver allograft and meet all other eligibility criteria.

Once consent has been obtained, the subject is considered enrolled in the study. In case of provisional enrollment due to pending CMV serology (if not performed in the last 6 month) pending pregnancy test (must be performed with-in 48 hours or randomization), or enrollment of CMV seronegative subjects prior to transplant (must receive donor positive liver), the subject will be randomized only after all eligibility criteria are met .

If eligibility cannot be met for any enrolled (consented) subject, the subject will not be randomized and instead will become a screen failure and will be replaced. Enrolled subjects that

are found to be ineligible will be recorded on the electronic screening and randomization form noting reasons for exclusion. Electronic data to be collected on enrolled subjects that become screen failures include; date of consent, and reasons for ineligibility based on study inclusion and exclusion criteria. Investigative sites will keep a list of all enrolled subjects separate from the research regulatory files that contain the subject's name, date of consent, and study ID. Study ID will be assigned by the electronic screening system when final determination of eligibility or ineligibility is verified.

7.3 Baseline research procedures pre-randomization (study day 0)

After consent is obtained but prior to randomization the following study laboratory procedures will be performed to verify eligibility. Results obtained as standard care within 48 hours prior to randomization are acceptable.

- WBC with differential for absolute neutrophil calculation (Subject will be withdrawn if ANC < 1000/μL at baseline) (May use results that have been obtained with-in 48 hours prior to randomization)
- Creatinine with Creatinine Clearance (CrCl) calculated (May use results that have been obtained with-in 48 hours prior to randomization)
- Serum or urine pregnancy test result for females of childbearing potential (May use results that have been obtained with-in 48 hours prior to randomization). Subject will be withdrawn if positive pregnancy test is detected

In addition, every effort should be made to reconfirm the subject's CMV serology on a preoperative blood prior to randomization. In cases which this is not possible (pre-operative blood is no longer available at the local lab at time of consent), the most recent CMV serology results obtained within 6 months of transplant will be acceptable for meeting inclusion criteria. Subject will be withdrawn if their CMV IgG is found to be positive.

7. 4 Randomization (Study day 1)

Subjects that have met all criteria for study enrollment and have given written informed consent will be randomized. Subjects must meet all inclusion and no exclusion criteria at the time of randomization. The mandatory eligibility check list, provided on the CAPSIL web Portal, will be completed by the study team at the time of randomization and kept in the subject study file.The day of subject randomization is considered study day 1.

7. 4.1 Randomization procedure

Subjects that have met all criteria for study enrollment and have given written informed consent will be randomized via a web-based randomization system. Full instructions for use of the web-portal randomization procedures can be found in the Manual of Procedures. Subjects will be randomized to either prophylaxis therapy for CMV disease prevention or preemptive therapy for CMV disease prevention. The study team member randomizing the subject will get an immediate message providing group assignment.

7. 4.2 Post-randomization baseline laboratory assessments

All subjects will have the following laboratory assessments completed at the transplant center on Study day 1 prior to administration of valganciclovir.

The following laboratory assessments are anticipated to have been obtained as standard of care (SOC) and will only be drawn specifically for the study if not completed as standard care within the allowable specified window. (Standard of care values obtained within 48 hours prior to randomization may be used for baseline hematology and chemistry assessments.)

Lab values that will be collected at study day 1:

- Hematology (SOC results within 48 hours prior to randomization acceptable)
  - Hemoglobin
  - Hematocrit
  - Platelets
  - WBC with differential for absolute neutrophil calculation (Subject will be withdrawn if ANC < 1000/μL at baseline)

- Chemistry (SOC results within 48 hours prior to randomization acceptable)
  - Blood urea nitrogen
  - Creatinine with Creatinine Clearance (CrCl) calculated Total bilirubin
  - AST
  - ALT

- Baseline blood sample for genetic testing (study specific evaluation)

All subjects that have agreed to genetic testing will have a onetime 10 ml sample of blood obtained at baseline. Specific consent for genetic testing will be requested at enrollment. Consent for genetic testing will be incorporated into the main consent form and will not

constitute a separate informed consent document. Subjects will have the choice to opt out of genetic testing although take part in all other aspects of the CAPSIL study. This blood sample will be obtained at the transplant center. Samples will be coded with subject study numbers. Sites will freeze samples per tube manufacturer's specifications and retain on site until shipped to the Boeckh Lab at Fred Hutchinson Cancer Research Facility.

Genetic samples are not time sensitive or dose dependent and therefore may be drawn at any time point after consent for this sampling is obtained. This genetic sample may therefore be drawn with eligibility labs (pre-randomization) or the next scheduled standard of care blood draw (pre or post-study drug administration) to avoid unnecessary blood draw. If a sample is drawn pre-randomization and the subject is not eligible for randomization (screen failure), the genetic sample should be destroyed as per the institutions policy.

7. 5 Treatment phase (study days 1-100)

7. 5.1 Prophylaxis group only

Subjects assigned to the prophylaxis group will initiate valganciclovir at a dose of 900 mg po qd (adjusted for renal dysfunction) on study day 1. For subjects randomized to the prophylaxis group that have been receiving prophylaxis therapy for CMV as part of their standard care prior to randomization, the prophylaxis dose will not be repeated on study day 1 if a dose has already been administered. However, if a dose was given as standard care, the dose will be counted as the 1st day of the 100 days of study treatment doses.

7. 5.2 Preemptive therapy group only

Subjects assigned to the preemptive therapy group will undergo weekly CMV PCR assessments beginning on study Day 7. Preemptive treatment group will begin preemptive antiviral drug therapy during study days 1-100 only when viremia is detected by weekly surveillance and will continue preemptive therapy until two consecutive weekly CMV PCRs are negative. Valganciclovir 900 mg po bid (dose adjusted for renal function) will be employed upon detection of CMV viremia (at any level) and started within one week of initial positive PCR and continued until two consecutive tests performed one week apart are negative. Therapy may be repeated if further detection of viremia is detected during the 100 day period.

In the event that a preemptive subject has already received a dose of valganciclovir as standard care prior to randomization on study Day 1: study Day 1 will still remain the day of randomization and the dose given prior to randomization will not be considered a protocol deviation.

7. 5.3 Laboratory assessments

7. 5.3.1 Drug safety labs (To be assessed during treatment period) all subjects- Study Days 7, 14, 21, 28, 42, 56, 70, 84, 98)
(For study days 7, 14, 21, and 28 sample windows are +/-3 days. For study visits on study days: 42, 56, 70, 84, 98, samples windows are +/-7 days.)

All subjects will have the following laboratory assessments completed at the transplant center while hospitalized. If discharged, laboratory assessments may be collected at routine care follow-up clinic appointments, at skilled nursing facilities, rehabilitation facilities, or outpatient laboratories.

The following laboratory assessments are anticipated to have been obtained as standard of care and will only be drawn specifically for the study if not completed as standard care within the allowable specified window.

Drug safety labs will be assessed and recorded for the entire treatment period in both the prophylaxis and preemptive group regardless whether the subject received valganciclovir/iv ganciclovir.

These labs will be continued at a bi-weekly interval (+/-7 days) in the preemptive subjects if receiving valganciclovir therapy after day 98 and continue until end of treatment.

- Hematology
  - Hemoglobin
  - Hematocrit %
  - Platelets
  - WBC with differential for absolute neutrophil calculation(ANC)

- Chemistry
  - Blood urea nitrogen
  - Serum creatinine with CrCl estimated
  - Total bilirubin
  - AST
  - ALT

ANC will be calculated at each safety lab visit. ANC < 1000/ µL will be reported as an adverse event (please see protocol section 9.3).

CrCl (based on serum creatinine and most recent weight recorded as standard of care) will be estimated and monitored at each safety lab visit to assist in decisions related to recommended renal adjustments. (Please refer to MOP for calculation instruction for ANC and CrCl)

7.5.3.2 CMV PCR surveillance monitoring – preemptive group only - Study days 7,14,21,28,35,42,49,56,63,70,77,84,91,98 (+/-3 days)
CMV PCR surveillance monitoring to be assessed in preemptive therapy subjects only

Subjects in the preemptive group of the CAPSIL study will have CMV PCR serum sample obtained specifically for the study at study days:

7,14,21,28,35,42,49,56,63,70,77,84,91,98. All sample windows are +/-3 days. CMV PCR samples will be obtained at the transplant center if hospitalized, or will be collected during routine care follow-up clinic appointments and outpatient laboratories, skilled nursing facilities, or rehabilitation facilities. CMV PCR analysis will be completed at the University of Washington Medical Center Virology lab.

CMV PCR results will be faxed to the study site within 24 hours of receipt of blood if received Monday through Saturday. Samples received on Sundays or holidays will be resulted within 48 hours or the next business day. CMV PCR surveillance must continue at weekly intervals in preemptive subjects if continuing valganciclovir therapy after day 98 until two consecutive negative results weekly.

7.6 Follow up laboratory procedures

7. 6.1 7 day Post End of Treatment

Drug Safety Assessment - all subjects (+/-7 days)

To be completed on all subjects one week after the last study dose of valganciclovir.

- Anticipated to be study day 107 in prophylaxis group
- To be scheduled one week post last study dose of valganciclovir in preemptive group (which may be beyond day 107)

All subjects will have the following laboratory assessments completed at the transplant center while hospitalized. If discharged, laboratory assessments may be collected at routine care follow-up clinic appointments, at skilled nursing facilities, rehabilitation facilities, or outpatient laboratories.

The following laboratory assessments are anticipated to have been obtained as standard of care and will only be drawn specifically for the study if not completed as standard care within the allowable specified window of +/-7 days.

- Hematology
  - Hemoglobin
  - Hematocrit %
  - Platelets
  - WBC with differential for absolute neutrophil calculation
- Chemistry
  - Blood urea nitrogen
  - Creatinine
  - Total bilirubin
  - AST
  - ALT

7.6.2 Immunologic studies (immune assays and lymphocyte count) All subjects

98 days, 6 month and 12month (+/- 14 days)

Subjects will have study specific serum samples obtained for Immune Assay testing and a lymphocyte count [proportion of white blood cells (hematology) that are lymphocytes] at study day 98, and at 6 months and 12 month (post-transplant) time point. Each sample may be obtained within +/- 14 day window. This blood sample may be obtained at the transplant center if hospitalized. If discharged, blood may be collected during routine follow-up clinic appointments, at outpatient laboratories, skilled nursing facilities, or rehabilitation facilities. Lymphocyte counts may be done as standard care at the local lab and recorded for the study purpose. If not completed as standard care, the site investigator will order the lymphocyte count to be drawn and processed locally and the results will be recorded.

Immune assays will be done by the Boeckh Lab at Fred Hutchinson Cancer Research Facility in Seattle Washington. Results of immune assays will be provided only to the coordinating center since these results do not impact subject care. All materials, shipping supplies, and instructions for immune assays will be provided to the remote location. Please see laboratory manual for all process and storage directions.

7.6.3 Pregnancy test for all females of childbearing potential

End of Treatment (+3 month)

All female subjects of childbearing potential will have a onetime post treatment pregnancy test. The pregnancy test may be performed on urine or blood and will be performed after the end of treatment up to 3 month post end of treatment.

All female subjects of childbearing potential will have a pregnancy test completed at the transplant center while hospitalized. If discharged, pregnancy test may be collected at routine care follow-up clinic appointments, at skilled nursing facilities, rehabilitation facilities, or outpatient laboratories.

7.6.4 Suspected CMV disease sample collection (Study day 1 – 12 month)

Subjects in either group suspected to have CMV disease based on the assessment of the clinician or the investigator will have a blood sample sent to the central lab for CMV DNA PCR testing. This sample collection may occur at any time point throughout the 12 month

duration of the study. In preemptive therapy group, this sample will be in addition to the weekly CMV testing sample. This blood sample will be obtained at the transplant center if hospitalized. If discharged, blood may be collected during routine follow-up clinic appointments, at outpatient laboratories, skilled nursing facilities, or rehabilitation facilities. Resistance testing will be completed at the University of Washington virology laboratory. All materials, shipping supplies, and instructions will be provided to the remote location. Please see laboratory manual for all process and storage directions. These results will be reported to the site via fax.

7.6.5 CMV resistance testing

Study day 1 – 12 month

(This one time study visit may occur at any time point throughout the 12 month duration of the study)

For those subjects who demonstrate no reduction in viral load after three weeks of ganciclovir/valganciclovir employed for CMV viremia in the preemptive therapy group or for the treatment of CMV disease in subjects in either study group, assessment of genotypic resistance for UL97 and UL54 mutations may be undertaken. This blood sample will be obtained at the transplant center if hospitalized. If discharged, blood may be collected during routine follow-up clinic appointments, at outpatient laboratories, skilled nursing facilities, or rehabilitation facilities. Resistance testing will be completed at the University of Washington virology laboratory. All materials, shipping supplies, and instructions will be provided to the remote location. Please see laboratory manual for all process and storage directions.

7. 7 Non-laboratory Procedures and Clinical Assessments Schedule (see also Schedule of Procedures appendix A)

7.7.1 Study day 1-100

Drug administration phase

Prophylaxis therapy group will begin to receive antiviral prophylaxis treatment on day 1 and will continue receiving a daily dose through day 100. Please see protocol section 6.2 for a complete description and instructions regarding of prophylaxis dosing.

Preemptive treatment group will begin preemptive antiviral drug therapy during study days 1-100 only when viremia is detected by weekly surveillance and will continue preemptive therapy until two consecutive weekly CMV PCRs are negative. Therapy may be repeated if further detection of viremia is detected during the 100 day period.

7.7.2 Study day 1-107

(Drug treatment safety labs phase)
Monitoring and managing neutropenia

Neutropenia is the most commonly encountered toxicity of ganciclovir formulations (IV ganciclovir and valganciclovir). Guidance for the evaluation and management of neutropenia are provided in the study manual of operations. If necessary, administration of valganciclovir may be interrupted temporarily and subsequently re-started in response to neutropenia. If the drug has to be interrupted for more than 14 consecutive days for a given interruption episode, the site investigator may contact the study PIs to determine if there are circumstances in which it may be appropriate to re-start the drug. Otherwise, valganciclovir should be discontinued permanently and subjects should be followed as discussed in the section on handling of discontinuations.

7.7.3 Study day 1 through one year

Outcome data recording

After randomization and throughout the entire study period, all subjects will be followed and data collected related to study outcomes. All evaluations monitored will be completed as standard of care and not specifically for the study. Data collection and follow-up will be primarily achieved by medical record review at each study visit time point. Record reviews will include transplant hospital, out-patient clinics, outside hospitals, skilled nursing centers, and rehabilitation hospital records. Non-study hospital physicians and/or subjects may be contacted to verify whether or not they have had any additional hospitalizations or clinic visits outside of the transplant hospital in order to confirm complete collection of all outcome data.

Specific outcome data to be followed and recorded include:

- Evaluations for suspected and confirmed CMV disease
- All treatments for suspected and confirmed CMV disease
- Evaluations for organ rejection
- All treatments for organ rejection
- Graft loss and need for liver retransplantation
- Fungal, major bacterial, and non-CMV viral infections
- All-cause mortality during the 12 month
- All immunosuppressant taken during 100 days
- Immunosuppressant given for the treatment of rejection episodes for the 12 month period
- Immunosuppressant medication levels taken as standard of care
- Hospitalization status

7.7.4 Adverse Event Monitoring and Reporting (Study day 1 through 7 days post end of study treatment phase)

All subjects will be assessed for adverse events throughout the administration of drug period plus 7 days. This is anticipated to be study day 1 through 107 for prophylaxis group subjects. Preemptive therapy arm monitoring of AEs timelines will be individually determined based on the length of treatment for viremia detected during study days 1-100.Please see adverse event section for complete description of adverse event monitoring and reporting.

7.7.5 Study Specific Medication Compliance Visit (Study day 30, 60, and end of treatment +14 days)

The Clinical Research Coordinator will track valganciclovir administration while the subject is in the hospital using hospital medication administration records. If the subject is discharged to a skilled nursing facility or rehabilitation facility, medication administration records will also be used to track valganciclovir administration. Upon discharge, if at home, the subject will be contacted and interviewed at day 30, 60 and end of treatment to assess drug compliance. These interviews may occur up to 14 days after the specified visit day.

A source document designed and provided by the coordinating center will be required to verify data acquired by interview with subjects regarding compliance with valganciclovir drug regimen. This interview may be conducted in person or by telephone. Using a non-judgmental approach, medication compliance will be assessed by interviewing subjects. Subjects will be requested to recall the number of missed doses in the previous reporting period. The coordinator will calculate percentage of compliance based on the subject's self- report. The compliance interview source document can be found on the CAPSIL website.

7.8 Early Termination Visit

7.8.1 Withdrawal of consent

Study subjects may withdraw voluntarily from participation in the study at any time. Study subjects may also withdraw voluntarily from receiving the study intervention for any reason. If a study subject withdraws or is discontinued from the study at any time prior to completion of the study, the study subject will be encouraged to complete safety follow-up visits and allow adverse event monitoring and reporting. All data collected up until date of withdrawal will be retained for study analysis.

7.8.2 Early termination for re-transplantation between study day 30 and 365

Subjects retransplanted after 30 days of randomization will be withdrawn from the study and receive standard care at that point since prolonged drug continuation can potentially impact the study outcomes. Subjects that require re-transplantation of their liver between study day 30 and 365 will be terminated from the study. Safety labs will be collected at 7 days post last dose of study therapy medication. Adverse events data will be collected for 7 days post last dose of study therapy medication. Mortality will be assessed at 1 year and study closure. No other endpoints or labs will be collected post-retransplant**.**

7.9 Therapy Discontinuation

Should a study subject's therapy be discontinued prematurely for any reason and consent has not been withdrawn, all clinical and laboratory evaluations will continue. Safety labs will be collected 1 week post last dose of therapy discontinuation. All key endpoints will be evaluated and all randomized study subjects will continue to be followed as long as possible and included in the final analysis.

7.10 Final Study Visit

The planned final study exit visit (with the exception of late outcome assessment at study closure, see 7.11) will take place 12 months post-transplant +/- 2 weeks.

7.11 Study Closure

All-cause mortality, rejection episodes, graft loss and retransplantation, opportunistic infections (bacterial, fungal), need for dialysis, and post-transplant malignancies will be assessed until study closure (7 years from enrollment maximum).

Study coordinators will review medical records at study closure to obtain study closure assessments.

7.12 Total blood volume collected

Approximately 295.5 ml (~20 tablespoons) of blood will be collected specifically for study purposes in the preemptive therapy group and 176.5 ml (~12 tablespoons) in the prophylaxis group over the 12 months of study participation. This is depicted in Appendix B. Including the samples for clinical care and study specific samples, the blood volume collected over the study period is within accepted limits of blood collection.

7.13 Future Use of Stored Specimens

Some of the specimens obtained from study participants during this study will be stored indefinitely at the University of Washington and Boeckh Lab Fred Hutchinson Cancer Research Center and may be used in future research. These specimens will be labeled with a code number and not with the study participant's name. At the time of consent for study participation, the study participant or legal guardian will have the opportunity to either agree to have their specimens used in future research or decline to have their specimens used in future research. The study participant or legal guardian will indicate his/her preference by initialing the appropriate line or checking the appropriate box of the Consent Form in the section entitled, "Future Use of Specimens". Non-protocol designated, future testing of samples will be performed only on samples from study participants who have consented for future testing of samples.

A repository for residual samples will be established according to OHRP guidelines ensuring that code or other personally identifying links will not be distributed to future researchers. The specimens will be stored indefinitely at the University of Washington and Boeckh Lab Fred Hutchinson Cancer Research Center. Samples from study participants will be labeled and coded without study participant's identifiers. If the study participant or legal guardian has indicated in the signed consent form that he/she does not agree to allow the future use of specimens for research, then his/her specimens will be destroyed at the completion of the study.

8 STUDY PROCEDURES/EVALUATIONS

The study procedures and evaluations are summarized in Appendix A

8.1 Clinical Evaluations

8.1.1 Baseline demographics/medical history

Information will be recorded at the baseline study visit day 1 following randomization. Data collected will include basic demographics (information in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html) gender; age, race; ethnicity. Subject weight will be recorded (weight recorded is the most recent weight obtained as standard care or obtained by the investigator). Also collected will be specific medical information including date of liver transplant, donor age and type (living or deceased), most recent standard of care MELD score prior to transplant, underlying liver diseases leading to transplant and co-morbid conditions (diabetes, cancer, cardiovascular disease, respiratory disease, renal disease). Specific baseline medications will be recorded including immunosuppressive agents, drugs with known hematologic toxicity, and any doses of antiviral agents. Standard of care clinical laboratory results, donor CMV serology, and study specific laboratory results will be recorded. All data will be abstracted from the medical records. Only current medical records from the transplant admission will be reviewed for baseline data collection. No past medical records will require review with the exception of confirmation of CMV serostatus with-in the allowable study window. No study specific physical examination will take place.

8.1.2 Adverse event assessment

All subjects will be assessed for adverse events throughout the administration of drug period plus 7 days. This is anticipated to be study day 1 through 107 for prophylaxis group subjects. Preemptive monitoring of AEs timelines will be individually determined by treatment for viremia detected during study days 1-100.Please see adverse event section for complete description of adverse event monitoring and reporting.

8.1.3 Neutropenia assessment

During active drug treatment, subjects will be monitored for neutropenia. Neutropenia with ANC $\leq$ 1000/µL will be documented and reported as adverse events. Guidance for the evaluation and management of neutropenia are provided in the study manual of operations.

8.1.4 Assessment of CMV disease

CMV disease includes **CMV syndrome** or **tissue invasive CMV disease**. The following definition will be used for the study's primary efficacy analysis:

8.1.4.1 CMV syndrome

The subject has CMV infection (viremia) identified by a nucleic acid based assay [CMV DNA PCR, pp67mRNA or digene hybridization (non-amplification assay)] or antigenemia assay or viral culture
AND at least one of the following clinical/laboratory findings:

- Fever of ≥ 38°C (100.4°F)
- Severe malaise
- Leukopenia defined as:
  White blood cell (WBC) count of <3,500/μL if the WBC count prior to the development of clinical symptoms is ≥ 4,000/μL **or**
  WBC decrease of > 20% if the WBC count prior to the development of clinical symptoms is < 4,000/μL
- Atypical lymphocytosis of ≥ 5%
- Thrombocytopenia defined as:
  Platelet count of < 100,000/μL if the platelet count prior to the development of clinical symptoms is ≥ 115,000/μL **or**
  Decrease of > 20% if the platelet count prior to the development of clinical symptoms is < 115,000/μL

8.1.4.2 Tissue invasive CMV disease

A diagnosis of organ-specific tissue invasive CMV disease detected by viral culture, histopathology/cytology (CMV inclusion cells), immunohistochemical analysis or *in situ* hybridization for CMV in a biopsy or other appropriate sample such as bronchoalveolar lavage (BAL), cerebrospinal fluid *AND* symptoms or signs of organ dysfunction. Detection of CMV by PCR in the tissue will be insufficient for the diagnosis of tissue invasive CMV disease.

Criteria to be met for diagnoses of specific organ CMV tissue invasive disease are as follows:

CMV hepatitis:

- Liver biopsy with CMV detected by viral culture, CMV inclusions by histopathology/cytology, immunohistochemical analysis or *in situ* hybridization
  (Other pathogens or etiologies of hepatic dysfunction such as rejection may be present and do not exclude the diagnosis of CMV hepatitis)

CMV gastrointestinal tract disease (includes esophagitis, gastritis, enteritis, colitis):

- Detection of CMV in tissue biopsy by viral culture, histopathology/cytology with CMV inclusions, immunohistochemical analysis or *in situ* hybridization
- In addition the subject has upper or lower gastrointestinal tract symptoms and/or signs such as nausea, vomiting, anorexia, dysphagia, odynophagia, cramping, diarrhea or abdominal pain. (Other pathogens for example, *C. difficile* may be present without excluding the diagnosis of CMV gastrointestinal disease)

CMV pneumonia:

- Presence of symptoms and/or signs of pulmonary disease
- AND the detection of CMV in the bronchoalveolar lavage (BAL) or lung biopsy. Detection of CMV in the BAL or biopsy may be performed by viral culture, histopathology/cytology with CMV inclusions, immunohistochemical analysis or *in situ* hybridization for CMV. Detection of CMV by PCR alone is insufficient for the diagnosis of CMV pneumonia. Other pathogens may coexist without excluding the diagnosis of CMV pneumonia

CMV retinitis:

- Dilated fundus examination and diagnosis of CMV retinitis by an ophthalmologist.

Central nervous system disease:

- Detection of CMV in the CSF by viral culture, CMV DNA/RNA PCR assay or in a biopsy sample by culture, histopathology/cytology (CMV inclusions) immunohistochemical analysis or *in situ* hybridization
- And Presence of central nervous system (CNS) symptoms

Other tissue invasive CMV disease:

- Detection of CMV by viral culture, histopathology/cytology with CMV inclusions, immunohistochemical analysis or *in situ* hybridization in a biologic specimen (e.g., tissue biopsy)
- AND the subject exhibits signs or symptoms of relevant organ dysfunction

8.1.4.3 Assessment and treatment of subjects with suspected CMV disease

Subjects who develop signs or symptoms of possible CMV disease within 12 months post-transplant will have a blood sample collected and sent to the central laboratory for CMV DNA PCR. CMV testing may also be performed at the local site on blood samples or any other relevant biologic samples using locally available assays for CMV detection. All aspects of diagnostic workup and CMV treatment will follow routine practices at the transplant center which

will include appropriate clinical evaluations and blood cultures to rule out bacterial, fungal or other opportunistic infections as a cause of the subject's symptoms which is a standard of care. Other diagnostic laboratory procedures and imaging studies will be performed as warranted by the subject's clinical status and standard medical practices at the site. The treatment for CMV disease will be initiated at the site investigator's or clinical team's discretion. The choice of anti-CMV agent, the duration of therapy and subject follow up will be in accordance with standard practice at the site

8.1.5 Secondary and Exploratory Outcome Assessments

All subjects will have data collection throughout the entire study period to capture assessments for the study's primary efficacy analysis, CMV disease. All aspects of diagnostic workup and CMV treatment will follow routine practices at the transplant center. All subjects will also have data collection throughout the study period to capture study outcomes including: rejection episodes and treatment, graft loss, retransplant opportunistic infections (bacterial, fungal, non-CMV viral), treatment with anti-viral medications, and hospitalization status.

8.1.6 Procedure for clinical data collection

Study staff will collect data by reviewing medical records at each study visit. These reviews will cover all intervals post-transplant with no omissions. Medical records will be reviewed for data pertaining to all study outcome measures, medical history, demographics (as described in protocol section 7.2.1), medications, procedures, lab results, and adverse events. Data will be entered into the CRF during each medical record review.

8.2 Laboratory Evaluations

Blood for study-specified laboratory evaluations may be obtained by methods such as the following: venipuncture, indwelling heparin-locked intravenous catheter, indwelling saline-locked intravenous catheter, etc. As detailed below, blood sample will be obtained for assessment of hematology safety labs, chemistry safety labs, whole blood CMV viral load/resistance testing, immune assays, and genetic analysis. With the exception of the pregnancy test, preemptive group PCR testing, and safety labs, those lab tests which are unable to be obtained due to lack of sufficient blood, or are obtained out of window study visits will not be reported as protocol deviations.

Initial labs will be obtained at the enrolling transplant center sites. Later labs (after the subjects have been discharged from the transplant center) may be obtained at the subject's local laboratory. It is typical for transplant patients to be released to the care of primary physician for routine standard care laboratory assessments when travel distance restricts return to the transplant center. These subjects will be having laboratory assessments at various remote out-patient clinics, hospitals, nursing facilities, or rehab centers, depending on their

home location. The subjects may have their blood drawn at any facility licensed to draw blood. It is not required for subjects to be seen by a doctor on the day of their blood draw

Lab analysis for immune assays, CMV PCR, genetic analysis and resistance will be done at study specific central labs as specified in the MOP for this study. All materials, shipping supplies, and instructions will be provided to the enrolling site by the coordinating center. The enrolling site will coordinate with any remote sites and provide shipping supplies and instructions. Please see laboratory manual for all process and storage directions.

8.2.1 Hematology safety labs

Hematology assessments will provide information on the safety of administering the valganciclovir. The following will be tested: white blood cell count, differential (ANC calculation), hemoglobin, and platelet count. Hematology results will be collected as standard care and data will be recorded from various CLIA certified labs in remote clinics for study purposes. If hematology labs are not ordered and drawn as standard care with-in the specified visit window, subjects will be required to have a study investigator prescribed hematology assessment at their local facility. Please see protocol; section 7.4.3.1 for allowable windows for the collection of lab values.

8.2.2 Chemistry safety labs

Chemistry assessments will provide information on the safety of administering the valganciclovir. The following will be tested: AST, ALT, total bilirubin, BUN and creatinine. Chemistry results will be collected as standard care and data will be recorded from various CLIA certified labs in remote clinics for study purposes. If chemistry labs are not ordered and drawn as standard care with-in the specified visit window, subjects will be required to have a study investigator prescribed chemistry assessment at their local facility. Please see protocol; section 7.4.3.1 for allowable windows for the collection of lab values.

8.2.3 CMV PCR surveillance monitoring (To be assessed in preemptive therapy subjects only)

Subjects in the preemptive group of the CAPSIL study will have CMV PCR serum sample (approximately 10ml) obtained specifically for the study at study days 7,14,21,28,35,42,49,56,63,70,77,84,91,98. All CMV PCR sample windows are +/- 3 days. CMV PCR samples will be obtained at the transplant center if hospitalized, or will be collected during routine care follow-up clinic appointments and outpatient laboratories, skilled nursing facilities, or rehabilitation facilities. CMV PCR analysis will be completed at the University of Washington Medical Center Virology lab. Results will be faxed to the enrolling site from University of Washington Medical Center Virology lab.

8.2.4 Special Assays or Procedures

8.2.4.1 CMV resistance to ganciclovir

(as needed during study day 1-365)

For those subjects who demonstrate no reduction in viral load after three weeks of ganciclovir/valganciclovir employed for CMV viremia in subjects in the preemptive therapy group or for the treatment of CMV disease in subjects in either study group, assessment of genotypic resistance for UL97 and UL54 mutations may be undertaken. Testing may also be undertaken within the 3 weeks at the request of the site investigators after discussion with the study PIs if the viral load fails to decline or if there is lack of response to the treatment of CMV disease. Please see section 7.5.5 for sample collection for resistance assays. Testing will be performed at the Central Virology lab (U. of Washington Virology Lab). Testing for UL97 and UL54 mutations conferring resistance to ganciclovir will be performed in a step-wise manner. If genotypic resistance to UL97 is not found, then no further testing will be undertaken. If UL97 mutation conferring resistance is found then reflexive UL 54 genotyping will be performed. Results of genotypic testing will be reported back to the investigators, approximately within one month of assessment. The decision to treat resistant CMV, the choice of anti-CMV agent for treatment, the duration of therapy and subject follow up will be in accordance with standard practice at the site

8.2.4.2 Immune assays and lymphocyte count

Subjects will have study specific serum samples obtained for Immune Assay testing and a lymphocyte count [proportion of white blood cells (hematology) that are lymphocytes] at study day 98, and at 6 months and 12 month (post-transplant) time point. . Immune assays will be done by the Boeckh Lab at Fred Hutchinson Cancer Research Facility in Seattle Washington. . Results of immune assays will be provided only to the coordinating center since these results do not impact subject care. Lymphocyte counts may be processed at the transplant center local laboratory or at the subject's local laboratory. Results of the Lymphocyte count will be recorded in the CRF.

8.2.4.3 Genetic testing

This clinically well characterized cohort with an anticipated high-incidence of outcomes of interest (CMV, other infectious and non-infectious complications) has the potential for significantly advancing our understanding of the pathogenesis of post-transplant complications. We propose to collect a blood sample for future testing to assess host immune response polymorphism to identify potential immunologic correlates of susceptibility (or protection) against CMV infection and/or disease. We will characterize immune and adaptive immune responses to CMV (for example CMV-specific T-cell responses, antibodies to CMV) as a potential explanation for protection against late-onset CMV disease in subjects who receive preemptive therapy compared to antiviral prophylaxis. Additionally, we propose to explore if there were any associations between polymorphisms in a range of genes and risk of other infectious and non-infectious complications after liver transplant.

We will obtain informed consent for 'future use of samples for genetic testing' at the time of enrollment into this study according to applicable local and other regulatory requirements. All proposed future genetic studies will be submitted for local IRB review and all applicable regulatory requirements will be met. However, no further subject consent will be sought for genetic studies related to infectious and non-infectious complications after liver transplant. Subjects will have the choice to opt out of future genetic testing at the time of initial consent although participates in all other aspects of the study. If consent is given for genetic testing a onetime sample of blood (10ml) will be obtained at the transplant center and shipped to Boeckh Lab at Fred Hutchinson Cancer Research Facility for future genetic analysis. The current award does not include funding to perform such studies so we propose to store samples indefinitely until such funding becomes available. Several safeguards will be implemented (Please see the MOP) to ensure that samples for genetic testing are stored only from subjects who have provided specific consent for such testing.

8.2.4.4 Pregnancy testing

All female subjects of child bearing potential will have a pregnancy test as follows: First, at baseline (within 48 hours prior to randomization) and at the end of treatment (window of up to 3 month post study treatment). This may be done on urine or blood sample. Positive results at baseline will result in exclusion from study.

8.2.4.5 Suspected CMV disease sample collection

Subjects in either group suspected to have CMV disease based on the assessment of the clinician or the investigator will have a blood sample sent to the central lab for CMV DNA PCR testing. This sample collection may occur at any time point throughout the 12 month duration of the study. In preemptive therapy group, this sample will be in addition to the weekly CMV testing sample. CMV PCR analysis will be completed at the University of Washington Medical Center Virology lab. Results will be faxed to the enrolling site from the University of Washington Medical Center Virology lab.

8.2.5 Specimen Preparation, Handling, and Shipping

Specific instructions on specimen preparation, handling and shipping will be provided in the Manual of Procedures for this study.

9 ASSESSMENT OF SAFETY

DMID Safety Reporting and Safety Monitoring

Regulatory requirements including the Food and Drug Administration (FDA) regulations, International Conference on Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP), and European Union (EU) Clinical Trials Directive set forth safety monitoring and reporting responsibilities of sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

9.1 Responsibilities

Investigators participating in this clinical trial are responsible for and will:

- Evaluate subject safety including assessment of adverse events (AEs) for seriousness, severity, expectedness, and causality;
- Notify the sponsor (DMID) of protocol defined serious adverse events (SAEs) with-in the protocol defined reporting requirement.
- Provide detailed written reports, including necessary documentation requested by the sponsor or institutional review board (IRB)/independent ethics committee (IEC), promptly following initial reports
- Inform the IRB/IEC of AEs as required by applicable local regulatory requirements.

9.2 Adverse Event (AE) Definitions

9.2.1 ICH E6 Definition

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor. (Please note: for the CAPSIL study AEs are reportable only if study related or unexpected, please see definition of reportable event for CAPSIL study 9.2.3)

9.2.2 FDA Definition

The FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. (Please note: for the CAPSIL study AEs are reportable only if study related or unexpected, please see definition of reportable event for CAPSIL study 9.2.3)

9.2.3 CAPSIL Definition

For the CAPSIL study, a reportable adverse event is defined as:

1. Any clinically important untoward medical occurrence in a subject receiving study drug that is different from what is expected in the clinical course of a patient with a liver transplant (see appendix C for events considered to be part of the expected course of liver transplant).

   OR

2. Any clinically important, untoward medical occurrence that is thought to be related to the study drug, regardless of the "expectedness" of the event for the course of a patient with a liver transplant**.** Expected events for liver transplant are untoward clinical occurrences that are deemed by the investigator to occur with reasonable frequency in the day-to-day care of patients with a liver transplant (see appendix C for events considered to be part of the expected course of liver transplant).

9.3 Documentation of Reportable AEs

All reportable AEs should be captured on the eCRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, and relationship to study product or procedure (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. Reportable AEs occurring while on study must be documented appropriately and will be followed to resolution or stabilization. AE events that are assessed as Serious require additional reporting as described in section 9.4.1.1.
Any medical condition that is present at the time that the subject is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study and meets reportable criteria it should be recorded as an AE.

9.4 Investigator's Assessment of Adverse Events

Complete review of each subject's medical records will take place on an ongoing basis by the site investigator while hospitalized at the transplant center. Although only AEs meeting reportable criteria will be documented in the eCRF, after discharge, the site investigator will assess all subjects' records at each study visit to verify that all adverse events that meet reportable AE criteria have been documented in the eCRF and reported per reporting guidelines discussed below. The investigator will provide documentation in the subject's study file confirming that all AEs have been assessed and those meeting reportable criteria have been reported and documented as required.

For reportable adverse events, the investigator is required to provide source documentation for the serious criteria, severity, relatedness, action taken, and outcome of each event.

The determination of seriousness, severity, and causality will be made by an on-site investigator who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

9.4.1 Serious Adverse Events (Assessment of Seriousness)

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event*
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the subject or/ and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

  * Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

9.4.1.1 Reportable SAEs for CAPSIL study

Liver transplant recipients represent a critically-ill population in whom a high rate of untoward medical events are commonly seen during the routine post-transplant course as part of their underlying medical condition, transplant surgery or postoperative state. In an effort to document only clinically-relevant untoward medical events that have a greater likelihood of being study-related (rather than the normal course of liver transplantation), study endpoints (CMV disease, opportunistic infections, rejection, graft loss, mortality, and hematologic toxicity) and certain pre-specified expected events commonly seen in this population (see appendix C for list of expected events for liver transplant patients) will not be reported as serious adverse events SAEs even if they meet the serious event criteria listed in 9.4.1. Reportable SAEs for CAPSIL study will be adverse events that are serious and unexpected [not expected to occur with a reasonable frequency in the typical clinical course of a patient following liver transplant (appendix C)].

Death will be recorded in the CRF although it will not be a reportable SAE as it is an endpoint for the CAPSIL study unless it meets the CAPSIL reportable criteria of related and/or unexpected (see appendix C for list of expected events for liver transplant patients)

Reportable SAEs will be:

- Recorded on the appropriate CRF
- Followed through resolution by a study clinician
- Reviewed and evaluated by a study clinician

9.4.1.2 Notification of the Sponsor, Coordinating Center and Local IRB of Serious Adverse Events (SAE)

A Reportable AE that meets the protocol-defined serious criterion must be reported with a completed SAE report within 24 hours of site awareness to the DMID pharmacovigilance contractor, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr., Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

A copy of the DMID SAE form will also be faxed to the Coordinating Center: Attention: CAPSIL Study Manager: Mary Stefanick, BSN RN CCRC. FAX: 412-647-6872

Other supporting documentation of the event may be requested by the DMID pharmacovigilance contractor and should be provided as soon as possible. The DMID pharmacovigilance contractor will notify the DMID medical monitor and clinical

protocol manager. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct. At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

Each individual enrolling site is responsible for adhering to the AE and SAE reporting requirements of their local IRB. The University of Pittsburgh will submit to the coordinating center IRB per their reporting requirements.

9.4.2 Assessment of Severity

Reportable AEs will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, published May 28, 2009 (v.4.03 June 14, 2010). The severity of each event will be classified into one of five defined categories as follows:

- Grade 1 Mild
- Grade 2 Moderate
- Grade 3 Severe
- Grade 4 Life Threatening or Disabling
- Grade 5 Death

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9.4.3 Assessment of Relationship to Study Product or Procedure

Relationship to Study: The clinician's assessment of an AE's relationship to study product or study procedures is part of the documentation process and may determine what is or is not reported in the study (Please see CAPSIL definition of an AE). If there is any doubt as to whether a clinical observation is a reportable AE, the event should be reported. Reportable AEs must have their relationship to study product or procedure assessed using the terms: related or not related. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product or study procedure caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product or study procedure and the adverse event.
- Not Related – There is not a reasonable possibility that the study product or study procedure caused the adverse event.

The investigator must provide an assessment of relationship of AEs to the study product based on:

- Temporal relationship of the event to the administration of study product or procedure
- Whether an alternative etiology has been identified

- Biological plausibility
- Existing therapy, and/or concomitant medications.

9.5 Reporting Interval

Site investigators will be responsible to monitor, document reportable AEs and SAEs, through study intervention period of the CAPSIL study plus one week. This is anticipated to be study day107 in the prophylaxis group and may vary in the preemptive group depending on treatment for viremia detected during study days 1-100.

Reportable AEs and SAEs will be followed until resolution, even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event as a SAE.

9.6 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All reportable serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.7 Reporting of Pregnancy

If pregnancy is discovered during the follow-up period the investigator must report information using the Pregnancy Report Form to the Coordinating Center. All pregnancies will be followed up to final outcome, using the pregnancy follow-up form. At that time, the status of the mother and infant will be noted, including the date of delivery and the infant's gender and weight.

The outcome, including any premature termination, must be reported to the Coordinating Center within calendar 5 days awareness of pregnancy on a pregnancy notification form. The pregnancy is not considered an AE; however pregnancy complications, including miscarriage or spontaneous abortion, are considered AEs. The report of any pregnancy and the outcomes of pregnancy as outlined above will be reported by the Coordinating Center to the DMID Medical Monitor within 7 business days of receiving pregnancy report form from the site.

9.8 Safety Monitoring By the DMID Safety Oversight Mechanism

9.8.1 Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB) will be established by the DMID. The DSMB members will consist of persons independent of the investigators or study team with no financial, scientific or other conflict of interest with the study and will be selected by the NIH. The initial responsibility of the DSMB will be to review and make recommendations regarding the initiation of the study. After the initiation of the study and during the course of the study at intervals determined by the DMID, the DSMB will:

1. Review the research protocol, template informed consent document and plans for data and safety monitoring.
2. Evaluate the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that can affect study outcome.
3. Review study endpoints for differences between groups
4. Consider factors external to the study when relevant information becomes available,
5. Monitor the confidentiality of the study data and the results of monitoring.

The coordinating center will provide all data, tables, unique and repeated listings, figures, descriptive statistics, and tests of significance requested by the NIH for all DSMB meetings, interim analysis plan and final analysis.

9.8.2 Discontinuation of Study Enrollment and Study Product Administration for all Subjects in the Study

DMID, when it is the study sponsor, may interrupt study dosing and/or study entry at any time if medically indicated. To minimize risk, the medical monitor and the DSMB will review cumulative safety data. The study enrollment and dosing will be stopped, and an ad hoc review will be performed if halting rules are met.

9.8.3 Halting rules

1. All-cause mortality compared at interim analysis will be used as safety parameter for halting the trial. With 60 subjects enrolled, a difference in survival probability of greater than 37% would be detected at the .05 level with 90% power. Thus an important difference in mortality between the two groups if documented will be detected at interim analysis and DSMB recommended action can be undertaken. The table below depicts minimal detectable hazard ratio with sample size of 60.

Safety endpoint At interim-Minimal detectable hazard ratio with sample size of 60 at alpha=.05, power=.9:			
Probability of survival (inferior regimen)	Probability of survival (superior regimen)	Events	Hazard ratio
.600	.976	13	.048
.550	.950	16	.089

2. Halting for a difference in reportable SAEs will be done upon the recommendation of the DSMB if there is a statistically significant difference in reportable SAEs at the .05 level after at least 20 subjects are enrolled.

3. A statistically significant difference (p=0.001) of more than 50% between the two groups in the incidence of CMV disease after 1 year follow of 60 subjects.

10 END-POINTS COMMITTEE (EPC)

10.1 Composition

An EPC will be established comprising three individuals with expertise in CMV and transplantation to adjudicate CMV disease events. The members of the EPC will be independent of the study investigators and will be selected by the DMID. The function of the committee will be to review the supporting clinical and laboratory data from all subjects identified as having developed CMV disease by the site investigators. Based on their review of the protocol definition of CMV disease and the clinical and laboratory documentation, the committee will identify those CMV disease events that should be included in the primary efficacy analysis. The EPC members will receive no financial incentives for their participation, but may be reimbursed for customary consultative or administrative support fee as determined appropriate by the DMID.

10.2 Responsibilities and Data Flow

The role of the EPC will be to evaluate and determine if the cases reported as having CMV disease by the investigators meet the protocol definition of CMV disease. In order to do so, the EPC will review all supporting clinical and laboratory data from such cases. The committee members will be blinded to the treatment allocation of all subjects in the study, including the subjects on which they are adjudicating. Subjects will be identified only with their study number. For the purposes of review, the following specific information will be provided to the EPC for the cases:

- Narrative summary that includes description of all signs and symptoms of the illness, and the reasons that the investigator diagnosed or suspected CMV disease.
- Type of CMV disease (viral syndrome or tissue invasive disease)
- Sites of CMV organ disease.
- Laboratory assay(s) that detected CMV disease
- Biopsy findings (if applicable)
- Other relevant laboratory, imaging studies or diagnostic procedures
- Outcome of disease and treatment employed.

The coordinating center will provide the required documents to the EPC. The EPC event adjudication will occur prior to interim analysis and prior to close out. It is anticipated that there will be approximately 30 CMV disease events to be reviewed.

The EPC will provide the coordinating center and the DMID with the conclusion for all cases that it has reviewed and whether the event meets the protocol definition of CMV disease (yes or no). When a unanimous decision cannot be made on the adjudication of the case by the three committee members, a vote will be taken and the majority vote will hold.

11 SITE MONITORING PLAN

Site monitoring will be conducted to ensure that human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, CRFs, informed consent forms, medical and laboratory reports, source documents, data collection forms , medical records and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

12 STATISTICAL CONSIDERATIONS

12.1 Sample size calculations

The primary endpoint is the incidence of CMV disease within 12 months post transplantation. The null hypothesis is that the incidence of CMV disease in the prophylaxis group is equal to that in the preemptive therapy group. The null hypothesis will be rejected if there is a significant difference between the two groups at 0.05. The sample size estimates have been calculated based on the numbers required to detect a disparity in two survivor functions using a two-sided log-rank test [stpower logrank, STATA version 10.1].The calculations are based on a realistic and conservative expectation in differences in event (CMV disease), feasibility and clinically relevant effect size. The mean CMV disease rate (weighted for study size) for prophylaxis and preemptive therapy studies (depicted in the Tables in the Background section of the protocol) is 30.6% (95% CI 24.1-37.1) and 0.78% (95% CI: 0-2.4%), respectively. Based on power calculations that coincide with the lowest observed rate for CMV disease in prophylaxis studies and highest rate observed in preemptive therapy studies, we assume that CMV disease will occur in 20% of the subjects in the prophylaxis and 5% in the preemptive therapy group. A sample size of 160 study subjects (80 per group) will detect this difference with power of 80% and alpha =0.05 using two-sided Fisher's exact test for two independent proportions. The Table below provides sample size estimates for CMV disease rates of 20-25% in the prophylaxis and 5-7% in the preemptive therapy group.

Prophylaxis group		**Preemptive therapy group**		**Hazard ratio**	**Power**	**Estimated events**	**Sample size each group**	**Total sample**
Estimated disease rate	**Disease free survival**	**Estimated disease rate**	**Disease free survival**					
25%	.75	7.1%	.929	.254	.83	25	75	150
25%	.75	7.1%	.929	.257	.85	26	80	160
22%	.78	7.0%	.930	.291	.80	27	90	180
20%	.80	5.0%	.950	.229	.80	20	80	160
20%	.80	6.0%	.941	.273	.80	25	93	186
20%	.80	5.4%	.946	.247	.80	22	85	170
20%	.80	7.0%	.930	.325	.80	31	112	224

Non-inferiority: Although the study sample size is powered to detect the superiority of one preventive strategy compared to the other, it is possible that the two approaches may be similar in efficacy with respect to CMV disease. Therefore a non-inferiority assessment will be done using a non-inferiority margin of 5%. This margin is based on what is known about the effectiveness of the two approaches in the literature and the potential that the two strategies may be equally efficacious in preventing CMV disease. As noted above, the success rate for the prophylaxis group is estimated at 75 to 80% based on previous studies with comparable success rates for the preemptive group. The null hypothesis for non-inferiority is that the success rate (percent of subjects remaining CMV disease free) for subjects in the prophylaxis group is better than the success rate for those in preemptive therapy group by 5%. The null hypothesis will be rejected and the preemptive therapy will be considered to be non-inferior if the success rate in the prophylaxis group is less than 5% greater than that in the preemptive therapy group. Sample size estimates for non-inferiority are given below at alpha=.05

Success in prophylaxis group	Success in preemptive therapy group	Non inferiority margin	Sample size each group [total sample]	Power
80%	85%	10%	80 [160]	80
80%	85%	8%	106 [212]	80
80%	90%	5%	96 [192]	90
80%	90%	5%	70 [140]	80
80%	89%	5%	80 [160]	80
80%	85%	5%	178 [356]	80
75%	85%	5%	87 [174]	80
75%	90%	5%	76 [152]	95
75%	90%	2%	83 [166]	90
75%	88%	5%	78 [156]	90
80%	80%	5%	792 [1584]	80

Note: for "equivalence" at 80% success rate in each group and a 5% equivalence limit, a sample of 1097 in each group would be required [2194 total subjects] at alpha=.05 and power=.80.

Our sample size is adequate to assess both superiority and non-inferiority (with a margin of 5%) without statistical penalty. In a sample size of 160 subjects, if there is a true difference of 9% in favor of the preemptive therapy group [9% higher success rate for CMV disease prevention]; we can be certain (with power equal to 80%) that the upper limit of a one-side 95% confidence interval will not include a difference of more than of 5% (non-inferiority margin) in favor of the prophylaxis group.

12.2 Treatment groups

Subjects will be randomized into one of the two groups in 1:1 ratio. Prophylaxis group will receive VGCV for 100 days and preemptive therapy group will undergo weekly CMV PCR screenings and receipt of VGCV following a positive CMV PCR test. A stratified, blocked randomization scheme will be utilized in order to keep the sample sizes similar in both groups.

Subjects at each site will be stratified by receipt of anti-lymphocyte induction therapy and renal dialysis at the time of randomization. Subjects will be consecutively entered.

12.3 Populations for analysis

There will be two analysis populations: Intent-to-treat (ITT) and a modified ITT. Limited summary information will be provided for screen failures. An ITT analysis will be conducted for the primary endpoint.

12.3.1 Intent-to-treat (ITT) population

All subjects who meet all eligibility criteria, sign an informed consent form, and are randomized to one of the treatment groups constitute the ITT population regardless of whether they received valganciclovir or had any post-baseline evaluations.

12.3.2 Modified intent-to-treat population

The modified ITT population will consist of those subjects in ITT population who were randomized and received at least one dose of valganciclovir in the prophylaxis group or had at least one CMV PCR test post-randomization in the preemptive therapy group.

12.3.3 Screen failures

Subjects that were screen failures will be summarized as to the reason for screen failure with no further characterization. Screen failures will include patients that never signed consent as well as those that signed consent but did not meet eligibility criteria and therefore were never randomized

12.4 Data Analyses

12.4.1 General considerations

All statistical analyses will be performed using STATA version 10.1 or later [Stata Corp, College Station, TX] or SAS version 9.2 or later [SAS Institute Inc., Cary, NC]. Cost efficacy analysis will use TreeAge Pro 2011 or later [TreeAge software, Williamstown, MA]. Tests of statistical significance will be two sided at an alpha of 0.05 unless otherwise specified.

12.4.2 Missing data

Sensitivity analysis employing imputation of missing values will be carried out for efficacy and safety endpoints as appropriate. The type of imputation is specified under the analysis section for each end point where imputation is employed. In addition, imputation will be employed for missing or incomplete dates for the onset of adverse events. For selected analyses, a complete date is necessary to determine whether or not an adverse event began after study treatment. In cases where an incomplete onset date is provided in the case report form (CRF), the missing components will be defined as follows:

- Missing day, month and year present: If month and year are the same as the first treatment dose month and year, the day will be assumed to be the same as the first dose day. Otherwise, the day will be assumed to be the 15th.
- Missing day, month and year: The date will be assumed to be the same as the first treatment dose date. If the imputed date is after the resolution date, the imputed onset date will be assumed to be the same as the resolution date.
- Severity and causality of adverse events occurring on or after the start of study drug are imputed when missing events for which causality was unknown or not recorded will be included in the summary of study drug related events.

12.4.3 Disposition of subjects

The number and percentage of subjects who were screened, enrolled, and completed the arm specific protocol, as well as the reason for discontinuation or withdrawal from the study will be presented in summary tables by treatment group and total subjects.

12.4.4 Baseline assessments

Baseline assessment summary tables will be presented by treatment group and total subjects for the ITT populations. Baseline demographics include age, gender, ethnicity, race, and transplant information including receipt of induction therapy. Baseline laboratory assessments include hematologic, renal and liver function tests. Continuous data will be presented with descriptive statistics—*N*, mean, standard deviation, median, range and interquartile range. Categorical data will be summarized by frequency distributions.

12.4.5 Duration of treatment, compliance, and participation

Duration of valganciclovir, length of study participation (while on therapy and during follow up), compliance as well as a distribution of the number of days from the date of transplantation to the date of the first dose of valganciclovir [prophylaxis arm] or date of first CMV PCR test [preemptive arm] will be summarized for the modified ITT population using descriptive statistics, including frequency distributions. For the preemptive arm the number of PCR tests, number of days between positive PCR test and the initiation of valganciclovir as well as well as the exposure to valganciclovir will be summarized.

Duration of valganciclovir is (date of the last dose of drug minus the date of first dose of drug plus 1 day).
Study participation as defined by the duration of the overall CMV evaluation period (while on therapy and during follow-up) will be summarized for the ITT and modified ITT populations.
Evaluation period length for CMV disease assessment is (date of last study contact minus the date of randomization plus 1 day).
Receipt and duration of generic or nongeneric valganciclovir will be recorded and summarized for each study population.

12.5 Primary outcome measures

12.5.1 Definition

The primary endpoint is the incidence of CMV disease within 12 months post-transplant. This endpoint will include all reported occurrences of CMV disease as adjudicated by the endpoint committee and/or investigator-determined CMV disease. The latter endpoint will include all reported occurrences if CMV disease by the investigator, regardless of whether they met the protocol definition of CMV disease.

12.5.2 Missing with respect to the primary endpoint

A subject in the ITT population will be defined as missing with respect to the primary endpoint (lost to follow up for the purposes of analysis of the primary endpoint) if the subject withdrew, discontinued consent or was lost to follow up prior to 3 month window for CMV disease without having reached the primary endpoint.

12.5.3 Primary outcome analysis

12.5.4 Primary end point-CMV disease [ITT group] includes all randomized subjects]

The primary endpoint is CMV disease. The primary analysis is the Cochran-Mantel-Haenszel [CMH]. Incidence rates will be compared and point estimates and confidence intervals for the incidence-rate ratio and difference, along with attributable or prevented fractions will be calculated for the two regimens. The analysis will be repeated, controlling for induction antilymphocyte therapy. Within-stratum statistics will be shown as well as the combined with Mantel-Haenszel estimate. Subjects that die or are lost to follow up without evidence of CMV disease will be considered as 'no disease' for this analysis. All randomized subjects will be

included in the denominator. An additional analysis will be performed in which all lost to follow up subjects will be considered as 'CMV disease'.

12.5.5 Primary end point-CMV disease [modified ITT group]

The primary analysis will be repeated in the modified ITT group [excludes subjects that withdrew prior to receipt of valganciclovir if in preemptive arm and subjects that have no post randomization PCR testing in the preemptive group]. An additional disease free survival model will be presented with censoring at retransplantation [return to standard care] if retransplantation occurs greater than 30 days post enrollment.

12.5.6 End point committee and investigator-determined CMV disease

The primary analysis will be repeated separately utilizing the adjudicated incidence of CMV disease and the investigator-determined CMV disease as the end point.

12.6 Secondary outcome measures

12.6.1 Clinical outcomes

12.6.1.1 Incidence of late onset CMV disease [adjudicated]

The incidence of adjudicated CMV disease will be determined at the 3 and 12-month time points (late-onset CMV disease) using an analysis similar to that specified for the primary analysis of the primary endpoint and, in addition, will include separate subset analyses for CMV syndrome and CMV tissue invasive disease. The analysis will be a Cochran-Mantel-Haenszel test of the significance of the odds ratio, while controlling for the use of induction antibody therapy and renal dialysis. In addition potential prognostic factors, such as allograft rejection will be examined with a Cox proportional hazards model applied to the ITT and modified ITT populations. This analysis will be used to test the presence of a treatment effect while controlling for the various prognostic factors entered into the model. Results will be reported as the hazard ratio comparing two groups.

12.6.1.2 Incidence of late onset of CMV disease [adjudicated and investigator-determined]

The above analysis will be repeated separately utilizing adjudicated CMV disease and investigator determined CMV disease as the respective endpoint.

12.6.1.3 Bacterial, fungal and non-CMV viral infections

Invasive fungal infections, major bacterial infections and non-CMV viral infections will be analyzed. These infections are outlined in the manual of operations. The analysis of incidence of an opportunistic infection will be performed using a Chi Square test or Fisher Exact in the modified ITT population. Results will be reported as the *p*-value and the 95% confidence interval for the difference in the rates of invasive fungal, major bacterial or viral infections; the analysis will be repeated using a CMH test stratified by receipt of induction therapy. The analysis will include an assessment of baseline comparability of the comparison groups and will incorporate variables not adequately controlled by randomization. The initial episode of major infection will be evaluated in a time dependent Cox model with the end point being the date of first major infection. The starting point will be date of transplantation and the infection free survival functions will be compared between the two regimens adjusted for cluster [site]. Subjects will be censored at death and study termination/withdrawal. Multiple infection episodes will be evaluated by comparing the total 1 year episodes per person day of follow up between the two regimens. The analysis will be repeated controlling for receipt of anti-lymphocyte induction and renal dialysis. The type of infection (major bacterial, fungal, non- CMV viral) will be compared between the two regimens using a CMH test, non-stratified and controlled for induction antilymphocyte therapy and renal dialysis. Summary tables listing the type of opportunistic infections for each regimen will be presented.

12.6.1.4 Allograft rejection

Rejection will be evaluated by several methods. Rejection rates at one year, between the two groups will be evaluated with a CMH test, both non-stratified and adjusted for receipt of antilymphocyte induction and renal dialysis. Initial rejection will be evaluated in a time dependent Cox model with the end point being the date first rejection episode. The starting point will be date of transplantation and the rejection free survival functions will be compared between the two regimens adjusted for cluster. Multiple rejection episodes will be evaluated by comparing the total 1 year episodes of rejection between the regimens using a regression model with the endpoint total number of rejection episodes, clustered by site, and adjusted for receipt of antilymphocyte induction therapy and renal dialysis. An additional model may be developed to adjust for inequalities not adequately controlled by the randomization process. Incidence of chronic graft rejection will be compared between the two study groups using a CMH test stratified for receipt of induction lymphocyte depleting therapy and controlling for renal dialysis.

Rejection episodes

Incidence rates will [number of rejection episodes per patient days of follow up] will be calculated and compared between the two groups. Patient days will be counted from the day of

randomization until the date of death, one year or last known follow up if subject is unable to be followed for one year.

12.6.1.5 Graft loss (loss due to retransplantation)

The incidence of retransplantation will be determined at the 3 and 12 month time points. The date of retransplantation will be considered the date of occurrence of the event. A Cochran-Mantel-Haenszel test stratified for receipt of antilymphocyte therapy and controlling for renal dialysis will be used to test the significance between the two treatment regimens.

12.6.1.6 All-cause mortality

A Kaplan-Meier survival probability will be calculated for each regimen. The starting point will be the date of randomization and the end point will be date of death. Subjects will be censored at date of last follow up if lost to follow up, or at one year post-transplant. The equality of the two survivor functions will be compared using a rank sum test for both the non-stratified data as well as the stratified log rank test for each stratum separately (receipt of lymphocyte depleting induction therapy and renal dialysis). Graphs of the Kaplan-Meier curves will be presented.

12.6.2 Hematologic toxicity

12.6.2.1 Incidence of neutropenia

The incidence of neutropenia will be summarized for each of the following definitions of neutropenia: ANC values < 1000/μL and < 500/μL. Incidence will be determined based on safety labs performed per protocol. The analysis will be a CMH test of the significance of the odds ratio, while controlling for anti-lymphocyte antibody induction and renal dialysis with the levels of stratification determined at the time of randomization, using the modified ITT population. Results will be reported, as the MH odds ratio with 95% confidence intervals in the modified ITT population. The analysis will include assessment of baseline comparability of the comparison groups and will incorporate relevant items not adequately controlled by randomization in additional models.

12.6.3 Adverse events

Severe, unexpected drug related adverse events will be compiled and compared between the two groups. Adverse events will be evaluated by comparing the total number of reportable adverse events per person day of follow up between the two regimens. The analysis

will be repeated stratified by receipt of anti-lymphocyte induction and renal dialysis using Mantel-Haenzel weights. The analysis will include assessment of baseline comparability of the comparison groups and if necessary, will adjust for items not adequately controlled by randomization in additional models.

12.7 Exploratory end-points

12.7.1 Clinical outcomes

12.7.1.1 Time to onset of CMV disease

Disease free survival will be evaluated by calculating the Kaplan-Meier survival probability for each regimen. The starting point will be the day of transplant; the end point will be the date of onset of CMV disease. Subjects will be censored at date of death if unrelated to CMV, date of last follow up if lost to follow up or at end o of study. Kaplan-Meier estimates of median time to onset of CMV disease and the associated 95% confidence interval will be presented. In addition, the Kaplan-Meier estimates and associated confidence intervals will be presented for specific time points (100-day, 6-month 12-months) and quartiles (25^{th} and 75^{th}). The study groups will be compared using the non-stratified log rank test, as well as the stratified log rank test for each stratum separately. Graphs of the Kaplan-Meier curves for each therapy group will be presented. The equality of the two survivor functions will also be compared using a Cox model adjusted for cluster [site] and controlled for receipt of anti-lymphocyte induction therapy and renal dialysis. The primary analysis will include assessment of baseline comparability between the two treatment groups. If needed, additional models will be developed to adjust for inequalities not adequately controlled by the randomization process. Results will be reported as the hazard ratio comparing the 2 groups. Survival analyses will also be controlled for unequal follow up periods between subjects. As such, the number of subjects available at annual time points will be reported, along with the Kaplan-Meier probability and associated 95% confidence intervals. It is assumed that subjects censored early will have the same probability of disease at each time point as those with longer follow up. To examine this assumption, relevant baseline characteristics will be compared between subjects enrolled in the first half of the study with those enrolled later. If the 2 cohorts are similar, we can expect that the survival probabilities are valid even with difference in follow up. Disease free survival will also be examined between the two cohorts.

Additional analysis will be presented separately for adjudicated CMV disease and investigator-determined CMV disease. For this analysis the entry point will be date of transplant and the endpoint will be the date of onset of CMV disease as determined by the endpoint committee and a similar comparison in which the end point is the onset of CMV disease as determined by the investigator. The study groups will be again be compared using the non-

stratified log rank test, as well as the stratified log rank test for each stratum separately (receipt of induction antilymphocyte antibodies and renal dialysis).

12.7.1.2 Composite disease free survival

A Kaplan-Meier survival probability will be calculated for each regimen. The starting point will be the day of transplant; the end point will be the date of onset of CMV disease or date of death. Subjects will be censored at date of last follow up if lost to follow up, withdrawal or at end of study. The equality of the two survivor functions will be compared using a rank sum test for both the nonstratified grouping and the stratified by receipt of antilymphocyte induction therapy and renal dialysis.

12.7.1.3 CMV resistance

For CMV resistance testing performed in subjects with CMV disease, the incidence of resistance as assessed by proportion of subjects with UL97 and UL54 mutations will be compared between the two study arms. The resistance will be categorized as present or absent and compared between the 2 groups using a Fisher exact test. In pre-emptive therapy subjects showing increase or no decline in CMV PCR quantitation following receipt of valganciclovir but no evidence of CMV disease, the incidence of resistance (UL97 and UL54 mutations) will be categorized.

12.7.1.4 Graft loss [retransplantation]

A Kaplan-Meier survival probability will be calculated for each regimen. The starting point will be the day of transplant; the end point will be the date of retransplant. Subjects will be censored at date of last follow up if lost to follow up, at date of death, or at study closure (and no longer than 7 years after enrollment). The equality of the two survivor functions will be compared using a rank sum test. Additional models will include adjustment for cluster [site] and receipt of antilymphocyte induction therapy and renal dialysis. The analysis will include assessment of baseline comparability between the two treatment groups. If needed, additional models will be developed to adjust for inequalities not adequately controlled by the randomization process.

12.7.1.5 Graft loss [death]

A Kaplan-Meier survival probability will be calculated for each regimen. The starting point will be the day of transplant; the end point will be the date of retransplant or date of death if no retransplantation occurs. Subjects will be censored at date of last follow up if lost to follow up, or at study closure (and no longer than 7 years after enrollment). The equality of the two survivor functions will be compared using a Cox model. The survival functions will also be presented adjusted for cluster [site] and stratified by receipt of antilymphocyte induction therapy and renal dialysis. The analysis will include assessment of baseline comparability between the

two treatment groups. If needed, additional models will be developed to adjust for inequalities not adequately controlled by the randomization process

12.7.1.6 All-cause mortality

A Kaplan-Meier survival probability will be calculated for each regimen. The starting point will be the date of randomization and the end point will be date of death. Subjects will be censored at date of last follow up if lost to follow up, or at study closure (and no longer than 7 years after enrollment).The equality of the two survivor functions will be compared using a rank sum test for both the non-stratified data as well as the stratified log rank test for each stratum separately (receipt of lymphocyte depleting induction therapy and renal dialysis). The number of subjects available at annual time points will be reported, along with the Kaplan-Meier probability and associated 95% confidence intervals. To validate the assumption of that all subjects have similar probability of survival at each time point despite different follow up, relevant baseline characteristics will be compared between subjects enrolled in the first half of the study with those enrolled later. If the 2 cohorts are similar, we can expect that the survival probabilities are valid irrespective of variable follow up. Survival will also be examined between the two cohorts.

12.7.1.7 Opportunistic infections

Incidence rates [number of infections per patient days of follow up] will be calculated. Patient days will be counted from the day of randomization until the date of death, end of study or last known follow up if subject is lost to follow up. The rates will be compared between the two groups using both the ITT population and modified ITT populations. The incidence rate will be calculated for all OI combined. Separate rates will also be calculated for bacterial and fungal infections. Similar analyses will be done using the date of study closure as the endpoint.

12.7.1.8 Need for dialysis

Renal failure will be evaluated between the 2 groups using both the ITT and modified ITT populations. Subjects requiring renal replacement therapy [RRT] more than 7 days post-transplant will be considered in the comparison. The proportions of subjects with RRT will be compared stratified by pre-transplant renal status. Timing to start of RRT will also be compared using a Kaplan-Meier probability function.

12.7.1.9 Post-transplant malignancies

Post-transplant malignancies will be compared in both the ITT and modified ITT populations. The timing to diagnosis will be compared using a Kaplan-Meier probability function and will be stratified by recurrence and new diagnosis. Descriptive statistics will be given on the location/type of malignancy in each study group.

12.7.2 Use of hematopoietic growth factors

12.7.2.1 Frequency of use

The number and percentage of subjects in the modified ITT population receiving any hematopoietic growth factor (e.g., G-CSF) during the study treatment period will be tabulated, by study group. The study groups will be compared using a CMH test of the odds ratio controlling for anti-lymphocyte induction and renal dialysis.

12.7.2.2 Number of dosages

Total doses of will be compared between the study groups using a Mann Whitney test. Time to use of G-CSF will also be evaluated with the starting point as date of randomization and the end point date of use of G-CSF. A rank sum test will be used to compare the two groups. Summary tables will be presented with descriptive statistics for percent use, timing and total dosage of G-CSF for each group.

12.7.3 Immunologic endpoints [CMV-specific immunity]

12.7.3.1 T-cell immunity

Initial sample [100 days]: The proportions of subjects with detectable CMV specific monofunctional CD4+ and CD8+ T-cells (that produce IFN-γ) and CMV-specific multifunctional CD4+ and CD8+ T-cells ($\geq$ 2 markers that include IFN-γ + additional cytokines such as IL-2 and TNF-α) will be compared between the two groups. It is hypothesized that T-cell immune responses will be greater in preemptive versus prophylaxis group and that positive responses will correlate with lack of development of CMV disease overall and late-onset CMV disease (69). A number of analyses will be carried out for these assessments. Proposed threshold levels that confer protection against CMV in transplant recipients are 0.4 CMV-specific T-cells/µl for both CD4+ and CD8+ T-cells (69). The percent of responders will be compared between the two treatment regimens using a Mann Whitney test.

The mean, median, range of multifunctional CD4+ and CD8+ T-cells will be summarized between the two regimens. The rate of CMV positive responders and the absolute number of CMV-specific T-cells will be compared between the subjects with adjudicated CMV disease and those without disease using GEE models. A receiver operator characteristic (ROC) will be used to estimate a cutoff for number of CMV-specific T-cells that appear to prevent the development of CMV disease.

Follow up samples [6 and 12 months] for immune assays will also be evaluated as above. In addition, a repeated measures model will be used to evaluate the relationship of each type of CMV response over time and the development of CMV disease.

12.7.3.2 Neutralization antibody

This will be measured as titer of CMV neutralizing antibody [dilution factor]. Currently, the precise relationship between CMV neutralizing antibody and CMV disease is uncertain, therefore a number of exploratory analyses will be performed.

Initial sample [100 days]: The percent of samples demonstrating any detectable CMV neutralizing antibody will be compared between the two groups using a Chi square test. This activity will also be quantitatively evaluated. The titers will be transformed to a log10 and the log mean titers will be compared between the two groups using a t-test. A generalized estimating equation [GEE] regression model will be used to examine the relationship of antibody to CMV disease. The log transformed levels will be compared between a dichotomized grouping [subjects that develop CMV disease and those that remain disease free] using a t-test. The timing to onset of late CMV disease will also be compared between subjects with and without CMV antibody activity using a Kaplan-Meier probability estimate. The two probability functions will be compared using a rank sum test.

Follow up samples [6 and 12 months]: The follow up samples will be evaluated similarly to the initial sample. In addition, the log difference between the initial sample and the follow up sample will be calculated and compared between the two regimens using a regression analysis. A repeated measures model will be used to evaluate the relationship of CMV antibody activity over time and the development of CMV disease.

12.7.4 Cost effectiveness analysis

CMV disease and the costs associated with treating it are estimated to add $25,000 to $50,000 to the cost of organ transplantation (70, 71). In addition to the direct effects of CMV disease, CMV in transplant recipients may contribute to an increased risk of rejection and opportunistic infection (OI). These events add a substantial economic burden to solid organ transplantation related to diagnosis, treatment and increased hospital length of stay. In addition to the economic considerations, CMV may also reduce graft and recipient survival. Treeage Pro 2009 [Treeage software, Williamstown, MA] will be used to develop a decision analysis model. The basic model will be similar in structure to those developed by Mauskopf et.al (72) for evaluating CMV in renal transplantation and Annemans et.al.(73) in liver transplantation. The tree will be utilized to estimate the costs associated with the 2 CMV intervention strategies, preemptive therapy and prophylaxis. Each strategy has the principle outcome of CMV disease, no disease. The disease state (CMV disease/no CMV disease) then transitions to 4 possible outcome states: rejection, OI, both rejection and OI, or no complications. Rejection can than transition to graft loss/no loss. Each outcome state terminates as alive/dead. Transition probabilities will be estimated post hoc. The model will allow for a range of sensitivity analyses

in which the parameter estimates can be varied [for example at the 10th and 90th percentile of the observed value].

Data already collected in this study will be used for cost effectiveness analyses. These include length of stay, drugs received for CMV prophylaxis/preemptive therapy, CMV disease, treatment for leukopenia, and other outcomes including rejection and retransplantation. Available data already captured will be used for the analyses; no additional items will be collected specifically for costs efficacy analyses. Cost estimates are obtained from average standard costs for drugs and procedures available in the literature. No site specific costs will be collected.

12.7.4.1 Total costs

Cost effectiveness will be estimated comparing the costs for each regimen. A cost-effectiveness ratio will be calculated as [Costs associated with preemptive therapy- costs associated with prophylaxis]/Effect of preemptive therapy-effect prophylaxis therapy]. Where the costs are the total composite costs derived from the analysis tree and the effect is CMV disease free success.

12.7.4.2 Cost utility analysis

A Cost-utility analysis will estimate the cost per quality-adjusted life-years (QALY). Quality of life will be estimated for possible outcomes where 0=death and 1=perfect health. The utility values will be estimated from the literature for patients with end stage liver disease and cirrhosis (74, 75). A fully functioning graft at one year, in a recipient with no major complications is estimated to have a utility value of about .83. Utility values for subjects with complications will be estimated from articles listed in the Cost-effectiveness analysis registry (76). Cost per QALY will be estimated for both regimens.

Cost utility analysis is based on estimated utilities found in the literature for items already collected such as retransplantation/disease/death. No additional data items will be collected.

12.8 Interim and Final Analysis

12.8.1 Interim analysis

There will be a single planned interim analysis for the primary endpoint of CMV disease. This analysis will be done after 1/3 of the subjects or 60 subjects have been enrolled and followed for 1 year. Additional endpoint analysis would introduce a multiple comparison bias with a resultant "alpha penalty". Each test done at alpha=.05 means that roughly 5% will generate a type 1 error [rejecting the null hypothesis when in fact it is true]. Repeated testing of interim data can therefore inflate false positive error. To account for this loss of alpha, the sample size would need to be up-adjusted making the study infeasible. For the planned interim analysis, we will use alpha preservation estimation for repeated assessments [Method of

Haybittle-Peto] so that the trial will be terminated only if there is a statistically significant difference in the incidence of CMV disease at the 0.001 level. A statistically significant difference in the incidence of CMV disease at this level [.001] would be detectable after the enrollment of 60 subjects with 1 year follow up, if the difference in the incidence of CMV diseases between the two groups is greater than 50%.

12.8.2 Interim analysis showing futility:

It is possible that the interim analysis could suggest the likelihood of futility i.e. the trial may result in equivalence or non-inferiority. However, there will be no stopping rule for futility. Even if the interim analysis shows that the two groups are equivalent in efficacy with regards to CMV disease rate, there are additional benefits of continuing the trial for example, cost effectiveness of the two approaches which has societal benefits and wider implications. Additionally, differences in immunologic parameters, opportunistic infections, survival, graft loss, and drug resistance have profound relevance for advancing the field. The study will therefore continue so that these additional assessments can be systematically conducted.

12.8.3 Final analysis:

This will be completed when all subjects who have not previously discontinued have completed the one year follow up study visit. Primary, secondary and selected exploratory analyses (outlined in section 12.7) will be performed after one year follow up with supplementary analyses of exploratory endpoints (outlined in section 12.7) performed at study closure (and no longer than 7 years after first enrollment).

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DOCUMENTS

Source data for CAPSIL are defined as all the information related to clinical findings, observation, or other activities in the study, written down or electronically entered in original records

In addition, medical records and laboratory data from all facilities (long-term nursing care facilities, rehab institutions, out-patient clinics) where subjects receive care throughout the study will be reviewed and serve as source documentation. Records to include all physician progress notes, laboratory results, diagnostic testing results, and medication records, will be requested from the institutions by the enrolling site for review and monitoring purposes.

Additionally, study specific source documents have been created to record data not routinely documented in the subject's medical record. Study specific documents created for the CAPSIL study. For each subject entered into the study, the investigator will keep a file that will record the specific data which are not part of the routine documentation. The investigator will permit trial-related monitoring, audits, IRB reviews, and regulatory inspections by providing direct access to source data according to the subject's consent.

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data for CAPSIL study. Participating sites should consult the MOP and the DMID/NIAID Source Document Standards for specific instructions/forms.

14 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the investigational site is responsible for conducting routine quality assurance (QA) and quality control (QC)) activities to internally monitor study progress and protocol compliance. The Principal Investigator will provide direct access to all trial-related sites source data/documents, data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site. DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The Statistical and Data Coordinating Center will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

15 ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

15.2 Institutional Review Board

Reviewing IRBs will be registered with the OHRP to conduct FDA-regulated studies. In the United States and in other countries, only institutions and will hold a current US Federal wide Assurance (FWA) issued by OHRP. Notification of the IRB's composition and the institutions FWA number will be provided to DMID. This protocol, informed consent documents, relevant supporting information, and all types of volunteer recruitment or advertisement information will be submitted to the Institutional Review Board (IRB) for review and must be approved before the study is initiated. Any amendments to the protocol must also be approved by the IRB prior to implementing changes in the study. The investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once per year. The investigator must also keep the IRB informed of any significant AEs. All IRB approved documents as well as relevant study correspondence should be copied and sent to U. Pitt (Coordinating Center).

15.3 Informed Consent Process

The process of obtaining informed consent must be documented in the medical records, clinic chart, and/or research chart. The consent form must be signed and dated by the study participant or legal guardian before participation in the study. A copy of the signed consent form must be provided to the study participant or legal guardian. Signed consent forms must remain in each study participants study file and must be available for verification by study monitors at any time.

The investigational nature and research objectives of this trial, the procedure, and its attendant risks and discomforts will be carefully explained to the study participant or legal guardian. A signed informed consent document will be obtained from each study participant or legal guardian prior to entry into this study. At any time during participation in the protocol, if new information becomes available relating to risks, AEs, or toxicities, this information will be provided orally or in writing to all enrolled or prospective study participant or legal guardian.

Documentation will be provided to the IRB and, if necessary, the informed consent will be amended to reflect any relevant information.

An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative.

The participant or legal guardian will sign the informed consent document prior to any procedures being done specifically for the study. The participant or legal guardian should have the opportunity to discuss the study with their family, friends or personal physician, or think about it prior to agreeing to participate. The participant or legal guardian may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participant or legal guardian for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.
For non-English speaking subjects, a translated informed consent form will be used according to institutional IRB specific procedures.

15.4 Exclusion of Women, Minorities and Children (Special Populations)

Subjects will be eligible for participation in the study, regardless of gender, race or ethnicity. All patients undergoing liver transplant are potentially eligible for inclusion. The expected percentage of women and minorities enrolled will reflect the local demographics of the liver transplant population. Only individuals who are greater than or equal to 18 years old will be included at this time.

Children will not be included in the trial because there are significant problems and concerns with the use and appropriate dosing of valganciclovir in pediatric setting. Unlike adult transplant recipients, the oral bioavailability of valganciclovir is considerably lower in children (77). Its use in pediatric patient population has been limited by low bioavailability necessitating large and frequent doses, a problem compounded by the increased weight-adjusted clearance of ganciclovir in children (78). Consequently, unexpectedly high inter- and intrapatient variability in ganciclovir levels has been observed (77) and dosing recommendations based on adult guidelines have the potential for overdosing in children with below normal serum creatinine, low body surface area or low body weight. The potential dosing variability in pediatric subjects could significantly confound the study. Thus, there are significant concerns related to the potential for inadvertently achieving low levels and the risk of ganciclovir resistance.

A powder for oral solution with bioequivalence to valganciclovir tablets exists (78). However, valganciclovir oral solution in pediatric liver and kidney transplant recipients using a dosing algorithm based on dose normalization for body surface area resulted in children aged <5 years being under exposed to ganciclovir by approximately 2-fold (79-80). Another study (WV 16726) that included 63 pediatric transplant recipients of whom 17 were liver

transplant patients assessed the pharmacokinetic, safety, and efficacy study of the valganciclovir oral solution using dosing algorithm based on creatinine clearance and body surface area (81). A majority of the patients (60%) belonged to groups other than R-/D+. Treatment failures defined as either the development of CMV disease up to day 100 post-transplant or discontinuation of study medication due to lack of efficacy or toxicity was observed in 7% of the patients; a higher number of these were liver transplant recipients. When only R-/D+ patients were considered, treatment failure was documented in 33%. Currently, oral solution is not approved for use in pediatric liver transplant recipients.

While, there are recipes available to compound liquid valganciclovir from the tablets, there is no standardization for these. Furthermore, unlike adult liver transplant recipients, there are very limited published data on the use of oral valganciclovir in children and there is no precedence for the use of valganciclovir as preemptive therapy in children (11). Given low bioavailability and the potential for erratic drug levels, use of valganciclovir for established viremia as in the setting of preemptive therapy is a major concern. Prolonged valganciclovir use in the form of prophylaxis is also theoretically worrisome given the known carcinogenicity in animals and unknown consequences of prolonged ganciclovir derivatives in children.

Thus, valganciclovir use in children in context of this study poses compelling and significant concerns related to bioavailability (with the potential for both underdosing and overdosing), efficacy and toxicity such that the scientific validity of the study results and the safety of the subjects could be compromised. Therefore until further data becomes available regarding the safety and appropriate dosing of valganciclovir, inclusion of children in this study was not deemed prudent.

15.5 Subject Confidentiality

Once a subject is randomized in the study, they will be assigned a study number and will be identified only by that number for the entire study period. Identity of the research subject will be kept confidential and any identifying information will be kept by the study personnel at the local site. This confidentiality will extend to cover testing of biological samples and any clinical information relating to participating subjects. The study protocol, documentation, data, and all other information generated will be held in strict confidence. The study monitor or other authorized representatives of the Institutional Review Boards of the participating Medical Center, National Institutes of Allergies and Infectious Disease (NIAID), U.S. Office for Human Research Protections (OHRP), and U.S. Food and Drug Administration (FDA) may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) for the subjects in this study. The clinical study site will permit access to such records.

15.6 Study Discontinuation

Under certain circumstances as noted in the protocol, the study or an individual subject's participation in the study may be terminated. If the study is terminated, safety and follow up procedures will be continued as described in the protocol unless consent is withdrawn.

15.7 Future Use of Stored Specimens

The investigators intend to store specimens from study participants .The prospectively collected samples and clinical parameters assessed in this study represent an important resource for future studies. Cryopreserved samples may be used to perform additional assays to support standardization and validation of laboratory assays, and to evaluate additional endpoints and associations of interest. These assays may include, but are not limited to PCR testing for other pathogens, additional cytokines and chemokines, proteomics, gene expression studies, and research related to furthering the understanding of CMV and other infections, and other transplant-related outcomes, to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. The Steering Committee (Drs. Singh, Limaye and Boeckh) in conjunction with the DMID will evaluate proposals for substudies utilizing either samples or clinical data collected for the primary study. Proposals will be reviewed and prioritized for scientific merit and compliance with applicable regulatory/consent requirements. Funding for substudies beyond those outlined in the contract will not be provided and will be the responsibility of substudy investigators.

The specimens will be labeled with a code number and not with the study participant's name or other subject-identifiable information. At the time of consent for study participation, study participants will have the opportunity to either agree or decline to have their specimens used in future research. The study participant will indicate his/her preference by initialing the appropriate line or checking the appropriate box of the Consent Form in the section entitled, "Future Use of Specimens". Non-protocol designated, future testing of samples will be performed only on samples from study participants who have consented for future testing of samples. A repository for residual samples will be established according to OHRP guidelines ensuring that codes or other personally identifying links will not be distributed to future researchers. If the study participant has indicated in the signed consent form that he/she does not agree to allow the future use of specimens for future research, then his/her specimens will be destroyed at the end of the study.

16 DATA MANAGEMENT PLAN

The investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the data reported.

16.1 Data management responsibilities

The University of Pittsburgh's Center for Research on Health Care (CRHC) Data Center will function as the Data Coordinating Center (DCC) for the CAPSIL study and will oversee all aspects of data management and quality review. Analyses and reports from the study data will be generated by the study statistician at the Coordinating Center. Electronic Case Report Forms (eCRF) will be created for use in a customized web application designed to meet CAPSIL protocol requirements. The web application allows secured user specific access to the eCRFs. Menus and reporting screens allow users to randomize, track, and enter data. To protect the privacy of the research subjects, only coded data will be entered. The web site will also act as a repository for CAPSIL study related documents including the Manual of Procedures, current protocol version, and additional general study information to keep sites updated on CAPSIL progress. Printable hardcopy versions of these forms will be available on the website in case of problems with the electronic system.

All data and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for seriousness, severity and causal relationship, and reviewed by the site principal investigator or designee. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator.

Data from all participating sites is merged upon transmission to the DCC server and will be used to generate files for data analysis. The DCC will also generate reports from the merged dataset as required by the DSMB to monitor study safety. Measures to ensure data security, quality and integrity are fully described in the Data Management Plan. Specific information on the use of the web-based data entry system is provided in the Data management section of the MOP.

16.2 Data capture methods

Clinical data will be abstracted from medical records and entered onto eCRFs provided on the study website. All eCRFs are fully HIPAA compliant. Data will be recorded directly from the subject's medical record or study specific source documents. Data form completion instructions will be provided by the coordinating center and can be found in this study's MOP. The data system is 21CFR part 11 compliant. Quality control computer checks will be run periodically to check for data completeness, ranges of data, consistency of data between forms

and logic reliability. All data changes will be automatically tracked with a change reason required and will be recorded on a change log.

16.3 Types of data

Data for this study include safety, clinical laboratory, virologic, outcome measures (clinical assessments of CMV disease, mortality, allograft rejection; graft loss, hematologic toxicity, co-infections) and exploratory immunologic assessments.

16.4 Timing of reports

The following reports will be generated during this study.

- DSMB reports at a minimum annually. Timing and frequency of these reports will be determined in conjunction with DMID and the DSMB, but will be generated no less frequently than annually. (See Section 9.8.1)
- Interim analysis will be conducted and provided to the DSMB after 60 subjects have been enrolled and followed for 1 year. (See Section 10)
- End point committee reports will be provided to the EPC prior to the interim analysis and prior to close out. (See Section 12.8.1)

16.5 Study record retention

Records and documents pertaining to the conduct of this study, including eCRFs, source documents, consent forms, laboratory test results, and medication inventory records, must be retained by the investigator for 2 years . No study records shall be destroyed without prior authorization from the University of Pittsburgh (Coordinating Center) and the DMID. All study records will be retained in compliance with CFR title 21 part 312.57

16.6 Protocol deviations

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. However, if the deviation increases study subject risk, the reporting timeline is expedited, requiring submission of deviation within 2 working days of identification. All deviations must be promptly reported to the University of Pittsburgh (Coordinating Center). Subject specific deviations will be reported on the electronic protocol deviation form of the eCRF. Non-subject specific protocol deviations will require a fax

transmittal of a protocol deviation form to the U. of Pitt (coordinating center). This form will be available at the CAPSIL web portal.

Each investigator must adhere to the protocol as detailed in this document and agree that any changes to the protocol must be approved by the University of Pittsburgh Coordinating Center and the DMID prior to seeking approval from the IRB/IEC. Each investigator will be responsible for randomizing only those study participants who have met protocol eligibility criteria.

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the study participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with GCP:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
5.1 Quality Assurance and Quality Control, Section 5.1.1
5.20 Noncompliance, Sections 5.20.1 and 5.20.

All deviations from the Protocol must be addressed in a source document. A completed copy of the Protocol Deviation (PD) Form must be maintained in the Regulatory File, as well as in the subject's data collection forms. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

17 PUBLICATION POLICY

Following completion of the study, the investigators may publish the results of this research in a scientific journal. One year end-points will also be analyzed and reported prior to study closure. Specific criteria for authorship will be reviewed and discussed with all investigators.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. This protocol will be registered on ClinicalTrials.gov by the Principal Investigator prior to onset of subject enrollment.

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication. Publications arising from this protocol will comply with this requirement.

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SUPPLEMENTS/APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

																Follow-up post drug administration		
Assessments/test for ALL subjects	Baseline	7d	14	21	28	35	42	49	56	63	70	77	84	91	98	107	6mo	12mo
Informed consent	x																	
Medical/ surgical history	x																	
Donor/recipient CMV serology status	x																	
Demographics/subject weight	x																	
Pregnancy testing (~5ml blood)[1]	x															x		
Hematology (~3ml of blood)[2,12]	x	x	x	x	x		x		x		x		x		x	x	x	x
Chemistry (~6ml of blood) [2,12]	x	x	x	x	x		x		x		x		x		x	x		
Genetic sample (10 ml of blood)[3,4]	x																	
Immune Assay (~45ml of blood) [4]															x		x	x
Outcome monitoring and data collection	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
AEs & SAEs [6]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Suspected CMV disease sample (~8.5 ml)[5,7]	----------x----------																	
CMV resistance assay (~8.5ml) [5,8]	----------x----------																	
Medication Compliance Interview [13]						x				x						x		
Prophylaxis group only																		
Valganciclovir administration [9]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Preemptive therapy group only																		
CMV PCR assay (~8.5ml of blood)[10]		x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Valganciclovir administration[11]		x	x	x	x	x	x	x	x	x	x	x	x	x	x			

1. Females of child bearing potential will be pregnancy tested prior to enrollment and after the end of treatment. SOC testing may be used if obtained during current hospitalization. The end of treatment pregnancy test may be obtained after end of treatment up to 3 months post end of treatment.
2. Hematology sample will be collected until day 107 as part of safety labs. Additional hematology samples at 6 and 12 months will be collected for lymphocyte count assessment for the immune assays. Lymphocyte count from day 98, 6 month, and 12 month sample will be recorded on the immune assay eCRF
3. Subject may opt out of genetic sampling. If consent for genetic testing is obtained, sample will be taken once at baseline.
4. Samples will be sent to Boeckh Lab at the Fred Hutchinson Cancer Research Center
5. Samples will be sent to the University of Washington Medical Center Virology Lab
6. AEs and SAEs will be recorded through study intervention period plus 7 days beyond last study drug administration
7. This sample will be collected only in subjects with suspected CMV disease (either group)
8. Resistance testing may be performed on on subjects who fail to achieve reduction in viral load after 3 weeks of valganciclovir or iv ganciclovir use for CMV viremia (in preemptive therapy group) or in subjects with CMV disease (in either group)
9. Valganciclovir will be given for 100 days

10. Samples will be sent to the University of Washington Medical Center Virology Lab
11. Study valganciclovir will only be employed upon detection of a positive CMV PCR during study days 1-100 and will continue until two negative consecutive weekly PCRs are obtained
12. Baseline, hematology and chemistry standard of care lab results will only be accepted if within 48 hours of enrollment. If not collected as part of standard of care within 48 hours, these must be drawn for research purposes.
13. Medication compliance interview to be completed at day 30, day 60, and end of treatment with a +1

APPENDIX B: BLOOD VOLUME COLLECTED

	Base-line	7d	14	21	28	35	42	49	56	63	70	77	84	91	98	6mo	12mo
Pregnancy **test**	5ml														5ml		
CMV PCR		8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5ml	8.5mll	8.5mll	8.5mll		
CMV disease		----------8.5ml----------															
CMV resistance		----------8.5ml----------															
Immune Assays															45ml	45ml	45ml
Genetic testing	8.5ml																
Lymphocyte count																3ml	3ml

Preemptive Therapy Group

Pregnancy testing	10	ml	
CMV PCR: (14 visits X 8.5mL)		119	ml
Suspected CMV disease (only if indicated)		8.5	ml
CMV Resistance testing (only if indicated)		8.5	ml
Immune Assays: (45 ml X3)	135	ml	
Genetic sample: (optional)	8.5	ml	
Lymphocyte count	6	ml	
Preemptive therapy total research blood draw		**~295.5**	**ml/12 month (~20 tablespoons)**

Prophylaxis Group

	Baseline	7d	14	21	28	35	42	49	56	63	70	77	84	91	98	6mo	12mo
Pregnancy test	5ml														5ml		
Immune Assays															45ml	45ml	45ml
Genetic	8.5ml																

testing																	
Lymphocyte count																3ml	3ml
CMV disease		----------8.5ml----------															
CMV resistance		----------8.5ml----------															

Pregnancy testing	10	ml	
Immune Assays: (45 ml X3)	135	ml	
Suspected CMV disease (only if indicated)		8.5	ml
CMV resistance testing: (only if indicated)		8.5	ml
Genetic sample: (optional)	8.5	ml	
Lymphocyte count	6	ml	
Prophylaxis total research blood draw ~		**~176.5**	**ml/12 month (~ 12 tablespoon)**

APPENDIX C: EXPECTED EVENTS FOR LIVER TRANSPLANT PATIENTS

Expected events for liver transplant patients: The following events are expected to occur with a reasonable frequency in the typical/expected clinical course of a patient following liver transplant:

- Hepatobiliary: Graft rejection, non-function of liver, delayed graft function, hepatic artery thrombosis, hepatic necrosis, liver abscess, biliary complications, biliary stricture or leak, biliary stenosis, cholecystitis, pancreatitis, liver failure, hepatic necrosis, viral hepatitis, graft versus host disease
- Surgical: Leaks (gastrointestinal, biliary or anastomotic), hemorrhage, tracheostomy, return to the operating room for surgery, retransplantation, vascular stenosis or thrombosis (inferior vena cava, portal vein), anastomotic problems, strictures, hernia, lymphocele, incision dehiscence, abdominal wall defect, wound infection, hernia, fluid collection, seroma, biloma, hematoma, abdominal wall defect, infarction of liver, vana caval tear
- Gastrointestinal: Nausea, vomiting, diarrhea, dyspepsia, abdominal distension or bloating, abdominal pain, anorexia, perforation, ischemic bowel, reflux gastritis, dyspepsia, ascites, ileus, bowel obstruction, GI bleed, achlasia
- Neurologic: Tremor, seizures, confusion, headache, dizziness, hallucinations, delusion, psychosis, insomnia, somnolence, lethargy, depressed level of consciousness, agitation, amnesia, anxiety, emotional lability, paralysis, vertigo, abnormal dreams, headache, cerebrovascular accident, subarachnoid hemorrhage, encephalopathy, posterior reversible encephalopathy syndrome, tacrolimus toxicity, progressive multifocal leukoencephalopathy, central pontine myelinolysis, neuropathy, abnormal vision, blindness, amblyopia, tinnitus, deafness
- Neuromuscular and skeletal: Hypertonia, incoordination, leg cramps, monoparesis, hemiparesis, myoclonus, nerve compression, quadriparesis
- Constitutional/ systemic : Fever, asthenia, failure to thrive, weight loss or weight gain, anasarca, embolism, multiorgan failure, malnutrition
- Infection: Fever, hypothermia, rigors, chills, systemic inflammatory response syndrome, infection (documented or suspected), sepsis, multisystem organ failure
- Pulmonary: Acute lung injury, respiratory distress syndrome, aspiration, asthma, atelectasis, mucus plugging, respiratory failure, dyspnea, hypoxia, pneumonia, pleural effusion, pneumothorax, pulmonary edema, sinusitis, need for mechanical ventilation, intubation or reintubation, chest tube insertion, pleural effusion, embolism, pulmonary hypertension
- Electrolyte and metabolic: Acidosis or alkalosis, low albumin, dehydration, gout, elevated liver function tests (bilirubin and liver enzymes), increase or decrease in the level of

sodium, potassium, blood urea nitrogen, creatinine, glucose, calcium, magnesium, phosphate, uric acid, cholesterol, lipids, iron

- Hematologic: Anemia, blood loss, prolonged PT or PTT, abnormalities in coagulation (DIC), hematoma, hemorrhage, bleeding, venous thrombosis, thrombocytopenia/thrombocytosis, leukopenia/leukocytosis, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, pancytopenia
- Cardiac: Arrythmias (atrial flutter, atrial fibrillation, ventricular fibrillation), prolonged QT interval, QRS or ST segment abnormal, tachycardia, bradycardia, torsade de pointes, hypertension, hypotension, pulmonary hypertension, myocardial ischemia or infarction, syncope, postural hypotension, phlebitis, thrombosis, myocardial hypertrophy, cardiomyopathy, anaphylaxis, shock, anaphylactoid reaction, angioedema, arterial dissection
- Renal: Acute renal failure, albuimuria, nephropathy, hematuria, renal tubular necrosis, interstitial nephritis, nephropathy
- Genitourinary: Bladder spasm, dysuria, nocturia, incontinence, urinary frequency, urinary retention, hemorrhagic cystitis
- Malignancies: Lymphoproliferative disorder, skin neoplasm, melanoma, hepatocellular carcinoma, hematologic or solid tumor.
- Dermatologic: Edema at anybody site, rash (petechiae, purpura, spider angiomata), ecchymosis, bruising, flushing, cellulitis, dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, decubitus ulcer, photosensitivity, skin ulcer, wound healing impaired.