Cytomegalovirus in Transplantation: Developing Drugs to Treat or Prevent Disease

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

May 2018
Clinical/Antimicrobial

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I. INTRODUCTION

The purpose of this guidance is to assist sponsors in the clinical development of drugs for the treatment or prevention of cytomegalovirus (CMV) disease in patients who have undergone solid organ (SOT) or hematopoietic stem cell transplantation (HSCT).[2] Specifically, this guidance addresses the Food and Drug Administration's (FDA's) current thinking regarding the overall development program and clinical trial designs for the development of drugs and biologics to support an indication for the treatment or prevention of CMV disease in post-transplant populations. This draft guidance is intended to serve as a focus for continued discussions among the Division of Antiviral Products (DAVP), pharmaceutical sponsors, the academic community, and the public.[3] This guidance does not address drug development for the prevention or treatment of congenital CMV infection or CMV infection in patients other than those undergoing SOT or HSCT.

This guidance also discusses the use of CMV viremia, measured as DNAemia (CMV deoxyribonucleic acid (DNA) in blood determined by polymerase chain reaction (PCR)), as a surrogate endpoint in clinical trials.

This guidance does not contain discussion of the general issues of statistical analysis or clinical trial design. Those topics are addressed in the ICH guidances for industry *E9 Statistical*

[1] This guidance has been prepared by the Division of Antiviral Products in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] For the purposes of this guidance, all references to *drugs* include both human drugs and therapeutic biological products unless otherwise specified.

[3] In addition to consulting guidances, sponsors are encouraged to contact the DAVP to discuss specific issues that arise during the development of anti-CMV drugs.

Principles for Clinical Trials and *E10 Choice of Control Group and Related Issues in Clinical Trials*, respectively.[4]

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

CMV is a member of the beta-herpes virus group that causes infection worldwide with variable geographic distribution linked to socioeconomic status. In the United States, CMV seroprevalence ranges from 40 percent to 80 percent (Cannon and Davis 2005; Bate et al. 2010). Primary infection occurs in CMV seronegative hosts and is usually acquired during the first decades of life. In most cases, primary infection is benign and self-limited. However, in patients with immature or compromised immune systems (e.g., transplant recipients, congenitally infected newborns, or patients with acquired immunodeficiency syndrome (AIDS)), primary CMV infection is often symptomatic and is associated with increased morbidity and mortality. As with all herpes viruses, CMV establishes lifelong latency after primary infection; thereafter, intermittent viral shedding and reactivation of disease can occur, particularly in hosts with compromised immune systems (Ramanan and Razonable 2013).

CMV is the single most frequent opportunistic pathogen in transplant recipients. The incidence of CMV infection and disease in this population depends on a number of factors such as transplant type, donor and recipient CMV serostatus, and the level of immunosuppression (Ramanan and Razonable 2013). A transplant recipient is described by nomenclature that first describes the donor's CMV serostatus followed by the recipient's CMV serostatus. For example, D+/R- refers to a seronegative individual who has received a transplant from a seropositive donor.[5] In SOT, observational studies have demonstrated an association between donor and recipient CMV serostatus and risk for CMV disease; D+/R- status is associated with a higher risk (with rates of 50 to 60 percent) for developing CMV disease than CMV seropositive recipients (D+/R+ or D-/R+) who have rates of 10 to 20 percent (Hartmann et al. 2006). The lowest rate of CMV infection (less than 5 percent) occurs in CMV seronegative SOT recipients who received a transplanted organ from a seronegative donor (D-/R-). In HSCT recipients, CMV seropositive recipients (R+) are at the highest risk for development of CMV infection regardless of the donor's CMV serostatus. Without intervention, approximately 80 percent of CMV seropositive HSCT patients will experience CMV infection (viremia) and approximately 30 percent of patients with CMV viremia will develop CMV disease (Ljungman et al. 2010).

[4] We update guidances periodically. To make sure you have the most recent version of a guidance, check the FDA Drugs guidance web page at https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

[5] CMV serostatus of donor (D) and recipient (R) is designated as D+ or D- and R+ or R-, respectively. The term *CMV seropositive* refers to a donor or recipient with antibodies to a previously acquired CMV infection and the term *CMV seronegative* denotes that anti-CMV antibodies are absent.

The clinical manifestations of CMV infection range from asymptomatic CMV viremia to tissue-invasive (end-organ) CMV disease. Any organ can be infected by CMV. However, CMV pneumonia is the most serious manifestation of CMV infection in HSCT recipients and has been associated with high mortality. In contrast, in SOT recipients CMV has a predilection to replicate in the allograft. CMV infection may also be associated with an increased risk of other opportunistic infections, graft failure, graft rejection, and mortality (Razonable et al. 2013).

Because of the increased morbidity and mortality associated with CMV infection in transplant recipients, it has been recognized that prevention of CMV disease may be a better strategy than treatment of established CMV disease. Prophylactic therapy (treatment administered to all patients at risk for developing CMV disease) and preemptive therapy (treatment of patients with evidence of CMV replication in blood) are the two major strategies used for prevention (Boeckh and Ljungman 2009; Tomblyn et al. 2009; Razonable et al. 2013; Kotton et al. 2013). Both strategies have been shown to be useful for prevention of CMV disease in SOT and HSCT recipients.

Although at present no large, randomized, controlled trials have directly compared the two approaches, prophylaxis with oral valganciclovir has emerged as the most commonly used clinical strategy for the prevention of CMV disease in high-risk SOT recipients in part because of the convenient once daily dosing with this drug (Kotton 2013; Razonable et al. 2013). Until recently, preemptive therapy rather than prophylaxis therapy was the preferred strategy in HSCT patients because of the bone marrow toxicities of the available anti-CMV drugs (Boeckh and Ljungman 2009). However, the approval of letermovir in late 2017 for prophylaxis of CMV infection in adult CMV-seropositive recipients of an allogeneic HSCT is anticipated to change the therapeutic approach in these patients (Marty et al. 2017).

Currently, there are limited therapeutic options for the treatment or prevention of CMV disease in transplant patients. Only five drugs have received FDA approval for systemic use for the treatment or prevention of CMV disease: letermovir, ganciclovir and its prodrug valganciclovir, foscarnet, and cidofovir. Letermovir was approved for CMV prophylaxis in CMV-seropositive recipients of an allogeneic HSCT; ganciclovir and valganciclovir were approved for the prevention of CMV disease in transplant recipients, and for the treatment of CMV retinitis in immunocompromised patients, including patients with AIDS. Foscarnet and cidofovir have received FDA approval only for the treatment of CMV retinitis in AIDS patients. Moreover, most of the existing treatments are associated with significant toxicity. These findings, coupled with the emergence of resistance to available drugs (Lurain and Chou 2010; Komatsu et al. 2014), strongly support the urgent need for new therapeutic agents that are effective and less toxic.

During the past 15 years, all phase 3 studies designed to support marketing applications for CMV drugs were prophylaxis studies in SOT and/or HSCT recipients. The primary endpoint used in these prophylaxis studies in SOT recipients was the incidence of CMV disease, including both symptomatic CMV infection (also called *CMV syndrome*) and/or tissue-invasive CMV disease (e.g., CMV colitis, hepatitis, or pneumonia). CMV syndrome is better defined in SOT than in HSCT patients, mainly because the symptoms associated with CMV syndrome can have several

other causes in the setting of HSCT, including other viral infections. Until recently, the primary endpoint used in prophylaxis studies in HSCT patients was the incidence of tissue-invasive CMV disease.

However, the results of recent trials revealed that in the current era of preemptive therapy for CMV viremia based on optimized PCR assays, the incidence of tissue-invasive CMV disease in HSCT recipients at 6 months post-transplantation was less than 5 percent (Marty et al. 2011). These results call into question whether trials with tissue-invasive CMV disease as an endpoint in HSCT patients are feasible, considering the sample sizes needed for such trials given the low frequency of CMV disease. The accumulated clinical literature supports the premise that CMV viremia predicts development of CMV disease in transplant patients (Gor et al. 1998; Emery et al. 1999; Emery et al. 2000; Jang et al. 2012; Natori et al. 2018), that prophylaxis or preemptive therapy prevents CMV disease (Green et al. 2016), and that the suppression of viremia is associated with clinical resolution of CMV disease (Åsberg et al. 2007).

These observations have prompted the FDA to consider CMV viremia (DNAemia) as a sufficiently validated endpoint to be used as a part of a composite endpoint to support traditional approval. Therefore, tradional approval for new drug applications (NDAs) for CMV prophylaxis trials in HSCT recipients can be based on a composite endpoint defined as the occurrence of either CMV tissue-invasive disease or the development of CMV DNAemia above a prespecified threshold. The consideration of CMV DNAemia as a part of a composite endpoint for other indications (e.g., treatment) is also discussed in this guidance.

III. DEVELOPMENT PROGRAM

A. General Drug Development Considerations

1. Early Phase Development Considerations

General considerations pertinent to nonclinical development and early clinical development are outlined in this section. Sponsors considering development of antiviral drugs for the treatment or prevention of CMV disease are encouraged to communicate with the FDA through the pre-investigational new drug application (pre-IND) consultation program.[6,7] Pre-IND consultation with the FDA is optional, although it may be particularly helpful for sponsors with limited experience in the IND process or to obtain FDA advice in the development of drugs with unique considerations based on mechanistic action, novel treatment approaches, or the use of novel biomarkers.

[6] https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/Overview/ucm077776.htm

[7] https://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/InvestigationalNewDrugINDApplication/Overview/ucm077546.htm

a. Pharmacology/toxicology development considerations

Pharmacology/toxicology development for CMV antivirals should follow existing guidance for drug development. For detailed recommendations regarding pharmacology/toxicology development for single antiviral drugs and for two or more new investigational drugs to be used in combination, sponsors should consult the following ICH guidance on nonclinical safety studies: For small molecules, see the ICH guidance for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals*; for biologics, see the ICH guidance for industry *S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*.

Carcinogenicity studies are recommended if the expected treatment duration, including intermittent use, is 6 months or longer (e.g., prevention indications).[8] Carcinogenicity studies can be submitted with an initial marketing application (i.e., NDA or biologics license application) or as required postmarketing studies.

For drugs to be used in combination, ICH M3(R2) includes a discussion of nonclinical safety studies appropriate in a combination drug development setting involving two early stage entities.[9] ICH M3(R2) defines early stage entities as compounds with limited clinical experience (i.e., phase 2 studies or earlier).

b. Nonclinical virology development considerations

Nonclinical virology studies can facilitate initial dose selection, enable the design of a clinical proof-of-concept study, and support an antiviral claim. Studies to support initial human trials should be conducted before submission of an IND. Virology development for CMV treatment or prevention should follow existing guidance for drug development.[10] Additional recommendations for nonclinical and clinical virology assessments specific to the development of drugs for the treatment or prevention of CMV infection are summarized throughout this guidance.

Mechanism of action

The mechanism by which a drug exhibits anti-CMV activity should be investigated using cell culture, biochemical, structural, and/or genetic studies that include evaluation of the effect of the drug on relevant stages of the virus life cycle and identification of the CMV target protein(s) for direct-acting antivirals. Mechanism of action investigations should include appropriate controls for assessing the specificity of anti-CMV activity, which may include assessments of activity against other CMV proteins, relevant host proteins, other viruses, and/or cells infected with investigational drug-resistant CMV variants. Biochemical or subcellular quantitative assays

[8] See the ICH guidance for industry *S1A The Need for Long-Term Rodent Carcinogenicity Studies of Pharmaceuticals*.

[9] See ICH M3(R2), section XVII., Combination Drug Toxicity Testing.

[10] See the guidance for industry *Antiviral Product Development — Conducting and Submitting Virology Studies to the Agency*.

supporting the mechanism of action should report the inhibitory concentration values (IC_{50} and IC_{90}).

Antiviral activity data from cell culture studies

The antiviral activity of an investigational drug should be characterized in cell culture to identify a target plasma concentration for evaluation in CMV-infected patients. Antiviral activity of investigational drugs should be assessed using CMV laboratory isolates as well as several (more than 20) geographically and temporally distinct isolates, the vast majority of which should be U.S. isolates. The 50 percent and 90 percent effective concentrations (EC_{50} and EC_{90} values) should be determined. These studies should include different CMV types (i.e., the four gB (UL55) genotypes (gB1 through gB4) and the two gH (UL75) genotypes (gH1 and gH2)). Additional analyses with worldwide isolates are encouraged. If differences in susceptibility are observed for different clinical isolates, additional genotypic and phenotypic characterizations should be conducted to identify genetic polymorphisms that may affect CMV susceptibility to the investigational drug. Sequestration of the drug by serum proteins should also be assessed and a serum-adjusted EC_{50} value determined. We recommend evaluation of the drug's antiviral activity at different concentrations of human serum and extrapolation of the EC_{50} value in the presence of 100 percent human serum.

Combination antiviral activity relationships

Early in development, cell culture combination antiviral activity relationships of the investigational drug and approved drugs for CMV should be characterized to identify any combinations where the antiviral activity is antagonistic if future combination therapy is anticipated. Each component of a drug that contains multiple novel agents (e.g., combinations of monoclonal antibodies) should be assessed individually for antagonism of approved drugs. For all combination antiviral activity assessments, sponsors should provide combination index values when the two agents are combined at their individual EC_{50} values, and studies should include controls for cytotoxicity. Combination antiviral activity relationships for nucleos(t)ide and deoxynucleos(t)ide CMV investigational drugs should also be assessed with approved nucleos(t)ide and deoxynucleos(t)ide antiviral drugs targeting other viruses (e.g., hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV-1)), as appropriate, before testing combinations of the agents in co-infected patients.

Cytotoxicity and mitochondrial toxicity

The cytotoxic effects of the drug should be quantified directly for the cells used to assess CMV antiviral activity and a 50 percent cytotoxic concentration (CC_{50}) should be determined. The therapeutic index (CC_{50} value/EC_{50} value) should be calculated. Cytotoxicity should also be assessed using various human cell lines and primary cells cultured under proliferating conditions for several cell divisions and nonproliferating conditions.

Mitochondrial toxicity should be assessed in glucose-containing and in galactose-containing medium (Marroquin et al. 2007). In addition for nucleoside analogs, inhibition of mitochondrial ribonucleic acid polymerase should be evaluated (Arnold et al. 2012). Positive controls for

mitochondrial toxicity studies should be relevant to the class of the investigational drug whenever possible.

These biochemical and cell-based assessments for potential cellular and mitochondrial toxicity should be conducted as a complement to in vivo toxicology assessments and not in lieu of in vivo studies. Results from these studies should be interpreted in the context of the in vivo toxicology, nonclinical, and clinical pharmacokinetic data to help assess clinical risk.

Considerations for antisense RNA and siRNA candidates

Knockdown of viral protein expression via antisense RNA and siRNA has shown promise for the development of antiviral drugs. Drugs of this nature, which bind to a nucleic acid target, present potential mismatch issues that could lead to species-specific toxicities not detected in classical toxicity studies. Therefore, we recommend that the following bioinformatic studies be conducted for drugs that target a nucleic acid:

- Potential off-target matches should be identified in the human transcriptome, regardless of tissue expression. For each of these, available information on mouse knockouts and human genetic diseases should be described. A plan for monitoring for significant off-target effects should be included in clinical trial protocols.

- The conservation among the candidate off-target human genes should be determined with their respective mouse genes that are three or fewer mismatched bases different from the drug to determine if these sites are sufficiently conserved in the mouse such that toxicities related to off-target matches would be present in mice.

- Potential off-target matches should be identified in the human mitochondrial transcriptome (e.g., https://omictools.com/the-mitochondrial-genome-browser-tool or http://www.mtdb.igp.uu.se/, as well as other public sources for mitochondrial genome information).

- The variation within the off-target matches should be determined in the transcriptomes of different populations in the United States to assess whether different populations would be more susceptible to off-target effects than others.

- The effect of different mismatches with respect to off-target effects should be determined (i.e., comparing purine to purine versus other mismatches).

Antiviral activity in animal models

Demonstration of CMV antiviral activity in an animal model is not required. However, if such studies are conducted and provided as part of nonclinical development, reported data should include the CMV type/subtype used (e.g., four gB (UL55) genotypes and two gH (UL75) genotypes), the EC_{50} value of the challenge virus, time course plots of viral load data for each animal, and an assessment of resistance development.

Resistance and cross-resistance

The ability of CMV to develop resistance when subjected to drug pressure should be examined in appropriate cell culture models selecting and characterizing genotypically and phenotypically several independent resistant isolates. Amino acid substitutions associated with the development of resistance to the investigational drug should be determined and validated by introducing the changes into the CMV genome (e.g., using bacterial artificial chromosome technology) and determining the fold-shift in susceptibility relative to the parental strain using appropriate cell culture and/or biochemical assays. Results from these studies should be used to: (1) determine whether the genetic barrier for resistance development is high or low; (2) predict whether the genetic barrier for resistance may vary as a function of concentration of the investigational drug; (3) reveal potential resistance pathways and the potential for cross-resistance with other anti-CMV drugs; (4) assess the potential effect of polymorphisms at amino acid positions associated with resistance using available sequence databases; (5) provide preliminary information on assays that may be used in clinical studies; and (6) support the drug's hypothesized mechanism of action. Resistant viruses selected in cell culture can provide important controls for assessing clinical isolates phenotypically.

Resistance studies should include evaluation of the potential for cross-resistance, both to approved drugs and to drugs in development (when possible), particularly focusing on those in the same drug class and other classes with the same viral target. The antiviral activity of approved drugs against viruses resistant to the investigational drug and the antiviral activity of the investigational drug against viruses resistant to approved drugs should be determined. The resistance and cross-resistance studies may be important to support studies in patients who have developed resistance to approved treatments.

Some deoxynucleoside analogs for the treatment of CMV have also been found to have antiviral activity against HIV-1 and can select for resistant variants (Tachedjian et al. 1995; McMahon et al. 2008; Lisco et al. 2008). Sponsors of such drugs should determine the cell culture antiviral activity of the active moiety against HIV-1 because these may be used in HIV-positive patients. If the drug demonstrates antiviral activity, development of resistance to the investigational drug should be determined genotypically and phenotypically by selecting resistant HIV-1 variants. Resistance studies should include evaluation of cross-resistance to approved nucleos(t)ide reverse transcriptase inhibitors for HIV-1.

Targeting host factors

For drugs targeting host factors, polymorphisms in the human population should be assessed to determine if the drug will be more or less effective against different populations. If a nonclinical assay to assess the drug effect is available, multiple samples from each of the key racial groups in the United States should be evaluated to determine whether or not race may be a factor in efficacy. Samples should be collected during clinical trials to determine the genotype of subjects who respond less favorably to treatment. We recommend that drugs targeting host functions be evaluated in animal models to demonstrate activity and assess for the potential for toxicities in infected animals.

Development of monoclonal antibodies

The development of monoclonal antibodies (mAbs) for CMV treatment or prevention should follow the same recommendations described above. In addition, the conservation (identity) at each amino acid position for the mAb binding site in available CMV sequence data for each CMV type/subtype should be assessed as well as the dependence of binding upon the target protein's conformation. The amino acid residues that may affect susceptibility for any isolates showing reduced susceptibility in cell culture studies should be identified. Sponsors developing monoclonal antibodies should evaluate the potential for antibody dependent enhancement of infection (Manley et al. 2011).

c. General considerations for phase 1 and phase 2 clinical development

In general, phase 1 trials should be conducted to assess pharmacokinetics and safety of the investigational drug and when possible, antiviral activity. Phase 2 trials should characterize doses of the investigational drug with regard to both antiviral activity and safety for further study in phase 3 trials. Specific study design issues for CMV drug development depend on the intended indication(s) (prevention or treatment of CMV disease) and the intended patient population(s) (SOT or HSCT recipients).

The following information provides recommendations and examples for potential phase 1 and phase 2 trial designs for CMV antivirals based on the current state of the field.

Phase 1a/first-in-human trials

For the first-in-human trials, we recommend single- and/or multiple-ascending-dose trials in healthy adult subjects to assess safety, pharmacokinetics, and the ability to achieve target concentrations based on cell culture antiviral activity studies. Single-dose and short-duration multiple-dose pharmacokinetic trials can also be conducted in subjects at risk for CMV disease (e.g., immunocompromised hosts), particularly if nonclinical data indicate that a drug may be genotoxic or otherwise unacceptable for studies in healthy volunteers.

Phase 2 proof-of-concept trials

For other antiviral drugs (e.g., drugs for treatment of HIV, HBV, or HCV infection), proof of concept for antiviral activity generally is demonstrated via short-term administration of the investigational drug to chronically infected patients with measurable levels of circulating virus. A reduction from baseline in plasma viral load over days or weeks is assessed to establish initial antiviral activity and to evaluate exposure-response relationships. For anti-CMV drugs, proof-of-concept trials may be somewhat more challenging because transplant recipients with CMV DNAemia are typically started immediately on antiviral treatment and generally would not be considered candidates for delaying approved treatments to participate in short-term monotherapy trials of investigational drugs without proven activity in humans.

Phase 2 trial design options to demonstrate proof of concept could include evaluation of reductions in CMV DNAemia (or by monitoring CMV replication in other compartments) in

patients with measurable virus with or without overt disease. In either category, selection of patients and concomitant treatment are key considerations to avoid situations in which patients would not receive adequate standard of care (SOC). Examples of such designs include:

- Randomized, placebo-controlled, dose-ranging trial in which the investigational drug or placebo is added to SOC treatment (e.g., ganciclovir) or, in some cases, could be directly compared to SOC treatment in patients being treated for CMV viremia. The treatment period would be short (2 to 3 weeks) with a switch to SOC for the remaining duration of therapy. Assessment of antiviral activity is the degree of reduction in plasma CMV DNAemia from baseline after 2 to 3 weeks of treatment, or proportion of patients with undetectable CMV DNAemia (less than the lower limit of quantitation (LLOQ)), at a specified time point, or rate of reduction of CMV DNA. A similar proof-of-concept trial could also be conducted in patients with CMV DNAemia that is resistant to SOC therapy.

- Assessment of antiviral activity in renal transplant patients at low risk for progression to tissue-invasive CMV disease (e.g., D-/R+) with CMV viruria or low-level CMV viremia in a placebo-controlled trial with switch to rescue therapy for progressive viremia above a prespecified threshold may be feasible in some settings.

- Randomized, placebo-controlled, dose-ranging trial to measure reductions in CMV shedding in semen or in urine in asymptomatic patients with underlying immune suppression such as HIV infection who generally would not be treated for asymptomatic CMV infection.

Before adding the investigational drug to other approved therapies, the potential for drug-drug interactions should be assessed and drug interaction trials may be needed if there is a likelihood of a pharmacokinetic interaction. Doses selected for early phase 2 trials should be predicted to provide plasma and/or tissue drug exposures that exceed by several-fold the protein binding-adjusted, cell culture EC_{50} value of the drug. The doses evaluated should also take into account any safety margins previously identified in animal toxicology studies and in trials conducted in healthy volunteers.

Results from proof-of-concept antiviral activity trials can be used to guide dose selection for subsequent phase 2b or phase 3 trials in which anti-CMV therapy is studied for longer durations.

Phase 2b trials

The same trial designs discussed for phase 3 (section III.B., Phase 3 Efficacy Trial Considerations) could be used for phase 2b; however, phase 2b trials generally should include more doses and fewer subjects per arm compared with the phase 3 trials. The primary goal in phase 2b trials is to determine doses and durations based on safety and efficacy considerations for further evaluation in phase 3 trials. Further dose discrimination for efficacy and safety can be evaluated in phase 3 trials with greater statistical power to detect smaller differences.

Trial randomization should be stratified according to baseline characteristics predicted to have a significant effect on treatment outcome (e.g., donor and recipient CMV serostatus). Initial trials

should include frequent CMV virologic monitoring and individual and study stopping rules for poor virologic outcomes (e.g., virologic breakthrough or relapse or progression to CMV disease). Protocols should include opportunities for patients with virologic failure or clinical progression to receive appropriate therapeutic *rescue* regimens. Final efficacy outcome data from all subjects, including those who received therapeutic *rescue* regimen(s), should be collected and reported in final trial reports and/or other appropriate regulatory submissions, as these data could be informative for future clinical trials. As safer and more tolerable and efficacious drugs become available, we anticipate that the risk-benefit considerations for patient populations will evolve.

Specific information recommended to support phase 3 trials includes:

- Single- and multiple-dose pharmacokinetics and safety in healthy subjects or other populations, as appropriate.

- Antiviral (anti-CMV) activity data from phase 2 clinical trials.

- Human safety data in approximately 100 patients for the highest dose that will be evaluated further in phase 3 trials.

- Data from clinical trials or other sources indicating that doses and duration of dosing chosen for study are likely to provide anti-CMV activity. Dose selection should take into consideration the potential for overlapping toxicities with other drugs likely to be used in the proposed patient population.

- Drug-drug interaction data if in vitro and in vivo study results suggest potential for a drug interaction with other drugs likely to be used concomitantly in phase 3 trials.

For an end-of-phase 2 meeting, efficacy and safety data from each of the regimens under study in phase 2 trials should be available to select drug regimens and patient populations for study in phase 3.

2. Drug Development Population

The drug development population for efficacy studies should be transplant recipients at risk for CMV disease, including:

- HSCT recipients
- SOT recipients, including kidney, liver, heart, lung, pancreas, and other SOT recipients

Supportive data may be needed before trials in specific subgroups to define safety and pharmacokinetics. This may include data from hepatic or renal impairment trials and drug-drug interaction trials (e.g., drug-drug interaction trials with immunosuppressants used post-transplantation).

Trials should include adequate U.S. subject representation to ensure the applicability of trial results to the U.S. population. An adequate representation of sexes, races, ages, and virus types is also recommended during drug development. Sponsors should share their pretrial initiation work with the FDA to ensure the sites selected have a sufficient number of subjects from these populations (e.g., women, Black/African Americans, Hispanic/Latinos, Asian Americans) to enroll in phase 2 and phase 3 clinical trials. Extending trial site enrollment caps to allow for enrollment of underrepresented populations can also help to increase trial diversity.

3. Efficacy Considerations

Sponsors can submit a marketing application to gain approval of a drug for a single indication (prophylaxis or treatment) in one or more populations, or can submit a marketing application for multiple indications. Generally, applications should include at least two adequate and well-controlled trials. However, two trials may not be needed for every indication and population. Trials for different indications (prophylaxis or treatment) and in different populations (HSCT or SOT recipients) generally would be considered supportive of each other. Sponsors should consult existing guidance regarding circumstances in which one phase 3 clinical trial may be supportive of approval.[11]

Because CMV disease in transplant recipients is considered serious and life-threatening and currently available treatments have limitations in terms of efficacy and safety, CMV investigational drugs may be eligible for fast track, priority review, or breakthrough therapy designation.

4. Safety Considerations

The FDA recommends that sponsors engage in early discussions with the DAVP on trial designs as well as on the proposed size of the safety database that depends upon the patient population and proposed indication. Because CMV disease is serious and life-threatening in immunocompromised patients, a safety database of 300 to 500 patients who received the proposed dose and duration (or greater) of the drug generally should be sufficient to assess risk-benefit for an initial marketing application. Flexibility in the size of the recommended safety database potentially could be considered for investigational drugs that demonstrate substantial improvement in efficacy and safety compared to currently available therapeutic options. On occasion, specific findings from nonclinical or clinical development may indicate the need for a larger safety database to adequately evaluate potential drug toxicity. If significant safety signals emerge during drug development, the safety database may need to be increased or specific safety studies may need to be conducted.

For marketing applications containing trials evaluating treatment of CMV disease in patients who have failed or developed resistance to approved treatments, a safety database of approximately 300 patients may be appropriate.

[11] See the guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products*.

Ideally, safety data from controlled and comparative trials are recommended to assess the safety of the investigational drug. We recommend that sponsors provide controlled and comparative safety data to an approved and clinically accepted SOC treatment (or placebo, if appropriate). In some situations, uncontrolled or historically controlled data may be appropriate as supportive data for marketing applications.

B. Phase 3 Efficacy Trial Considerations

1. Trial Design

Phase 3 trial design depends on the proposed indication(s) and the intended population(s) for use. The following are examples of trial designs that could be considered for evaluation of CMV antiviral therapy in transplant patients. All trial designs should include considerations for rescue therapy in case of treatment or prophylaxis failure.

a. Prevention of CMV disease

Prevention of CMV in transplant recipients includes both prophylaxis (administration of anti-CMV drug to at-risk subjects with no evidence for CMV DNAemia or CMV disease) and preemptive therapy (prevention of CMV disease by treatment of subjects with CMV DNAemia). The following sections discuss trial designs for CMV prophylaxis or preemptive therapy in SOT or HSCT populations.

CMV prophylaxis trials in SOT recipients

The following clinical trial designs can be considered for evaluation of CMV prophylaxis in SOT recipients:

- *Noninferiority Trials.* In a randomized, double-blinded, active-controlled trial, high-risk (D+/R-) SOT recipients would be randomized to receive the SOC regimen (currently valganciclovir) or the investigational drug for at least 100 days (200 days for kidney transplant recipients) post-transplantation. The primary endpoint would be the proportion of subjects who develop CMV disease (CMV syndrome or tissue-invasive CMV disease). The duration of follow-up depends on the duration of prophylaxis, type of organ transplant, and other factors such as expected timing of immune recovery post-transplantation. In general, subjects need to be followed for an adequate time to ensure they are not at increased risk for late-onset CMV disease. Longer term follow-up potentially could be performed as a part of a postmarketing commitment.

  The size of the noninferiority margin depends on the specific patient population being studied as well as other factors. Sponsors should discuss with the DAVP their justification for the proposed noninferiority margin, the proposed study design, the data analysis plan, and plans for long-term follow-up postmarketing. See the Appendix for additional considerations regarding clinical trials to evaluate CMV prophylaxis in liver transplant recipients.

- *Superiority Trials.* In a randomized, double-blinded, superiority trial, valganciclovir (or other drug considered SOC for the indication) would be used as comparator. Alternatively, in an add-on superiority trial, transplant recipients would be randomized to receive the investigational drug plus valganciclovir versus valganciclovir alone. The primary endpoint would be the incidence of CMV disease (CMV syndrome or tissue-invasive CMV disease).

CMV prophylaxis trials in HSCT recipients

The following clinical trial designs can be considered for evaluation of CMV prophylaxis in HSCT recipients:

- *Noninferiority Trials:* In a randomized, double-blinded, active-controlled trial, high-risk (CMV seropositive) HSCT recipients would be randomized to receive the SOC regimen (currently letermovir) or the investigational drug for at least 100 days post-transplantation. The primary endpoint would be a composite endpoint defined as the occurrence of either tissue-invasive CMV disease or the development of CMV DNAemia above a prespecified threshold. It is expected that the endpoint will be driven by the incidence of CMV DNAemia. The FDA considers CMV viremia (DNAemia) as a sufficiently validated endpoint to grant traditional approval for NDAs for prophylaxis trials in HSCT recipients.

- *Superiority Trials:* A superiority trial of the investigational drug in a blinded comparison against the SOC may be appropriate in CMV seropositive HSCT recipients. Enrolled patients should be randomized to receive SOC or the investigational drug for at least 100 days post-transplantation or until a time when most patients are expected to achieve immune recovery.[12] The primary endpoint would be a composite endpoint, as defined above.

  A dose-ranging or duration of prophylaxis superiority trial in which shorter and longer duration of prophylaxis or a range of doses are compared may also be appropriate in this population. Efficacy is supported by demonstrating superiority of the longer duration over the shorter duration or of the higher dose over the lower dose.

Preemptive therapy in SOT or HSCT recipients

Preemptive therapy (antiviral therapy initiated when CMV DNAemia is detected at a level above a predetermined threshold without evidence of tissue-invasive CMV disease or CMV syndrome) depends on frequent and regular monitoring for CMV DNAemia. The goal of preemptive therapy is to prevent tissue-invasive CMV disease. In the past, establishing universal quantitative viral thresholds for initiation of preemptive therapy has been difficult because of differences in assay performance and source (whole blood versus plasma), but may now be

[12] Other treatment durations may be proposed based on scientific rationale.

feasible with the publication of the World Health Organization standard for CMV DNA quantification (Fryer et al. 2010) and with the availability of approved assays.[13]

Some examples of preemptive therapy study designs that could be used in these populations include:

- *Superiority Trials.* Superiority trials of the investigational drug versus intravenous ganciclovir or oral valganciclovir, or add-on superiority trial in which subjects are randomized to the investigational drug or placebo added to an SOC background therapy (e.g., intravenous ganciclovir or oral valganciclovir) may be feasible. In superiority trials for this indication, efficacy can be assessed using the clinical endpoint of the occurrence of CMV disease (tissue-invasive disease or CMV syndrome in SOT recipients or tissue-invasive CMV disease in HSCT recipients) or by using a composite endpoint (undetectability of CMV DNAemia at a specific time point, or time to undetectability of CMV DNAemia and absence of CMV disease).

  Other trial design considerations could include duration of treatment or dose-ranging superiority trials in which shorter and longer durations of treatment or higher versus lower doses are compared. Superiority of the longer duration or of the higher dose demonstrates efficacy of the investigational drug.

- *Noninferiority Trials.* For a noninferiority trial, the treatment effect of the SOC comparator, ganciclovir or valganciclovir, over placebo should be determined to support an appropriate noninferiority margin for this indication. Detailed justification should be provided for proposed noninferiority margins, and proposals should be discussed with the DAVP.

b. Treatment of CMV disease

The following section discusses considerations for clinical trial design for treatment of CMV disease in SOT or HSCT recipients, including treatment of CMV infections resistant or refractory to current SOC therapy.

Treatment of CMV disease in SOT and HSCT recipients

In the SOT setting, CMV disease refers to either tissue-invasive disease or CMV syndrome, as defined in section III.B.8., Efficacy Endpoints. In HSCT recipients, CMV disease refers only to tissue-invasive CMV disease.

Options for trial designs for CMV disease treatment trials in either SOT or in HSCT recipients include:

- *Superiority Trials.* Trials to demonstrate superiority to SOC therapy, or add-on superiority trials in which subjects are randomized to the investigational drug or placebo

[13] https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm

added to an SOC therapy (e.g., intravenous ganciclovir or oral valganciclovir) are feasible and appropriate. The primary endpoints should include both resolution or improvement of clinical signs and symptoms of CMV disease and undetectable CMV DNAemia.

- *Noninferiority Trials.* No antiviral drugs have been approved for the treatment of CMV disease in SOT or HSCT recipients. Therefore, noninferiority trials are not feasible for this indication unless the treatment effect for the SOC anti-CMV therapy over placebo can be determined for treatment of CMV disease in these populations to support a noninferiority margin.

Treatment of CMV infections resistant or refractory to CMV antiviral drugs in transplant recipients

Trials for treatment of CMV infections resistant or refractory to treatment with available drugs (i.e., ganciclovir/valganciclovir, foscarnet) could include treatment of CMV disease or treatment of CMV viremia. The term *resistant* refers to CMV infection having documented resistance-associated amino acid substitutions and documented failure to achieve greater than 1 $\log_{10}$ decline in CMV DNA level in plasma after an interval of at least 2 weeks of treatment. The term *refractory* refers to CMV infection that has documented failure to achieve greater than 1 $\log_{10}$ decline in CMV DNA level in plasma after an interval of at least 2 weeks of treatment despite the absence of documented resistance-associated amino acid substitutions to SOC drugs. It should be noted for trials that include both groups of patients (resistant and refractory to treatment) that statistical significance should be demonstrated in the overall population. Efficacy in the key subgroups of patients who are refractory or resistant to CMV antiviral drugs should be consistent with the overall treatment effect.

Trial design options for these populations can include superiority trial versus SOC therapy or add-on superiority trial comparing the investigational drug plus SOC versus SOC treatment alone (if the two drugs did not demonstrate antagonism in combination antiviral activity assessments). Rescue therapy options for subjects failing therapy should be proposed as part of the protocol.

2. Trial Population

As mentioned, this guidance focuses on treatment or prevention of CMV disease in SOT and HSCT recipients. Some of the specific issues with regard to trial population for these indications are discussed below.

- *CMV Prophylaxis in SOT Recipients.* For trials evaluating an investigational drug for CMV prophylaxis in SOT recipients, patients should be high risk based on CMV serostatus (D+/R-).

- *CMV Prophylaxis in HSCT Recipients.* Trials of investigational drug versus SOC should be conducted in CMV seropositive (R+) HSCT recipients who are at the highest risk for CMV infection and disease.

- *Preemptive Therapy in SOT or HSCT Recipients.* Preemptive therapy can be studied in any transplant recipient who has evidence of CMV DNAemia at levels above a prespecified threshold.

- *Treatment of CMV Disease.* Any SOT or HSCT recipient with CMV disease, regardless of CMV serostatus of donor and recipient, could be included in treatment trials. However, in trials evaluating treatment in SOT recipients, a sufficient number of subjects with tissue-invasive CMV disease should be enrolled (and not just those with CMV syndrome) to support an indication for treatment of CMV disease.

- *Treatment of CMV Infections Resistant or Refractory to CMV Antiviral Drugs in Transplant Recipients.* Any SOT or HSCT recipient with CMV infection resistant or refractory to available CMV antiviral drugs could be included in these trials.

3. Entry Criteria

The following are specific considerations for trial entry criteria for CMV treatment or prevention trials:

- *Prophylaxis Trials in SOT or HSCT Recipients*. To be enrolled in a CMV prophylaxis trial, the patient should have no detectable CMV infection post-transplantation as documented by CMV DNA testing with PCR in plasma (less than LLOQ), within 5 days before initiation of therapy.

- *Preemptive Therapy Trials in SOT or HSCT Recipients*. In clinical practice, virologic thresholds for initiation of preemptive therapy in HSCT recipients have been based on preestablished risks for CMV disease (Boeckh and Ljungman 2009). For clinical trials, optimal virologic thresholds for initiation of preemptive therapy have not been established. Proposed virologic thresholds for initiation of preemptive therapy for CMV viremia in clinical trials should be discussed and agreed upon with the DAVP.

- *Treatment Trials in SOT or HSCT Recipients*. To be enrolled in a CMV treatment trial, transplant recipients should have virological evidence of CMV replication with signs and symptoms of CMV syndrome or tissue-invasive CMV disease (SOT recipients) or with clinical evidence of tissue-invasive CMV disease (HSCT recipients).

- *Treatment Trials in Patients With CMV Infections Resistant or Refractory to CMV Antiviral Drugs*. CMV isolates at baseline should have evidence of resistance to CMV antiviral drugs by genotypic analysis. Patients with CMV disease refractory to treatment can be included, but the inclusion criteria for subjects refractory to therapy should be rigorously defined in the protocol.

4. Randomization, Stratification, and Blinding

Sponsors should conduct randomized, double-blinded trials whenever feasible. For add-on superiority trials of an investigational drug added to SOC therapy compared to SOC therapy alone, subjects randomized to the latter should receive a matching placebo.

Sponsors designing trials in which blinding may be difficult or infeasible should discuss their proposals with the DAVP in advance to review potential modifications that might facilitate blinding and to discuss the potential effect of open-label therapy on interpretation of results.

Sponsors should consider stratification of subjects by important baseline risk factors for CMV infection/disease in HSCT recipients, such as CMV serostatus of donor and recipient and other factors associated with risk of CMV disease. For SOT recipients, consideration should be given to stratification by CMV serostatus of donor and recipient and the type of transplant (e.g., kidney, liver, lung).

In trials that include both SOT and HSCT recipients, stratification by type of transplant (SOT or HSCT) should be considered.

5. Pediatric Populations

Sponsors are encouraged to begin discussions about their pediatric formulation and clinical development plan early in development because pediatric clinical trials are a required part of the overall drug development program. Under the Pediatric Research Equity Act, sponsors must submit an initial pediatric study plan to the FDA no later than 60 days after the end-of-phase 2 meeting.[14]

Inclusion of pediatric patients in clinical trials generally can be initiated after sufficient safety, pharmacokinetic, and efficacy data are available from adults. If clinical trials in adults have demonstrated no significant safety concern that would preclude study in children, evaluation of adolescents using the adult dose and formulation is encouraged (Momper et al. 2013). However, initial pediatric pharmacokinetic data and results of available modeling and simulation should be discussed with the DAVP before dose selection for pediatric treatment trials. Depending on results of the adult clinical trials, and on whether efficacy in adults can be extrapolated to pediatric patients (i.e., if the course of disease and the effect of the drug are sufficiently similar in adults and pediatric patients), either comparative or single-arm trials may be appropriate in pediatric subjects.[15] The sponsor's pediatric study plan should include information to support pediatric extrapolation, as needed.

[14] See the draft guidance for industry *Pediatric Study Plans: Content of and Process for Submitting Initial Pediatric Study Plans and Amended Initial Pediatric Study Plans*. When final, this guidance will represent the FDA's current thinking on this topic.

[15] For additional information on pediatric extrapolation, see the draft guidance for industry *General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products*. When final, this guidance will represent the FDA's current thinking on this topic.

779 *6. Dose Selection*
780
781 To guide optimal selection of doses and treatment durations in phase 3 trials, sponsors should
782 consider safety and efficacy results from previous trials and exposure-response relationships for
783 safety and efficacy. For treatment studies, we recommend that sponsors develop a mechanistic
784 model of the kinetics of viral load reduction that can assist the optimization of dose and
785 treatment duration, and reduce the risk of selecting for resistant virus caused by subtherapeutic
786 exposures. Such a model should include a mechanistically appropriate targeted drug effect,
787 components to describe virologic breakthrough and virologic response, and contain relevant
788 covariates for describing differences in response. When applicable, these mechanistic modeling
789 approaches can use viral kinetic model structures and corresponding disease progression
790 parameter values from the literature.
791
792 A range of doses and treatment durations can be selected for phase 3 trials if there are
793 uncertainties on the optimal regimen or the model indicated a different dose or treatment
794 duration to be better for certain subpopulations such as patients having CMV with baseline
795 ganciclovir resistance. An adaptive design for the dose selection can also be considered.
796
797 *7. Use of Active Comparators*
798
799 In general, the active comparator in a noninferiority trial should be an FDA-approved drug that is
800 considered the SOC for the specific indication and population being studied. Proposed
801 noninferiority margins should be justified and discussed with the DAVP. See the guidance for
802 industry *Non-Inferiority Clinical Trials to Establish Effectiveness* for additional information on
803 determining noninferiority margins.
804
805 *8. Efficacy Endpoints*
806
807 The preferred definitions for CMV infection and disease for use in clinical trials are those
808 advocated by Ljungman and colleagues (Ljungman et al. 2017).
809
810 a. CMV prophylaxis trials in SOT recipients
811
812 The recommended primary endpoint for trials of CMV prophylaxis in SOT recipients is a clinical
813 endpoint of CMV disease, and includes both CMV syndrome and tissue-invasive CMV disease
814 measured at 6 or 12 months post-transplantation depending on duration of prophylaxis. The
815 diagnosis of CMV syndrome and tissue-invasive CMV disease should be confirmed by an
816 independent, blinded, clinical adjudication committee.
817
818 Secondary endpoints in CMV prophylaxis trials for SOT recipients could include some of the
819 following. However, only a limited number of such endpoint(s) should be considered for testing
820 using appropriate statistical methods for multiplicity:
821
822 • The proportion of subjects with CMV disease at time points other than the time point
823 used for the primary endpoint
824

- The time to development of CMV disease

- The proportion of subjects with investigator-determined CMV disease

- The initiation of other anti-CMV therapy

- The proportion of subjects with CMV DNAemia at different time points

- The time to development of CMV DNAemia

- Survival at different time points

- The proportion of subjects experiencing biopsy-proven acute rejection

- The proportion of subjects with graft loss

- The proportion of subjects with opportunistic infections

- The proportion of subjects developing genotypic changes associated with CMV resistance to investigational drug

b. CMV prophylaxis trials in HSCT recipients

The recommended primary endpoint for a phase 3 prophylaxis trial in HSCT recipients is the incidence of CMV infection or disease within 6 months post-transplantation. This is a composite endpoint that includes both a clinical component (tissue-invasive CMV disease) and a surrogate endpoint (CMV DNAemia).

Initiation of anti-CMV preemptive treatment in prophylaxis trials should be based on documented CMV DNAemia (as measured by a central virology laboratory). Viral load thresholds for initiation of preemptive therapy should be based on the risks for CMV disease (Boeckh and Ljungman 2009). Virologic thresholds for initiation of preemptive therapy will depend on the assay and specimen (whole blood versus plasma), as well as the risk of CMV infection/disease in the population under study, and individual patient risk factors. Virologic thresholds should be agreed upon with the DAVP before trial initiation.

Secondary endpoints in CMV prophylaxis trials in HSCT recipients could include, but are not limited to:

- The proportion of subjects with tissue-invasive CMV disease

- The proportion of subjects with CMV DNAemia

- The time to onset of CMV infection (DNAemia)/tissue-invasive disease through 6 months or 12 months post-transplantation

- Survival at 6 and 12 months post-transplantation

- The proportion of subjects with opportunistic infections other than CMV infection

- The proportion of subjects developing resistance to the investigational drug

c. CMV preemptive therapy trials in SOT or HSCT recipients

The recommended primary endpoint for phase 3 trials of preemptive therapy in either SOT or HSCT patients is the proportion of subjects with undetectable CMV DNA (less than LLOQ) without evidence of CMV disease at a prespecified time point after treatment initiation.

d. Treatment of CMV disease in SOT or HSCT recipients

The recommended primary endpoint in a phase 3 trial in either SOT or HSCT recipients with tissue invasive CMV disease (for SOT or HSCT) or CMV syndrome (for SOT) is the proportion of responders at a prespecified time point after treatment initiation. Response should include the following elements:

- Substantial improvement/resolution of signs and symptoms of tissue-invasive CMV disease or CMV syndrome

- Undetectable CMV DNAemia (defined as two consecutive negative tests taken at least 5 to 7 days apart)

- No new occurrence of CMV disease at other sites

- No evidence for relapse (CMV disease or DNAemia) within a prespecified time frame after stopping therapy

Specific details regarding the primary endpoint should be discussed with and agreed upon by the DAVP.

Secondary endpoints can include, but are not limited to:

- The time to undetectable CMV DNA (less than LLOQ)

- The time to resolution of signs and symptoms of tissue-invasive disease or CMV syndrome

- Survival

- The development of opportunistic infections, graft rejection, or failure

- The development of antiviral resistance

9. Trial Procedures and Timing of Assessments

For trials of investigational drugs for treatment or prophylaxis of CMV in the post-transplant setting, rescue therapy for development of CMV disease or CMV viremia should be included in the protocol. Quantitative CMV DNA should be measured frequently during clinical trials. For treatment of CMV disease, treatment should continue at least until CMV DNAemia is less than LLOQ for at least two consecutive measurements performed at a prespecified interval, and duration of treatment should be recorded. Sponsors should consider longer treatment based on the kinetics of viral load reduction because several logs of CMV may be present when an assay reports less than LLOQ. In prophylaxis trials, CMV DNA should be monitored routinely during the trial and subjects should be monitored for development of signs and symptoms of CMV disease. In treatment trials (including preemptive therapy), frequent monitoring of CMV DNA should continue after discontinuation of therapy to detect relapse of CMV viremia during the risk period.

10. Endpoint Adjudication

Determination of CMV tissue-invasive disease and CMV syndrome endpoints should be adjudicated by an independent endpoint assessment committee conducting a blinded review of clinical source data (Ljungman et al. 2017).

11. Statistical Considerations

In general, a detailed statistical analysis plan stating the trial hypotheses and analysis methods should be submitted before trial initiation. Statistical analysis topics and issues are discussed in detail in the guidances for industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products* and *Non-Inferiority Clinical Trials to Establish Effectiveness* and the FDA white paper "Statistical Considerations on Subgroup Analysis in Clinical Trials" (Alosh et al. 2015).

a. Analysis populations

All subjects who are randomized and receive at least one dose of assigned therapy during the trial generally should be included in the primary efficacy analysis. However, if a substantial proportion of randomized subjects do not receive treatment in either or both arms, then additional analyses may be needed.

b. Efficacy analyses

The primary efficacy analyses in prophylaxis trials in SOT recipients should compare the incidence of CMV disease within 6 or 12 months post-transplantation across treatment arms.

The primary efficacy analyses in prophylaxis trials in HSCT recipients should compare the incidence of tissue-invasive CMV disease and CMV DNAemia above a prespecified threshold within 6 months post-transplantation across treatment arms.

The primary efficacy analyses in preemptive therapy trials should compare the proportion of SOT recipients or HSCT recipients with undetectable CMV DNA in the absence of CMV disease at a prespecified time point across treatment arms.

For subgroup analyses, the analysis of the primary efficacy endpoint should be performed within important demographic and baseline characteristics (e.g., geographic region (United States, non-United States), sex, race, age group, high- versus low-risk group, donor CMV serostatus (D+ or D-), recipient CMV serostatus (R- or R+)). The purpose of these analyses is to explore the consistency of the primary efficacy endpoint result across these subgroups.

c. Handling of missing data

Sponsors should make every attempt to limit loss of subjects from the trial. We recommend that sponsors collect detailed data on reasons for trial discontinuation (e.g., opportunity to enter another trial offering a promising new treatment, death or events leading to death, disease progression, adverse events, loss to follow-up, withdrawal of consent, noncompliance, pregnancy, protocol violations, not discontinued or not known to be discontinued but data were missing at the final visit). For subjects who discontinue treatment early, investigators should determine if these subjects switched treatments or added additional therapy.

Analyses excluding subjects with missing data or other post-treatment outcomes can be biased because subjects who do not complete the trial may differ substantially in both measured and unmeasured ways from subjects who remain in the trial. The method of how missing data will be handled should be prespecified in the protocol or the statistical analysis plan. Sensitivity analyses may be needed to demonstrate that the primary analysis results are robust to the assumptions regarding missing data.

12. Accelerated Approval (Subpart H/E) Considerations

CMV viremia (DNAemia) is considered a sufficiently validated endpoint for use as part of a composite endpoint that includes a clinical component to support traditional approval; therefore, accelerated approval regulations generally are not applicable for CMV treatment and prevention indications.

C. Other Considerations

1. Clinical Virology Considerations

An FDA-approved assay should be used to quantify CMV DNA in plasma. We recommend that CMV DNA in whole blood also be quantified for short-term monotherapy studies because this may improve sensitivity to detect antiviral activity. Additionally, plasma CMV DNA has been shown to be highly fragmented, so care should be taken when interpreting the CMV DNA levels (Boom et al. 2002). Virology analyses should be conducted at a central virology laboratory.

Proof-of-concept and efficacy trials should assess the development of CMV genotypic resistance to the investigational drug. In prophylaxis studies, resistance testing should be performed for

subjects who have detectable CMV DNA at any time point or confirmed diagnosis of CMV disease, regardless of viral load. Observations of particular interest that should be reported include multiple occurrences of substitutions from the reference sequence(s) at highly conserved amino acid residues, substitutions at positions identified in cell culture selection studies and treatment studies, and multiple occurrences of unusual substitutions at polymorphic residues.

In treatment studies, resistance testing should be performed for subjects who demonstrate virologic breakthrough (defined as a greater than or equal to 1 $\log_{10}$ increase in CMV DNA above nadir, or detectable CMV DNA, while on treatment, after an initial drop to undetectable), an incomplete antiviral response (e.g., detectable CMV DNA at end of treatment or slower rate of decline than the average response), decline to a plateau viral load decay phase, or virologic relapse after treatment cessation. Sponsors should include a proposal of the subjects to be evaluated for resistance in their resistance analysis plans. Any amino acid changes, including mixtures, in the coding sequence of the targeted genome region present in on-treatment or follow-up samples, but not in the baseline sample, should be reported as having developed during therapy. In addition, baseline samples should be analyzed to identify CMV genetic polymorphisms that are associated with differential antiviral activity with the new investigational drug.

Sponsors should consider genotyping regions outside the direct CMV genome target depending on the characteristics of the antiviral drug and interactions of the target with other viral proteins or whole genome sequencing, if viral loads are adequate. In cases when resistance is suspected based on viral DNA kinetics, but genotypic evidence of resistance is not detected, sponsors should also consider performing additional genotypic analyses using a method sufficiently sensitive to detect minority variants (e.g., next generation sequencing). GCV/vGCV resistance-associated substitutions have been detected in specific compartments exclusively and not in blood. Therefore, sponsors should also consider genotyping samples collected from specific compartments.

Viral resistance-associated substitutions and baseline polymorphisms affecting response observed in clinical trials but not identified and characterized in nonclinical virology experiments should be evaluated phenotypically by introducing the changes into the CMV genome, and determining the conferred fold-shift in susceptibility to the drug using appropriate cell culture and/or biochemical assays. In addition, phenotypic analyses should be performed using baseline and on-treatment clinical isolates from a subset of trial subjects representative of the CMV genetic diversity and virologic responses observed in clinical trials. Phenotypic assays should include wild-type reference virus and resistant virus (initially from cell culture selection studies) controls.

For quantification of CMV DNA, we recommend that sponsors use an FDA-approved PCR assay(s) using a central laboratory. Sponsors should collect results from local laboratory tests, identifying the assay(s) used. If investigational assays are used, performance characteristics with geographically and temporally distinct isolates should be provided. Values that are less than LLOQ should be reported as “less than LLOQ, target not detected” or “less than LLOQ, target detected,” as appropriate.

The FDA performs independent assessments of virologic and resistance data. Before submitting virology datasets, sponsors should consult with the DAVP to obtain information on the most recent format and, in the case of Next Generation Sequence analysis, the procedure for submitting FASTQ files.

2. Pharmacokinetic/Pharmacodynamic Considerations

Pharmacokinetics and the relationship between exposure and virologic or clinical endpoints and toxicity should be assessed. Virologic or clinical endpoints to be used for analyses depend on the proposed indication and study designs.

Sponsors can use a combination of intensive and sparse sampling throughout development to characterize the pharmacokinetics of the investigational drug. An intensive sampling schedule is recommended in early phase trials. In longer term trials, however, an intensive sampling schedule might not be feasible, or may be feasible only in a subset of subjects or over a limited period of time. Sparse pharmacokinetic samples should be obtained from as many subjects in longer duration trials as possible, and the pharmacokinetic samples from these trials can be combined with intensive pharmacokinetic data from earlier trials for analysis.

Pharmacokinetics and the relationship between exposure and virologic or clinical responses in early phase trials (i.e., proof-of-concept studies) can be used to aid the design of phase 2b or phase 3 trials (e.g., dose selection and treatment duration). When sufficient efficacy and pharmacokinetic data are available, a simplified analysis relating proportion of subjects with treatment failure and appropriate exposure variable (e.g., minimum concentration or area under the plasma drug concentration versus time curve) can be used to support evidence of effectiveness of different dosage regimens. Analyses of the exposure-safety relationship(s) using similar approaches also should be performed to assist in evaluating the balance between effectiveness and toxicity of different dosage regimens.

GLOSSARY OF ACRONYMS

AIDS	acquired immune deficiency syndrome
CC	cytotoxic concentration
CMV	cytomegalovirus
DAVP	the Division of Antiviral Products
DNA	deoxyribonucleic acid
EC	effective concentration
FDA	the Food and Drug Administration
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HSCT	hematopoietic stem cell transplantation
LLOQ	lower limit of quantitation
mAb	monoclonal antibody
NDA	new drug application
PCR	polymerase chain reaction
pre-IND	pre-investigational new drug application
SOC	standard of care
SOT	solid organ transplantation

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APPENDIX: CLINICAL TRIAL DESIGN CONSIDERATIONS FOR CMV PROPHYLAXIS IN LIVER TRANSPLANT RECIPIENTS

At this time, a noninferiority trial with valganciclovir as comparator cannot be used to evaluate efficacy in liver transplant recipients as the sole population in the trial because the efficacy of valganciclovir in this population has not been adequately demonstrated. In a randomized controlled trial in solid organ transplant recipients submitted for marketing authorization, valganciclovir was noninferior to oral ganciclovir in the overall trial population for prevention of cytomegalovirus (CMV) disease (CMV syndrome and tissue-invasive CMV disease) post-transplantation.[16] However, among liver transplant recipients who made up the largest subgroup (approximately 50 percent of patients enrolled), approximately three times more tissue-invasive CMV disease (as determined by an adjudication committee) was reported with valganciclovir than with oral ganciclovir as prophylaxis (valganciclovir package insert).

These findings remain unexplained, and currently no antiviral drugs other than oral ganciclovir have been approved in the United States for CMV prophylaxis in liver transplant recipients. However, because valganciclovir generally is considered the standard of care in this population (Levitsky et al. 2008; Kotton et al. 2013) and because oral ganciclovir currently is not available in the United States, valganciclovir could be used as a comparator in a superiority trial. Additionally, a noninferiority trial including recipients of different types of organ transplants (e.g., liver, heart, kidney, kidney-pancreas) using valganciclovir as comparator may be appropriate to demonstrate efficacy in liver transplant recipients if noninferiority is demonstrated for the overall trial population and the rate of CMV disease is similar between the liver transplant recipients and the other subpopulations for both the new treatment and the valganciclovir comparator. Definitions for success in subpopulations in this type of study design should be defined in the statistical analysis plan. If the rate of tissue-invasive CMV disease is higher for liver transplant recipients than for other organ transplant recipients in the valganciclovir comparator arm, then noninferiority could not be concluded for liver transplant recipients.

[16] In a placebo-controlled trial, oral ganciclovir was shown to decrease the incidence of CMV disease in liver transplant recipients during the first 6 months post-transplantation (ganciclovir capsules package insert). However, oral ganciclovir is currently not available in the United States.