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Definitions of Resistant and Refractory Cytomegalovirus Infection and Disease in Transplant Recipients for Use in Clinical Trials

Roy F. Chemaly,[1] Sunwen Chou,[2] Hermann Einsele,[3] Paul Griffiths,[4] Robin Avery,[5] Raymund R. Razonable,[6] Kathleen M. Mullane,[7] Camille Kotton,[8] Jens Lundgren,[9] Takashi E. Komatsu,[10] Peter Lischka,[11] Filip Josephson,[12] Cameron M. Douglas,[13] Obi Umeh,[14] Veronica Miller,[15] and Per Ljungman[16,17]; for the Resistant Definitions Working Group of the Cytomegalovirus Drug Development Forum

[1]Department of Infectious Diseases, Infection Control, and Employee Health, University of Texas MD Anderson Cancer Center, Houston; [2]Division of Infectious Diseases, Oregon Health and Science University, and Research and Development Service, Veterans Affairs Portland Health Care System; [3]Department of Internal Medicine II, University Hospital Wuerzburg, Germany; [4]Institute for Immunity and Transplantation, University College London Medical School, United Kingdom; [5]Division of Infectious Diseases, Department of Medicine, Johns Hopkins University, Baltimore, Maryland; [6]Division of Infectious Diseases, Department of Medicine, William J. von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic, Rochester, Minnesota; [7]Section of Infectious Diseases and Global Health, Department of Medicine, University of Chicago, Illinois; [8]Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston; [9]Centre for Health and Infectious Disease Research, Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Denmark; [10]Division of Antiviral Products, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, Maryland; [11]AiCuris Anti-infective Cures GmbH, Wuppertal, Germany; [12]Swedish Medical Products Agency, Uppsala; [13]Merck & Co, Inc, Kenilworth, New Jersey; [14]Shire Global Clinical Development (Immunology Therapeutic Area), Lexington, Massachusetts; [15]Forum for Collaborative Research, University of California, Berkeley; and [16]Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital, and [17]Division of Hematology, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden

Despite advances in preventive strategies, cytomegalovirus (CMV) infection remains a major complication in solid organ and hematopoietic cell transplant recipients. CMV infection may fail to respond to commercially available antiviral therapies, with or without demonstrating genotypic mutation(s) known to be associated with resistance to these therapies. This lack of response has been termed "resistant/refractory CMV" and is a key focus of clinical trials of some investigational antiviral agents. To provide consistent criteria for future clinical trials and outcomes research, the CMV Resistance Working Group of the CMV Drug Development Forum (consisting of scientists, clinicians, regulatory officials, and industry representatives from the United States, Canada, and Europe) has undertaken establishing standardized consensus definitions of "resistant" and "refractory" CMV. These definitions have emerged from the Working Group's review of the available virologic and clinical literature and will be subject to reassessment and modification based on results of future studies.

Keywords. cytomegalovirus; resistance; refractory; definitions; clinical trials.

Despite advances in preventive strategies, cytomegalovirus (CMV) infection remains a major complication in solid organ transplant (SOT) and hematopoietic cell transplant (HCT) recipients. A number of anti-CMV agents are commercially available, and others are in development. However, CMV infection can fail to respond, with or without the presence of genotypic mutation(s) known to be associated with resistance to these therapies. Refractory or resistant CMV infection in transplant recipients remains challenging due to a limited number of available antiviral drugs, their associated serious toxicities, and an increased number of vulnerable patients [1–4]. The lack of consistent definitions for "resistant/refractory CMV" in clinical practice and the literature hampers assessment of clinical trial results. In this article, we aim to define refractory and/or resistant CMV infections after SOT or HCT to provide consistent criteria for future clinical trials and outcomes research.

Clinical suspicion of drug-resistant CMV infections is usually based on suboptimal responses to antiviral agents leading to a treatment-refractory condition. Laboratory testing may or may not confirm a drug-resistant virus, as treatment failure may also result from other causes, such as adverse host factors or inadequate drug delivery. We divide the following definitions into 2 categories: refractory CMV infection (a clinical definition based on criteria for suboptimal response to therapy) and CMV antiviral drug resistance (a laboratory definition of a drug-resistant phenotype or the presence of mutations known to confer resistance to antiviral agents). These definitions are not comprehensive enough to cover all clinical scenarios but can serve as a starting point toward justification of enrolled subjects in future clinical trials for management of drug-resistant or refractory CMV infections. In addition, these definitions may serve to guide clinicians on when to suspect resistance, the optimal laboratory testing to diagnose resistance, and how to interpret the results of the assays employed.

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Correspondence: R. F. Chemaly, Department of Infectious Diseases, Infection Control, and Employee Health, Unit 402, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030-4009 (rfchemaly@mdanderson.org).

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METHODS

The CMV Resistance Working Group is a subgroup of the CMV Drug Development Forum [5] and includes representation from US and European experts on transplantation, transplant infectious disease, and clinical virology; regulators from the US Food and Drug Administration and the European Medicines Agency; and representatives from the pharmaceutical and diagnostics industries. This forum is a program of the Forum for Collaborative Research, which operates as an independent and neutral venue for experts from all stakeholder categories to advance the regulatory science for diseases of unmet medical need [6] through a process of dialogue and deliberation.

The CMV Resistance Working Group reviewed previously published articles related to CMV resistance in SOT and HCT recipients. The PubMed Central Database was used to identify published studies of interest. We used the following search terms: "cytomegalovirus," "transplant," "antiviral or drug or pre-emptive or foscarnet or ganciclovir or cidofovir or valganciclovir," "resistance or resistant or refractory." Thereafter, a draft proposal for definitions was developed, and this proposal was discussed and approved at a meeting of the entire CMV Drug Development Forum on 6 October 2017. The Working Group addressed the received comments, and the updated document was circulated to all members of the Working Group for final approval.

RISK FACTORS AND OUTCOMES OF DRUG-RESISTANT CMV INFECTION

Recognizing the risk factors for the development of drug-resistant CMV infection may prompt early diagnosis and management of antiviral resistance (Table 1). Drug-resistant CMV infections, particularly in donor-seropositive, recipient-seronegative SOT recipients and high-risk HCT recipients, are associated with high morbidity and mortality [7–12]. A high rate of CMV drug resistance was reported for patients undergoing haploidentical and cord blood HCT [13]. CMV drug resistance mutations may eventually be selected for, if the antiviral treatment allows for ongoing replication (either because of inadequate dosage, dose-limiting toxicities, poor absorption, poor penetration into the body compartment where replication is occurring, or overwhelming replication).

The highest-risk group for drug-resistant CMV infection in the SOT setting is the donor CMV–seropositive, recipient-seronegative combination. Other risk factors for resistant CMV infection include the type of organ transplanted (ie, lung transplant recipients are at the highest risk), lower doses or longer duration of (val)ganciclovir prophylaxis, peak CMV viral loads, and the intensity of immunosuppression [10, 14–16].

Data on the harmful impact of refractory or resistant CMV infections on clinical outcomes, including mortality after SOT and HCT, are scarce. In one study of HCT recipients, persistent CMV reactivation, which was defined as persistent CMV antigenemia for >3 weeks despite treatment with available antivirals, occurred in 29% of T-cell–depleted HCT recipients who had CMV reactivation, and the maximum CMV level was significantly associated with persistent CMV reactivation [17]. In a recent study [18], allogeneic HCT (allo-HCT) recipients with refractory CMV reactivation (defined as CMV DNAemia lasting for >2 weeks despite administration of a full dose of antiviral drug therapy) within 100 days of transplantation had a higher incidence of CMV disease and nonrelapse mortality (11.9% and 17.1%, respectively) than did those without refractory CMV (0.8% and 8.3%, respectively).

In SOT recipients, several studies on the outcomes of ganciclovir-resistant CMV infections showed an association with longer hospitalization [19], serious toxicities from alternative therapy [7, 8, 16], and increased mortality [7, 8, 12, 16, 19, 20]. In a recent study [21], when compared to patients with ganciclovir-sensitive CMV infections, SOT recipients with ganciclovir-resistant infections had worse outcomes including higher mortality (11% vs 1%; $P = .004$), with fewer days alive and non-hospitalized (73 vs 81 days; $P = .039$).

Table 1. Risk Factors for Cytomegalovirus Resistance in Hematopoietic Cell Transplant Recipients[a,b]

Risk Factor
Host factors
Prolonged antiviral CMV drug exposure (>3 mo)
Previous antiviral CMV drug exposure
Recurrent CMV infection
Inadequate antiviral CMV drug absorption and bioavailability
Inadequate antiviral CMV oral prodrug conversion
Variation in antiviral CMV drug clearance
Subtherapeutic antiviral CMV drug level
Poor patient compliance with antiviral drug regimen
T-cell depletion
Haploidentical, allogeneic, or cord blood HCT
Delayed immune reconstitution
CMV-seropositive recipient and CMV-seronegative donor
Treatment with antithymocyte antibodies
Active GVHD
Young age
Congenital immunodeficiency syndromes
Viral factors
CMV viral load rise while receiving treatment (after >2 wk of adequate dosing)
Failure of CMV viral load to fall despite appropriate treatment
Rise in CMV viral load after initial decline while receiving appropriate treatment
Intermittent low-level CMV viremia
High CMV viral loads

Abbreviations: CMV, cytomegalovirus; GVHD, graft-vs-host disease; HCT, hematopoietic cell transplantation.

[a]Modified with permission from El Chaer et al 1.

[b]Most of the risk factors for CMV resistance pertain to solid organ transplant recipients as well, in addition to graft rejection (instead of GVHD) and CMV-seropositive donor and CMV-seronegative recipient.

DEFINITIONS OF REFRACTORY CMV INFECTION FOR USE IN CLINICAL TRIALS

Table 2 summarizes our proposed definitions for refractory and resistant CMV infection and disease. For clarity and simplicity, we categorized refractory CMV infection into 4 definitions related to clinical and laboratory characteristics indicating suboptimal response to therapy; these characteristics encompass signs, symptoms, and measurements of viral load:

1. Refractory CMV infection is defined as CMV viremia (DNAemia or antigenemia) that increases (ie, >1 $\log_{10}$ increase in CMV DNA levels in blood or serum between peak viral load within the first week and the peak viral load at ≥2 weeks as measured in the same laboratory with the same assay) after at least 2 weeks of appropriately dosed antiviral therapy.

Limitations and caveats: To increase the sensitivity of this definition, it is important to emphasize that CMV viral load monitoring should be done regularly and not less than once per week. On the other hand, a modest quantitative increase in viral load (as defined above but a <1 $\log_{10}$ increase) during the first 2 weeks of anti-CMV therapy may occur in some patients, and it may not be an indication of refractoriness or resistance unless it persists at the same level or higher at ≥2 weeks (see below).

2. Probable refractory CMV infection is defined as a persistent viral load (CMV viral load at the same level or higher than the peak viral load within 1 week but <1 $\log_{10}$ increase in CMV DNA titers done in the same laboratory and with the same assay) after at least 2 weeks of appropriately dosed antiviral therapy.

Limitation: Persistent CMV DNA titers <1000 IU/mL, and in particular detected but not quantifiable (<137 IU/mL), should not be considered refractory CMV infection.

3. Refractory CMV end-organ disease is defined by a worsening in signs and symptoms or progression into end-organ disease after at least 2 weeks of appropriately dosed antiviral therapy (CMV end-organ disease is defined as per Ljungman et al [5].

Limitations: Signs and symptoms of CMV end-organ disease may be difficult to discern, and reporting of symptoms is often subjective and varies with patient and provider. Certain CMV end-organ diseases (eg, CMV retinitis and gastrointestinal diseases) are not always associated with measurable viral loads in serum or whole blood; in these cases, CMV may be replicating locally at tissue sites and may not be recovered for resistance testing.

4. Probable refractory CMV end-organ disease is defined by the lack of improvement in signs and symptoms after at least 2 weeks of appropriately dosed antiviral therapy.

Limitations: Signs and symptoms of CMV end-organ disease may be difficult to discern, and reporting of symptoms is often subjective and varies with patient and provider. In addition, other factors have to be weighed into the determination of probable refractory CMV end-organ disease such as the site (ie, gastrointestinal tract vs retina) and the presence of other contributing causes to the signs and symptoms such as graft-vs-host disease.

Patients who meet any of these 4 definitions should be eligible for enrollment in clinical trials for resistant/refractory CMV infection or end-organ disease.

DEFINITION OF CMV ANTIVIRAL DRUG RESISTANCE FOR USE IN CLINICAL TRIALS

Antiviral drug resistance is commonly defined as a viral genetic alteration that decreases susceptibility to 1 or more antiviral drugs. The alteration typically involves genes involved in antiviral drug anabolism (eg, UL97-mediated phosphorylation of ganciclovir [22], the antiviral drug target (eg, UL54, UL97, UL56/89/51), or compensation for antiviral inhibition of biological function (eg, UL27 [23]).

Table 2. Summary of the Definitions of Refractory Cytomegalovirus Infection and Disease and Antiviral Drug Resistance for Use in Clinical Trials

Term	Definition
Refractory CMV infection	CMV viremia that increases[a] after at least 2 wk of appropriately dosed antiviral therapy
Probable refractory CMV infection	Persistent viral load[b] after at least 2 wk of appropriately dosed antiviral therapy
Refractory CMV end-organ disease	Worsening in signs and symptoms or progression into end-organ disease after at least 2 wk of appropriately dosed antiviral therapy
Probable refractory CMV end-organ disease	Lack of improvement in signs and symptoms after at least 2 wk of appropriately dosed antiviral drugs
Antiviral drug resistance	Viral genetic alteration that decreases susceptibility to one or more antiviral drugs[c]

Abbreviation: CMV, cytomegalovirus.

[a]More than 1 $\log_{10}$ increase in CMV DNA levels in blood or serum and determined by $\log_{10}$ change from the peak viral load within the first week to the peak viral load at ≥2 weeks as measured in the same laboratory with the same assay.

[b]CMV viral load at the same level or higher than the peak viral load within 1 week but <1 $\log_{10}$ increase in CMV DNA titers done in the same laboratory and with the same assay.

[c]Known examples involve genes involved in antiviral drug anabolism (eg, UL97-mediated phosphorylation of ganciclovir), the antiviral drug target (eg, UL54, UL97, UL56/89/51), or compensation for antiviral inhibition of biological function (eg, UL27).

Decreased susceptibility is defined by assay of the drug concentration required to reduce viral growth in cell culture by 50% (EC_{50}). All such phenotypic assays require careful standardization using control baseline and resistant strains, attention to cell culture conditions, viral inocula, range of drug concentrations, a reproducible growth readout [24, 25], and adequate replicates of testing. The level of resistance detected can range from <2-fold to >8000-fold increases in EC_{50} values [26–28]. For ganciclovir, the most common UL97 mutations confer a 3- to 10-fold increase in the EC_{50}, but combinations of UL97 and UL54 mutations result in higher levels of resistance. Because EC_{50} assays using CMV isolates from treated patients are technically impractical for reasons of standardization, timeliness, and availability, the level of drug resistance in clinical practice is usually estimated from genotypic assays. Antiviral dose escalation may be an option for treatment of viral mutants with low to moderate levels of drug resistance [29].

Viral genetic alterations that decrease drug susceptibility are identified by genotypic assays that involve sequence analysis of gene regions relevant to the drug in question. Quality control is necessary to exclude sequencing artifacts unlikely to represent viral mutations present in treated subjects [30]. Conversely, an accurate genotypic assay should detect emerging subpopulations of resistant mutants after drug exposure. Resistance-associated mutations require phenotypic confirmation. Poorly authenticated mutations may lead to unwise clinical decisions to switch to alternative therapy, with associated adverse consequences.

Recommendations for CMV genotypic testing are as follows:

Specimen Requirements

Genotypic testing is commonly done on the same plasma or whole-blood specimens used for viral load assays. There must be sufficient CMV DNA in the sample; the accuracy of detection of variant subpopulations was less at 1000 copies/mL than at 10 000 copies/mL [31]. Fragmented viral DNA in plasma samples may be unsuitable for amplification of the longer sequences used for genotypic testing [32]. In some cases where drug-resistant viral genomes and disease have become localized to a particular body site without significant viremia, a tissue-specific sample (ie, intravitreal fluid, cerebrospinal fluid, or biopsy) may be more informative [33]. During clinical trials, it is advisable to collect and store specimens for genotypic analysis at baseline, every few weeks thereafter, at visits for treatment or prophylaxis failure, and at posttreatment follow-up.

Analytical Coverage

Viral gene regions for sequence determination depend on the antiviral target and observations of the mutations that emerge after exposure to the drug in cell culture or in vivo. For clinical trials, the entire coding sequences of viral genes known to be relevant to each drug should be determined: UL97 and UL54 for ganciclovir; UL54 for foscarnet and cidofovir; UL97 and UL27 for maribavir; and UL56, UL89, and UL51 for letermovir (Table 3).

Sequencing Technology

Standard genotyping currently involves amplification of the target gene regions (often using nested polymerase chain reaction [PCR] assays) followed by dideoxy (Sanger) sequencing of the PCR product [34]. The main technical limitations are dependence on a representative PCR product, and insensitivity in detecting variant sequence subpopulations of <20%, as demonstrated by parallel analysis using newer deep-sequencing technologies [35]. Newer sequencing technologies offer the potential of detecting much smaller mutant viral sequence subpopulations [31, 36, 37], but at present are limited by inadequate standardization of the technical platform, sample preparation, breadth and depth of sequencing, and calibration of the accuracy and significance of detection of small variant subpopulations.

Recombinant Phenotyping

This is a research tool that involves the transfer of 1 or more mutations into a drug-susceptible baseline cloned strain of CMV (marker transfer), followed by testing of the mutagenized recombinant virus for drug susceptibility [25]. Recombinant phenotyping correlates the mutation(s) present in the specimen (genotype) with the associated level of drug resistance (phenotype).

Interpretation of Mutations

Sequence variants detected in clinical specimens have been characterized to varying degrees [38, 39]. They can be categorized as follows:

Mutations That Confer a Known Level of Drug Resistance

Most of the mutations supporting a diagnosis of drug-resistant CMV are in this category. For established drugs, canonical mutations are repeatedly detected in individuals failing therapy and confer a consistent level of drug resistance in well-controlled recombinant phenotyping assays [25, 38–40]. Examples are UL97 amino acid substitutions M460V/I, H520Q, C592G, A594V, L595S, and C603W for ganciclovir; UL54 E756K and A809V for foscarnet; UL54 N408K and A987G for cidofovir;

Table 3. Cytomegalovirus Genes Associated With Novel or Commercially Available Antiviral Agents

CMV Gene	Role	Associated Drug Resistance
UL97	Kinase	Ganciclovir, valganciclovir, maribavir
UL54	Polymerase	Ganciclovir, valganciclovir, cidofovir, foscarnet, brincidofovir
UL27	Cell cycle regulation	Maribavir (low level)
UL51/UL56/UL89	Cleavage and packaging	Letermovir

and UL97 T409M and H411Y/N for maribavir. Many other resistance mutations have been confirmed for these drugs [25, 38, 39]. Some phenotype data based on limited replicates of testing and accompanying controls, or on viruses resulting from older, nonclonal marker transfer techniques, may have quality control concerns. Borderline or low-grade changes in susceptibility are more likely to require reevaluation [41], as traditional plaque reduction assays may give fluctuating baseline EC_{50} values that can greatly affect the calculation of the fold-change in EC_{50} value of a tested mutant [24].

Sequence Polymorphisms Detected in Isolates Not Previously Exposed to Antiviral Drugs, Whether In Vitro or In Vivo

There is considerable baseline sequence polymorphism among circulating CMV strains. Baseline polymorphism in target and nontarget genes may affect susceptibility to antiviral drugs. While this is not well documented for the licensed DNA polymerase inhibitors, this possibility needs ongoing evaluation, especially with newer antiviral drugs and targets. During clinical trials, statistical correlation of particular sequence variants with treatment failure can be used to identify candidates for further phenotypic analysis.

Treatment-emergent Mutations That Have Not Been Phenotypically Characterized

These are changes from a baseline pretreatment sequence and have a high priority for phenotyping once it is verified that the detected mutations are not an artifact of the genotypic assay [30]. An alternative explanation for treatment-emergent sequence variants is the appearance of an unrelated CMV strain with a different set of polymorphisms, since CMV reinfections or mixed-strain infections are not uncommon [42]. An informed guess as to the phenotypic significance of uncharacterized mutations can be made based on relative conservation of the locus among strains and on proximity and similarity to known resistance mutations, although this speculation cannot substitute for accurate phenotype data and may be misleading.

Mutations Detected in Treated Individuals Without a Prior Baseline Sequence and Not Previously Phenotyped or Established as Baseline Sequence Polymorphisms

These mutations usually occur in clinical treatment settings or in prophylaxis trials where a baseline sequence is not available. In general, such sequence variants should be verified by retesting and considered for phenotyping to facilitate the interpretation of future genotypic test results, especially if the sequence variant was detected in the context of treatment or prophylaxis failure.

Limitations and Unresolved Issues in Genotypic Resistance Testing

Resistance mutations may be undetected because the specimen tested has too low a viral load or is not collected from a tissue site where mutations have localized. They can also exist as subpopulations too small to be detectable or involve previously uncharacterized genetic loci.

Fundamental to the definition of drug resistance is a phenotype of reduced drug susceptibility. There are significant issues of standardization and interassay variability in determining the drug susceptibility phenotype. The same mutation may confer resistance in some assays but not others, and the measured level of resistance may vary. Thus, the classification of mutations may evolve as more experimental data become available and confidence in the published data rises in proportion to the number of independent studies of the same mutation.

FUTURE DIRECTIONS AND RESEARCH AGENDA

Resistance of CMV to antiviral drugs is clearly a problem of increasing importance. While this manuscript provides a snapshot of the current situation, we can anticipate many changes in the near future.

First, we will gain access to more antiviral drugs active against CMV, with letermovir a relevant example. Its reduced bone marrow toxicity, when compared to ganciclovir, will allow this drug to be given prophylactically without having to wait for engraftment of bone marrow. Yet, this benefit may bring with it emergence of resistance to letermovir. Furthermore, it may help high-risk patients to survive longer, allowing time for them to develop resistance to >1 anti-CMV drug if CMV reactivation has to occur. In the future, maribavir, shown to be effective and well tolerated in a phase 2 trial [43] and currently under development in phase 3 trials, could be a potential agent for treatment of refractory/resistant CMV infections.

Second, we will see increasing use of next-generation sequencing to detect mutations associated with failure to suppress viremia and perhaps resistance. This modality offers the potential of improved sensitivity, yet may turn out to be too sensitive in some clinical situations. We will need careful laboratory studies to confirm through marker transfer into laboratory-adapted strains that the observed mutations do indeed confer resistance. Third, we can anticipate the potential use of anti-CMV drugs in combination, especially when drug-resistant CMV infection is suspected. Randomized controlled trials will be required to demonstrate that increased efficacy for patients is sufficient to outweigh added side effects, cost, complexity, and potential drug interactions of using drugs in combination. Whatever the future holds, the principles and practices reviewed in this publication will undoubtedly be applied to a series of new situations. Those currently planning randomized controlled clinical trials of novel antiviral drugs should therefore consider these concepts in their trial designs.

Notes

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