The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation

Camille N. Kotton, MD,[1] Deepali Kumar, MD,[2] Angela M. Caliendo, MD, PhD,[3] Shirish Huprikar, MD,[4] Sunwen Chou, MD,[5] Lara Danziger-Isakov, MD, MPH,[6] and Atul Humar, MD[7]
on behalf of the The Transplantation Society International CMV Consensus Group

Abstract: Despite recent advances, cytomegalovirus (CMV) infections remain one of the most common complications affecting solid organ transplant recipients, conveying higher risks of complications, graft loss, morbidity, and mortality. Research in the field and development of prior consensus guidelines supported by The Transplantation Society has allowed a more standardized approach to CMV management. An international multidisciplinary panel of experts was convened to expand and revise evidence and expert opinion-based consensus guidelines on CMV management including prevention, treatment, diagnostics, immunology, drug resistance, and pediatric issues. Highlights include advances in molecular and immunologic diagnostics, improved understanding of diagnostic thresholds, optimized methods of prevention, advances in the use of novel antiviral therapies and certain immunosuppressive agents, and more savvy approaches to treatment resistant/refractory disease. The following report summarizes the updated recommendations.

(*Transplantation* 2018;102: 900–931)

The past 5 years has seen exciting advances related to the understanding, diagnosis, and treatment of Cytomegalovirus (CMV). We currently stand on the cusp of modernizing the management of CMV infection posttransplant. Despite these advances, CMV remains one of the most common complications affecting solid organ transplant recipients (SOTR), still befitting the designation: "a transplantation troll."[1] In addition to the direct effects of CMV infection and disease, there are "indirect effects," both general and transplant-specific, and higher rates of all types of infection, graft loss, morbidity, and mortality.[2,3] A panel of experts on CMV and solid organ transplantation (SOT) was previously convened in 2008 and 2012 by The Infectious Diseases Section of The Transplantation Society to develop consensus guidelines on CMV management, subsequently published in 2010[4] and 2013.[5] Topics included diagnostics, immunology, prevention, treatment, resistance, and pediatrics. Given numerous recent advances in the field, a third meeting of experts was convened in March 2017 to update these guidelines.

The expert panel rated the quality of evidence on which recommendations are based by following a process used in the development of other guidelines, including those by the Infectious Diseases Society of America. The Grading of Recommendations Assessment, Development and Evaluation

Received 15 January 2018. Revision received 12 March 2018.

Accepted 13 March 2018.

[1] Transplant and Immunocompromised Host Infectious Diseases Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

[2] Transplant Infectious Diseases and Multi-Organ Transplant Program, University Health Network, Toronto, Ontario, Canada.

[3] Rhode Island Hospital, Providence, RI.

[4] Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY.

[5] Division of Infectious Diseases, Oregon Health & Science University, Portland, OR.

[6] Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

[7] Multi Organ Transplant Program, University Health Network, Toronto, Ontario, Canada.

C.N.K. received funding for serving on scientific advisory boards for Roche Molecular and Merck; adjudication boards for Astellas, Merck, and Shire; and consultancy fees from Qiagen and Oxford Immunotec. A.M.C. received funding for serving on a scientific advisory board for Roche Molecular. D.K. has received research grants from Roche, Shire, Qiagen, Oxford Immunotec as well as consultancy fees from Qiagen and Oxford Immunotec. S.C. performed contracted CMV phenotyping research for Merck and Shire. S.H. and L.D.-I. have no conflicts to declare. A.H. received research support from Qiagen, Astellas and Roche.

All authors participated in the consensus meeting, review and summary of available data, and in the writing of the article.

The CMV Consensus Conference was organized by the Infectious Diseases Section of The Transplantation Society. Independent, nonrestricted grants from Hoffman LaRoche, Merck, Oxford Immunotec, Qiagen, Shire, CSL Behring, Lophius Biosciences, Chimerix, Roche Molecular Systems, and Abbott Laboratories made this conference possible. At no time did the funding sources have input into the list of attendees, discussion, or content.

Correspondence: Camille N. Kotton, MD, Transplant and Immunocompromised Host Infectious Diseases Infectious Diseases Division, Massachusetts General Hospital, Harvard Medical School 55 Fruit Street, Cox 5, Boston, MA 02114. (ckotton@mgh.harvard.edu).

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

ISSN: 0041-1337/18/10206-0900

DOI: 10.1097/TP.0000000000002191

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

system allows for a systematic weighting of the strength of recommendation (eg, "high, moderate, low, very low") and quality of evidence (eg, "strong, weak") (Table 1).[6-10,13]

We used the following definitions, consistent with the American Society of Transplantation and the CMV Drug Development Forum recommendations for use in clinical trials[14,15]:

- CMV infection: evidence of CMV replication regardless of symptoms (differs from latent CMV); "defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen"[15]
- CMV disease: evidence of CMV infection with attributable symptoms. CMV disease can be further categorized as a viral syndrome (ie, fever, malaise, leukopenia, and/or thrombocytopenia), or as tissue invasive ("end organ") disease.

As in our prior versions, the term deoxyribonucleic acid in blood (DNAemia) will be used instead of viremia, to reflect the detection of CMV DNA in blood or plasma (whether actively replicating virus or not). For accuracy, the phrases "viral load" or "quantitative nucleic acid amplification testing (QNAT)" are used instead of "polymerase chain reaction (PCR)."

DIAGNOSTICS

Pretransplant Testing

Given that the CMV serostatus of donor and recipient (D/R) are key predictors of the risk of CMV after transplant and guide decisions on antiviral prophylaxis or preemptive

TABLE 1.

GRADE strength of recommendations and quality of the evidence[6-12]

Strength of recommendation and quality of evidence	Clarity of balance between desirable and undesirable effects	Methodological quality of supporting evidence (examples)	Implications
Strong recommendation, high-quality evidence	Desirable effects clearly outweigh undesirable effects, or vice versa	Consistent evidence from well-performed RCTs or exceptionally strong evidence from unbiased observational studies	Recommendation can apply to most patients in most circumstances. Further research is unlikely to change our confidence in the estimate of effect
Strong recommendation, moderate-quality evidence	Desirable effects clearly outweigh undesirable effects, or vice versa	Evidence from RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from unbiased observational studies	Recommendation can apply to most patients in most circumstances. Further research (if performed) is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Strong recommendation, low-quality evidence	Desirable effects clearly outweigh undesirable effects, or vice versa	Evidence for at least 1 critical outcome from observational studies, RCTs with serious flaws or indirect evidence	Recommendation may change when higher-quality evidence becomes available. Further research (if performed) is likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate
Strong recommendation very-low-quality evidence (very rarely applicable)	Desirable effects clearly outweigh undesirable effects, or vice versa	Evidence for at least 1 critical outcome from unsystematic clinical observations or very indirect evidence	Recommendation may change when higher-quality evidence becomes available; any estimate of effect for at least 1 critical outcome is very uncertain.
Weak recommendation, high-quality evidence	Desirable effects closely balanced with undesirable effects	Consistent evidence from well-performed RCTs or exceptionally strong evidence from unbiased observational studies	The best action may differ depending on circumstances or patients or societal values. Further research is unlikely to change our confidence in the estimate of effect.
Weak recommendation, moderate-quality evidence	Desirable effects closely balanced with undesirable effects	Evidence from RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from unbiased observational studies	Alternative approaches likely to be better for some patients under some circumstances. Further research (if performed) is likely to have an important impact on our confidence in the estimate of effect and may change the estimate
Weak recommendation, low-quality evidence	Uncertainty in the estimates of Desirable effects, harms, and burden; Desirable effects, harms, and burden may be closely balanced	Evidence for at least 1 critical outcome from observational studies, from RCTs with serious flaws or indirect evidence	Other alternatives may be equally reasonable. Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Weak recommendation very low-quality evidence	Major uncertainty in the estimates of desirable effects, harms, and burden; Desirable effects may or may not be balanced with undesirable effects may be closely balanced	Evidence for at least 1 critical outcome from unsystematic clinical observations or very indirect evidence	Other alternatives may be equally reasonable. Any estimate of effect, for at least 1 critical outcome, is very uncertain.

GRADE, Grading of Recommendations Assessment, Development and Evaluation; RCT, randomized controlled trials.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

treatment, serologic tests with high sensitivity and high specificity are recommended. A test measuring CMV-specific IgG should be used, as serologic tests measuring IgM or IgG and IgM combined have poorer specificity and may cause false-positive results. Neither of the latter 2 should be used for screening.[16-18] Not all serologic tests are equivalent, and performance characteristics of the specific test used should be understood.[19] Discordant CMV IgG serology results have been reported for 1.0% to 2.6% of samples tested by commonly used assays.[16,17] A change of the serologic assay requires evaluation of its performance, including comparison with the previously used assay.

If the donor or recipient is seronegative during the pretransplant evaluation, serology should be repeated at the time of the transplantation. Interpretation of serology results can be difficult in donors and recipients with recent transfusion of IVIG and other blood products (platelets, plasma, red blood cells), given the potential for passive transfer of antibody,[20] and a pretransfusion sample should be tested when possible. In seropositive children younger than 12 months, passive transfer of antibody can lead to transient false-positive serologic results.[20] Cell-mediated immunity (CMI) assays may be useful in assisting in establishing true immunologic status in individuals who may have passive antibody.[21,22] Testing for CMV-specific CMI appears to be less useful in deceased donors because of a high prevalence of indeterminate results.[23] In infants younger than 12 months, nucleic acid amplification tests (NAT) of urine or oral samples may be helpful to identify infected patients, as children with postnatal primary infection appear to persistently shed virus for long periods,[24] whereas a negative CMV NAT cannot exclude past exposure. Thus, if equivocal serologic assay results are obtained in the donor or in the recipient, we recommend assigning the highest appropriate CMV risk group for posttransplantation management decisions.

Posttransplant Testing

Serology has no role in the diagnosis of active CMV replication and disease posttransplantation. Serology may be used to determine ongoing susceptibility to community-acquired disease in patients seronegative before transplantation who do not develop infection or disease after transplantation.

Using CMV-antibody binding assays, seroconversion at the end of antiviral prophylaxis was not a useful predictor of future CMV disease in CMV-mismatched patients,[25,26] although seroconversion at 6 months after transplant in D+/R– patients given 100 days of antiviral therapy (ie, 3 months after the end of prophylaxis) conveyed a reduced risk for late CMV disease.[26] In a pilot study in CMV-mismatched patients managed using preemptive therapy, measurement of antibodies neutralizing epithelial cell infection, particularly when combined with CMI studies was identified as a possible predictor of future CMV disease risk.[27]

Viral culture of blood for detecting CMV has limited clinical utility due to poor sensitivity for diagnosis of disease. Conversely, there is no role for CMV culture of urine or oral secretions due to poor specificity for CMV disease (versus viral shedding).[28] Quantitative nucleic acid amplification testing in blood is the cornerstone for diagnosis and monitoring for CMV infection and disease; QNAT is preferred over antigenemia because of standardization and other technical reasons, as previously described.[5]

Quantitative nucleic acid amplification testing is the preferred method for diagnosis of CMV infection, guiding preemptive strategies, and monitoring response to therapy.[29-35] There are numerous commercial reagents and automated platforms available for quantitative CMV DNA testing, as well as increasing data on the performance characteristics of these tests. Plasma and whole-blood specimens both provide prognostic and diagnostic information regarding CMV disease.[36-40] CMV DNA is generally detected earlier and in greater quantitative amounts per unit volume (ie, mL) in whole blood compared with plasma. Persistent plasma DNAemia has been shown to be a better predictor of relapse at day 21 of treatment compared with persistent whole-blood DNAemia.[38] There is evidence that CMV DNA exists predominantly as small fragments in plasma,[41] this may not to be true to the same extent for whole blood as both intracellular and extracellular DNA are detected. More work is needed to understand the form of DNA in whole blood samples; paired sample studies are required to establish correlation of results between the 2 sample types. Thus, when serially monitoring patients, 1 specimen type should be used.

Calibration of tests with the World Health Organization (WHO) international standard[42] has led to improved agreement of viral load values,[43] therefore all results should be reported as IU/mL. Important differences still exist due to a variety of factors[44-46] including extraction method, amplification target, probe, nonstandardized quantification of secondary standards[45,47,48] and amplicon size.[41] After calibration, commutability needs to be demonstrated, meaning that patient samples and calibrators behave similarly in a given quantitative test. Commutability of current assays calibrated to the WHO international standard varies widely.[49] The increased use of commercial assays that address these factors should decrease variability; a recent study showed good reproducibility in viral load values across multiple laboratories when using a commercial test calibrated to the WHO standard.[50] Such agreement allows comparison of data across different transplant centers using the same test.

The QNAT results should be demonstrated to be linear in the clinically important range between the lower and upper limits of quantification (LLOQ, ULOQ). The LLOQ varies amongst assays with newer, highly sensitive assays demonstrating LLOQ less than 200 IU/mL. Assays results reported as detectable below the LLOQ represent very low levels of CMV DNA that may not be clinically significant. The precision of QNAT results is such that changes in values should be at least threefold (0.5 $\log_{10}$ IU/mL) to represent biologically important changes in viral replication.[50-53] The QNAT variability is greatest for viral loads of 1000 IU/mL (3 $\log_{10}$) and below, where changes may need to be greater than fivefold (0.7 $\log_{10}$ IU/mL) to be considered significant. Reporting results as both integers and $\log_{10}$-transformed data may help clinicians avoid overinterpreting small changes in viral load.

It remains imperative that laboratories use quantitative external standard materials (independent of that provided by the manufacturer) to monitor quantification across different lots of reagents to ensure consistency in assay performance. Quantitative nucleic acid amplification testings should accurately quantify the common CMV genotypes. If the laboratory changes QNAT or extraction methods, a comparison must be performed documenting the performance characteristics of the new versus old tests. Until QNATs have been

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

better harmonized, a single test should be used for clinical trials and for monitoring patients over time. Given the rapid replication dynamics, CMV QNAT results should be available within 24 to 48 hours for optimal clinical decision making.

Consensus viral load thresholds to initiate preemptive therapy need to be defined, but widespread use of laboratory-developed tests and paucity of assays calibrated to the WHO International Standard has limited the ability to perform such studies. Moreover, the D/R risk group and exposure to lymphocyte-depleting antibodies need to be taken into account. Early work has shown that higher viral load values correlate with increased risk for disease[30,31] (see Prevention section for further discussion). It is important to note that these recent studies were done with different laboratory developed assays, specimen types (plasma, whole blood), and patient populations, so these thresholds cannot simply be adapted. This leaves individual centers with the task of determining appropriate thresholds in their patient populations, and emphasizes the urgent need to perform collaborative studies to determine consensus threshold in IU/mL, which should be increasingly practical with the availability of commercial assays.

The viral load kinetics (rapid doubling time) in high-risk groups suggests that the frequency of viral load testing will impact the effectiveness of a preemptive strategy (ie, more frequent testing will be more effective). The half-life of viral loads is such that monitoring patients on therapy should be done at least weekly.[32] Dynamics of CMV loads over time may be more important in predicting disease than any absolute viral load value,[30] especially when close to the LLOQ. However, the LLOQ varies among the different viral load tests; an LLOQ of greater than 1000 IU/mL (using either whole blood or plasma) may be too insensitive and hence inadequate for preemptive strategies aiming at antiviral treatment before disease.[54] Conversely, a very sensitive test (LLOQ <10 IU/mL) may detect latent virus particularly if whole-blood specimens are used, which limits the clinical utility of an extremely sensitive test.

When monitoring a response to treatment, there is concern that highly sensitive tests may increase the time to reach an undetectable viral load, thus unnecessarily exposing patients to prolonged periods of antiviral therapy. A recent study showed that using a more sensitive test does increase the time to reach an undetectable viral load; however, there was a trend towards shorter duration of therapy with the more sensitive assay.[55] Finally, treatment until viral load values are less than 137 IU/mL is predictive of a clinical response.[56] Though there is value to using highly sensitive assays, the practice of continuing therapy until successive viral load tests are undetectable may not be necessary when using such tests (ie, with highly sensitive assays, multiple very low results might be sufficient before stopping therapy).

Diagnostics for Tissue-invasive Disease

The definitive diagnosis of tissue-invasive disease relies on detection of CMV in the tissue specimen. Identification of CMV cytopathic changes or CMV antigens by immunohistochemistry[15,57,58] represents the gold standard for the diagnosis of tissue-invasive disease. Not all antibodies and staining procedures have equal sensitivity and the performance may differ between fresh and formalin-fixed, paraffin-embedded tissue.[59]

Gastrointestinal disease in all organ transplants and pneumonitis in lung transplant recipients may have undetectable or low DNAemia.[60,61] Plasma QNAT has a high sensitivity for diagnosis of gastrointestinal disease in D+/R– patients, which decreases substantially in R+ patients.[62,63]

Viral culture of tissue samples, though not available in most laboratories today, may improve sensitivity compared to histopathology eg, in gastrointestinal disease.[63] However, in patients with viremia the specificity of culture results of tissue for end-organ CMV disease may be limited. Similar considerations apply to the more sensitive molecular testing. Some studies have shown positive QNAT tissue results in cases with a clinical suspicion of gastrointestinal CMV disease, however, in which histopathology and DNAemia were negative.[64,65] Tissue QNAT may be preferable to qualitative NAT due to higher specificity, but studies are needed to define thresholds and to provide a standardized approach for QNAT in tissue-invasive CMV disease.

In lung transplant recipients, the detection of the CMV by QNAT in bronchoalveolar lavage (BAL) reflects CMV replication in the lung rather than contamination with oropharygeal fluids.[66] Qualitative NAT detecting CMV-DNA in BAL specimens in lung and nonlung transplant recipients may not be specific for a diagnosis of CMV pneumonitis.[67,68] Quantitative NAT is preferred on BAL specimens and increased DNA levels may better correlate with symptomatic CMV disease.[61,69–71] Further work is needed to standardize QNAT on BAL samples including development of thresholds using normalized reporting units.[69,72]

Central nervous system disease in SOTRs is extremely rare. In the absence of further clinical studies, the presence of CMV DNA in the cerebrospinal fluid likely represents CMV disease necessitating treatment.

The diagnosis of retinitis is based on ophthalmologic examination; CMV DNAemia is rarely useful as a predictor of CMV retinitis, although it may be positive before and at the time of diagnosis. A positive QNAT in vitreous fluid may be helpful in guiding the diagnosis of retinitis.

Consensus Statements and Recommendations

- The availability of the WHO-approved CMV international standard has led to improved agreement of plasma viral load values between various assays.[43] Differences still exist due to a variety of factors,[44-46] including matrix, extraction method, amplification target, amplicon size, hybridization/detection probe, nonstandardized quantification of secondary standards,[45,47,48] and use of noncommutable standards.
- Global harmonization and development of universal thresholds will be facilitated by using assays where all steps are highly standardized. The increased availability of appropriately designed, affordable commercial assays should accelerate these processes.
- We recommend performing donor and recipient CMV IgG serology pretransplantation for risk stratification (strong, high).
- We do not recommend IgM testing (strong, low).
- We recommend repeat serologic testing at the time of transplant if pretransplantation serology is negative (strong, low).
- We recommend that in adults, an equivocal serologic assay result in the donor be assumed to be positive, whereas in the recipient this result be interpreted to assign the recipient to the highest appropriate CMV risk group for posttransplantation management decisions (strong, low) (for guidance on infants and children younger than 12 months, see Pediatrics section).

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

- We do not recommend viral culture of blood, urine, or oral secretions for the diagnosis of active CMV infection or disease (strong, high). Positive cultures of BAL samples may not always correlate with disease.
- We do not recommend QNAT on urine and oral secretions for surveillance or diagnosis of CMV disease (strong, low).
- We recommend using QNAT calibrated to the WHO standard for diagnosis, surveillance to guide preemptive antiviral treatment, and for therapeutic monitoring due to the ability to harmonize and standardize these tests (strong, high). Results must be reported as IU/mL and termed as DNAemia rather than viremia (strong, high). If QNAT is not available, antigenemia is a less desirable alternative.
- We recommend either plasma or whole blood specimens for QNAT, with an appreciation for the differences in viral load values, viral kinetics and assay performance characteristics. (strong, high). Neither the specimen type nor the assay should be changed when monitoring patients.
- Despite reporting in IU/mL, we recommend that viral load values are not directly compared across centers and/or laboratories unless identical testing reagents and procedures can be assured or equivalence has been documented (strong, high).
- We recommend that only changes in viral load exceeding 0.5 $\log_{10}$ IU/mL (threefold) are considered to represent clinically significant differences in DNAemia (strong, low).
- Although harmonization of QNAT has improved, universal thresholds for therapy or treatment endpoints have not been established and current published thresholds remain assay-specific. Accordingly, we recommend that centers establish their own thresholds and audit clinical outcomes to verify the thresholds used (strong, moderate).
- We do not recommend surveillance of CMV DNAemia during routine prophylaxis.
- We recommend when monitoring response to antiviral therapy, that QNAT is performed weekly (strong, moderate).
- With the use of highly sensitive QNAT (LLOQ <200 IU/mL), we suggest discontinuing therapy after 1 result is less than the LLOQ. If this approach is used, confirmatory testing should be done 1 week after discontinuing therapy. If the assay is not highly sensitive, then 2 consecutive undetectable (negative) results are needed to discontinue therapy (weak and moderate).
- We recommend histology coupled with immunohistochemistry for the diagnosis of tissue-invasive disease. Histopathologic examination of tissue should routinely include immunohistochemistry for CMV (strong, moderate).

Future Directions

- Directly compare QNAT monitoring in plasma, whole blood, and BAL specimens with respect to disease prediction and monitoring response to therapy with an emphasis on using commercially available testing systems.
- Determine commutability and harmonization using the WHO International Standard for whole blood and BAL.
- To improve harmonization of QNAT, determine the viral form (virions, fragmented, or genomic CMV) and viral kinetics in whole blood.
- Assess the role of digital QNAT to improve standardization of copy number assignment for secondary standards.
- Once viral load tests are harmonized, establish thresholds and kinetics for DNAemia for initiating preemptive therapy.
- Compare the performance characteristics of the different serologic tests and assess the utility of CMI assays and QNAT using a variety of sample types for the interpretation of passive immunity.
- Standardized/optimize/harmonize tests measuring neutralizing antibody in epithelial cells and fibroblasts when used to evaluate vaccine responses, characterize immunoglobulin preparations and evaluate antibody levels as a biomarker for predicting disease risk.
- Further validate nonseroconversion at 12 to 18 months after antiviral prophylaxis as a marker of nontransmission of donor-derived CMV infection in CMV mismatched patients.
- Further evaluate the usefulness of binding and neutralizing antibody (epithelial and fibroblast) measurements pretransplant in CMV seropositive patients and posttransplant in CMV mismatched patients as a predictor of disease risk.

IMMUNOLOGIC MONITORING FOR CMV

Innate Factors

Both innate and adaptive immune mediators are necessary for control of CMV after transplantation.[73,74] Innate immune factors include Toll-like receptors in which single nucleotide polymorphisms are associated with an increased risk of CMV disease.[75] Polymorphisms in genes for mannose binding lectin (MBL) associated with low MBL production and ficolin-2 may also be associated with increased risk of CMV disease.[76-78] A multicenter study has recently demonstrated that patients with lower complement C3 levels (<80 mg/dL) early (7 days) after transplantation have a greater risk for CMV disease.[79] Transplant recipients with an IL-28B single nucleotide polymorphism were found to be at significantly less risk of CMV replication.[80] Conversely those with a CCL8 promoter polymorphism showed increased risk of CMV replication.[81] In addition, lower levels of natural killer (NK) cells early after transplantation are a risk factor for CMV disease in heart and liver recipients.[82,83] Inhibitory NK cell KIR genotypes have been described as a predisposing factor during CMV reactivation in kidney transplantation while B-type NK cell haplotypes containing activating NK cell KIR genes were shown to be protective in organ recipients.[84-86] γδ (gamma-delta) T lymphocytes that have both innate and adaptive characteristics have been reported to have a role in the immune response to CMV in transplantation.[87,88] Longitudinal kinetic surveillance of $V\delta2^{-}$ γδ T cells in kidney recipients has been suggested to predict CMV infection resolution.[89] Although the above associations are important for the pathogenesis of CMV, changes in CMV management based on these factors have not been studied.

Nonpathogen-specific Adaptive Immunity

Adaptive or acquired immune responses of B and T lymphocytes are critical in controlling CMV replication. Methods to monitor the adaptive immune response to CMV may allow for early identification of patients at increased risk of viral replication. B cells are important in the humoral response to CMV, producing neutralizing antibodies that primarily target glycoprotein B (gB) and gH^{90} and the pentameric gH/gL/UL128/UL130/UL131A complex.[91-93] There is emerging evidence that a significant number of posttransplant patients develop hypogammaglobulinemia (26%-70% in some series[94-97]). A recent meta-analysis demonstrated that severe hypogammaglobulinemia during the first year after transplant significantly increased the risk of CMV disease. Moderate and severe (IgG, < 400 mg/dL) hypogammaglobulinemia have been demonstrated to be a risk factor for CMV infection in heart and lung transplant recipients in single-center studies

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

performed in patients with various induction immunosuppressive protocols.[79,98,99] A multicenter prospective study showed that heart recipients with moderate hypogammaglobulinemia at day 30 after transplantation were at higher risk of CMV disease.[79] Severe IgG hypogammaglobulinemia has been associated with CMV disease refractory to antiviral therapy.[100] Interventional studies in heart transplant recipients using CMV immunoglobulin (CMV Ig) or intravenous immunoglobulin replacement have shown that IgG replacement may prevent CMV disease.[101-103] Retrospective studies have suggested that replacement of IgG levels by use of IVIG in heart recipients with IgG hypogammaglobulinemia detected at the time of CMV disease including refractory cases was associated with better outcomes.[104,105] Despite the evidence of IgG replacement therapy in thoracic transplant recipients, the link of hypogammaglobulinemia with CMV risk remains controversial in kidney and liver recipients.[96,106]

The potential role of lymphopenia, a commonly used biomarker in clinical practice, as a risk factor for development of CMV infection has been evaluated. In a multivariate analysis, pretransplant lymphopenia was the strongest independent predictor of CMV disease among 276 liver transplant patients.[107] Lymphocyte counts also tended to be lower in patients who have recurrent CMV infections.[108]

Nonpathogen-specific immune function assays are also available. The ImmuKnow (Cylex/Viracor-Eurofins, USA) assay is not specific for CMV. This assay, which is commercially available in the United States and in some European countries, measures overall immune function and serves as a marker of immunosuppression by determining the amount of ATP produced by CD4+ T cells in response to whole blood stimulation by phytohemagglutinin. However, there are no studies indicating whether this assay is predictive of CMV DNAemia or disease. Therefore, there is insufficient evidence to recommend this assay for CMV prediction. Another immune function assay under development is the QuantiFERON-Monitor (Qiagen, USA) which measures global immune function after stimulation of whole blood with a lyosphere containing R848 and anti-CD3.[109] In general, however, CMV-specific immune-based assays are likely to have greater clinical utility than a nonspecific assay.

The complexity of the immunological response to control CMV infection makes it unlikely that any single marker will be highly predictive of risk. The combined use of distinct biomarkers has been suggested to be a better approach than the use of single markers including biomarker combinations and gene profiles.[110] Immunological scores including distinct biomarkers have been suggested as other option to identify patients at higher risk of CMV infection.[98]

CMV-specific Immune Monitoring Assays

Immune monitoring of CMV-specific T-cell responses can predict individuals at increased risk of CMV disease posttransplant and may be useful in guiding prophylaxis and preemptive therapies. There is a variety of CMV-specific T-cell assays. Many assays have now moved from the experimental to the clinical setting. The majority of assays rely on the detection of IFN-γ after stimulation of whole blood or peripheral blood mononuclear cells (PBMC) with CMV-specific antigens or overlapping peptides.[111,112] In addition to IFN-γ, other markers, including IL-2, TNF-α, CD107, programmed death-1 (PD-1), and CD154 have been used to correlate CMV-specific T-cell responses with the risk of CMV infection. The expression of chemokines CCL8 and CXCL10 has also been associated with CMV control in patients with CMV DNAemia whereas expression of CCR6, a chemokine receptor, predicted CMV reactivation.[81,90,113]

The expert panel was of the opinion that an ideal assay should provide both quantitative and functional information on CMV-specific $CD4^+$ and $CD8^+$ T cells. For clinical application, an assay should ideally be simple to perform, inexpensive, highly reproducible, and amenable to either widely available platforms or shipping to specialized reference laboratories. Each of the immune monitoring assays has specific advantages and limitations and has been studied in various clinical applications to predict disease or viremia (Table 2).

The QuantiFERON-CMV assay is a commercially available kit (CE marked in Europe) and is an enzyme-linked immunosorbent assay (ELISA)-based IFN-γ release assay detecting CD8 T cells after peptide stimulation. The assay has been evaluated in clinical studies of transplant patients at high risk of CMV and shown to be predictive of disease.[114-117] Moreover, a negative test before transplantation may aid in predicting viremia in the posttransplant period[118] or the dynamics of T-cell responses may be used as a monitoring tool in a preemptive setting.[119] In a small cohort of transplant recipients with low level CMV DNAemia, a positive assay was predictive of spontaneous clearance.[120] A main drawback stems from difficulties in interpretation if a patient does not respond to the mitogen control. Nonresponse to mitogen may potentially be a marker for global immunosuppression, and has been associated with a subsequent higher incidence of CMV disease.[116] Test sensitivity decreases in lymphopenic patients because an adequate number of cells are required for the production of IFN-γ.

The enzyme-linked immunosorbent spot (ELISpot) assay quantifies both $CD4^+$ and $CD8^+$ T cells producing IFN-γ in response to CMV. Purified PBMCs are stimulated with CMV-specific peptides or whole antigen lysates; IFN-γ is then captured, detected, and quantified using a labeled antibody. As with the QuantiFERON assay, a mitogen control may indicate general T-cell responsiveness. The ELISpot assay cannot differentiate between $CD4^+$ and $CD8^+$ T cells. Various in-house ELISpot assays have been evaluated and shown to be predictive of disease and viremia.[121-125] Studies have used arbitrary cutoffs for defining positive responses that range between 5 and 50 spot-forming cells per 200 000 PBMC. Others have suggested that kinetics of ELISpot responses are better for risk stratification.[126] ELISpot assays have also been used in the pretransplant setting to predict posttransplant outcomes.[126,127] One study showed that stimulation with an overlapping peptide pool of IE-1 in the pretransplant period predicted CMV in the posttransplant period in CMV seropositive patients.[127] However, another smaller study found no predictive value of pretransplant CD4 and CD8 responses.[128] A commercial ELISpot assay (T-Track CMV, Lophius Biosciences, Germany) has recently received CE marking in Europe.[129,130] Another assay (T-SPOT.CMV, Oxford Immunotec, UK, CE marked in Europe) is used as a laboratory developed test (LDT) in the United States.

Many studies that have analyzed CMV-specific T-cell responses have used intracellular cytokine staining (ICS) for IFN-γ using flow cytometry. Whole-blood or isolated PBMCs are stimulated with CMV peptides or CMV lysate. If whole antigen lysate is used, the assay is not HLA-restricted and

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 2.

Advantages and limitations of various assays for immune monitoring of CMV

Assay	Advantages	Limitations	Comments	Predict viremia	Predict disease
ICS	Whole blood assay with low blood volume (1 mL) or PBMC Short incubation time Results available after 8 hours Identification of CD4+ and CD8+ T cells Knowledge of HLA not necessarily required Quantitative and qualitative characterization	Needs access to a flow cytometer Not standardized	Most data available with this technique Potential to freeze PBMCs and ship to reference lab for testing	Yes	Yes
QuantiFERON-CMV (Qiagen, USA)	Whole blood assay with low blood volume (3 mL) Simple to perform Results available after 30-40 hours Can be done in any center and stimulated plasma can be sent to reference lab	CD8+ responses only. Sensitive to lymphopenia. Rare patients whose HLA types are not covered in assay	Approved in Europe	Yes	Yes
ELISpot	Identifies both CD4+/CD8+ T cells Knowledge of HLA not necessarily required Results available after 30-40 hours	Need for purified PBMC from 10 mL blood (in reality 5-10 mL) Cannot differentiate CD4+ and CD8+ T cells Not standardized	Potential to freeze PBMCs and ship to reference lab for testing; Commercial availability (T-Track CMV, Lophius CE marked in Europe; T-SPOT.CMV is LDT in U.S.) and CE marked in Europe	Yes	Yes
MHC multimer staining	Fast assay (1-2 h) Whole blood assay with low blood volume (0.5-1 mL) or PBMC	CD8+ responses only Needs access to a flow cytometer HLA and epitope-specific. No information about function unless combined with ICS Not standardized	Unlikely to be used on a widespread basis	No, Only in combination with functional or phenotypical markers	No

knowledge of patient HLA type is not required. Stimulated cells are stained with monoclonal antibodies directed against IFN-γ. This technique is fast, versatile, and can be expanded to include other cytokines and cell surface molecules. Unlike ELISpot or QuantiFERON assays, ICS can thus provide both quantitative and qualitative characteristics of CMV-specific T cells. Clinical studies have shown that this technique can predict both CMV disease and viremia. Several studies in SOTRs showed an increased risk of CMV disease in patients with low levels of specific T-cell immunity.[131-134] Similarly, the absence of anti-CMV T-cell response by this technique correlates with the inability to clear viremia.[131,134,135] Stable levels of CMV-specific CD4+ T cells were associated with lower risk of CMV replication.[131,134] The development of T-cell immunity especially polyfunctionality has also been shown to be associated with freedom from CMV disease after lung transplantation.[136-138] The predictive value for viremia may be improved when the analysis of IFN-γ is combined with other cytokines, such as IL-2, and additional markers, such as PD-1.[139,140] Advances in flow cytometry, such as the ability to test for several markers at once (eg, with CyTOF technology), can increase our understanding of immune control although these are available in the research setting only.

Major histocompatibility complex (MHC)-multimer based assays directly stain peptide-specific T cells using peptide-conjugated MHC class I tetramers or pentamers. They can determine CD8 T-cell responses but are epitope-specific and require knowledge of the patients' HLA type. Multimer assays have only been shown to predict CMV viremia when combined with analysis of surface markers, such as PD-1.[141,142] Both ICS and MHC-multimer staining require a fluorescence-activated cell sorting facility, which may limit widespread use in transplant centers.

Several clinical studies have now been published that have used immune monitoring to determine risk of CMV disease and viremia. The majority of studies have measured IFN-γ alone or in combination with other cytokines or cell-surface molecules and included both seropositive and seronegative recipients. The frequency of monitoring in these studies has been variable; high-risk patients were generally monitored starting at the end of prophylaxis and those undergoing preemptive therapy were screened weekly to monthly for up to 1 year posttransplant. Therefore, data are accumulating that suggest immune monitoring may be considered in combination with viral load monitoring to improve assessment of the individual's ability to control CMV. Data are also accumulating for measuring immune function in the pretransplant setting. Donor testing for CMV has also been performed using immune monitoring platforms although indeterminate results are frequently seen depending on the assay.[23] Clinical utility studies demonstrate that alteration of patient management based on the results of an immune-based assay is feasible, safe, and cost-effective are

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

ongoing. Recently, an interventional study using the QuantiFERON-CMV assay to stratify patients in receiving secondary prophylaxis showed that CMV-specific immune function could be measured in real time and used to make clinical decisions.[143] This study showed that it was safe to discontinue treatment antivirals in patients with CMV-specific immunity without the need for secondary prophylaxis. Interventional studies of primary prophylaxis are underway. Potential clinical applications for immune-based assays are summarized in Table 3.

Adoptive T-cell Therapy

Several case reports now exist demonstrating the clinical use of CMV-specific T cells for resistant/refractory CMV infection in organ transplant recipients.[144-148] In general, CMV-specific T cells undergo expansion with CMV synthetic peptides or viral lysate and are then infused into the recipient. This therapy has led to reconstitution of viral immunity, reduction in CMV viral loads or resolution of CMV disease. Cells can be obtained from the transplant recipient (autologous); however, the process of generating effector cells can take several weeks. Therefore, there is increasing interest in "off the shelf" T cells using HLA-matched third-party banked cells. This method could also allow generation of cells active against multiple viruses including CMV, Epstein-Barr virus (EBV), and adenovirus. There is a larger breadth of experience using CMV-specific T cells in the hematopoietic stem cell transplant (HSCT) field, which has shown varying degrees of efficacy with no association with graft versus host disease.[149,150] Commercialization of third-party CMV-specific T cells may lead to increasing use of this modality for resistant/refractory CMV in SOTRs.[151,152] Safety and efficacy studies will be needed.

CMV Vaccine

Several CMV vaccines are under development, but none are currently available for routine clinical use. Types of vaccines include live attenuated, recombinant/chimeric viral vectors, recombinant subunit, or gene-based vaccines.[153] A live attenuated vaccine based on the Towne strain of CMV was found to be safe during clinical testing but had a suboptimal antibody response and while CMV disease was attenuated, the vaccine failed to prevent infection.[154] A recombinant gB vaccine with MF59-adjuvant was shown to induce neutralizing antibodies[155] and prevent infection.[156] In a recent study, this vaccine was administered in a 3-dose schedule to both CMV seropositive and seronegative transplant candidates.[157] During follow-up, the vaccine reduced overall days of CMV viremia and number of days of antiviral therapy. A trial with a gB/pp65-based DNA plasmid vaccine in HSCT recipients was completed. This vaccine was administered as 1 pretransplant dose and 5 posttransplant doses. It showed a significant reduction in viremia versus placebo as well as a reduction in the number of CMV episodes.[158] A multicenter clinical trial for the gB/pp65-based DNA vaccine was conducted in CMV mismatched transplant recipients but did not show a significant reduction in CMV viremia needing antiviral therapy.[159] Recently, a peptide-based CMV vaccine (CMVPepVax) has been used in clinical trials. This vaccine is developed using the HLA-A*201-restricted pp65 CD8 T cell peptide epitope which is fused with the P2 peptide epitope of tetanus toxin and mixed with TLR9 agonist just before administration. This vaccine was given using a 2-dose schedule to HSCT patients in a small randomized trial and showed a significant reduction in CMV viremia.[160] An alphavirus replicon vector system has been used to produce viral particles expressing gB and pp65/IE-1 fusion protein; initial studies in mice and rabbits have shown the development of neutralizing antibodies[161] and a phase I trial has been done. Other vaccines include canarypox gB and pp65 vaccines that produce T-cell responses and neutralizing antibodies.[162,163] An adenoviral chimeric vaccine-based replication deficient

TABLE 3.

Potential clinical uses and management based on CMV-specific immune monitoring

Clinical settings	Viral load	Immune monitoring result[a]	Action	Interpretation
Pretransplant				
Pretransplant R+		Neg	Prophylaxis or surveillance	Indicates low level protection
Pretransplant Seropositive patients with potential passive antibodies		Neg		Passive immunity; T cells are not transferred
		Pos		True Infection
Posttransplant prophylaxis				
End of prophylaxis		Pos	Stop prophylaxis	Indicates protection
		Neg	Continue prophylaxis or stop prophylaxis and do surveillance	Indicates lack of protection
Posttransplant preemptive therapy				
Asymptomatic R+ patients	Neg	Pos	Continue surveillance	Low risk, indicates protection
(>1 month posttransplant)	Neg	Neg	Close surveillance	Increased risk, indicates lack of protection
	Pos	Pos	No treatment; close monitoring	Low risk, indicates sufficient immunity
	Pos	Neg	Treatment	Indicates lack of protection
End of treatment	Neg	Pos	Stop treatment	Low risk of relapse, sufficient immunity
	Neg	Neg	Secondary Prophylaxis	High risk of relapse, lack of protection

[a] Positive (or Reactive) immune monitoring result suggests a threshold has been established; viral load negative means below lower limit of quantitation. Limited information in heart and lung transplant recipients and pediatric recipients.
Neg, negative; Pos, positive.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

adenovirus encoding gB and multiple CMV epitopes was able to produce a robust cellular response and neutralizing antibodies in mice.[164] In general, CMV vaccines have reached human studies with clinical endpoints. However, because CMV vaccines are still in early stages of clinical development, no specific recommendation for vaccine use are made.

Consensus Statements and Recommendations

- Host genetic markers and immune parameters, such as low lymphocyte counts, complement levels and/or NK cell counts as well as T cell immune functional assays, have shown association with higher incidence of CMV infection. Measurement of such parameters could potentially be used to inform the risk of infection although no interventional studies using these have been performed.
- CMV vaccines are in preclinical, phase I and phase II trials. The primary goal of a CMV vaccine should be to prevent or modulate CMV replication and/or CMV disease. Surrogate endpoints (eg, reduction in viral replication, T-cell immunity) can be used to evaluate vaccine immunogenicity and efficacy.
- T-cell therapies should be further evaluated for resistant/refractory CMV.
- IgG hypogammaglobulinemia is associated with an increased risk of CMV disease after transplantation. Measurement of total immunoglobulins is suggested in situations where CMV is difficult to control (weak, low).
- CMV-specific cellular immune monitoring has been shown to predict CMV infection in the pretransplant and posttransplant settings in prospective observational, multicenter studies. Accordingly, assessment of CMI can be used to inform risk of CMV (strong, moderate). Interventional studies to determine precise clinical utility of CMI are ongoing.

Future Directions

The following future research directions are important for the further development of immune monitoring and CMV vaccines:

- Immune monitoring assays should continue to be standardized. Cut-off values need to be established for ELISpot and ICS assays. In addition, regulatory T cells and γδ T cells may have predictive value for DNAemia and should continue to be studied for incorporation into immune monitoring strategies.
- Clinical utility studies of immune monitoring are needed that demonstrate that alteration of patient management based on the results of an immune-based assay is feasible, safe, and cost-effective.
- Studies are also needed to determine the comparative performance of immune monitoring assays in the prediction of CMV viremia/disease.
- Further data are needed for the safety and feasibility of adaptive T-cell therapy in organ transplantation.
- For further development of CMV vaccines, the expert panel was of the opinion that (i) given the high frequency of disease in D+/R– transplant recipients, vaccines should be evaluated specifically in this group; (ii) vaccination may also reduce burden of disease or impact the course of latent CMV infection in seropositive patients; vaccination trials should therefore focus on this group also; and (iii) vaccine studies should include an evaluation of both humoral and cellular immunities where applicable as well as longevity of responses.

PREVENTION

Given the high frequency of primary CMV infection and reactivation, prevention strategies are of paramount importance as they augment transplant clinical success and outcomes, by decreasing the risk of CMV infection and disease, as well as the associated "indirect effects." Universal prophylaxis and preemptive therapy are the main approaches for prevention. An additional strategy that combines both of these approaches is "surveillance after prophylaxis" (termed "hybrid approach" in prior guidelines). Herein, we cover both standard prevention methods (Table 4), as well as additional tactics. Additional information is available on thresholds for initiating treatment, and we have a better understanding of the lower risk of CMV infection with mammalian target of rapamycin (mTOR) inhibitors in CMV seropositive recipients.

Universal Prophylaxis

Universal prophylaxis entails the administration of antiviral medication to all patients or a subset of "at-risk" patients, starting within 10 days after transplant and continuing for a finite period (ie, 3-6 months). Acyclovir, valacyclovir, intravenous ganciclovir (GCV), oral GCV, and valganciclovir (VGCV) have all been studied for universal prophylaxis. Valganciclovir is currently the most commonly used drug for prophylaxis. In early studies, GCV prophylaxis was found to be more effective than acyclovir.[165] Equivalent efficacy was found in a large study of D+/R– transplant patients comparing oral GCV to VGCV (PV16000); however, in small subgroup analysis, tissue invasive disease was noted at increased incidence in liver transplant patients who received VGCV.[166] Oral GCV is no longer available. High-dose

TABLE 4.

Comparison of prophylaxis versus preemptive therapy

	Prophylaxis	Preemptive therapy
Early CMV DNAemia/ infection	Rare	Common
Prevention of CMV disease	Good efficacy	Good efficacy
Late CMV (infection/disease)	Common	Rare
Resistance	Uncommon	Uncommon (with weekly testing)
Ease of implementation	Relatively easy	More difficult
Prevention of other herpes viruses	Prevents HSV, VZV	Does not prevent
Other opportunistic infections	May prevent	Unknown
Costs	Drug costs	Monitoring costs
Safety	Drug side effects	Less drug toxicity
Prevention of rejection	May prevent	Unknown
Graft survival	May improve	May improve

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

valacyclovir has been shown to be effective for prophylaxis in kidney transplant recipients.[167]

Late-onset CMV disease, or disease occurring after the discontinuation of prophylaxis, has occurred in all studies evaluating universal prophylaxis. Late CMV disease is likely related to a lack of CMV-specific CMI in the setting of ongoing immunosuppression. Risk factors for late-onset disease include D+/R– serostatus, shorter courses of prophylaxis, higher levels of immunosuppression, certain types of transplant (eg, lung), and allograft rejection.[168,169] In the PV16000 study, where 3 months of prophylaxis was used, 18% had late-onset CMV disease by 12 months (~30% when including investigator treated disease).[166] This has led to studies evaluating longer durations of prophylaxis (see below).[25]

Preemptive Therapy

Effective preemptive therapy (PET) involves monitoring for CMV in blood at regular intervals (ie, CMV viral load checked weekly) to detect early viral replication. Once a predetermined assay threshold is achieved (optimally before the development of symptoms), antiviral treatment is begun, which should prevent progression to clinical disease. Better availability and standardization of assays have made this approach more feasible. Nonetheless, given the variability among diagnostic specimens (whole blood versus plasma) and testing platforms,[43,170] a universal threshold for starting therapy cannot be defined. It is likely that optimal thresholds are different among different at-risk groups. There was strong consensus that a lower threshold should be used with D+/R–, as the use of higher thresholds may result in insufficient time to begin treatment for CMV and higher rates of disease.[171] For example, 1 study demonstrated a median viral load doubling time of 1.54 days (range, 0.55-5.5) in D+/R– compared with 2.67 days (range, 0.27-26.7) in the D+/R+ recipients ($P < 0.0001$).[172] Although prior guidelines mentioned that some experts, given the unpredictable viral kinetics (especially in D+/R–), recommended starting treatment with any detectable DNAemia,[172,173] with increasingly sensitive assays many experts felt that very low results should not always result in initiation of treatment, even in D + R– recipients (see Diagnostics).

Advantages of preemptive therapy include a reduced rate of late CMV, selective drug use, and decreased drug cost and toxicities. Preemptive therapy can be difficult to coordinate, given the logistics of weekly testing, reviewing results, initiating therapy rapidly after positive assays, and performing subsequent monitoring and management. Preferably, 1 assay and specimen type, (whole blood or plasma) should be used for an individual patient to ensure comparability of results. Preemptive therapy may not prevent the indirect effects of CMV infection, including effects on graft and patient survival; studies have demonstrated conflicting data.[174-177] Second episodes of replication are observed in about 30% of those treated for CMV DNAemia,[178] so the specter of late CMV remains. Patients managed with the preemptive approach should receive oral acyclovir (or similar) for the prevention of disseminated herpes simplex infections.[179]

Universal Prophylaxis Versus Preemptive Therapy

Four randomized trials directly compared universal prophylaxis with preemptive therapy in renal transplant recipients.[174-177] In studies using weekly monitoring for 4 months posttransplant with reported high compliance rates, no difference in the incidence of CMV disease or intragraft CMV infection was found compared to universal prophylaxis.[174,175,180] Comparable long-term graft survival was observed with preemptive therapy with a similar incidence of other CMV indirect effects[181]; PET was better when compared with valacyclovir prophylaxis.[182] It should be noted that less frequent screening (ie, less than weekly) resulted in higher rates of CMV disease and inferior long-term graft survival compared to prophylaxis.[176,177]

There is no randomized study directly comparing preemptive approach and prophylaxis in liver transplantation. Several meta-analyses with significant numbers of liver transplant recipients have confirmed similar efficacy in prevention of CMV disease and no difference in mortality, graft loss, and acute rejection. As expected, CMV DNAemia is more common with preemptive therapy, whereas late-onset CMV DNAemia or disease and neutropenia is more common with prophylaxis.[183-185] Other herpes viral infections are more common with the preemptive strategy.[185] A large prospective national cohort study found an increased risk of graft loss in patients managed by preemptive strategy, with notably short follow-up (~1 year).[186] In contrast, the 4-year pooled analysis of patients included in 2 randomized studies did not identify the mode of CMV prevention (preemptive vs valacyclovir prophylaxis vs VGCV prophylaxis) as an independent risk factor of graft loss. Cytomegalovirus DNAemia with viral load of more than ~2000 IU/mL in whole blood was a strong predictor of graft loss.[187]

Comparison of approaches among D+/R– patients is limited by a low proportion of the D+/R– group in randomized trials.[174-176] Nevertheless, the results seemed to be comparable even in higher risk patients. Both a subanalysis of a cohort study[186] and meta-analysis[185] showed a similar incidence of CMV disease in D+/R– group. Repeated courses of preemptive therapy are sometimes required in high-risk patients.[172] In summary, using a preemptive approach with once weekly CMV surveillance for 3 to 4 months and universal prophylaxis are both comparable methods of CMV disease prevention for D+/R– and/or R+ kidney and liver transplant recipients.

The preemptive approach is not well studied in nonrenal and nonliver transplant recipients, thus we suggest universal prophylaxis in D+/R– heart and lung transplant recipients, given the high rates of CMV disease and CMV indirect effects.[188,189] There are no studies available to prove efficacy of preemptive approach in R+ lung transplant thus universal prophylaxis is preferred in the majority of lung transplant centers.[190]

Surveillance After Prophylaxis

For prompt detection of evolving CMV infection (and to decrease the risk of late CMV), the majority of the experts utilize surveillance after prophylaxis at least sometimes. Thus far, research does not support the use of a surveillance after prophylaxis approach,[191-194] although studies are limited by short monitoring periods, long interassay intervals, and other methodological issues. Use of surveillance after prophylaxis may be considered in patients at increased risk for postprophylaxis CMV disease. Based on the experience with preemptive therapy, the value is probably greatest if done

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

weekly for 8 to 12 weeks after the end of prophylaxis (weak, low).

Thresholds for Triggering Preemptive Therapy (or Surveillance After Prophylaxis)

Specific viral load thresholds for triggering therapy in asymptomatic patients have not yet been defined, in part because of the significant interassay and interinstitutional variations seen with CMV DNA testing, along with the variable impact of immunity (primary versus reactivation infection, intensity of immunosuppression, impact of various immunosuppressive agents used for induction and maintenance). Although low viral loads are likely to be more clinically significant in those at higher risk for infection, using too low of a viral load threshold can result in unnecessary treatment. Programs may wish to define their own local thresholds based on their assay, specimen type, and patient risk factors. Table 5 highlights recent thresholds used in various research studies.

Some groups have suggested that the kinetics of the CMV DNA doubling time may be a valuable diagnostic parameter for PET, given that real-time quantitative reverse transcription PCR (qRT-PCR) assays show linearity above their limit of quantification, and may allow for direct comparison of results obtained across centers using similar or different qRT-PCR assay.[199-201] Viral replication kinetics have shown that viral load in the first surveillance sample and the rate of increase defines the risk of subsequent CMV disease; this risk is different in CMV-naïve patients undergoing primary CMV infection versus those with prior immunity.[30,202] This approach requires more frequent testing (at least biweekly).

Optimal Duration of Prophylaxis

Seropositive recipients generally need shorter courses of prophylaxis compared with D+/R–. Risk factors for late-onset disease include D+/R– serostatus, shorter courses of prophylaxis, higher levels of immunosuppression, and allograft rejection.[168,169,203] In the Improved Protection Against Cytomegalovirus in Transplantation study, a decreased risk of CMV disease was seen in patients given 200 days of prophylaxis (21.3%) in D+/R– kidney recipients compared with those given 100 days of prophylaxis (36.8%).[169] Extending CMV prophylaxis from 6 to 12 months in pediatric kidney-transplant patients, however, did not prevent CMV infection or disease.[204] Long courses of prophylaxis with VGCV are associated with higher rates of leukopenia and greater cost.

Longer prophylaxis may be warranted in D+R– lung recipients. In a study of 136 lung transplant recipients (including both D+/R– and R+), VGCV prophylaxis for 12 months versus 3 months was associated with a significantly lower CMV infection and disease incidence.[205] In a study of 6 months of prophylaxis, almost 50% of D+/R– lung transplant patients developed late-onset CMV infection or disease.[203] Many programs have moved towards 6 to 12 months of prophylaxis.[188,203,205-207] Serostatus of both donor and recipient at the time of transplant may help guide the duration of prophylaxis; after lung transplant and 6 months of prophylaxis, 34% of D+/R+ and only 6% of D–/R+ developed infection or disease.[203] Some programs continue prophylaxis indefinitely after lung transplant, although there is insufficient data to support this approach.[208,209]

Valacyclovir

High-dose valacyclovir is effective for prevention of CMV disease and CMV DNAemia in both D+/R– and D±/R+ renal transplant recipients.[167,210] Apart from comparable efficacy to oral GCV prophylaxis,[211,212] a recent randomized study showed similar rates of CMV DNAemia compared with VGCV prophylaxis.[213] A lower incidence of acute rejection and higher rate of polyomavirus viremia was observed with VGCV.[213,214] Disadvantages of valacyclovir include high pill burden and neuropsychiatric side effects, which may be decreased if valacyclovir initiation is postponed in patients with

TABLE 5.

Thresholds used in various research publications (published since last guidelines)

Population	Threshold	Comments	Reference
High risk D+R–			
39 D+R– SOT (23 kidney, 15 liver, 1 heart)	1500 IU/mL in plasma	No episodes of symptomatic CMV disease were diagnosed in patients with viral loads below 1500 IU/mL. Very high rate of infection (36/39).	195
Mixed risk D+R– and R+			
689 kidney (n = 368) and liver (n = 321), 11% D+R–, 71% R+	3000 copies/mL in whole blood, twice a week (same group later converted this to 2520 IU/mL,[196] see below)	More of a study of preemptive therapy and effect of immunity than analysis of threshold	172
3/45 D+R– 42/45 D+R+ SOTR	2275 IU/mL (2500 copies/mL) in plasma	This threshold allowed for discrimination between self-clearing infections and those requiring therapy. Focus of study on use of CMV DNAemia vs antigenemia.	197
59 Kidney, liver, HSCT patients (minority were higher risk)	2520 IU/mL (3000 copies/mL) of whole blood	Whether antiviral treatment needed for PET @ 2520 IU/mL (yes); not an analysis of best threshold, but whether 2520 IU/mL is an effective threshold (yes).	196
Lower risk R+			
252 R+ SOTR	3983 IU/mL threshold resulted in 99.6% NPV, "the great majority of patients at lower risk will not develop CMV disease without specific antiviral therapy"	Analysis of best threshold; single center and only seropositive recipients	198

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

delayed graft function. Advantages of valacyclovir include less myelotoxicity and lower cost.[210,213,215]

New Drugs for CMV Prevention

New potent oral drugs would be useful additions to the currently available therapies for CMV prevention. Three compounds are in various stages of late development. Letermovir inhibits the viral terminase enzyme complex encoded by UL56, does not share cross-resistance with GCV,[216] and has both an oral and intravenous formulations. Letermovir has demonstrated efficacy and safely in HSCT patients[217,218] and is commercially available for use as prophylaxis in HSCT recipients. A phase 3 trial for letermovir prophylaxis in kidney transplant recipients is just beginning. Maribavir is an oral drug that inhibits CMV UL97 and plays a role in inhibition of viral maturation and egress. It also appears to have a good safety profile with no evidence of myelosuppression or nephrotoxicity. Early studies for use in prophylaxis after SOT failed to demonstrate efficacy[219]; ongoing studies are evaluating higher doses for treatment, either for DNAemia or for patients with refractory/resistant CMV. Neither letermovir nor maribavir covers HSV and VZV, so additional antiviral prophylaxis may be needed. Brincidofovir is a lipid conjugated prodrug of cidofovir (CDV) with broad antiviral efficacy, including potent antiherpesvirus activity, through inhibition of viral DNA polymerase. It has not been evaluated for prophylaxis in SOT patients. Although there was preliminary evidence of benefit in HSCT patients,[220] significant gastrointestinal toxicity in the phase III trial led to the discontinuation of a planned prophylaxis trials in kidney transplant recipients. An intravenous formulation is being developed in hopes of reducing toxicity.

Prevention Strategies for CMV D–/R–

When both donor and recipient are seronegative for CMV, there is minimal risk of CMV infection, and routine prevention of CMV is not recommended. Antiviral prophylaxis against other herpes infections (especially disseminated varicella and herpes simplex) with acyclovir, famciclovir, or valacyclovir should be considered.

Prevention During (Massive) Blood Transfusion

To avoid transfusion-transmitted CMV, we recommend the use of leukoreduced or CMV-seronegative blood products (strong, moderate). The highest risk is in D–/R–, although there may be a contribution in R+. Additional clinical benefit of combining these 2 strategies is not available.[221] Extensive transfusion of blood products increases the risk of CMV disease (especially if not CMV screened or leukoreduced), and transplant centers may wish to monitor such recipients with weekly viral load testing or give CMV prophylaxis (weak, very low).

Secondary Prophylaxis and Recurrent Infection

There have been no prospective randomized trials of secondary prophylaxis for prevention of recurrent CMV infection in transplant recipients following resolution of CMV infection and/or disease. Risk factors for recurrent CMV among study populations have not been adequately defined. In particular, donor and recipient serostatus, organ transplanted, choice of immunosuppression, rejection and its treatment, initial prophylactic strategies, the presence of renal insufficiency, severity of disease, and the presence of immune reconstitution have varied in those individuals experiencing recurrent infection.[108,178,222-224] Notably, risk factors for recurrent infection may differ from those associated with primary infection or disease.[223,224] Retrospective analyses conducted in diverse transplant populations have not demonstrated a benefit to secondary prophylaxis.[108,222,223]

Recurrent DNAemia upon completion of treatment and/or preemptive therapy occurs, particularly in high-risk transplant patients,[173,195] although viral load replication may be slower and subsequent peak viral load significantly lower than the initial episode,[172] possibly due to an evolving immune response. Although many transplant centers treat all recurrent episodes of CMV DNAemia, irrespective of a relevant threshold,[173] some episodes of CMV DNAemia may resolve spontaneously.[195]

Prevention During Treatment of Rejection

Prophylaxis may be preferred over preemptive therapy in certain high-risk patients, including those who have received recent antilymphocyte therapy, potent immunosuppression including desensitization or ABO incompatible protocols (including those on rituximab, bortezomib, eculizumab, and plasmapheresis/immunoadsorption), and those with human immunodeficiency virus (HIV) (weak, moderate).[225] Although a retrospective cohort study (2007-2012) found no significant difference in the incidence of CMV infection in patients with or without acute rejection (13% vs 10%, $P = 0.37$) 6 months after kidney transplant, the incidence of tissue-invasive CMV disease (8% vs 3%, $P = 0.04$), particularly gastrointestinal CMV disease, was significantly greater in patients with acute rejection.[226] In a retrospective cohort of 15 848 adult kidney transplant recipients between 2004 and 2010, early-onset CMV disease was identified in 1.2% and delayed-onset CMV disease in 4.0% of the kidney transplant recipients; risk factors for delayed-onset CMV disease included transplant failure or rejection (HR 3.2).[227]

Prevention During Critical Illness

Critical illness (including mechanical ventilation and septic shock) conveys an increased risk of CMV infection in nontransplant patients, with associated increased mortality.[228,229] The duration of acute illness (not severity) plays a role in CMV reactivation.[230] A randomized trial of GCV prophylaxis in CMV-seropositive adults with critical illness due to sepsis or trauma did not support its routine clinical use in that population.[231] Another trial in a critical illness population found that valacyclovir or low-dose VGCV prophylaxis suppressed CMV reactivation, but demonstrated higher mortality in the valacyclovir arm.[232] Transplant clinicians should be aware of the increased risk of CMV infection with critical illness in normal hosts, as there may be a similar effect in transplant recipients; however at this time there is insufficient data to support routine use of antiviral prophylaxis or enhanced monitoring in critically ill transplant recipients.

Lower-dose VGCV

Some centers, in hopes of improving tolerability and reducing costs, employ half the recommended dose of VGCV for prophylaxis (ie, 450 mg daily in patients with normal renal function, sometimes called "mini-dosing."[233] This is based on pharmacokinetic data showing comparable GCV exposure with VGCV 450 mg and oral GCV 3 g daily.[234,235] Published comparisons of low-dose versus standard-dose

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

VGCV are limited to retrospective observational studies in kidney transplant recipients.[236-238] These studies suggest similar CMV outcomes in intermediate risk patients (D+R+); however, data are less clear in D+R– who are at higher risk for breakthrough disease and development of resistance.[236] Whether reduced VGCV exposure was achieved in any of these studies is unclear because compliance with renal dosing protocols cannot be assumed, and immunosuppression may not have been standardized. There are no comparative studies of low versus standard-dose VGCV in nonrenal transplant recipients.

CMV Immune Globulin

Cytomegalovirus immune globulin was first licensed for use in prevention of primary CMV disease in renal transplant recipients.[239,240] Its role in prophylaxis has been limited in the era of antivirals, primarily due to limited effectiveness as a single agent compared to GCV or VGCV, as well as expense and infusion related toxicity (weak, moderate). It may be useful for prevention in combination with antivirals in higher risk (CMV D+/R–) lung or small bowel transplant recipients, based on data in cohorts compared to historical controls (weak, low).[206,241,242] The combination demonstrated reduced incidence of CMV disease, and bronchiolitis obliterans syndrome (chronic rejection), with improved survival in a cohort of lung transplant recipients (weak, low).[243] Use of the combination has been based, in part, on the demonstration of synergy between antibody (serum) and GCV in animal models of lethal CMV infection.[244] Adequately powered, randomized trials measuring the additive benefit have not yet been performed. Cytomegalovirus immune globulin has no role for CMV prophylaxis in lung transplant recipients when given alone. Cytomegalovirus immune globulin has been used in patients for prophylaxis with prolonged neutropenia who are intolerant of GCV. It has also been used in patients with refractory CMV disease and hypogammaglobinemia.[101] In summary, CMV Ig is not generally recommended for use, although there may be specific circumstances, especially in thoracic organs, when used in combination with antivirals, in which some benefit has been demonstrated.

There may be subtle in vitro differences between selected immune globulin products with respect to titers of CMV antibody but clinical differences have never been evaluated or demonstrated. In vitro studies show consistently enhanced functional antibody against CMV compared to regular intravenous immune globulin.[245,246] Monoclonal antibodies are in development and show potential promise for reduction of CMV DNAemia and disease.[247,248]

Role of mTOR Inhibitors

Two systematic reviews and meta-analyses showed that the incidence of CMV infection/disease is lower among patients receiving immunosuppressive regimens containing mTOR inhibitors, suggesting that less prevention may be needed in such patient.[249,250] These findings are consistent in kidney (high, strong),[251,252] and in liver,[253] heart,[254-256] lung,[257-259] and pediatric kidney transplant recipients[260] (all moderate, strong).[261] For example, in a single-center, prospective study, among 288 patients randomized to receive 1 of 3 immunosuppression regimens, the lowest incidence of CMV infection and disease was observed in patients who received either of 2 everolimus-based regimens, with a proportional reduction in CMV of 90% and 75%.[262]

Consensus Statements and Recommendations

- We recommend either universal prophylaxis or preemptive therapy for prevention of CMV disease (Table 6) (strong, high).

TABLE 6.

Recommended approaches for CMV prevention in different organs for adult SOTR

Organ	Serostatus	Risk level	Recommended	Alternate
All	D–/R–	Low	Monitoring for clinical symptoms; consider antiviral prophylaxis against other herpes infections	Preemptive therapy (if higher risk, ie, significant transfusions)
Kidney	D+/R–	High	6 months of GCV/VGCV OR Preemptive therapy	
	R+	Intermediate	3 months of VGCV OR Preemptive therapy	
Liver	D+R–	High	3 -6 months of VGCV (VGCV not FDA approved in liver) OR Preemptive therapy	
	R+	Intermediate	3 months of VGCV (VGCV not FDA approved in liver) OR Preemptive therapy	
Pancreas	D+R–	High	3 -6 months of VGCV	Preemptive therapy
	R+	Intermediate	3 months of VGCV OR Preemptive therapy	
Islet	D+R–	Intermediate	3 months of VGCV	Preemptive therapy
	R+	Intermediate	3 months of VGCV OR Preemptive therapy	
Heart	D+/R–	High	3-6 months of GCV/VGCV	-Preemptive therapy -Some experts add CMV Ig to prophylaxis
	R+	Intermediate	3 months of GCV/VGCV OR Preemptive therapy	
Lung	D+/R–	High	6-12 months of GCV/VGCV -Some experts add CMV Ig to prophylaxis	-Preemptive therapy
	R+	Intermediate	Minimum 6 months of GCV/VGCV	
Intestinal, composite tissue	D+/R–	High	Minimum 6 months GCV/VGCV + – surveillance after prophylaxis	-Preemptive therapy -Some experts add CMV Ig
	R+	High	3-6 months GCV/VGCV + – surveillance after prophylaxis	

When a range is given, the duration of prophylaxis may depend on degree of immunosuppression, including the use of antilymphocyte antibodies for induction.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

- For D+/R–, we recommend the use of either prophylaxis or preemptive therapy after kidney and liver transplant (strong, high). For programs or patients unable to meet the stringent logistic requirements required with a preemptive therapy strategy, prophylaxis is preferred.
- For D+/R–, we suggest the use of prophylaxis over preemptive therapy after heart and lung transplant, based on the available data suggesting better graft survival and clinical outcomes (weak, low). Preemptive therapy has not been well studied in pancreas, islet, intestinal, and vascularized composite allotransplantation (ie, hand, face) such that prophylaxis may be preferable over preemptive therapy until more data are available (weak, very low).
- For seropositive recipients (R+) after kidney or liver transplant, we recommend either strategy (strong, high). Preemptive therapy has not been well studied in some seropositive populations including lung, heart, vascularized composite, pancreas, islet, and intestinal transplant; we suggest prophylaxis may be preferable (weak, low).
- We suggest prophylaxis may be preferred in donor and/or recipient seropositive patients whose risk for CMV may be increased, including those on recent antilymphocyte therapy, potent immunosuppression including desensitization or ABO incompatible protocols (including those on rituximab, bortezomib, eculizumab, and plasmapheresis/immunoadsorption), and those with HIV; a longer duration of prophylaxis (ie, 6 months) may be more effective (weak, moderate).
- The choice of CMV prevention method should be determined by the individual center, based on CMV disease incidence, immunosuppression, logistics of CMV surveillance, and economic aspects.
- We recommend against routinely monitoring patients for CMV DNAemia while they are receiving standard-dose prophylactic therapy based on renal function (Table 7) (strong, moderate).
- Use of surveillance after prophylaxis may be considered in patients considered at increased risk for postprophylaxis CMV disease (weak, low). The value is probably greatest if done weekly for 8 to 12 weeks. Biweekly or monthly monitoring is insufficient for preemptive interventions (low, weak).
- With preemptive therapy, we recommend monitoring at least once weekly for 3 to 4 months after transplant; longer monitoring would be indicated if they are perceived to be at ongoing increased risk for CMV disease (strong, moderate).
- Programs should develop their own thresholds for initiating treatment in preemptive therapy; this value may be different for various populations.
- Once DNAemia is at a positive threshold, for asymptomatic patients, we recommend VGCV (treatment dose) be started [strong, high] and continued until resolution of DNAemia (as per the Diagnostic section), with a minimum of 2 weeks of treatment. After resolution, discontinue antiviral and continue weekly surveillance. Intravenous GCV is a less preferred option unless concerns about absorption exist.
- If symptomatic, see Treatment section of these guidelines.

Prophylaxis Strategy D+/R–: Recommended Durations

- For D+/R– kidney recipients, prophylaxis for 6 months is preferable (strong, high).
- In D+/R– liver, heart, and pancreas recipients, prophylaxis should be 3 months (strong, moderate) to 6 months (strong, low).
- For D+/R– islet recipients, prophylaxis for 3 months is recommended (weak, low).
- Between 6 and 12 months prophylaxis is recommended for D+/R– lung transplant recipients (strong, moderate).
- A minimum of 6 months of prophylaxis is recommended for D+/R– vascularized composite (ie, hand and face) and intestinal transplant recipients (weak, low).

TABLE 7.

Dosage recommendations for ganciclovir and valganciclovir and valacyclovir for adult patients with impaired renal function (using Cockcroft-Gault formula)

Intravenous ganciclovir (adapted from[265])

CrCl, mL/min	Treatment dose	Maintenance/prevention dose
≥70	5.0 mg/kg q12 h	5.0 mg/kg q24 h
50-69	2.5 mg/kg q12 h	2.5 mg/kg q24 h
25-49	2.5 mg/kg q24 h	1.25 mg/kg q24 h
10-24	1.25 mg/kg q24 h	0.625 mg/kg q24 h
<10	1.25 mg/kg 3 times a week after hemodialysis	0.625 mg/kg 3 times a week after hemodialysis

Valganciclovir (adapted from[263,264])

CrCl, mL/min	Treatment dose	Maintenance/prevention dose
≥60	900 mg every 12 h	900 mg once daily
40-59	450 mg every 12 h	450 mg once daily
25-39	450 mg once daily	450 mg every 2 d
10-24	450 mg every 2 d	450 mg twice weekly
<10	200 mg 3 times a week after hemodialysis[a]	100 mg 3 times a week after hemodialysis[a]

Valacyclovir (high dose)[167]

CrCr, mL/min	Prevention dose (kidney only)
>75	2000 mg 4 times per day
51-75	1500 mg 4 times per day
26-50	1500 mg 3 times per day
10-25	1500 mg twice daily
<10 or dialysis	1500 mg once daily

[a] Oral solution must be used in this instance (as VGCV tablets cannot be split).

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

- When a range is given, the duration of prophylaxis may depend on degree of immunosuppression, including the use of antilymphocyte antibodies for induction.

Prophylaxis Strategy R+: Recommended Durations

- When a prophylaxis strategy is used for prevention in R+ patients (with either D+ or D–), a majority of the experts felt that 3 months of antiviral medication should be used for routine kidney, pancreas, liver, and heart transplant recipients (strong, high/moderate) and islet (weak, low).
- For those receiving more potent immunosuppression (antilymphocyte antibody therapy, desensitization protocols) or vascularized composite and intestinal transplant recipients, between 3 and 6 months of prophylaxis can be used (weak, low).
- In R+ lung transplant recipients, a minimum of 6 months prophylaxis is recommended (strong, moderate).

Additional Consensus Recommendations in Prevention

- In CMV D–/R–, antiviral prophylaxis against other herpes infections (varicella and herpes simplex) with acyclovir, famciclovir, or valacyclovir should be considered (strong, high).
- To avoid transfusion-transmitted CMV, we recommend the use of leukoreduced or CMV-seronegative blood products (strong, moderate) especially in the highest risk group, D–/R–.
- Given the potential toxicity and cost, we do not recommend the routine use of secondary prophylaxis after treatment of CMV infection or disease (low, weak). We would consider either secondary prophylaxis or preemptive therapy in certain higher risk situations, that is, potent immunosuppression, augmented risk of complications from recurrent CMV, or inability to monitor closely due to extenuating circumstances (weak, low).
- Local practices should guide management of recurrent episodes of DNAemia or utilization of relevant thresholds to initiate therapy given that there is insufficient evidence for an optimal or cutoff or threshold (weak, low).
- We recommend that treatment of rejection with antilymphocyte antibodies in at-risk recipients should result in reinitiation of prophylaxis or preemptive therapy for 1 to 3 months (weak, moderate)[266-268]; a similar strategy may be considered during treatment of rejection with high dose steroids or plasmapharesis (weak, very low).
- CMV Ig is not generally recommended for use, although there may be specific circumstances, especially in thoracic organs, when used in combination with antivirals, some benefit has been demonstrated (weak, low).
- We do not recommend the routine use of low-dose VGCV (weak, low).
- CMV seropositive recipients receiving mTOR inhibitors have a significantly lower incidence of CMV infection/disease. We suggest the use of mTOR inhibitors as a potential approach to decrease CMV infection and disease in CMV seropositive kidney transplant recipients (high/strong) and in liver, heart, and lung transplant recipients (moderate/strong). Cytomegalovirus risk is only one of the factors to consider when deciding on the optimal immunosuppression regimen. The impact of mTOR inhibitors on CMV in D+R– recipients is less clear.

Future Directions

- Despite many centers successfully deploying preemptive therapy strategies, no clear universal thresholds have been defined. In the absence of natural history studies, these may be difficult to define in the current era.
- Future studies should assess potential ways to improve the efficacy of the surveillance after prophylaxis strategy such as more stringent monitoring, optimal thresholds for initiating antiviral therapy, use of viral kinetics, and the adjunctive use of immunodiagnostic assays.
- Studies investigating the incidence of opportunistic infections, including CMV, after treatment of acute rejection are needed. While the majority of early acute rejection episodes occur during the period of CMV prevention, a growing number of patients diagnosed with late acute rejection (cellular, antibody mediated or mixed rejection) treated with various effective immunosuppressive strategies (thymoglobulin, rituximab, plasmapheresis, bortezomib, eculizumab) requires reevaluation of CMV prevention strategies.
- We await more data on the use of novel antiviral agents for CMV prevention, including maribavir, letermovir, and brincidofovir.
- Pathogen inactivation techniques may further reduce the risk of transfusion-related CMV transmission.
- More data are needed in nonkidney solid organ transplant and in high risk (D+/R–) recipients, both on the incidence of primary and reactivation CMV episodes and on the efficacy and safety of conversion to mTOR inhibitors after the first episode of CMV infection and disease. A large independent confirmatory trial is underway in kidney transplant recipients (TRANSFORM trial).

CMV TREATMENT

Initial Treatment

Oral VGCV and intravenous GCV treatment are associated with similar long-term outcomes in SOTRs with CMV syndrome and tissue-invasive CMV disease based on the VICTOR study conducted in adult renal, liver, heart, and lung transplant recipients.[178] Although both may be used for non–life-threatening CMV disease, VGCV is preferred when feasible due to its oral formulation which may prevent or reduce hospital stays and minimize the infectious and vascular complications associated with intravenous therapy. However, intravenous GCV is preferred as initial treatment of life-threatening CMV disease when optimal drug exposure is essential. Furthermore, there are limited pharmacokinetic data to confirm adequate VGCV bioavailability in patients with gastrointestinal CMV disease.

Management

Appropriate antiviral dosing is essential in the management of CMV disease (Table 7). Suboptimal dosing may increase the risk for clinical treatment failure and the development of resistance,[269] whereas supratherapeutic doses may increase toxicity.[270] Renal function should be assessed with regular monitoring of serum creatinine. Although the PV16000[166] and VICTOR[271] trials used the Cockcroft-Gault formula for (val)ganciclovir dosing, as does the package insert, other methods to estimate renal function are more commonly used clinically (such as the Modified Diet in Renal Disease formula and Chronic Kidney Disease Epidemiology Collaboration), and clinicians should be cognizant of this difference. Antiviral dose adjustment based on a population pharmacokinetics Bayesian prediction model may further optimize (val)ganciclovir exposure.[272] In addition, complete blood counts should also be monitored in regular intervals to assess for hematologic toxicity (eg, leukopenia).

The intensity of immunosuppressive therapy can impact the treatment outcomes associated with CMV disease.[273,274]

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Multiple studies include a reduction in immunosuppression as a component of therapy. In addition, double (versus triple) immunosuppressive therapy and lower blood concentrations of calcineurin inhibitors are significantly associated with eradication of CMV DNA at 21 days.[273]

Treatment Duration

CMV viral load should be monitored at weekly intervals to guide the duration of therapy. Although whole blood is more sensitive than plasma in detecting residual DNAemia, it is not a better predictor of recurrent CMV disease.[38] Failure to eradicate plasma DNAemia at the end of treatment is the major significant predictor of virologic recurrence.[178] Suppression of plasma CMV DNAemia measured with an assay calibrated to the WHO predicts clinical response to (val)ganciclovir.[56] Reduction in antiviral dosing in the setting of persistent CMV DNAemia at day 21 is associated with a significant risk of GCV resistance by day 49.[275] Therefore, treatment doses of (val)ganciclovir are recommended until eradication of CMV DNAemia below a specific threshold and resolution of all clinical signs of CMV disease. Eradication of CMV DNAemia is defined as below LLOQ on 1 highly sensitive assay (LLOQ < 200 IU/mL; see Diagnostic section), or lack of detection on 2 consecutive less sensitive assays. With use of highly sensitive assays, a completely undetectable viral load may not always be achievable. DNAemia may not accurately reflect the clinical disease status in all situations. Therefore, longer courses of treatment may be needed in the treatment of tissue-invasive gastrointestinal disease, pneumonitis in lung transplant recipients, and central nervous system or retinal disease. Secondary prophylaxis, defined as continuing prophylactic doses after discontinuing treatment dosing, is not associated with fewer relapses after suppression of CMV DNA.[60,108,222,275a]

Consensus Statements and Recommendations

- For initial and recurrent episodes of CMV disease, VGCV (900 mg every 12 hours) or intravenous GCV (5 mg/kg every 12 hours) are recommended as first-line treatment in adults with normal kidney function (strong, moderate).
- Valganciclovir is recommended in patients with mild to moderate CMV disease who can tolerate and adhere to oral medication (strong, moderate).
- Intravenous GCV is recommended in life-threatening and severe disease (strong, low).
- Oral GCV, acyclovir or valacyclovir are not recommended for the treatment of CMV disease (strong, moderate).
- Adjunctive immunoglobulin therapy is not routinely recommended (strong, low).
- In patients without concomitant rejection, reduction of immunosuppression is suggested in the following settings: severe CMV disease, inadequate clinical response, high viral loads, and cytopenia (weak, very low).
- During the treatment phase, weekly plasma CMV DNA testing is recommended using an assay calibrated to the WHO standard to monitor response (strong, high).
- During the treatment phase, frequent monitoring of renal function is recommended to guide dosage adjustments (strong, moderate).
- Antiviral dosage reductions are only recommended during the treatment phase to adjust for worsening renal function as suboptimal dosing may be associated with increased risk of clinical failure and/or resistance (strong, low).
- In the setting of leukopenia, changing (val)ganciclovir to another agent is not recommended before the addition of granulocyte colony stimulating factor and/or discontinuation of other myelosuppressive therapies (strong, low).
- In patients who are intolerant to (val)ganciclovir during the treatment phase, foscarnet (FOS) is the recommended second-line agent (strong, very low).
- After clinical response, intravenous GCV may be transitioned to VGCV in patients who are able to tolerate oral therapy (strong, moderate).
- Due to individual variation in virologic response, change in antiviral agent is not recommended in clinically improving patients with unchanged or rising DNAemia during the first few weeks of therapy (strong, moderate).
- Drug resistance should be suspected in patients with a prior cumulative (val)ganciclovir exposure that exceeds 6 weeks and clinical treatment failure despite at least 2 weeks of antiviral treatment or development of CMV DNAemia during prophylaxis (see Resistance section)(strong, moderate).
- Antiviral treatment dosing should be continued for a minimum of 2 weeks, until clinical resolution of disease and eradication of CMV DNAemia below a specific threshold (LLOQ < 200 IU/mL) on 1 or 2 consecutive weekly samples (strong, moderate).
- Secondary prophylaxis is not routinely recommended (low, weak).

Future Directions

Prospective studies in the following areas are needed to evaluate novel strategies to optimize the treatment of CMV disease:

- The application of CMV immune assays to guide duration of treatment.
- The role for measuring and repleting immunoglobulins.
- The role for switching to an mTOR inhibitor.
- The role of therapeutic drug monitoring of GCV.

ANTIVIRAL DRUG RESISTANCE

Drug resistance is defined as a viral genetic alteration that decreases susceptibility to 1 or more antiviral drugs. It may manifest as a persistent or increasing viral load or symptomatic disease after a normally effective dosage and duration of antiviral therapy. These clinical features do not in themselves imply that drug resistance is present, but are used in the context of relevant host factors to assess the need for diagnostic testing and alternative therapy.

Risk Factors, Frequency, and Clinical Consequences

Risk factors for drug resistance include prolonged antiviral drug exposure (median, 5 months for GCV) and ongoing active viral replication due to factors such as the lack of prior CMV immunity (D+/R–), strongly immunosuppressive therapy, or inadequate antiviral drug delivery.[276,277] Among solid organ recipients the usual incidence of resistance after GCV therapy is 5% to 12%,[278-280] but up to 18% in lung,[275,281] and 31% in intestinal and multivisceral organ transplant recipients.[282,283] The incidence of resistance is lower, in the 0% to 3% range, for 100 to 200 days of GCV or VGCV prophylaxis in D+/R– kidney recipients.[284] A higher incidence of GCV resistance has sometimes been reported with preemptive therapy as compared with prophylaxis where no viral load is expected at baseline.[171,279] The incidence of FOS and CDV resistance in transplant recipients

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

is not well defined, but expected to be comparable to GCV based on early studies in the AIDS setting.[285] The relative incidence of resistance to currently experimental drugs, such as maribavir and letermovir, is unclear from the few case reports so far.[216,286,287] Drug-resistant CMV infection may range from asymptomatic and resolving without treatment change (eg, during antiviral prophylaxis[284]) to severe or fatal end-organ disease.[288] The development of drug resistance is correlated with increased morbidity and mortality.[277,289,290]

Diagnosis of Drug Resistance

When to Test

Antiviral drug resistance should be suspected and tested for when there is persistent or recurrent CMV DNAemia or disease during prolonged antiviral therapy. For GCV, prolonged therapy generally means 6 or more weeks of cumulative drug exposure, including longer than 2 weeks of ongoing full dose therapy at the time of evaluation.[277,278] With adverse host factors and/or high starting viral loads, drug resistance can occasionally be detected after less than 6 weeks of drug exposure, as reported in several lung recipients.[277] In a large GCV and VGCV treatment trial,[54] the viral load declined with a median half-life of ~11 days after an initial lag of ~6 days before any reduction was noted. The median time to viral load below 200 copies/mL was 21 days; longer with starting viral loads of more than 50 000 copies/mL. Thus, persistent viral loads in the first 2 weeks of treatment are not predictive of emerging drug resistance,[173,275] but failure to achieve significant viral load reduction beyond this period may reasonably be interpreted as an unsatisfactory response. The kinetics of viral load response and risk for early emergence of resistance may differ with newer antiviral drugs.

How to Test

Testing of CMV culture isolates for antiviral susceptibility by reduction of plaque counts under increasing drug concentrations[291] is technically impractical because of poor assay standardization, slow turnaround and decreasing availability of culture isolates. Instead, genotypic assays for viral drug resistance mutations are performed on viral sequences directly amplified from blood (whole blood, plasma or leukocytes), fluids (urine, cerebrospinal, lung, eye), or tissue specimens. The same blood specimens used for CMV QNAT are usually tested by amplification and sequencing of viral DNA. Results are more reliable if the CMV copy number in the specimen is at least 1000 IU/mL.[292] Quality control concerns include false positive detection of mutations, especially as mixed populations from low viral load specimens,[293] and false negatives due to insensitivity in detecting mutant subpopulations comprising less than 20% to 30% of the total.[292,294] Evolving deep sequencing technologies offer the potential of detecting far smaller mutant subpopulations.[292,294-296] These early reports have not yet established properly calibrated, adequately sensitive, reproducible and stable technical platform to serve as a base for clinical validation, including comparison with existing technology. There are reports of discordant findings of resistance mutations at different body sites (eye, cerebrospinal fluid).[287,297,298] Indications of progressive disease at tissue sites despite negative findings in blood specimens may warrant the genotypic testing of tissue-specific specimens.

Gene Regions to Test

These will evolve as new mutations are better characterized and drug targets are developed. There is an increasing database of CMV mutations associated with drug resistance.[299-301]

In patients initially treated with (val)ganciclovir, UL97 kinase gene mutations appear first in about 90% of cases, affecting drug phosphorylation that is necessary for antiviral action.[276-278,281] UL54 DNA polymerase gene mutations usually evolve later, conferring increased GCV resistance and likely cross-resistance to CDV and/or FOS, but may uncommonly be the first mutation detected. Given increasing availability, genotypic testing after GCV exposure should now routinely include both the UL97 and UL54 genes. Ideally, testing should cover the entire UL97 and UL54 coding sequence, but many diagnostic laboratories test for more limited codon ranges, such as 450 to 650 for UL97, and 300 to 1000 for UL54, which cover the most common mutations but omit some unusual loci where resistance mutations have been reported (Figure 1, Table 8).

Interpretation of Genotypic Data

Test results are reported by diagnostic laboratories as a list of mutations detected (sometimes as a mixture of mutant and wild type sequences) and usually an indication of whether the detected mutation confers resistance to particular drug(s).[303] There is lack of standardization as to the level of drug resistance conferred by the listed mutations, and whether reports include sequence variants that are not in a list of resistance mutations maintained by the laboratory.

Most UL97 mutations conferring GCV resistance are strongly clustered at codons 460, 520, or 590 to 607, although atypical loci exist.[276,299,301] The 7 most common ("canonical") mutations (Table 8) account for over 80% of cases. Other UL97 mutations may confer varying degrees of GCV resistance (Table 8). UL97 mutations do not affect FOS or CDV susceptibility. UL54 drug resistance mutations tend to occur in the conserved functional domains and usually confer cross-resistance to other drugs (Figure 1), such as the GCV-CDV dual resistance of exonuclease domain mutations and the low-grade GCV-FOS cross-resistance of DNA polymerization (palm and finger) domain mutations. A few single UL54 mutations can confer resistance to all 3 drugs.[276]

Individual mutations are matched to their associated levels of drug resistance by recombinant phenotyping, a research technique whereby a mutation is transferred by recombination into a baseline laboratory strain (marker transfer) and the resulting virus is tested against antiviral drugs (phenotyping) by a standardized assay calibrated to baseline and drug-resistant control strains.[276] The level of resistance is reported as the change in the drug concentration that reduces viral growth by 50% (EC50). For GCV, EC50 increases of twofold to fivefold may be considered low-grade, fivefold to 15-fold considered moderate (a level that may result from a single UL97 mutation), and greater than 15-fold considered a high level that suggests the combined effect of UL97 and UL54 mutations. When both UL97 and UL54 mutations are present, the combined level of GCV resistance may be approximated by multiplying the fold changes in resistance conferred by each mutation alone.[304] Mutations that confer slight decreases in susceptibility may significantly increase the overall level of resistance when combined with other mutations.[305] Foscarnet resistance mutations typically confer

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

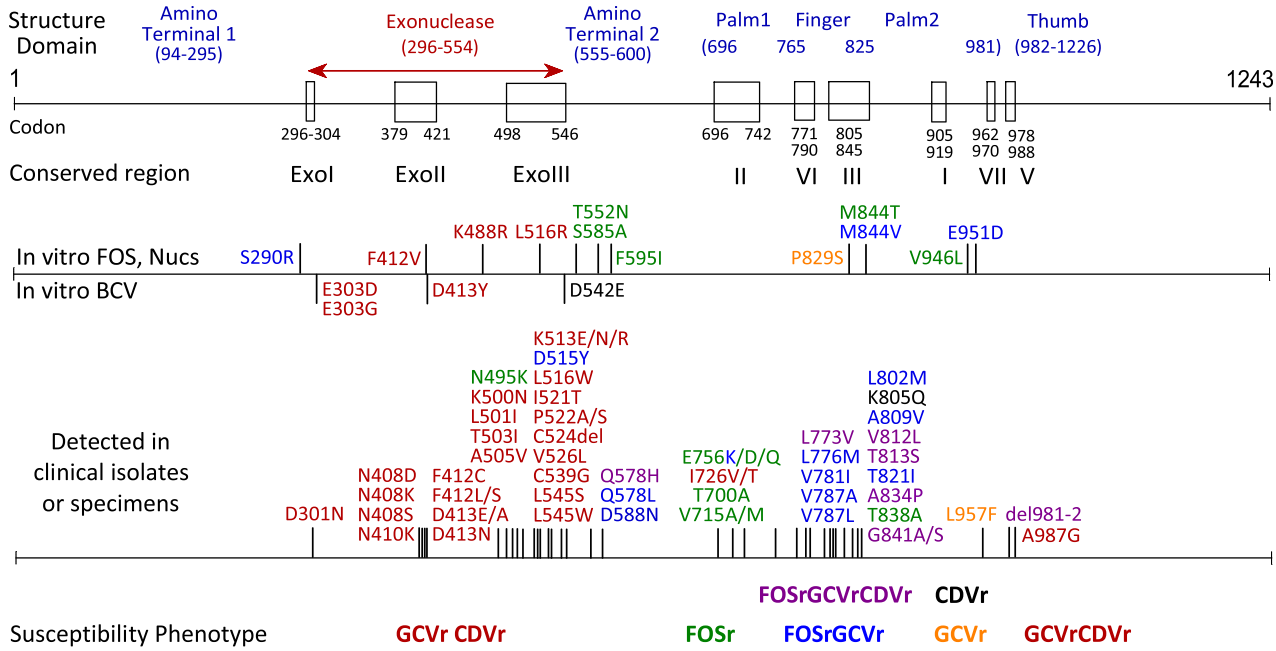

FIGURE 1. CMV UL54 DNA polymerase gene mutation map. Shown are the structure domains and regions of amino acid sequence conservation in herpesvirus polymerases, where resistance mutations are clustered. Corresponding resistance phenotypes are color coded for the involved drugs. Adapted and updated from prior publications.[5,276] BCV, brincidofovir; Nucs, various nucleoside analogs.

twofold to fivefold increases in EC50 and frequently attenuate viral growth.[276] Some common UL54 exonuclease domain mutations confer 10- to 20-fold increases in CDV EC50.[276]

Uncharacterized sequence variants without a documented phenotype cannot be presumed to be resistance-related without careful analysis of such factors as sequencing quality control, evolution in serial specimens, treatment history, proximity to known gene mutations, and corroboration by recombinant phenotyping.[293]

Alternate Therapy for Drug-resistant CMV

No controlled trial data define a best practice for selection of alternate therapy when suspected or confirmed drug resistance is present based on clinical risk factors or genotypic testing. An updated algorithm (Figure 2) is based on consensus expert opinion but its application to individual cases must take into account host factors that strongly influence outcomes and the urgency of treatment changes. Depending on the severity of the CMV disease (whether life or sight threatening) and host risk factors (D+/R–, severe immunosuppression), empiric changes in therapy can be made when drug resistance is suspected, pending return of genotypic resistance data. Only a fraction of cases with clinical suspicion of drug resistance will be genotypically confirmed.[278] If laboratory testing returns no evidence supporting drug resistance, emphasis should be given to optimization of host factors and drug delivery rather than switching antiviral medications. Therapeutic drug monitoring may be helpful in adjusting doses to maintain effective drug levels in relation to viral inhibitory concentrations and minimize toxicity,[306] although timely availability of antiviral drug assays is limited. Subtherapeutic GCV levels may increase the selection of resistant mutants and risk of treatment failure.[307-309] Additional genotypic testing over time, including specimens from diseased body sites or use of validated deep sequencing technology when available, may increase the sensitivity of detection of

TABLE 8.

GCV resistance levels associated with selected UL97 genotypes

Genotype frequency	Fold change in GCV EC50[a]		
	5-15×	2-5×	<2×
Most common	M460V/I, H520Q, A594V, L595S, C603W	C592G	
Less common at codons 460, 590-607	M460T, A594G, 595del[b], L595F/W, E596Y, 597del2[b], 599del, K599T, 600del, 601del, 601del2, C603R, C607Y, del(≥3)[c]	A591V, A594E/T, E596G, C603S, 596del[b], 600del2, C607F	E596D, N597D, K599E/R, L600I, T601M, D605E[d]
Atypical loci	F342S[e], K355M[e],V356G[e], V466G[e], C480R[e], C518Y, P521L[e]	L405P, I610T, A613V	M615V, Y617H, A619V, L634Q, E655K, A674T

[a] Moderate resistance (5-15×), low-grade resistance (2-5×), or insignificant resistance (<2×).
[b] del = in frame deletion of codon.
[c] In frame deletion of ≥3 codons in the 590-607 range can be assumed to confer moderate GCV resistance (eightfold to 15-fold). Deletion of less than 3 codons may confer varying degrees of GCV resistance (fourfold to 10-fold).[302]
[d] D605E is a baseline sequence polymorphism common in east Asia, unrelated to drug resistance.
[e] Maribavir cross-resistance documented; all except F342S are markedly growth-inhibited.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

resistance mutations. Immunosuppressive therapy should be reduced to the lowest feasible amount, and adjunctive measures described in the next section can be considered.

Some UL97 mutations confer lower levels of GCV resistance by themselves (Tables 8 and 2[72]) and may be amenable to GCV dose escalation (up to 10 mg/kg every 12 hours) combined with optimization of host factors, if severe disease is not present.[310] This is double the standard dose and needs monitoring for bone marrow suppression and dose adjustment for renal function.

Switching to FOS is recommended if a mutation confers higher level GCV resistance, or UL97 and UL54 mutations combine to confer high level GCV resistance and usually CDV cross-resistance. Foscarnet salvage therapy is often successful at least initially,[279,290] but metabolic and renal toxicities may impair eventual treatment outcomes.[311] There is insufficient information on the efficacy of CDV as salvage therapy in SOT.[312,313] Nephrotoxicity is dose-limiting. Cidofovir can be considered when GCV and FOS dual resistance is detected without CDV resistance, but the rapid development of viral load relapse and new CDV resistance mutations has been repeatedly documented in case reports,[287,294,314] probably related to undetected subpopulations of cross-resistant mutants selected during prior GCV therapy.[294] High-dose GCV may also be an option for situations where FOS resistance is present without associated high-level GCV resistance.

Adjunctive Therapy

Adjunctive treatments, defined as those without a specific CMV antiviral drug target, have not been adequately evaluated. Cytomegalovirus Ig (or IVIG) and adaptive infusions of CMV-specific T cells[146,315] may improve antiviral host defenses. Several drugs used for other purposes, including mTOR inhibitors (sirolimus and everolimus), leflunomide, and artesunate, have anti-CMV effects in vitro that may sometimes act synergistically with conventional antivirals.[316,317]

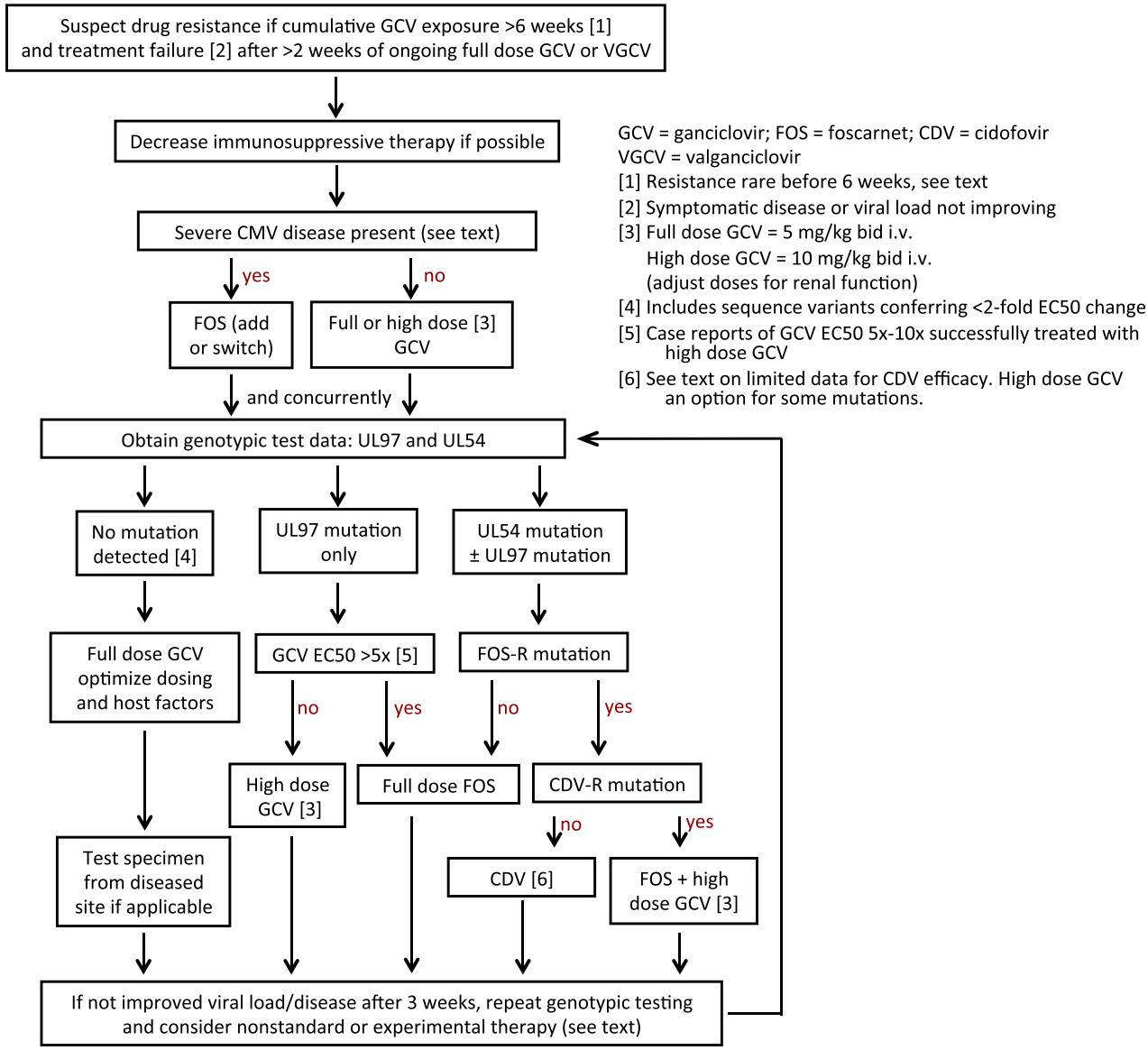

FIGURE 2. Proposed algorithm for management of suspected antiviral drug resistance, based on consensus expert opinion. There are no controlled trials that define clinical outcomes according to genotypic diagnosis and selection of alternative therapy.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

Switching immunosuppressive therapy to an mTOR inhibitor may be reasonable if otherwise tolerated, based on studies showing a lower incidence of CMV infection and disease.[318-320] Leflunomide has been advocated in case reports and small series.[321-324] Evidence for efficacy is limited and caution is advised when used for cases of severe disease or with high viral loads. Monitoring for drug metabolite levels and liver toxicity is recommended. Use of artesunate has been the subject of case reports,[325,326] with mixed outcomes suggesting a similar degree of caution as with leflunomide.

Experimental CMV Antiviral Agents

Brincidofovir (CMX001) is an orally bioavailable derivative of CDV with improved intracellular active drug delivery and in vitro antiviral potency. Although effective in a phase 2 trial as CMV prophylaxis in HSCT recipients,[220] the follow-up phase 3 trial failed primarily because of severe gastrointestinal toxicity,[327] and the drug is not currently available or being developed for CMV treatment. If eventually marketed for some other indication, case reports of successful brincidofovir salvage therapy[328] may prompt further study. Resistance is expected to involve similar UL54 mutations as CDV.[329]

Maribavir is an oral benzimidazole L-riboside inhibitor of the CMV UL97 kinase.[330] After promising early phase clinical trials, phase 3 trials in HSCT and liver transplant recipients demonstrated no prophylactic efficacy of low-dose (100 mg BID) maribavir.[219,331] At higher doses, it has been used as salvage therapy for drug resistant CMV infection, with mixed results.[332,333] A phase 2 trial of maribavir (400, 800, or 1200 mg bid) for salvage treatment of refractory and resistant CMV infection has been completed, with indications of success in that approximately 67% achieved clearance of DNAemia within 6 weeks, but infection recurred in approximately 35% of those who cleared.[334] Salvage efficacy did not vary significantly among the 3 doses tried. Resistance to maribavir involves mutations in the UL97 kinase that confer moderate to high level resistance, and assorted mutations in the gene UL27 that confer low-level resistance.[335] No maribavir resistance was observed in the phase 3 prophylaxis trials,[335] but UL97 mutations T409M and H411Y/N appear to be the most commonly detected after maribavir therapy.[286,287,333] These mutations confer approximately 80-fold and approximately 12-fold increased maribavir EC50 without GCV cross-resistance. Although the UL97 mutations preferentially elicited by GCV or maribavir do not confer cross-resistance, there are atypical UL97 mutations (Table 8), notably the p-loop mutation F342S that confers GCV and maribavir resistance without severely impairing viral growth fitness.[336]

Letermovir is a CMV UL56 terminase inhibitor with high in vitro potency, which showed antiviral efficacy in placebo-controlled phase 2 and phase 3 prophylaxis trials in HSCT recipients.[217,218] A case report suggests that letermovir salvage therapy and reduced immunosuppression successfully cleared a decreasing viral load after FOS therapy was limited by toxicity,[314] but the efficacy of letermovir in treating active CMV infection is unknown. A concern is that high-level resistance to letermovir is readily elicited in vitro with little impact on viral growth fitness, associated with mutations clustered at codons 231 to 261, 325 and 369 of the UL56 gene.[337,338] Mutations at codon 325 confer absolute resistance. No cross-resistance with current antivirals is expected. Letermovir resistance mutation UL56 V236M was encountered once in each of the phase 2 and phase 3 prophylaxis trials,[216,218] along with other mutations[339]; additional incidence data are needed to enable comparison with the current DNA polymerase inhibitors.

Consensus Statements and Recommendations

- Interpretation of genotypic resistance testing is based on moderate- to high-quality evidence (depending on the mutation and number of times tested) where recombinant phenotyping data are available, and low-quality evidence where a phenotype is inferred from properties of mutations other than the one being detected.
- We propose a management algorithm (Figure 2); given the lack of controlled trial data to define a best practice for selection of alternate therapy when suspected or confirmed drug resistance is present, the recommendations should be considered "strong, low" for the initial approach to diagnosis, "moderate, low" for selection of alternate therapy, and "weak, low" for CDV and other nonstandard salvage therapies.

Future Directions

- Prospective studies are needed to define the outcomes of drug-resistant CMV under various management options.
- New therapeutic options are needed, with adequate potency, bioavailability, and lack of toxicity and cross-resistance with current drugs, including consideration of drug combinations directed at different viral targets.
- Genotypic resistance testing needs improved quality control and standardized reporting, including documentation of the level of drug resistance and cross-resistance conferred by various mutations.
- The role of next generation genotyping technology remains to be defined.

PEDIATRIC ISSUES IN CMV MANAGEMENT

Prevention and treatment of CMV infection and disease in pediatric and adolescent SOTRs present several unique issues described here, incorporating and expanding on previous guidelines.[4,5,340]

Burden of CMV Disease in Pediatric SOT

There are limited data on the precise disease burden in pediatric SOTRs. Nonuniform approaches to diagnosis, varying definitions of disease, and inconsistent durations of monitoring hamper data interpretation. Epidemiological studies conducted before the advent of prophylactic or preemptive therapy indicated that as many as 40% of pediatric liver and 15% of pediatric kidney transplant recipients developed CMV disease.[341,342] With various antiviral prevention strategies, CMV disease initially decreased to 10% to 20% in pediatric liver transplant recipients[343] with further declines to 0% to 10% in more recent reports.[344-349] Late-onset CMV disease occurred in 6% of pediatric liver transplant recipients. Declines in CMV disease from 24% to 12% also have been documented in pediatric intestinal transplant recipients after the introduction of antiviral prophylaxis.[350,351]

The incidence of CMV DNAemia after pediatric renal transplantation is approximately 20%, with disease in 1-10%[352–354] and late-onset disease in 14%.[204] CMV was detected in the blood in 29-32% of pediatric lung transplant recipients in the first year, with CMV pneumonitis in 20%. Late-onset CMV data has not been reported after pediatric lung transplantation. DNAemia occurred in 38% of a small

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

cohort of pediatric heart transplant recipients, with 8-9% developing CMV disease in the Pediatric Heart Transplant Study cohort over the first 5 years posttransplant.[355,356]

Primary Risk Factors for the Development of CMV Disease in Children

As with adults, the risk of CMV disease in pediatric transplant recipients varies with donor and recipient serostatus. Interpretation of donor and recipient serostatus for infants less than 12 months of age is confounded by the potential presence of transplacentally-acquired maternal CMV antibodies, and by the fact that CMV shedding in saliva or urine among infected infants is intermittent. It is known that CMV DNAemia may occur before transplantation in infants with perinatal or postnatally acquired CMV infection, but it is not known if the presence of CMV DNAemia before transplantation impacts the risk of CMV disease posttransplantation.

Adult and pediatric patients share similar risk factors for CMV disease after SOT, but there are a number of factors that specifically influence the risk of CMV exposure and infection among children. Children are more often CMV naïve at the time of transplant and are therefore more likely to acquire primary CMV infection post transplantation. CMV D–/R– pediatric SOTRs are also more likely to acquire de novo CMV infection from community exposures, such as daycare attendance. As many as 7% of pediatric CMV D–/R– recipients developed primary CMV infection in the first year after transplantation.[357]

Indirect Effects of CMV in Pediatrics

The nature and definition of the indirect effects of CMV may differ between children and adults. Studies performed in the adult SOT population suggest significant indirect effects including increased risk of fungal and other opportunistic infections, coronary artery vasculopathy and chronic allograft rejection.[358] Evidence does seem to support an association between CMV infection and long-term graft function in pediatric kidney transplant recipients. Early studies in pediatric kidney transplant recipients suggested an association between CMV DNAemia and an increased risk of histological graft rejection.[353,359] One study was potentially confounded by the presence of EBV coinfection in half of the small sample size.[353] A recent multicenter cohort analysis in pediatric renal transplant patients showed a significant association between CMV DNAemia and the decline of graft function at 3 years posttransplant.[354] Multivariate analysis of variance investigating the impact of various factors on eGFR revealed that, besides recipients' age at transplantation, CMV replication and acute rejection episodes were significantly associated with a lower eGFR, whereas the type or overall intensity of immunosuppressive therapy was not. The use of antiviral prophylaxis in CMV D+/R– and D+/R+ patients was accompanied by a higher CMV-free survival and a lower eGFR loss than preemptive therapy.[354]

Studies have not established a clear association between CMV replication and negative outcomes in nonrenal pediatric transplant recipients. In pediatric lung transplant recipients, CMV is associated with increased mortality within the first year of transplantation,[357] but an association with chronic allograft rejection and opportunistic infections has not been demonstrated.[360,361] In heart transplantation, CMV prophylaxis with either CMV Ig or antiviral agents was associated with decreased mortality.[362] Others have reported association between CMV seropositivity and coronary artery vasculopathy,[363] but data from the multicenter Pediatric Heart Transplant Study did not demonstrate this association.[356] In studies of pediatric liver transplant recipients, CMV replication was not associated with immediate transplant outcome, graft loss, death or with other potential indirect CMV effects such as acute cellular rejection, sepsis, EBV infection, biliary strictures and leakages or vascular complications.[344,345,348,364]

In summary, available data suggest a significant negative impact of CMV replication on pediatric renal graft function, but this association has not been established in nonrenal pediatric grafts. The lack of evidence linking CMV to substantial indirect deleterious effects in nonrenal pediatric SOTRs, coupled with the potential hematological toxicities associated with antiviral therapy in children,[354,365,366] provide a less compelling rationale for prolonged antiviral prophylaxis or avoidance of preemptive therapy in most pediatric SOT.

Immunologic Monitoring in Pediatrics

The potential role of monitoring for general or CMV-specific immune reconstitution has been explored only in uncontrolled and small studies in pediatric SOT.[367] Children under 2 years of age with both congenital and postnatal CMV acquisition show decreased CMV-specific CD4 responses,[368] although another study reported CMV-specific responses in this cohort.[21] Accordingly, it is clear that assays assessing CMV cellular immunity must be validated across the spectrum of ages in the pediatric population. Pediatric-specific studies of T-cell responses and their potential role as biomarkers of risk for CMV disease are needed before introducing these assays into clinical practice (see Immunology section).

Prevention of Pediatric CMV Disease

As a first step in the prevention of CMV disease, the use of leukodepleted or CMV-negative blood products is suggested for special populations (eg, bowel, lung, and heart transplants) and in CMV D–/R– patients. Prevention strategies in pediatric SOTRs include antiviral prophylaxis, preemptive therapy, or a sequential approach of brief prophylaxis followed by viral load surveillance. Although no pediatric trial has directly compared the relative efficacies of these 3 strategies, favorable outcomes for each have been reported among pediatric SOTRs. There is broad collective evidence to support the use of antiviral prophylaxis in pediatric recipients, but its use in young children may be limited by bone marrow suppression and limited VGCV pharmacokinetic data. Conversely, preemptive therapy may avoid the toxicities of antiviral exposure but requires intensive viral load surveillance, and the threshold viral load to prompt antiviral therapy is unknown. The rate of CMV disease has been reported to be as low as 5% in relatively small studies of children managed preemptively.[369,370] The sequential approach of a short course prophylaxis followed by viral load surveillance, previously designated the "hybrid approach," limits the duration of prophylaxis to the period of most intense immunosuppression. This approach has been used successfully in pediatric liver and heart transplant recipients, with reported CMV disease rates of 8-10%.[346,355] The reported duration of prophylaxis in this setting typically ranges from 2 to

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

4 weeks; however, the optimum duration of prophylaxis for a sequential approach has not been defined.[346,355,371]

Prophylactic intravenous GCV is usually dosed at 5 mg/kg per day. Some centers start with an initial dose of 10 mg/kg in 2 divided doses based on the rationale that a higher (treatment) dose may reduce viral replication within the graft,[346,371] but no data support superiority of this approach. In contrast to adults, the standard duration of prophylaxis varies significantly between individual centers and among different organs. In addition to reversible bone marrow toxicity, concerns for prolonged exposure to GCV or VGCV in the very young recipient have been raised due to animal toxicity studies demonstrating carcinogenesis or an adverse effect on spermatogenesis.[372] These effects have not been observed in humans, and prolonged IV GCV (12 weeks) has been used safely in pediatric transplant recipients.[373]

Compared with adults, less data are available to define the role of VGCV in pediatric SOTRs. Studies have addressed pharmacokinetics (PK) in older children,[374,375] and emerging data address PK in younger SOT populations, including infants. Current models support body surface area-based dosing to reach targeted GCV AUC as opposed to weight-based dosing.[366,376-379] Data evaluating the efficacy of VGCV for the prevention and treatment of CMV in pediatric SOTRs are needed, particularly given concerns for lower than anticipated plasma (and presumably intracellular) GCV levels and the potential subsequent risk for GCV resistance. Absorption issues might be of particular concern in small-bowel transplant recipients. The efficacy and safety of prolonged VGCV prophylaxis has not been the subject of randomized studies in children.

The choice of immunosuppressive agent choice may impact the risk for CMV disease. Subjects who received everolimus with low-dose cyclosporine in a recent multicenter study in pediatric kidney transplant recipients experienced a decreased risk of both CMV infection and disease compared to standard therapy with cyclosporine or tacrolimus with mycophenolate.[260]

Cytomegalovirus Ig and IVIG are sometimes used in combination with antivirals or preemptive therapy to prevent CMV. Data do not exist to enable a definitive recommendation for or against this practice. Evidence in support of this strategy has been extrapolated from data derived mostly from adult populations; however, some recent pediatric studies have been published with variable results.[369,370,380,381] In adult and pediatric heart transplant recipients, Scientific Registry of Transplant Recipients data showed an improvement in recipient and graft survival for those who received CMV Ig with or without antivirals; however, this improvement was not different from that demonstrated with antivirals alone.[362,382] Krampe et al[369] found a low incidence of CMV disease in 28 pediatric liver transplant recipients receiving IVIG and preemptive therapy but did not have a comparison group. In a retrospective review of 329 pediatric lung transplant recipients, of whom 62 (19%) received CMV Ig in addition to at least 3 weeks of IV GCV, CMV Ig was associated with a decreased risk of CMV infection but did not impact the incidence of CMV disease, acute rejection or early morbidity.[380] A beneficial effect of CMV Ig on CMV infection rates was also suggested by a combined pediatric and adult study of intestinal/multivisceral transplant recipients.[383] In 1 prospective randomized pediatric study that primarily targeted EBV, CMV Ig did not appear to have a significant impact on the development of CMV disease, although there was a trend toward a higher 2-year CMV disease-free rate in R+ children.[343]

Treatment of Pediatric CMV Disease

Many of the principles that guide therapy in children are similar to those among adults. There is a significant lack of published data on which to base firm recommendations for the treatment of CMV disease in children, particularly regarding intravenous versus oral therapy. Valganciclovir has been shown to be effective treatment of asymptomatic CMV DNAemia in the setting of preemptive therapy, providing rationale for consideration of its use in mild to moderate CMV disease.[354]

GCV Resistance in Pediatric Organ Transplantation

Due to the high likelihood of CMV D+/R– status in pediatric SOTRs, GCV resistance is of significant potential concern,[384] although published reports from pediatric cohorts report a low incidence of GCV resistance of only 2% to 4%.[366,385,386] It is unclear if the reported incidence is due to low resistance burden, lack of generated data, or underreporting. The currently available agents for the treatment of GCV-resistant CMV in children are similar to those used in adults.

Consensus Statements and Recommendations

- In general, the principles that guide the use of prophylaxis in adults are similar in children as defined by the organ transplanted and CMV donor and recipient serostatus.
- Significant updates from prior recommendations relate to body surface area-based dosing for younger children and infants and consideration for initial oral VGCV therapy for CMV infection and mild to moderate CMV disease.
- Some experts perform surveillance during prophylaxis due to concern for breakthrough DNAemia. If surveillance during prophylaxis is performed, the frequency of surveillance should take into account the immunosuppressive regimen (including T cell–depleting induction) and the likelihood of nonadherence with the prophylactic regimen. Adherence can be a particular problem with adolescents.
- Surveillance for CMV DNAemia among patients being managed preemptively or with surveillance after prophylaxis regimens should follow adult recommendations of weekly testing and continue for at least 3 to 4 months posttransplant.
- Children with recurrent CMV DNAemia or disease (at least 2 episodes) may benefit from secondary antiviral prophylaxis. The duration of secondary prophylaxis depends on immunosuppression regimen, age, presence of other opportunistic infections, and other risk factors. Conversion to mTOR inhibitor based immunosuppression in pediatric kidney transplant recipients may provide some protection against recurrent CMV.
- Given the challenge of characterizing donor and recipient serostatus in those less than 12 months of age (due to the possible presence of maternal antibodies), we recommend that risk assessment in this age group should assume the highest risk level for purposes of CMV prevention (strong, moderate) (Table 9). It should be noted that the negative predictive value of a culture or NAT is limited by intermittent CMV shedding.
- No pediatric trials have adequately evaluated the comparative efficacy of prophylaxis, preemptive therapy or surveillance after prophylaxis strategies. Retrospective data provide equal support for each these 3 prevention strategies, and as such

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 9.

Assignment of donor/recipient serostatus in infants < 12 months of age

Donor	Recipient	Highest risk categorization
+	+ or –	D+/R–[a]
–	+	D–/R+
–	–	D–/R–

[a]If recipient confirmed positive by CMV culture or NAT, assign D+/R+.

all 3 are recommended (strong, moderate). The decision to pursue specific strategies is dependent on organ type and risk stratification (Table 10).

- Use of the VGCV-dosing algorithm that adjusts for body surface area and renal function using the updated Schwartz formula[387] provides GCV exposures similar to those established as safe and effective in adults and is recommended in infants and children for prophylaxis (strong, moderate) (Table 11). Recent data strongly supports body surface area (BSA)-based dosing algorithm over the prior suggestion of 16 mg/kg dosing for young infants (strong, moderate).
- The risk of DNAemia is greatest during the 2 to 3 months after discontinuation of antiviral agents (for prevention or treatment). Accordingly, we recommend recipients undergo frequent monitoring for CMV DNAemia for at least this time period (strong, moderate).
- For the treatment of asymptomatic DNAemia, we suggest the use of oral VGCV (strong, low). In addition to patient age, antiviral choice should be guided by early clinical assessment for subtle CMV signs/symptoms, adherence, stable creatinine clearance and oral absorption.
- We recommend the initial treatment of severe CMV disease in children with IV GCV at a dose of 5 mg/kg every 12 hours with appropriate adjustments for renal function (strong, moderate). Some experts consider switching to oral therapy towards the end of their treatment courses (strong, low). The differential effectiveness of IV GCV or oral VGCV for the initial treatment of mild to moderate CMV disease in children younger than 12 years has not been established. Treatment decisions regarding delivery method should be individualized based on age, adherence, and other modifying factors (weak, very low).
- In the management of CMV infection and disease, immunosuppression should be reduced where feasible (strong, low).
- CMV Ig therapy is not routinely recommended for CMV disease (weak, low).
- In children at risk for CMV who receive significantly intensified immunosuppression (eg, antilymphocyte therapy, intravenous steroids) for rejection, primary disease recurrence, or other complicating condition, we recommend either prophylaxis with (val)ganciclovir or preemptive therapy (strong, low). There are no data to suggest a specific duration of prophylaxis in these circumstances.

Future Directions

- Reporting of the epidemiology and outcomes including CMV infection and disease rates with current preventative strategies and delineation of the short- and long-term indirect effects of CMV in pediatric transplant recipients is encouraged. Such reporting should be done using uniform criteria to enable comparisons across studies.
- Additional investigation into the use of biomarkers to aid in risk stratification for purposes of prevention and diagnosis should be explored.
- Additional work is required on the utility of immunogenetic biomarkers and adjunctive immunologic monitoring to guide treatment strategies. Such work should consider age-related immune maturity issues that might influence the optimal performance of assays (eg, interferon gamma release assays).
- Investigation into optimal CMV prevention strategies should include appreciation for the impact on different age groups and the potential consequences of antiviral side effects in pediatric-aged patients.

TABLE 10.

Recommended regimens for CMV prevention in children

Organ	Serostatus[a]	Risk level	Recommended	Alternate
All except small bowel	D–/R–	Low[b]	Monitoring for clinical symptoms	Preemptive therapy
Kidney	R+	Intermediate	3-6 mo of VGCV as recommended in adults[c] OR Preemptive therapy	2-4 wk IV/PO with surveillance after prophylaxis[c]
	D+/R–	High	3-6 mo of GCV/VGCV as recommended in adults[c]	
Liver	D–/R+, D+/R+, D+/R–	Intermediate to high	2-4 wk of GCV/VGCV with surveillance after prophylaxis[c] (VGCV not FDA approved in liver) OR 3-4 month of VGCV OR Preemptive therapy[c]	
Heart	R+	Intermediate	2-4 wk GCV/VGCV with surveillance after prophylaxis OR 3 months of GCV/VGCV[c]	
	D+/R–	High	4 wk GCV/VGCV with surveillance after prophylaxis OR3 months of GCV/VGCV[c]	Some experts add CMV Ig
Lung	R+	High	6-12 mo of GCV/VGCV	Shorter courses (3 mo) have been used with surveillance after prophylaxis
	D+/R–	High	6-12 mo of GCV/VGCV	Some experts add CMV Ig
Small bowel[d]	D–/R–	Low	Preemptive therapy OR 2 wk GCV with surveillance after prophylaxis	
	R+	High	2 wks GCV with surveillance after prophylaxis OR3-12 mo GCV/VGCV	
	D+/R–	High	3-12 mo GCV/VGCV	Some experts add CMV Ig

[a]Refer to serostatus recommendation for infants < 12 months of age.
[b]Risk of CMV infection in D–/R– is ~5-7% within 12 months of transplantation.
[c]T cell–depleting induction is associated with increased risk of CMV DNAemia and disease; consider prolonged prophylaxis or more intensive monitoring.
[d]VGCV should be used with extreme caution due to concerns for malabsorption in small bowel transplant recipients.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

TABLE 11.

Calculation of pediatric dosing for VGCV for prevention of CMV disease in kidney or heart transplant patients (4 months to 16 years of age)

Step 1	Calculate BSA	Mosteller BSA (m^2) = $\sqrt{\frac{\text{Height(cm)} \times \text{Weight(kg)}}{3600}}$
Step 2	Calculate CrCl[a]	Schwartz CrCL (mL/min/1.73 m^2) = k × Height (cm) / Serum Creatinine (mg/dL)
Step 3	Calculate the starting dose of VGCV oral solution[b]	7 × BSA × CrCl
		If the calculated Schwartz CrCl exceed 150 mL/min per 1.73 m^2, then a maximum CrCl value of 150 mL/min per 1.73 m^2 should be used in the equation

[a] Where k = 0.413.
[b] Maximum dose is 900 mg per dose.

- Pediatric data should be obtained for emerging antiviral agents to expand the opportunities to prevent and treat CMV.

CONCLUSIONS

We have seen major advances in CMV management in SOT (both adult and pediatric) over the past decade, with improved molecular and immunologic diagnostics, reductions in CMV infection and disease, better understanding of treatment, and enhanced knowledge of treatment of resistant virus. Nonetheless, major gaps in our ability to provide optimal care continue to exist, including viral load thresholds to trigger antiviral therapy in asymptomatic individuals, best use of immunodiagnostics and other methods to allow more personalized approaches to CMV prevention and treatment, ideal durations for prophylaxis in individual patients and how to avoid the specter of late CMV, and determination of best candidates for prophylaxis versus PET. Resistant CMV remains challenging, with among the worst outcomes of all patients afflicted by CMV infection. We look forward to more advances, and to the day when we overcome the "transplantation troll."[1]

ACKNOWLEDGMENTS

The authors thank Danielle Viau of The Transplantation Society for her administrative support.

REFERENCES

1. Griffiths PD, Emery VC. Taming the transplantation troll by targeting terminase. *N Engl J Med*. 2014;370:1844–1846.
2. Stern M, Hirsch H, Cusini A, et al. Cytomegalovirus serology and replication remain associated with solid organ graft rejection and graft loss in the era of prophylactic treatment. *Transplantation*. 2014;98:1013–1018.
3. Kotton CN. CMV: prevention, diagnosis and therapy. *Am J Transplant*. 2013;13(Suppl 3):24–40. quiz 40.
4. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation*. 2010;89:779–795.
5. Kotton CN, Kumar D, Caliendo AM, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation*. 2013;96:333–360.
6. Guyatt GH, Oxman AD, Kunz R, et al. Going from evidence to recommendations. *BMJ*. 2008;336:1049–1051.
7. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ*. 2008;336:924–926.
8. Jaeschke R, Guyatt GH, Dellinger P, et al. Use of GRADE grid to reach decisions on clinical practice guidelines when consensus is elusive. *BMJ*. 2008;337:a744.
9. Guyatt GH, Oxman AD, Kunz R, et al. Incorporating considerations of resources use into grading recommendations. *BMJ*. 2008;336:1170–1173.
10. Schunemann HJ, Oxman AD, Brozek J, et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. *BMJ*. 2008;336:1106–1110.
11. de Man RA, M Balk AH, Jonkman FA, et al. Patient to patient hepatitis B transmission during heart biopsy procedures. A report of the European Working Party on Viral Hepatitis in Heart Transplant Recipients. *J Hosp Infect*. 1996;34:71–72.
12. Guyatt GH, Oxman AD, Kunz R, et al. What is "quality of evidence" and why is it important to clinicians? *BMJ*. 2008;336:995–998.
13. Kish MA. Infectious Diseases Society of America. Guide to development of practice guidelines. *Clin Infect Dis*. 2001;32:851–854.
14. Humar A, Michaels M. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. *Am J Transplant*. 2006;6:262–274.
15. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. *Clin Infect Dis*. 2017;64:87–91.
16. Delforge ML, Desomberg L, Montesinos I. Evaluation of the new LIAISON(®) CMV IgG, IgM and IgG Avidity II assays. *J Clin Virol*. 2015;72:42–45.
17. Lagrou K, Bodeus M, Van Ranst M, et al. Evaluation of the new architect cytomegalovirus immunoglobulin M (IgM), IgG, and IgG avidity assays. *J Clin Microbiol*. 2009;47:1695–1699.
18. Seed CR, Piscitelli LM, Maine GT, et al. Validation of an automated immunoglobulin G-only cytomegalovirus (CMV) antibody screening assay and an assessment of the risk of transfusion transmitted CMV from seronegative blood. *Transfusion*. 2009;49:134–145.
19. Weber B, Fall EM, Berger A, et al. Screening of blood donors for human cytomegalovirus (HCMV) IgG antibody with an enzyme immunoassay using recombinant antigens. *J Clin Virol*. 1999;14:173–181.
20. Preiksaitis JK, Sandhu J, Strautman M. The risk of transfusion-acquired CMV infection in seronegative solid-organ transplant recipients receiving non-WBC-reduced blood components not screened for CMV antibody (1984 to 1996): experience at a single Canadian center. *Transfusion*. 2002;42:396–402.
21. Ritter M, Schmidt T, Dirks J, et al. Cytomegalovirus-specific T cells are detectable in early childhood and allow assignment of the infection status in children with passive maternal antibodies. *Eur J Immunol*. 2013;43:1099–1108.
22. Schmidt T, Ritter M, Dirks J, et al. Cytomegalovirus-specific T-cell immunity to assign the infection status in individuals with passive immunity: a proof of principle. *J Clin Virol*. 2012;54:272–275.
23. Schmidt T, Schub D, Wolf M, et al. Comparative analysis of assays for detection of cell-mediated immunity toward cytomegalovirus and *M. tuberculosis* in samples from deceased organ donors. *Am J Transplant*. 2014;14:2159–2167.
24. Mayer BT, Matrajt L, Casper C, et al. Dynamics of persistent oral cytomegalovirus shedding during primary infection in ugandan infants. *J Infect Dis*. 2016;214:1735–1743.
25. Humar A, Limaye AP, Blumberg EA, et al. Extended valganciclovir prophylaxis in D+/R– kidney transplant recipients is associated with long-term reduction in cytomegalovirus disease: two-year results of the IMPACT study. *Transplantation*. 2010;90:1427–1431.
26. Humar A, Mazzulli T, Moussa G, et al. Clinical utility of cytomegalovirus (CMV) serology testing in high-risk CMV D+/R– transplant recipients. *Am J Transplant*. 2005;5:1065–1070.
27. Blanco-Lobo P, Bulnes-Ramos A, McConnell MJ, et al. Applying lessons learned from cytomegalovirus infection in transplant patients to vaccine design. *Drug Discov Today*. 2016;21:674–681.
28. Pillay D, Ali AA, Liu SF, et al. The prognostic significance of positive CMV cultures during surveillance of renal transplant recipients. *Transplantation*. 1993;56:103–108.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

29. Caliendo AM, St George K, Allega J, et al. Distinguishing cytomegalovirus (CMV) infection and disease with CMV nucleic acid assays. *J Clin Microbiol*. 2002;40:1581–1586.
30. Emery VC, Sabin CA, Cope AV, et al. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. *Lancet*. 2000;355:2032–2036.
31. Humar A, Gregson D, Caliendo AM, et al. Clinical utility of quantitative cytomegalovirus viral load determination for predicting cytomegalovirus disease in liver transplant recipients. *Transplantation*. 1999;68:1305–1311.
32. Humar A, Kumar D, Boivin G, et al. Cytomegalovirus (CMV) virus load kinetics to predict recurrent disease in solid-organ transplant patients with CMV disease. *J Infect Dis*. 2002;186:829–833.
33. Lao WC, Lee D, Burroughs AK, et al. Use of polymerase chain reaction to provide prognostic information on human cytomegalovirus disease after liver transplantation. *J Med Virol*. 1997;51:152–158.
34. Razonable RR, van Cruijsen H, Brown RA, et al. Dynamics of cytomegalovirus replication during preemptive therapy with oral ganciclovir. *J Infect Dis*. 2003;187:1801–1808.
35. Rollag H, Sagedal S, Kristiansen KI, et al. Cytomegalovirus DNA concentration in plasma predicts development of cytomegalovirus disease in kidney transplant recipients. *Clin Microbiol Infect*. 2002;8:431–434.
36. Hamprecht K, Steinmassl M, Einsele H, et al. Discordant detection of human cytomegalovirus DNA from peripheral blood mononuclear cells, granulocytes and plasma: correlation to viremia and HCMV infection. *J Clin Virol*. 1998;11:125–136.
37. Koidl C, Bozic M, Marth E, et al. Detection of CMV DNA: is EDTA whole blood superior to EDTA plasma? *J Virol Methods*. 2008;154:210–212.
38. Lisboa LF, Asberg A, Kumar D, et al. The clinical utility of whole blood versus plasma cytomegalovirus viral load assays for monitoring therapeutic response. *Transplantation*. 2011;91:231–236.
39. Razonable RR, Brown RA, Wilson J, et al. The clinical use of various blood compartments for cytomegalovirus (CMV) DNA quantitation in transplant recipients with CMV disease. *Transplantation*. 2002;73:968–973.
40. Tang W, Elmore SH, Fan H, et al. Cytomegalovirus DNA measurement in blood and plasma using Roche LightCycler CMV quantification reagents. *Diagn Mol Pathol*. 2008;17:166–173.
41. Tong Y, Pang XL, Mabilangan C, et al. Determination of the biological form of human cytomegalovirus DNA in the plasma of solid-organ transplant recipients. *J Infect Dis*. 2017.
42. Fryer JF HA, Anderson R, Minor PD, and the collaborative study group. Collaborative study to evaluate the proposed 1st WHO International Standard for human cytomegalovirus (HCMV) for nucleic acid amplification (NAT)-based assays. 2010; WHO ECBS Report 2010; WHO/BS/2010.2138. Available at www.who.int/biologicals/expert_committee/TRS_978_61st_report.pdf?ua=1.
43. Preiksaitis JK, Hayden RT, Tong Y, et al. Are we there yet? Impact of the first international standard for cytomegalovirus DNA on the harmonization of results reported on plasma samples. *Clin Infect Dis*. 2016;63:583–589.
44. Hayden RT, Sun Y, Tang L, et al. Progress in quantitative viral load testing: variability and impact of the WHO quantitative international standards. *J Clin Microbiol*. 2017;55:423–430.
45. Hayden RT, Yan X, Wick MT, et al. Factors contributing to variability of quantitative viral PCR results in proficiency testing samples: a multivariate analysis. *J Clin Microbiol*. 2012;50:337–345.
46. Jones S, Webb EM, Barry CP, et al. Commutability of cytomegalovirus WHO international standard in different matrices. *J Clin Microbiol*. 2016;54:1512–1519.
47. Hayden RT, Gu Z, Sam SS, et al. Comparative evaluation of three commercial quantitative cytomegalovirus standards by use of digital and real-time PCR. *J Clin Microbiol*. 2015;53:1500–1505.
48. Tsai HP, Tsai YY, Lin IT, et al. Comparison of two commercial automated nucleic acid extraction and integrated quantitation real-time PCR platforms for the detection of cytomegalovirus in plasma. *PLoS One*. 2016;11:e0160493.
49. Hayden RT, Preiksaitis J, Tong Y, et al. Commutability of the first World Health Organization international standard for human cytomegalovirus. *J Clin Microbiol*. 2015;53:3325–3333.
50. Hirsch HH, Lautenschlager I, Pinsky BA, et al. An international multicenter performance analysis of cytomegalovirus load tests. *Clin Infect Dis*. 2013;56:367–373.
51. Caliendo AM, Schuurman R, Yen-Lieberman B, et al. Comparison of quantitative and qualitative PCR assays for cytomegalovirus DNA in plasma. *J Clin Microbiol*. 2001;39:1334–1338.
52. Lilleri D, Lazzarotto T, Ghisetti V, et al. Multicenter quality control study for human cytomegalovirus DNAemia quantification. *New Microbiol*. 2009;32:245–253.
53. Abbate I, Piralla A, Calvario A, et al. Nation-wide measure of variability in HCMV, EBV and BKV DNA quantification among centers involved in monitoring transplanted patients. *J Clin Virol*. 2016;82:76–83.
54. Åsberg A, Humar A, Rollag H, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant*. 2007;7:2106–2113.
55. Dioverti MV, Lahr B, Razonable RR. Treatment of cytomegalovirus infection and disease pre- and post-quantitative nucleic acid test standardization: does use of a more sensitive assay lead to longer treatment duration? *Clin Transplant*. 2016;30:154–160.
56. Razonable RR, Asberg A, Rollag H, et al. Virologic suppression measured by a cytomegalovirus (CMV) DNA test calibrated to the World Health Organization international standard is predictive of CMV disease resolution in transplant recipients. *Clin Infect Dis*. 2013;56:1546–1553.
57. Chemaly RF, Yen-Lieberman B, Castilla EA, et al. Correlation between viral loads of cytomegalovirus in blood and bronchoalveolar lavage specimens from lung transplant recipients determined by histology and immunohistochemistry. *J Clin Microbiol*. 2004;42:2168–2172.
58. Solans EP, Yong S, Husain AN, et al. Bronchioloalveolar lavage in the diagnosis of CMV pneumonitis in lung transplant recipients: an immunocytochemical study. *Diagn Cytopathol*. 1997;16:350–352.
59. Halme L, Lempinen M, Arola J, et al. High frequency of gastroduodenal cytomegalovirus infection in liver transplant patients. *Apmis*. 2008;116:99–106.
60. Eid AJ, Arthurs SK, Deziel PJ, et al. Clinical predictors of relapse after treatment of primary gastrointestinal cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant*. 2010;10:157–161.
61. Westall GP, Michaelides A, Williams TJ, et al. Human cytomegalovirus load in plasma and bronchoalveolar lavage fluid: a longitudinal study of lung transplant recipients. *J Infect Dis*. 2004;190:1076–1083.
62. Durand CM, Marr KA, Arnold CA, et al. Detection of cytomegalovirus DNA in plasma as an adjunct diagnostic for gastrointestinal tract disease in kidney and liver transplant recipients. *Clin Infect Dis*. 2013;57:1550–1559.
63. Fisher CE, Alexander J, Bhattacharya R, et al. Sensitivity of blood and tissue diagnostics for gastrointestinal cytomegalovirus disease in solid organ transplant recipients. *Transpl Infect Dis*. 2016;18:372–380.
64. McCoy MH, Post K, Sen JD, et al. qPCR increases sensitivity to detect cytomegalovirus in formalin-fixed, paraffin-embedded tissue of gastrointestinal biopsies. *Hum Pathol*. 2014;45:48–53.
65. Mills AM, Guo FP, Copland AP, et al. A comparison of CMV detection in gastrointestinal mucosal biopsies using immunohistochemistry and PCR performed on formalin-fixed, paraffin-embedded tissue. *Am J Surg Pathol*. 2013;37:995–1000.
66. Kerschner H, Jaksch P, Zweytick B, et al. Detection of human cytomegalovirus in bronchoalveolar lavage fluid of lung transplant recipients reflects local virus replication and not contamination from the throat. *J Clin Microbiol*. 2010;48:4273–4274.
67. Buffone GJ, Frost A, Samo T, et al. The diagnosis of CMV pneumonitis in lung and heart/lung transplant patients by PCR compared with traditional laboratory criteria. *Transplantation*. 1993;56:342–347.
68. Storch GA, Ettinger NA, Ockner D, et al. Quantitative cultures of the cell fraction and supernatant of bronchoalveolar lavage fluid for the diagnosis of cytomegalovirus pneumonitis in lung transplant recipients. *J Infect Dis*. 1993;168:1502–1506.
69. Bauer CC, Jaksch P, Aberle SW, et al. Relationship between cytomegalovirus DNA load in epithelial lining fluid and plasma of lung transplant recipients and analysis of coinfection with Epstein-Barr virus and human herpesvirus 6 in the lung compartment. *J Clin Microbiol*. 2007;45:324–328.
70. Gerna G, Lilleri D, Rognoni V, et al. Preemptive therapy for systemic and pulmonary human cytomegalovirus infection in lung transplant recipients. *Am J Transplant*. 2009;9:1142–1150.
71. Riise GC, Andersson R, Bergström T, et al. Quantification of cytomegalovirus DNA in BAL fluid: a longitudinal study in lung transplant recipients. *Chest*. 2000;118:1653–1660.
72. Puchhammer-Stöckl E. Herpesviruses and the transplanted lung: looking at the air side. *J Clin Virol*. 2008;43:415–418.
73. Kaminski H, Fishman JA. The cell biology of cytomegalovirus: implications for transplantation. *Am J Transplant*. 2016;16:2254–2269.
74. Carbone J. The immunology of posttransplant CMV infection: potential effect of CMV immunoglobulins on distinct components of the immune response to CMV. *Transplantation*. 2016;100(Suppl 3):S11–S18.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

75. Fernandez-Ruiz M, Corrales I, Arias M, et al. Association between individual and combined SNPs in genes related to innate immunity and incidence of CMV infection in seropositive kidney transplant recipients. *Am J Transplant*. 2015;15:1323–1335.
76. de Rooij BJ, van der Beek MT, van Hoek B, et al. Mannose-binding lectin and ficolin-2 gene polymorphisms predispose to cytomegalovirus (re)infection after orthotopic liver transplantation. *J Hepatol*. 2011;55:800–807.
77. Cervera C, Lozano F, Linares L, et al. Influence of mannose-binding lectin gene polymorphisms on the invasiveness of cytomegalovirus disease after solid organ transplantation. *Transplant Proc*. 2009;41:2259–2261.
78. Kwakkel-van Erp JM, Paantjens AW, van Kessel DA, et al. Mannose-binding lectin deficiency linked to cytomegalovirus (CMV) reactivation and survival in lung transplantation. *Clin Exp Immunol*. 2011;165:410–416.
79. Sarmiento E, Jaramillo M, Calahorra L, et al. Evaluation of humoral immunity profiles to identify heart recipients at risk for development of severe infections: a multicenter prospective study. *J Heart Lung Transplant*. 2017;36:529–539.
80. Egli A, Levin A, Santer DM, et al. Immunomodulatory function of interleukin 28B during primary infection with cytomegalovirus. *J Infect Dis*. 2014; 210:717–727.
81. Lisboa LF, Egli A, Fairbanks J, et al. CCL8 and the immune control of cytomegalovirus in organ transplant recipients. *Am J Transplant*. 2015;15: 1882–1892.
82. Fernández-Ruiz M, Silva JT, López-Medrano F, et al. Post-transplant monitoring of NK cell counts as a simple approach to predict the occurrence of opportunistic infection in liver transplant recipients. *Transpl Infect Dis*. 2016;18:552–565.
83. Sarmiento E, del Pozo N, Gallego A, et al. Decreased levels of serum complement C3 and natural killer cells add to the predictive value of total immunoglobulin G for severe infection in heart transplant recipients. *Transpl Infect Dis*. 2012;14:526–539.
84. de Rham C, Hadaya K, Bandelier C, et al. Expression of killer cell immunoglobulin-like receptors (KIRs) by natural killer cells during acute CMV infection after kidney transplantation. *Transpl Immunol*. 2014;31: 157–164.
85. Gonzalez A, Schmitter K, Hirsch HH, et al. KIR-associated protection from CMV replication requires pre-existing immunity: a prospective study in solid organ transplant recipients. *Genes Immun*. 2014;15:495–499.
86. van Duin D, Avery RK, Hemachandra S, et al. KIR and HLA interactions are associated with control of primary CMV infection in solid organ transplant recipients. *Am J Transplant*. 2014;14:156–162.
87. Ravens S, Schultze-Florey C, Raha S, et al. Human γδ T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. *Nat Immunol*. 2017;18:393–401.
88. Knight A, Madrigal AJ, Grace S, et al. The role of Vδ2-negative γδ T cells during cytomegalovirus reactivation in recipients of allogeneic stem cell transplantation. *Blood*. 2010;116:2164–2172.
89. Kaminski H, Garrigue I, Couzi L, et al. Surveillance of γδ T cells predicts cytomegalovirus infection resolution in kidney transplants. *J Am Soc Nephrol*. 2016;27:637–645.
90. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. *Clin Microbiol Rev*. 2009;22:76–98. Table of Contents.
91. Lilleri D, Kabanova A, Lanzavecchia A, et al. Antibodies against neutralization epitopes of human cytomegalovirus gH/gL/pUL128-130-131 complex and virus spreading may correlate with virus control in vivo. *J Clin Immunol*. 2012;32:1324–1331.
92. Fouts AE, Chan P, Stephan JP, et al. Antibodies against the gH/gL/UL128/UL130/UL131 complex comprise the majority of the anti-cytomegalovirus (anti-CMV) neutralizing antibody response in CMV hyperimmune globulin. *J Virol*. 2012;86:7444–7447.
93. Genini E, Percivalle E, Sarasini A, et al. Serum antibody response to the gH/gL/pUL128-131 five-protein complex of human cytomegalovirus (HCMV) in primary and reactivated HCMV infections. *J Clin Virol*. 2011;52:113–118.
94. Doron S, Ruthazer R, Werner BG, et al. Hypogammaglobulinemia in liver transplant recipients: incidence, timing, risk factors, and outcomes. *Transplantation*. 2006;81:697–703.
95. Ohsumi A, Chen F, Yamada T, et al. Effect of hypogammaglobulinemia after lung transplantation: a single-institution study. *Eur J Cardiothorac Surg*. 2014;45:e61–e67.
96. Mozer-Glassberg Y, Shamir R, Steinberg R, et al. Hypogammaglobulinemia in the early period after liver transplantation in children. *Clin Transplant*. 2013;27:E289–E294.
97. Fernandez-Ruiz M, Lopez-Medrano F, Varela-Pena P, et al. Monitoring of immunoglobulin levels identifies kidney transplant recipients at high risk of infection. *Am J Transplant*. 2012;12:2763–2773.
98. Sarmiento E, Navarro J, Fernandez-Yanez J, et al. Evaluation of an immunological score to assess the risk of severe infection in heart recipients. *Transpl Infect Dis*. 2014;16:802–812.
99. Goldfarb NS, Avery RK, Goormastic M, et al. Hypogammaglobulinemia in lung transplant recipients. *Transplantation*. 2001;71:242–246.
100. Sarmiento E, Fernàndez-Yáñez J, Muñoz P, et al. Hypogammaglobulinemia after heart transplantation: use of intravenous immunoglobulin replacement therapy in relapsing CMV disease. *Int Immunopharmacol*. 2005;5:97–101.
101. Yamani MH, Avery R, Mawhorter SD, et al. The impact of CytoGam on cardiac transplant recipients with moderate hypogammaglobulinemia: a randomized single-center study. *J Heart Lung Transplant*. 2005;24: 1766–1769.
102. Yamani MH, Avery R, Mawhorter S, et al. Hypogammaglobulinemia after heart transplantation: impact of pre-emptive use of immunoglobulin replacement (CytoGam) on infection and rejection outcomes. *Transpl Infect Dis*. 2001;3 Suppl 2:40–43.
103. Sarmiento E, Diez P, Arraya M, et al. Early intravenous immunoglobulin replacement in hypogammaglobulinemic heart transplant recipients: results of a clinical trial. *Transpl Infect Dis*. 2016;18:832–843.
104. Carbone J, Sarmiento E, Del Pozo N, et al. Restoration of humoral immunity after intravenous immunoglobulin replacement therapy in heart recipients with post-transplant antibody deficiency and severe infections. *Clin Transplant*. 2012;26:E277–E283.
105. Carbone J, Sarmiento E, Palomo J, et al. The potential impact of substitutive therapy with intravenous immunoglobulin on the outcome of heart transplant recipients with infections. *Transplant Proc*. 2007;39: 2385–2388.
106. Augusto JF, Garnier AS, Demiselle J, et al. Hypogammaglobulinemia and risk of severe infection in kidney transplant recipients. *Transpl Infect Dis*. 2016;18:741–751.
107. Nierenberg NE, Poutsiaka DD, Chow JK, et al. Pretransplant lymphopenia is a novel prognostic factor in cytomegalovirus and noncytomegalovirus invasive infections after liver transplantation. *Liver Transpl*. 2014;20:1497–1507.
108. Natori Y, Humar A, Husain S, et al. Recurrence of CMV infection and the effect of prolonged antivirals in organ transplant recipients. *Transplantation*. 2017;101:1449–1454.
109. Sood S, Testro AG. Immune monitoring post liver transplant. *World J Transplant*. 2014;4:30–39.
110. Carbone J, Lanio N, Gallego A, et al. Simultaneous monitoring of cytomegalovirus-specific antibody and T-cell levels in seropositive heart transplant recipients. *J Clin Immunol*. 2012;32:809–819.
111. Egli A, Humar A, Kumar D. State-of-the-art monitoring of cytomegalovirus-specific cell-mediated immunity after organ transplant: a primer for the clinician. *Clin Infect Dis*. 2012;55:1678–1689.
112. Sester M, Leboeuf C, Schmidt T, et al. The "ABC" of virus-specific T cell immunity in solid organ transplantation. *Am J Transplant*. 2016;16: 1697–1706.
113. Camargo JF, Resende MR, Zamel R, et al. Potential role of CC chemokine receptor 6 in prediction of late-onset cytomegalovirus infection following solid organ transplant. *Clin Transplant*. 2015;29:492–498.
114. Walker S, Fazou C, Crough T, et al. Ex vivo monitoring of human cytomegalovirus-specific CD8+ T-cell responses using QuantiFERON-CMV. *Transpl Infect Dis*. 2007;9:165–170.
115. Westall GP, Mifsud NA, Kotsimbos T. Linking CMV serostatus to episodes of CMV reactivation following lung transplantation by measuring CMV-specific CD8+ T-cell immunity. *Am J Transplant*. 2008;8: 1749–1754.
116. Manuel O, Husain S, Kumar D, et al. Assessment of cytomegalovirus-specific cell-mediated immunity for the prediction of cytomegalovirus disease in high-risk solid-organ transplant recipients: a multicenter cohort study. *Clin Infect Dis*. 2013;56:817–824.
117. Kumar D, Chernenko S, Moussa G, et al. Cell-mediated immunity to predict cytomegalovirus disease in high-risk solid organ transplant recipients. *Am J Transplant*. 2009;9:1214–1222.
118. Cantisán S, Lara R, Montejo M, et al. Pretransplant interferon-γ secretion by CMV-specific CD8+ T cells informs the risk of CMV replication after transplantation. *Am J Transplant*. 2013;13:738–745.
119. Weseslindtner L, Kerschner H, Steinacher D, et al. Prospective analysis of human cytomegalovirus DNAemia and specific CD8+ T cell responses in lung transplant recipients. *Am J Transplant*. 2012;12:2172–2180.
120. Lisboa LF, Kumar D, Wilson LE, et al. Clinical utility of cytomegalovirus cell-mediated immunity in transplant recipients with cytomegalovirus viremia. *Transplantation*. 2012;93:195–200.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

121. Bestard O, Lucia M, Crespo E, et al. Pretransplant immediately early-1-specific T cell responses provide protection for CMV infection after kidney transplantation. *Am J Transplant*. 2013;13:1793–1805.
122. Mattes FM, Vargas A, Kopycinski J, et al. Functional impairment of cytomegalovirus specific CD8 T cells predicts high-level replication after renal transplantation. *Am J Transplant*. 2008;8:990–999.
123. Abate D, Fiscon M, Saldan A, et al. Human cytomegalovirus-specific T-cell immune reconstitution in preemptively treated heart transplant recipients identifies subjects at critical risk for infection. *J Clin Microbiol*. 2012;50:1974–1980.
124. Abate D, Saldan A, Fiscon M, et al. Evaluation of cytomegalovirus (CMV)-specific T cell immune reconstitution revealed that baseline antiviral immunity, prophylaxis, or preemptive therapy but not antithymocyte globulin treatment contribute to CMV-specific T cell reconstitution in kidney transplant recipients. *J Infect Dis*. 2010;202:585–594.
125. Costa C, Astegiano S, Terlizzi ME, et al. Evaluation and significance of cytomegalovirus-specific cellular immune response in lung transplant recipients. *Transplant Proc*. 2011;43:1159–1161.
126. Schachtner T, Stein M, Reinke P. CMV-specific T cell monitoring offers superior risk stratification of CMV-seronegative kidney transplant recipients of a CMV-seropositive donor. *Transplantation*. 2017;101:e315–e325.
127. Lucia M, Crespo E, Melilli E, et al. Preformed frequencies of cytomegalovirus (CMV)-specific memory T and B cells identify protected CMV-sensitized individuals among seronegative kidney transplant recipients. *Clin Infect Dis*. 2014;59:1537–1545.
128. Shabir S, Kaul B, Pachnio A, et al. Impaired direct priming of CD8 T cells by donor-derived cytomegalovirus following kidney transplantation. *J Am Soc Nephrol*. 2013;24:1698–1708.
129. Barabas S, Spindler T, Kiener R, et al. An optimized IFN-γ ELISpot assay for the sensitive and standardized monitoring of CMV protein-reactive effector cells of cell-mediated immunity. *BMC Immunol*. 2017;18:14.
130. Banas B, Böger CA, Lückhoff G, et al. Validation of T-Track® CMV to assess the functionality of cytomegalovirus-reactive cell-mediated immunity in hemodialysis patients. *BMC Immunol*. 2017;18:15.
131. Sester M, Sester U, Gartner B, et al. Levels of virus-specific CD4 T cells correlate with cytomegalovirus control and predict virus-induced disease after renal transplantation. *Transplantation*. 2001;71:1287–1294.
132. Gerna G, Lilleri D, Fornara C, et al. Monitoring of human cytomegalovirus-specific CD4 and CD8 T-cell immunity in patients receiving solid organ transplantation. *Am J Transplant*. 2006;6:2356–2364.
133. Gerna G, Lilleri D, Chiesa A, et al. Virologic and immunologic monitoring of cytomegalovirus to guide preemptive therapy in solid-organ transplantation. *Am J Transplant*. 2011;11:2463–2471.
134. Sester U, Gartner BC, Wilkens H, et al. Differences in CMV-specific T-cell levels and long-term susceptibility to CMV infection after kidney, heart and lung transplantation. *Am J Transplant*. 2005;5:1483–1489.
135. Radha R, Jordan S, Puliyanda D, et al. Cellular immune responses to cytomegalovirus in renal transplant recipients. *Am J Transplant*. 2005;5:110–117.
136. Snyder LD, Chan C, Kwon D, et al. Polyfunctional T-cell signatures to predict protection from cytomegalovirus after lung transplantation. *Am J Respir Crit Care Med*. 2016;193:78–85.
137. Snyder LD, Medinas R, Chan C, et al. Polyfunctional cytomegalovirus-specific immunity in lung transplant recipients receiving valganciclovir prophylaxis. *Am J Transplant*. 2011;11:553–560.
138. Egli A, Binet I, Binggeli S, et al. Cytomegalovirus-specific T-cell responses and viral replication in kidney transplant recipients. *J Transl Med*. 2008;6:29.
139. Sester U, Presser D, Dirks J, et al. PD-1 expression and IL-2 loss of cytomegalovirus-specific T cells correlates with viremia and reversible functional anergy. *Am J Transplant*. 2008;8:1486–1497.
140. Nebbia G, Mattes FM, Smith C, et al. Polyfunctional cytomegalovirus-specific CD4+ and pp65 CD8+ T cells protect against high-level replication after liver transplantation. *Am J Transplant*. 2008;8:2590–2599.
141. La Rosa C, Krishnan A, Longmate J, et al. Programmed death-1 expression in liver transplant recipients as a prognostic indicator of cytomegalovirus disease. *J Infect Dis*. 2008;197:25–33.
142. Sund F, Lidehall AK, Claesson K, et al. CMV-specific T-cell immunity, viral load, and clinical outcome in seropositive renal transplant recipients: a pilot study. *Clin Transplant*. 2010;24:401–409.
143. Kumar D, Mian M, Singer L, et al. An interventional study using cell-mediated immunity to personalize therapy for cytomegalovirus infection after transplantation. *Am J Transplant*. 2017;17:2468–2473.
144. Brestrich G, Zwinger S, Fischer A, et al. Adoptive T-cell therapy of a lung transplanted patient with severe CMV disease and resistance to antiviral therapy. *Am J Transplant*. 2009;9:1679–1684.
145. Pierucci P, Malouf M, Glanville AR, et al. Novel autologous T-cell therapy for drug-resistant cytomegalovirus disease after lung transplantation. *J Heart Lung Transplant*. 2016;35:685–687.
146. Macesic N, Langsford D, Nicholls K, et al. Adoptive T cell immunotherapy for treatment of ganciclovir-resistant cytomegalovirus disease in a renal transplant recipient. *Am J Transplant*. 2015;15:827–832.
147. Gupta MP, Coombs P, Prockop SE, et al. Treatment of cytomegalovirus retinitis with cytomegalovirus-specific T-lymphocyte infusion. *Ophthalmic Surg Lasers Imaging Retina*. 2015;46:80–82.
148. Holmes-Liew CL, Holmes M, Beagley L, et al. Adoptive T-cell immunotherapy for ganciclovir-resistant CMV disease after lung transplantation. *Clin Transl Immunology*. 2015;4:e35.
149. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. *Blood*. 2013;121:5113–5123.
150. Blyth E, Clancy L, Simms R, et al. Donor-derived CMV-specific T cells reduce the requirement for CMV-directed pharmacotherapy after allogeneic stem cell transplantation. *Blood*. 2013;121:3745–3758.
151. Lugthart G, Albon SJ, Ricciardelli I, et al. Simultaneous generation of multivirus-specific and regulatory T cells for adoptive immunotherapy. *J Immunother*. 2012;35:42–53.
152. Gerdemann U, Katari UL, Papadopoulou A, et al. Safety and clinical efficacy of rapidly-generated trivirus-directed T cells as treatment for adenovirus, EBV, and CMV infections after allogeneic hematopoietic stem cell transplant. *Mol Ther*. 2013;21:2113–2121.
153. McVoy MA. Cytomegalovirus vaccines. *Clin Infect Dis*. 2013;57(Suppl 4):S196–S199.
154. Plotkin SA, Starr SE, Friedman HM, et al. Effect of Towne live virus vaccine on cytomegalovirus disease after renal transplant. A controlled trial. *Ann Intern Med*. 1991;114:525–531.
155. Pass RF, Duliege AM, Boppana S, et al. A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant. *J Infect Dis*. 1999;180:970–975.
156. Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med*. 2009;360:1191–1199.
157. Griffiths PD, Stanton A, McCarrell E, et al. Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. *Lancet*. 2011;377:1256–1263.
158. Kharfan-Dabaja MA, Boeckh M, Wilck MB, et al. A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis*. 2012;12:290–299.
159. Vincenti F, Budde K, Merville P, et al. *Efficacy and Safety of ASP0113 versus Placebo in CMV-Seronegative Kidney Transplant Patients Receiving an Organ from a CMV-Seropositive Donor.* Chicago, USA: Paper presented at: American Transplant Congress; 2017.
160. Nakamura R, La Rosa C, Longmate J, et al. Viraemia, immunogenicity, and survival outcomes of cytomegalovirus chimeric epitope vaccine supplemented with PF03512676 (CMVPepVax) in allogeneic haemopoietic stem-cell transplantation: randomised phase 1b trial. *Lancet Haematol*. 2016;3:e87–e98.
161. Reap EA, Morris J, Dryga SA, et al. Development and preclinical evaluation of an alphavirus replicon particle vaccine for cytomegalovirus. *Vaccine*. 2007;25:7441–7449.
162. Bernstein DI, Schleiss MR, Berencsi K, et al. Effect of previous or simultaneous immunization with canarypox expressing cytomegalovirus (CMV) glycoprotein B (gB) on response to subunit gB vaccine plus MF59 in healthy CMV-seronegative adults. *J Infect Dis*. 2002;185:686–690.
163. Berencsi K, Gyulai Z, Gonczol E, et al. A canarypox vector-expressing cytomegalovirus (CMV) phosphoprotein 65 induces long-lasting cytotoxic T cell responses in human CMV-seronegative subjects. *J Infect Dis*. 2001;183:1171–1179.
164. Zhong J, Rist M, Cooper L, et al. Induction of pluripotent protective immunity following immunisation with a chimeric vaccine against human cytomegalovirus. *PLoS One*. 2008;3:e3256.
165. Rubin RH, Kemmerly SA, Conti D, et al. Prevention of primary cytomegalovirus disease in organ transplant recipients with oral ganciclovir or oral acyclovir prophylaxis. *Transpl Infect Dis*. 2000;2:112–117.
166. Paya C, Humar A, Dominguez E, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant*. 2004;4:611–620.
167. Lowance D, Neumayer HH, Legendre CM, et al. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. *N Engl J Med*. 1999;340:1462–1470.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

168. Razonable RR, Rivero A, Rodriguez A, et al. Allograft rejection predicts the occurrence of late-onset cytomegalovirus (CMV) disease among CMV-mismatched solid organ transplant patients receiving prophylaxis with oral ganciclovir. *J Infect Dis*. 2001;184:1461–1464.
169. Humar A, Lebranchu Y, Vincenti F, et al. The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in high-risk kidney transplant recipients. *Am J Transplant*. 2010;10:1228–1237.
170. Pang XL, Fox JD, Fenton JM, et al. Interlaboratory comparison of cytomegalovirus viral load assays. *Am J Transplant*. 2009;9:258–268.
171. Couzi L, Helou S, Bachelet T, et al. High incidence of anticytomegalovirus drug resistance among D+R– kidney transplant recipients receiving preemptive therapy. *Am J Transplant*. 2012;12:202–209.
172. Atabani SF, Smith C, Atkinson C, et al. Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy. *Am J Transplant*. 2012;12:2457–2464.
173. Sun HY, Cacciarelli TV, Wagener MM, et al. Preemptive therapy for cytomegalovirus based on real-time measurement of viral load in liver transplant recipients. *Transpl Immunol*. 2010;23:166–169.
174. Khoury JA, Storch GA, Bohl DL, et al. Prophylactic versus preemptive oral valganciclovir for the management of cytomegalovirus infection in adult renal transplant recipients. *Am J Transplant*. 2006;6:2134–2143.
175. Reischig T, Jindra P, Hes O, et al. Valacyclovir prophylaxis versus preemptive valganciclovir therapy to prevent cytomegalovirus disease after renal transplantation. *Am J Transplant*. 2008;8:69–77.
176. Kliem V, Fricke L, Wollbrink T, et al. Improvement in long-term renal graft survival due to CMV prophylaxis with oral ganciclovir: results of a randomized clinical trial. *Am J Transplant*. 2008;8:975–983.
177. Witzke O, Hauser IA, Bartels M, et al. Valganciclovir prophylaxis versus preemptive therapy in cytomegalovirus-positive renal allograft recipients: 1-year results of a randomized clinical trial. *Transplantation*. 2012;93:61–68.
178. Asberg A, Humar A, Jardine AG, et al. Long-term outcomes of CMV disease treatment with valganciclovir versus IV ganciclovir in solid organ transplant recipients. *Am J Transplant*. 2009;9:1205–1213.
179. Ramanan P, Razonable RR. Cytomegalovirus infections in solid organ transplantation: a review. *Infect Chemother*. 2013;45:260–271.
180. Reischig T, Nemcová J, Vanecek T, et al. Intragraft cytomegalovirus infection: a randomized trial of valacyclovir prophylaxis versus pre-emptive therapy in renal transplant recipients. *Antivir Ther*. 2010;15:23–30.
181. Spinner ML, Saab G, Casabar E, et al. Impact of prophylactic versus preemptive valganciclovir on long-term renal allograft outcomes. *Transplantation*. 2010;90:412–418.
182. Reischig T, Hribova P, Jindra P, et al. Long-term outcomes of preemptive valganciclovir compared with valacyclovir prophylaxis for prevention of cytomegalovirus in renal transplantation. *J Am Soc Nephrol*. 2012;23:1588–1597.
183. Owers DS, Webster AC, Strippoli GF, et al. Pre-emptive treatment for cytomegalovirus viraemia to prevent cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev*. 2013:CD005133.
184. Florescu DF, Qiu F, Schmidt CM, et al. A direct and indirect comparison meta-analysis on the efficacy of cytomegalovirus preventive strategies in solid organ transplant. *Clin Infect Dis*. 2014;58:785–803.
185. Mumtaz K, Faisal N, Husain S, et al. Universal prophylaxis or preemptive strategy for cytomegalovirus disease after liver transplantation: a systematic review and meta-analysis. *Am J Transplant*. 2015;15:472–481.
186. Manuel O, Kralidis G, Mueller NJ, et al. Impact of antiviral preventive strategies on the incidence and outcomes of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant*. 2013;13:2402–2410.
187. Reischig T, Kacer M, Hruba P, et al. The impact of viral load and time to onset of cytomegalovirus replication on long-term graft survival after kidney transplantation. *Antivir Ther*. 2017.
188. Paraskeva M, Bailey M, Levvey BJ, et al. Cytomegalovirus replication within the lung allograft is associated with bronchiolitis obliterans syndrome. *Am J Transplant*. 2011;11:2190–2196.
189. Snyder LD, Finlen-Copeland CA, Turbyfill WJ, et al. Cytomegalovirus pneumonitis is a risk for bronchiolitis obliterans syndrome in lung transplantation. *Am J Respir Crit Care Med*. 2010;181:1391–1396.
190. Zuk DM, Humar A, Weinkauf JG, et al. An international survey of cytomegalovirus management practices in lung transplantation. *Transplantation*. 2010;90:672–676.
191. Lisboa LF, Preiksaitis JK, Humar A, et al. Clinical utility of molecular surveillance for cytomegalovirus after antiviral prophylaxis in high-risk solid organ transplant recipients. *Transplantation*. 2011;92:1063–1068.
192. Montejo M, Montejo E, Gastaca M, et al. Prophylactic therapy with valgancyclovir in high-risk (cytomegalovirus D+/R–) liver transplant recipients: a single-center experience. *Transplant Proc*. 2009;41:2189–2191.
193. Boillat Blanco N, Pascual M, Venetz JP, et al. Impact of a preemptive strategy after 3 months of valganciclovir cytomegalovirus prophylaxis in kidney transplant recipients. *Transplantation*. 2011;91:251–255.
194. van der Beek MT, Berger SP, Vossen AC, et al. Preemptive versus sequential prophylactic-preemptive treatment regimens for cytomegalovirus in renal transplantation: comparison of treatment failure and antiviral resistance. *Transplantation*. 2010;89:320–326.
195. Martin-Gandul C, Perez-Romero P, Blanco-Lobo P, et al. Viral load, CMV-specific T-cell immune response and cytomegalovirus disease in solid organ transplant recipients at higher risk for cytomegalovirus infection during preemptive therapy. *Transpl Int*. 2014;27:1060–1068.
196. Griffiths PD, Rothwell E, Raza M, et al. Randomized controlled trials to define viral load thresholds for cytomegalovirus pre-emptive therapy. *PLoS One*. 2016;11:e0163722.
197. Boaretti M, Sorrentino A, Zantedeschi C, et al. Quantification of cytomegalovirus DNA by a fully automated real-time PCR for early diagnosis and monitoring of active viral infection in solid organ transplant recipients. *J Clin Virol*. 2013;56:124–128.
198. Martín-Gandul C, Pérez-Romero P, Sánchez M, et al. Determination, validation and standardization of a CMV DNA cut-off value in plasma for preemptive treatment of CMV infection in solid organ transplant recipients at lower risk for CMV infection. *J Clin Virol*. 2013;56:13–18.
199. Vinuesa V, Gimenez E, Solano C, et al. Would kinetic analyses of plasma cytomegalovirus DNA load help to reach consensus criteria for triggering the initiation of preemptive antiviral therapy in transplant recipients? *Clin Infect Dis*. 2016;63:1533–1535.
200. Solano C, Gimenez E, Pinana JL, et al. Preemptive antiviral therapy for CMV infection in allogeneic stem cell transplant recipients guided by the viral doubling time in the blood. *Bone Marrow Transplant*. 2016;51:718–721.
201. Gimenez E, Munoz-Cobo B, Solano C, et al. Early kinetics of plasma cytomegalovirus DNA load in allogeneic stem cell transplant recipients in the era of highly sensitive real-time PCR assays: does it have any clinical value? *J Clin Microbiol*. 2014;52:654–656.
202. Emery VC, Hassan-Walker AF, Burroughs AK, et al. Human cytomegalovirus (HCMV) replication dynamics in HCMV-naive and -experienced immunocompromised hosts. *J Infect Dis*. 2002;185:1723–1728.
203. Schoeppler KE, Lyu DM, Grazia TJ, et al. Late-onset cytomegalovirus (CMV) in lung transplant recipients: can CMV serostatus guide the duration of prophylaxis? *Am J Transplant*. 2013;13:376–382.
204. Hashim F, Gregg JA, Dharnidharka VR. Efficacy of extended valganciclovir prophylaxis in preventing cytomegalovirus infection in pediatric kidney transplantation. *Open Urol Nephrol J*. 2014;7(Suppl 2 M7):152–157.
205. Palmer SM, Limaye AP, Banks M, et al. Extended valganciclovir prophylaxis to prevent cytomegalovirus after lung transplantation: a randomized, controlled trial. *Ann Intern Med*. 2010;152:761–769.
206. Zamora MR, Nicolls MR, Hodges TN, et al. Following universal prophylaxis with intravenous ganciclovir and cytomegalovirus immune globulin, valganciclovir is safe and effective for prevention of CMV infection following lung transplantation. *Am J Transplant*. 2004;4:1635–1642.
207. Jaksch P, Zweytick B, Kerschner H, et al. Cytomegalovirus prevention in high-risk lung transplant recipients: comparison of 3- vs 12-month valganciclovir therapy. *J Heart Lung Transplant*. 2009;28:670–675.
208. Beam E, Lesnick T, Kremers W, et al. Cytomegalovirus disease is associated with higher all-cause mortality after lung transplantation despite extended antiviral prophylaxis. *Clin Transplant*. 2016;30:270–278.
209. Wiita AP, Roubinian N, Khan Y, et al. Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis. *Transpl Infect Dis*. 2012;14:248–258.
210. Hodson EM, Ladhani M, Webster AC, et al. Antiviral medications for preventing cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev*. 2013:CD003774.
211. Reischig T, Jindra P, Mares J, et al. Valacyclovir for cytomegalovirus prophylaxis reduces the risk of acute renal allograft rejection. *Transplantation*. 2005;79:317–324.
212. Pavlopoulou ID, Syriopoulou VP, Chelioti H, et al. A comparative randomised study of valacyclovir vs. oral ganciclovir for cytomegalovirus prophylaxis in renal transplant recipients. *Clin Microbiol Infect*. 2005;11:736–743.
213. Reischig T, Kacer M, Jindra P, et al. Randomized trial of valganciclovir versus valacyclovir prophylaxis for prevention of cytomegalovirus in renal transplantation. *Clin J Am Soc Nephrol*. 2015;10:294–304.
214. Reischig T, Prucha M, Sedlackova L, et al. Valganciclovir prophylaxis against cytomegalovirus impairs lymphocyte proliferation and activation in renal transplant recipients. *Antivir Ther*. 2011;16:1227–1235.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

215. Kacer M, Kielberger L, Bouda M, et al. Valganciclovir versus valacyclovir prophylaxis for prevention of cytomegalovirus: an economic perspective. *Transpl Infect Dis*. 2015;17:334–341.
216. Lischka P, Michel D, Zimmermann H. Characterization of cytomegalovirus breakthrough events in a phase 2 prophylaxis trial of letermovir (AIC246, MK 8228). *J Infect Dis*. 2016;213:23–30.
217. Chemaly RF, Ullmann AJ, Stoelben S, et al. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. *N Engl J Med*. 2014;370:1781–1789.
218. Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med*. 2017.
219. Winston DJ, Saliba F, Blumberg E, et al. Efficacy and safety of maribavir dosed at 100 mg orally twice daily for the prevention of cytomegalovirus disease in liver transplant recipients: a randomized, double-blind, multicenter controlled trial. *Am J Transplant*. 2012;12:3021–3030.
220. Marty FM, Winston DJ, Rowley SD, et al. CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation. *N Engl J Med*. 2013;369:1227–1236.
221. AABB, Clinical Transfusion Medicine Committee, Heddle NM, Boeckh M, Grossman B, et al. AABB Committee Report: reducing transfusion-transmitted cytomegalovirus infections. *Transfusion*. 2016;56(6 Pt 2): 1581–1587.
222. Sullivan T, Brodginski A, Patel G, et al. The role of secondary cytomegalovirus prophylaxis for kidney and liver transplant recipients. *Transplantation*. 2015;99:855–859.
223. Helantera I, Lautenschlager I, Koskinen P. The risk of cytomegalovirus recurrence after kidney transplantation. *Transpl Int*. 2011;24:1170–1178.
224. Nafar M, Roshan A, Pour-Reza-Gholi F, et al. Prevalence and risk factors of recurrent cytomegalovirus infection in kidney transplant recipients. *Iran J Kidney Dis*. 2014;8:231–235.
225. Moreno A, Cervera C, Fortún J, et al. Epidemiology and outcome of infections in human immunodeficiency virus/hepatitis C virus-coinfected liver transplant recipients: a FIPSE/GESIDA prospective cohort study. *Liver Transpl*. 2012;18:70–81.
226. Lee YM, Kim YH, Han DJ, et al. Cytomegalovirus infection after acute rejection therapy in seropositive kidney transplant recipients. *Transpl Infect Dis*. 2014;16:397–402.
227. Santos CA, Brennan DC, Fraser VJ, et al. Delayed-onset cytomegalovirus disease coded during hospital readmission after kidney transplantation. *Transplantation*. 2014;98:187–194.
228. Ong DS, Klein Klouwenberg PM, Verduyn Lunel FM, et al. Cytomegalovirus seroprevalence as a risk factor for poor outcome in acute respiratory distress syndrome*. *Crit Care Med*. 2015;43:394–400.
229. Walton AH, Muenzer JT, Rasche D, et al. Reactivation of multiple viruses in patients with sepsis. *PLoS One*. 2014;9:e98819.
230. Ong DS, Spitoni C, Klein Klouwenberg PM, et al. Cytomegalovirus reactivation and mortality in patients with acute respiratory distress syndrome. *Intensive Care Med*. 2016;42:333–341.
231. Limaye AP, Stapleton RD, Peng L, et al. Effect of ganciclovir on IL-6 levels among cytomegalovirus-seropositive adults with critical illness: a randomized clinical trial. *JAMA*. 2017;318:731–740.
232. Cowley NJ, Owen A, Shiels SC, et al. Safety and efficacy of antiviral therapy for prevention of cytomegalovirus reactivation in immunocompetent critically ill patients: a randomized clinical trial. *JAMA Intern Med*. 2017; 177:774–783.
233. Le Page AK, Jager MM, Kotton CN, et al. International survey of cytomegalovirus management in solid organ transplantation after the publication of consensus guidelines. *Transplantation*. 2013;95:1455–1460.
234. Chamberlain CE, Penzak SR, Alfaro RM, et al. Pharmacokinetics of low and maintenance dose valganciclovir in kidney transplant recipients. *Am J Transplant*. 2008;8:1297–1302.
235. Pescovitz MD, Rabkin J, Merion RM, et al. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. *Antimicrob Agents Chemother*. 2000;44:2811–2815.
236. Stevens DR, Sawinski D, Blumberg E, et al. Increased risk of breakthrough infection among cytomegalovirus donor-positive/recipient-negative kidney transplant recipients receiving lower-dose valganciclovir prophylaxis. *Transpl Infect Dis*. 2015;17:163–173.
237. Gabardi S, Asipenko N, Fleming J, et al. Evaluation of low- versus high-dose valganciclovir for prevention of cytomegalovirus disease in high-risk renal transplant recipients. *Transplantation*. 2015;99:1499–1505.
238. Heldenbrand S, Li C, Cross RP, et al. Multicenter evaluation of efficacy and safety of low-dose versus high-dose valganciclovir for prevention of cytomegalovirus disease in donor and recipient positive (D+/R+) renal transplant recipients. *Transpl Infect Dis*. 2016;18:904–912.
239. Snydman DR, Werner BG, Heinze-Lacey B, et al. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. *N Engl J Med*. 1987;317:1049–1054.
240. Snydman DR, Werner BG, Dougherty NN, et al. Cytomegalovirus immune globulin prophylaxis in liver transplantation. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med*. 1993;119:984–991.
241. Valantine HA, Luikart H, Doyle R, et al. Impact of cytomegalovirus hyperimmune globulin on outcome after cardiothoracic transplantation: a comparative study of combined prophylaxis with CMV hyperimmune globulin plus ganciclovir versus ganciclovir alone. *Transplantation*. 2001; 72:1647–1652.
242. Florescu DF, Abu-Elmagd K, Mercer DF, et al. An international survey of cytomegalovirus prevention and treatment practices in intestinal transplantation. *Transplantation*. 2014;97:78–82.
243. Ruttmann E, Geltner C, Bucher B, et al. Combined CMV prophylaxis improves outcome and reduces the risk for bronchiolitis obliterans syndrome (BOS) after lung transplantation. *Transplantation*. 2006;81:1415–1420.
244. Rubin RH, Lynch P, Pasternack MS, et al. Combined antibody and ganciclovir treatment of murine cytomegalovirus-infected normal and immunosuppressed BALB/c mice. *Antimicrob Agents Chemother*. 1989;33: 1975–1979.
245. Miescher SM, Huber TM, Kühne M, et al. In vitro evaluation of cytomegalovirus-specific hyperimmune globulins vs. standard intravenous immunoglobulins. *Vox Sang*. 2015;109:71–78.
246. Germer M, Herbener P, Schüttrumpf J. Functional properties of human cytomegalovirus hyperimmunoglobulin and standard immunoglobulin preparations. *Ann Transplant*. 2016;21:558–564.
247. Ishida JH, Patel A, Mehta AK, et al. Phase 2 randomized, double-blind, placebo-controlled trial of RG7667, a combination monoclonal antibody, for prevention of cytomegalovirus infection in high-risk kidney transplant recipients. *Antimicrob Agents Chemother*. 2017;61.
248. Dole K, Segal FP, Feire A, et al. A first-in-human study to assess the safety and pharmacokinetics of monoclonal antibodies against human cytomegalovirus in healthy volunteers. *Antimicrob Agents Chemother*. 2016;60:2881–2887.
249. Andrassy J, Hoffmann VS, Rentsch M, et al. Is cytomegalovirus prophylaxis dispensable in patients receiving an mTOR inhibitor-based immunosuppression? A systematic review and meta-analysis. *Transplantation*. 2012;94:1208–1217.
250. Su L, Tam N, Deng R, et al. Everolimus-based calcineurin-inhibitor sparing regimens for kidney transplant recipients: a systematic review and meta-analysis. *Int Urol Nephrol*. 2014;46:2035–2044.
251. Radtke J, Dietze N, Spetzler VN, et al. Fewer cytomegalovirus complications after kidney transplantation by de novo use of mTOR inhibitors in comparison to mycophenolic acid. *Transpl Infect Dis*. 2016;18:79–88.
252. Cervera C, Cofan F, Hernandez C, et al. Effect of mammalian target of rapamycin inhibitors on cytomegalovirus infection in kidney transplant recipients receiving polyclonal antilymphocyte globulins: a propensity score-matching analysis. *Transpl Int*. 2016;29:1216–1225.
253. Sheng L, Jun S, Jianfeng L, et al. The effect of sirolimus-based immunosuppression vs. conventional prophylaxis therapy on cytomegalovirus infection after liver transplantation. *Clin Transplant*. 2015;29:555–559.
254. Kobashigawa J, Ross H, Bara C, et al. Everolimus is associated with a reduced incidence of cytomegalovirus infection following de novo cardiac transplantation. *Transpl Infect Dis*. 2013;15:150–162.
255. Díaz Molina B, Velasco Alonso E, Lambert Rodríguez JL, et al. Effect of early conversion to everolimus together with prophylaxis with valganciclovir in the prevention of cytomegalovirus infection in heart transplant recipients. *Transplant Proc*. 2015;47:130–131.
256. Durante-Mangoni E, Andini R, Pinto D, et al. Effect of the immunosuppressive regimen on the incidence of cytomegalovirus infection in 378 heart transplant recipients: a single centre, prospective cohort study. *J Clin Virol*. 2015;68:37–42.
257. Ghassemieh B, Ahya VN, Baz MA, et al. Decreased incidence of cytomegalovirus infection with sirolimus in a post hoc randomized, multicenter study in lung transplantation. *J Heart Lung Transplant*. 2013;32:701–706.
258. Rittà M, Costa C, Solidoro P, et al. Everolimus-based immunosuppressive regimens in lung transplant recipients: impact on CMV infection. *Antiviral Res*. 2015;113:19–26.
259. Strueber M, Warnecke G, Fuge J, et al. Everolimus versus mycophenolate mofetil de novo after lung transplantation: a prospective, randomized, open-label trial. *Am J Transplant*. 2016;16:3171–3180.
260. Hocker B, Zencke S, Pape L, et al. Impact of everolimus and low-dose cyclosporin on cytomegalovirus replication and disease in pediatric renal transplantation. *Am J Transplant*. 2016;16:921–929.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

261. Pascual J, Royuela A, Fernandez AM, et al. Role of mTOR inhibitors for the control of viral infection in solid organ transplant recipients. *Transpl Infect Dis*. 2016;18(6):819–831.
262. Tedesco-Silva H, Felipe C, Ferreira A, et al. Reduced incidence of cytomegalovirus infection in kidney transplant recipients receiving everolimus and reduced tacrolimus doses. *Am J Transplant*. 2015;15:2655–2664.
263. Product Monograph Valcyte® Hoffmann-La Roche Limited (USA). *Patient Information and Instructions for Use*. 2017; https://www.gene.com/download/pdf/valcyte_prescribing.pdf. Accessed December 17, 2017.
264. Product Monograph Valcyte® Hoffmann-La Roche Limited (Canada). 2017; https://www.gene.com/download/pdf/valcyte_prescribing.pdf. Accessed December 17, 2017.
265. Product Monograph Cytovene® Hoffmann-La Roche Limited/CYTOVENE®-IV (ganciclovir sodium for injection) 2017; https://www.gene.com/download/pdf/cytovene_prescribing.pdf. Accessed December 17, 2017.
266. Hibberd PL, Tolkoff-Rubin NE, Conti D, et al. Preemptive ganciclovir therapy to prevent cytomegalovirus disease in cytomegalovirus antibody-positive renal transplant recipients. A randomized controlled trial. *Ann Intern Med*. 1995;123:18–26.
267. LeFevre PG, Jung CY, Chaney JE. Glucose transfer by red cell membrane phospholipids in H2O-CHCl-3-H2O three-layer systems. *Arch Biochem Biophys*. 1968;126:677–691.
268. Toyoda M, Shin BH, Ge S, et al. Impact of desensitization on antiviral immunity in HLA-sensitized kidney transplant recipients. *J Immunol Res*. 2017;2017:5672523.
269. Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. *Proc Natl Acad Sci U S A*. 2000;97:8039–8044.
270. McGavin JK, Goa KL. Ganciclovir: an update of its use in the prevention of cytomegalovirus infection and disease in transplant recipients. *Drugs*. 2001;61:1153–1183.
271. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron*. 1976;16:31–41.
272. Padulles A, Colom H, Bestard O, et al. Contribution of population pharmacokinetics to dose optimization of ganciclovir-valganciclovir in solid-organ transplant patients. *Antimicrob Agents Chemother*. 2016;60:1992–2002.
273. Asberg A, Jardine AG, Bignamini AA, et al. Effects of the intensity of immunosuppressive therapy on outcome of treatment for CMV disease in organ transplant recipients. *Am J Transplant*. 2010;10:1881–1888.
274. Asberg A, Humar A, Rollag H, et al. Lessons learned from a randomized study of oral valganciclovir versus parenteral ganciclovir treatment of cytomegalovirus disease in solid organ transplant recipients: the VICTOR trial. *Clin Infect Dis*. 2016;62:1154–1160.
275. Boivin G, Goyette N, Rollag H, et al. Cytomegalovirus resistance in solid organ transplant recipients treated with intravenous ganciclovir or oral valganciclovir. *Antivir Ther*. 2009;14:697–704.
275a. Gardiner BL, Chow JK, Price LL. Role of secondary prophylaxis with valganciclovir in the prevention of recurrent cytomegalovirus disease in solid organ transplant recipients. *Clin Infect Dis*. 2017;65:2000–2007.
276. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. *Clin Microbiol Rev*. 2010;23:689–712.
277. Fisher CE, Knudsen JL, Lease ED, et al. Risk factors and outcomes of ganciclovir resistant cytomegalovirus infection in solid organ transplant recipients. *Clin Infect Dis*. 2017;65:57–63.
278. Hantz S, Garnier-Geoffroy F, Mazeron MC, et al. Drug-resistant cytomegalovirus in transplant recipients: a French cohort study. *J Antimicrob Chemother*. 2010;65:2628–2640.
279. Myhre HA, Haug Dorenberg D, Kristiansen KI, et al. Incidence and outcomes of ganciclovir-resistant cytomegalovirus infections in 1244 kidney transplant recipients. *Transplantation*. 2011;92:217–223.
280. Young PG, Rubin J, Angarone M, et al. Ganciclovir-resistant cytomegalovirus infection in solid organ transplant recipients: a single-center retrospective cohort study. *Transpl Infect Dis*. 2016;18:390–395.
281. Lurain NS, Bhorade SM, Pursell KJ, et al. Analysis and characterization of antiviral drug-resistant cytomegalovirus isolates from solid organ transplant recipients. *J Infect Dis*. 2002;186(6):760–768.
282. Ambrose T, Sharkey LM, Louis-Auguste J, et al. Cytomegalovirus infection and rates of antiviral resistance following intestinal and multivisceral transplantation. *Transplant Proc*. 2016;48:492–496.
283. Timpone JG, Yimen M, Cox S, et al. Resistant cytomegalovirus in intestinal and multivisceral transplant recipients. *Transpl Infect Dis*. 2016;18:202–209.
284. Boivin G, Goyette N, Farhan M, et al. Incidence of cytomegalovirus UL97 and UL54 amino acid substitutions detected after 100 or 200 days of valganciclovir prophylaxis. *J Clin Virol*. 2012;53:208–213.
285. Jabs DA, Enger C, Forman M, et al. Incidence of foscarnet resistance and cidofovir resistance in patients treated for cytomegalovirus retinitis. The Cytomegalovirus Retinitis and Viral Resistance Study Group. *Antimicrob Agents Chemother*. 1998;42:2240–2244.
286. Schubert A, Ehlert K, Schuler-Luettmann S, et al. Fast selection of maribavir resistant cytomegalovirus in a bone marrow transplant recipient. *BMC Infect Dis*. 2013;13:330.
287. Strasfeld L, Lee I, Villano S, et al. Virologic characterization of multi-drug-resistant cytomegalovirus infection in two transplant recipients treated with maribavir. *J Infect Dis*. 2010;202:104–108.
288. Eid AJ, Arthurs SK, Deziel PJ, et al. Emergence of drug-resistant cytomegalovirus in the era of valganciclovir prophylaxis: therapeutic implications and outcomes. *Clin Transplant*. 2008;22:162–170.
289. Bonatti H, Sifri CD, Larcher C, et al. Use of cidofovir for cytomegalovirus disease refractory to ganciclovir in solid organ recipients. *Surg Infect (Larchmt)*. 2017;18:128–136.
290. Avery RK, Arav-Boger R, Marr KA, et al. Outcomes in transplant recipients treated with foscarnet for ganciclovir-resistant or refractory cytomegalovirus infection. *Transplantation*. 2016;100:e74–e80.
291. Landry ML, Stanat S, Biron K, et al. A standardized plaque reduction assay for determination of drug susceptibilities of cytomegalovirus clinical isolates. *Antimicrob Agents Chemother*. 2000;44:688–692.
292. Sahoo MK, Lefterova MI, Yamamoto F, et al. Detection of cytomegalovirus drug resistance mutations by next-generation sequencing. *J Clin Microbiol*. 2013;51:3700–3710.
293. Chou S, Boivin G, Ives J, et al. Phenotypic evaluation of previously uncharacterized cytomegalovirus DNA polymerase sequence variants detected in a valganciclovir treatment trial. *J Infect Dis*. 2014;209:1209–1216.
294. Chou S, Ercolani RJ, Sahoo MK, et al. Improved detection of emerging drug-resistant mutant cytomegalovirus subpopulations by deep sequencing. *Antimicrob Agents Chemother*. 2014;58:4697–4702.
295. Garrigue I, Moulinas R, Recordon-Pinson P, et al. Contribution of next generation sequencing to early detection of cytomegalovirus UL97 emerging mutants and viral subpopulations analysis in kidney transplant recipients. *J Clin Virol*. 2016;80:74–81.
296. Houldcroft CJ, Bryant JM, Depledge DP, et al. Detection of low frequency multi-drug resistance and novel putative maribavir resistance in immunocompromised pediatric patients with cytomegalovirus. *Front Microbiol*. 2016;7:1317.
297. Hamprecht K, Eckle T, Prix L, et al. Ganciclovir-resistant cytomegalovirus disease after allogeneic stem cell transplantation: pitfalls of phenotypic diagnosis by in vitro selection of an UL97 mutant strain. *J Infect Dis*. 2003;187:139–143.
298. Liu W, Kuppermann BD, Martin DF, et al. Mutations in the cytomegalovirus UL97 gene associated with ganciclovir-resistant retinitis. *J Infect Dis*. 1998;177:1176–1181.
299. Campos AB, Ribeiro J, Boutolleau D, et al. Human cytomegalovirus antiviral drug resistance in hematopoietic stem cell transplantation: current state of the art. *Rev Med Virol*. 2016;26:161–182.
300. Chevillotte M, von Einem J, Meier BM, et al. A new tool linking human cytomegalovirus drug resistance mutations to resistance phenotypes. *Antiviral Res*. 2010;85:318–327.
301. Komatsu TE, Pikis A, Naeger LK, et al. Resistance of human cytomegalovirus to ganciclovir/valganciclovir: a comprehensive review of putative resistance pathways. *Antiviral Res*. 2014;101:12–25.
302. Chou S, Ercolani RJ, Vanarsdall AL. Differentiated levels of ganciclovir resistance conferred by mutations at codons 591 to 603 of the cytomegalovirus UL97 kinase gene. *J Clin Microbiol*. 2017;55:2098–2104.
303. Kleiboeker S, Nutt J, Schindel B, et al. Cytomegalovirus antiviral resistance: characterization of results from clinical specimens. *Transpl Infect Dis*. 2014;16:561–567.
304. Chou S, Marousek GI, Van Wechel LC, et al. Growth and drug resistance phenotypes resulting from cytomegalovirus DNA polymerase region III mutations observed in clinical specimens. *Antimicrob Agents Chemother*. 2007;51:4160–4162.
305. Fischer L, Imrich E, Sampaio KL, et al. Identification of resistance-associated HCMV UL97- and UL54-mutations and a UL97-polymporphism with impact on phenotypic drug-resistance. *Antiviral Res*. 2016;131:1–8.
306. Perrottet N, Decosterd LA, Meylan P, et al. Valganciclovir in adult solid organ transplant recipients: pharmacokinetic and pharmacodynamic characteristics and clinical interpretation of plasma concentration measurements. *Clin Pharmacokinet*. 2009;48:399–418.
307. Gagermeier JP, Rusinak JD, Lurain NS, et al. Subtherapeutic ganciclovir (GCV) levels and GCV-resistant cytomegalovirus in lung transplant recipients. *Transpl Infect Dis*. 2014;16:941–950.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

308. Smith JP, Weller S, Johnson B, et al. Pharmacokinetics of acyclovir and its metabolites in cerebrospinal fluid and systemic circulation after administration of high-dose valacyclovir in subjects with normal and impaired renal function. *Antimicrob Agents Chemother*. 2010;54:1146–1151.
309. Bedino G, Esposito P, Bosio F, et al. The role of therapeutic drug monitoring in the treatment of cytomegalovirus disease in kidney transplantation. *Int Urol Nephrol*. 2013;45:1809–1813.
310. Gracia-Ahufinger I, Gutierrez-Aroca J, Cordero E, et al. Use of high-dose ganciclovir for the treatment of cytomegalovirus replication in solid organ transplant patients with ganciclovir resistance-inducing mutations. *Transplantation*. 2013;95:1015–1020.
311. Minces LR, Nguyen MH, Mitsani D, et al. Ganciclovir-resistant cytomegalovirus infections among lung transplant recipients are associated with poor outcomes despite treatment with foscarnet-containing regimens. *Antimicrob Agents Chemother*. 2014;58:128–135.
312. Bonatti H, Sifri CD, Larcher C, et al. Use of cidofovir for cytomegalovirus disease refractory to ganciclovir in solid organ recipients. *Surg Infect (Larchmt)*. 2016.
313. Patel SJ, Kuten SA, Knight RJ, et al. Resolution of mild ganciclovir-resistant cytomegalovirus disease with reduced-dose cidofovir and CMV-hyperimmune globulin. *J Transplant*. 2014;2014:342319.
314. Kaul DR, Stoelben S, Cober E, et al. First report of successful treatment of multidrug-resistant cytomegalovirus disease with the novel anti-CMV compound AIC246. *Am J Transplant*. 2011;11:1079–1084.
315. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. *Sci Transl Med*. 2014;6:242ra283.
316. Chou S, Van Wechel LC, Marousek GI. Effect of cell culture conditions on the anticytomegalovirus activity of maribavir. *Antimicrob Agents Chemother*. 2006;50:2557–2559.
317. Drouot E, Piret J, Boivin G. Artesunate demonstrates in vitro synergism with several antiviral agents against human cytomegalovirus. *Antivir Ther*. 2016;21:535–539.
318. Nashan B, Gaston R, Emery V, et al. Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. *Transplantation*. 2012;93:1075–1085.
319. Ozaki KS, Camara NO, Nogueira E, et al. The use of sirolimus in ganciclovir-resistant cytomegalovirus infections in renal transplant recipients. *Clin Transplant*. 2007;21:675–680.
320. Sabe N, Gonzalez-Costello J, Rama I, et al. Successful outcome of ganciclovir-resistant cytomegalovirus infection in organ transplant recipients after conversion to mTOR inhibitors. *Transpl Int*. 2012;25:e78–e82.
321. Avery RK, Mossad SB, Poggio E, et al. Utility of leflunomide in the treatment of complex cytomegalovirus syndromes. *Transplantation*. 2010;90:419–426.
322. Chon WJ, Kadambi PV, Xu C, et al. Use of leflunomide in renal transplant recipients with ganciclovir-resistant/refractory cytomegalovirus infection: a case series from the University of Chicago. *Case Rep Nephrol Dial*. 2015;5:96–105.
323. Morita S, Shinoda K, Tamaki S, et al. Successful low-dose leflunomide treatment for ganciclovir-resistant cytomegalovirus infection with high-level antigenemia in a kidney transplant: a case report and literature review. *J Clin Virol*. 2016;82:133–138.
324. Verkaik NJ, Hoek RA, van Bergeijk H, et al. Leflunomide as part of the treatment for multidrug-resistant cytomegalovirus disease after lung transplantation: case report and review of the literature. *Transpl Infect Dis*. 2013;15:E243–E249.
325. Germi R, Mariette C, Alain S, et al. Success and failure of artesunate treatment in five transplant recipients with disease caused by drug-resistant cytomegalovirus. *Antiviral Res*. 2014;101:57–61.
326. Shapira MY, Resnick IB, Chou S, et al. Artesunate as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation. *Clin Infect Dis*. 2008;46:1455–1457.
327. Marty FM, Winston DJ, Chemaly RF, et al. Brincidofovir for prevention of cytomegalovirus after allogeneic hematopoietic cell transplantation in CMV-seropositive patients. *BMT Tandem Meetings, Honolulu HI*. 2016.
328. El-Haddad D, El Chaer F, Vanichanan J, et al. Brincidofovir (CMX-001) for refractory and resistant CMV and HSV infections in immunocompromised cancer patients: a single-center experience. *Antiviral Res*. 2016;134:58–62.
329. Chou S, Ercolani RJ, Lanier ER. Novel cytomegalovirus UL54 DNA polymerase gene mutations selected in vitro that confer brincidofovir resistance. *Antimicrob Agents Chemother*. 2016;60:3845–3848.
330. Biron KK, Harvey RJ, Chamberlain SC, et al. Potent and selective inhibition of human cytomegalovirus replication by 1263 W94, a benzimidazole L-riboside with a unique mode of action. *Antimicrob Agents Chemother*. 2002;46:2365–2372.
331. Marty FM, Ljungman P, Papanicolaou GA, et al. Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplants: a phase 3, double-blind, placebo-controlled, randomised trial. *Lancet Infect Dis*. 2011;11:284–292.
332. Avery RK, Marty FM, Strasfeld L, et al. Oral maribavir for treatment of refractory or resistant cytomegalovirus infections in transplant recipients. *Transpl Infect Dis*. 2010;12:489–496.
333. Alain S, Gomes M, Moulinas R, et al. Bridging the gap? Benefits and emergence of resistance using maribavir as rescue therapy in European transplant recipients. *15th International CMV/Betaherpesvirus Workshop, Brisbane, Australia*. 2015: Abstract O59.
334. Papanicolaou G, Silveira FP, Langston A, et al. Maribavir for treatment of cytomegalovirus infections resistant or refractory to ganciclovir or foscarnet in hematopoietic stem cell transplant or solid organ transplant recipients: a randomized, dose-ranging double-blind, Phase 2 study. *ID Week, New Orleans, LA*. 2016: Abstract 78.
335. Chou S, Hakki M, Villano S. Effects on maribavir susceptibility of cytomegalovirus UL97 kinase ATP binding region mutations detected after drug exposure in vitro and in vivo. *Antiviral Res*. 2012;95:88–92.
336. Chou S, Ercolani RJ, Marousek G, et al. Cytomegalovirus UL97 kinase catalytic domain mutations that confer multidrug resistance. *Antimicrob Agents Chemother*. 2013;57:3375–3379.
337. Chou S. Rapid in vitro evolution of human cytomegalovirus UL56 mutations that confer letermovir resistance. *Antimicrob Agents Chemother*. 2015;59:6588–6593.
338. Goldner T, Hempel C, Ruebsamen-Schaeff H, et al. Geno- and phenotypic characterization of human cytomegalovirus mutants selected in vitro after letermovir (AIC246) exposure. *Antimicrob Agents Chemother*. 2014;58:610–613.
339. Product Monograph PREVYMIS™ (letermovir) Merck (USA). *PRESCRIBING INFORMATION* 2017. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209939Orig1s000,209940Orig1s000lbl.pdf. Accessed December 28, 2017.
340. Razonable RR, Humar A. AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplantation. *Am J Transplant*. 2013;13(Suppl 4):93–106.
341. Bowman JS, Green M, Scantlebury VP, et al. OKT3 and viral disease in pediatric liver transplant recipients. *Clin Transplant*. 1991;5:294–300.
342. Iragorri S, Pillay D, Scrine M, et al. Prospective cytomegalovirus surveillance in paediatric renal transplant patients. *Pediatr Nephrol*. 1993;7:55–60.
343. Green M, Michaels MG, Katz BZ, et al. CMV-IVIG for prevention of Epstein Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. *Am J Transplant*. 2006;6:1906–1912.
344. Gerna G, Lilleri D, Callegaro A, et al. Prophylaxis followed by preemptive therapy versus preemptive therapy for prevention of human cytomegalovirus disease in pediatric patients undergoing liver transplantation. *Transplantation*. 2008;86:163–166.
345. Nicastro E, Giovannozzi S, Stroppa P, et al. Effectiveness of preemptive therapy for cytomegalovirus disease in pediatric liver transplantation. *Transplantation*. 2017;101:804–810.
346. Madan RP, Campbell AL, Shust GF, et al. A hybrid strategy for the prevention of cytomegalovirus-related complications in pediatric liver transplantation recipients. *Transplantation*. 2009;87:1318–1324.
347. Saitoh A, Sakamoto S, Fukuda A, et al. A universal preemptive therapy for cytomegalovirus infections in children after live-donor liver transplantation. *Transplantation*. 2011;92:930–935.
348. Furuichi M, Fujiwara T, Fukuda A, et al. Fulminant hepatic failure as a risk factor for cytomegalovirus infection in children receiving preemptive therapy after living donor liver transplantation. *Transplantation*. 2016;100:2404–2409.
349. Verma A, Palaniswamy K, Cremonini G, et al. Late cytomegalovirus infection in children: high incidence of allograft rejection and hepatitis in donor negative and seropositive liver transplant recipients. *Pediatr Transplant*. 2017;21.
350. Bueno J, Green M, Kocoshis S, et al. Cytomegalovirus infection after intestinal transplantation in children. *Clin Infect Dis*. 1997;25:1078–1083.
351. Nayyar N, Mazariegos G, Ranganathan S, et al. Pediatric small bowel transplantation. *Semin Pediatr Surg*. 2010;19:68–77.
352. Kranz B, Vester U, Wingen AM, et al. Acute rejection episodes in pediatric renal transplant recipients with cytomegalovirus infection. *Pediatr Transplant*. 2008;12:474–478.

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.

353. Smith JM, Corey L, Bittner R, et al. Subclinical viremia increases risk for chronic allograft injury in pediatric renal transplantation. *J Am Soc Nephrol*. 2010;21:1579–1586.
354. Höcker B, Zencke S, Krupka K, et al. Cytomegalovirus infection in pediatric renal transplantation and the impact of chemoprophylaxis with (val-) ganciclovir. *Transplantation*. 2016;100:862–870.
355. Lin A, Worley S, Brubaker J, et al. Assessment of cytomegalovirus hybrid preventative strategy in pediatric heart transplant patients. *J Pediatric Infect Dis Soc*. 2012;1:278–283.
356. Mahle WT, Fourshee MT, Naftel DM, et al. Does cytomegalovirus serology impact outcome after pediatric heart transplantation? *J Heart Lung Transplant*. 2009;28:1299–1305.
357. Danziger-Isakov LA, Worley S, Michaels MG, et al. The risk, prevention, and outcome of cytomegalovirus after pediatric lung transplantation. *Transplantation*. 2009;87:1541–1548.
358. Paya CV. Indirect effects of CMV in the solid organ transplant patient. *Transpl Infect Dis*. 1999;1(Suppl 1):8–12.
359. Yamada A, Tashiro A, Hiraiwa T, et al. Long-term outcome of pediatric renal transplantation: a single center study in Japan. *Pediatr Transplant*. 2014;18:453–462.
360. Danziger-Isakov LA, Worley S, Arrigain S, et al. Increased mortality after pulmonary fungal infection within the first year after pediatric lung transplantation. *J Heart Lung Transplant*. 2008;27:655–661.
361. Liu M, Worley S, Arrigain S, et al. Respiratory viral infections within one year after pediatric lung transplant. *Transpl Infect Dis*. 2009;11:304–312.
362. Snydman DR, Kistler KD, Ulsh P, et al. Cytomegalovirus prevention and long-term recipient and graft survival in pediatric heart transplant recipients. *Transplantation*. 2010;90:1432–1438.
363. Hussain T, Burch M, Fenton MJ, et al. Positive pretransplantation cytomegalovirus serology is a risk factor for cardiac allograft vasculopathy in children. *Circulation*. 2007;115:1798–1805.
364. Indolfi G, Heaton N, Smith M, et al. Effect of early EBV and/or CMV viremia on graft function and acute cellular rejection in pediatric liver transplantation. *Clin Transplant*. 2012;26:E55–E61.
365. Camacho-Gonzalez AF, Gutman J, Hymes LC, et al. 24 weeks of valganciclovir prophylaxis in children after renal transplantation: a 4-year experience. *Transplantation*. 2011;91:245–250.
366. Varela-Fascinetto G, Benchimol C, Reyes-Acevedo R, et al. Tolerability of up to 200 days of prophylaxis with valganciclovir oral solution and/or film-coated tablets in pediatric kidney transplant recipients at risk of cytomegalovirus disease. *Pediatr Transplant*. 2017;21.
367. Patel M, Stefanidou M, Long CB, et al. Dynamics of cell-mediated immune responses to cytomegalovirus in pediatric transplantation recipients. *Pediatr Transplant*. 2012;16:18–28.
368. Lidehall AK, Engman ML, Sund F, et al. Cytomegalovirus-specific CD4 and CD8 T cell responses in infants and children. *Scand J Immunol*. 2013;77:135–143.
369. Krampe K, Briem-Richter A, Fischer L, et al. The value of immunoprophylaxis for cytomegalovirus infection with intravenous immunoglobulin in pediatric liver transplant recipients receiving a low-dose immunosupressive regimen. *Pediatr Transplant*. 2010;14:67–71.
370. Florescu DF, Langnas AN, Grant W, et al. Incidence, risk factors, and outcomes associated with cytomegalovirus disease in small bowel transplant recipients. *Pediatr Transplant*. 2012;16:294–301.
371. Green M, Kaufmann M, Wilson J, et al. Comparison of intravenous ganciclovir followed by oral acyclovir with intravenous ganciclovir alone for prevention of cytomegalovirus and Epstein-Barr virus disease after liver transplantation in children. *Clin Infect Dis*. 1997;25:1344–1349.
372. Neyts J, Jähne G, Andrei G, et al. In vivo antiherpesvirus activity of N-7-substituted acyclic nucleoside analog 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine. *Antimicrob Agents Chemother*. 1995;39:56–60.
373. Spivey JF, Singleton D, Sweet S, et al. Safety and efficacy of prolonged cytomegalovirus prophylaxis with intravenous ganciclovir in pediatric and young adult lung transplant recipients. *Pediatr Transplant*. 2007;11:312–318.
374. Pescovitz MD, Ettenger RB, Strife CF, et al. Pharmacokinetics of oral valganciclovir solution and intravenous ganciclovir in pediatric renal and liver transplant recipients. *Transpl Infect Dis*. 2010;12:195–203.
375. Vaudry W, Ettenger R, Jara P, et al. Valganciclovir dosing according to body surface area and renal function in pediatric solid organ transplant recipients. *Am J Transplant*. 2009;9:636–643.
376. Bradley D, Moreira S, Subramoney V, et al. Pharmacokinetics and safety of valganciclovir in pediatric heart transplant recipients 4 months of age and younger. *Pediatr Infect Dis J*. 2016;35:1324–1328.
377. Asberg A, Bjerre A, Neely M. New algorithm for valganciclovir dosing in pediatric solid organ transplant recipients. *Pediatr Transplant*. 2014;18:103–111.
378. Villeneuve D, Brothers A, Harvey E, et al. Valganciclovir dosing using area under the curve calculations in pediatric solid organ transplant recipients. *Pediatr Transplant*. 2013;17:80–85.
379. Launay E, Theoret Y, Litalien C, et al. Pharmacokinetic profile of valganciclovir in pediatric transplant recipients. *Pediatr Infect Dis J*. 2012;31:405–407.
380. Ranganathan K, Worley S, Michaels MG, et al. Cytomegalovirus immunoglobulin decreases the risk of cytomegalovirus infection but not disease after pediatric lung transplantation. *J Heart Lung Transplant*. 2009;28:1050–1056.
381. Renoult E, Clermont MJ, Phan V, et al. Prevention of CMV disease in pediatric kidney transplant recipients: evaluation of pp67 NASBA-based pre-emptive ganciclovir therapy combined with CMV hyperimmune globulin prophylaxis in high-risk patients. *Pediatr Transplant*. 2008;12:420–425.
382. Snydman DR, Kistler KD, Ulsh P, et al. The impact of CMV prevention on long-term recipient and graft survival in heart transplant recipients: analysis of the Scientific Registry of Transplant Recipients (SRTR) database. *Clin Transplant*. 2011;25:E455–E462.
383. Nagai S, Mangus RS, Anderson E, et al. Cytomegalovirus infection after intestinal/multivisceral transplantation: a single-center experience with 210 cases. *Transplantation*. 2016;100:451–460.
384. Gilbert C, Boivin G. Human cytomegalovirus resistance to antiviral drugs. *Antimicrob Agents Chemother*. 2005;49:873–883.
385. Kim YJ, Boeckh M, Cook L, et al. Cytomegalovirus infection and ganciclovir resistance caused by UL97 mutations in pediatric transplant recipients. *Transpl Infect Dis*. 2012;14:611–617.
386. Martin M, Goyette N, Ives J, et al. Incidence and characterization of cytomegalovirus resistance mutations among pediatric solid organ transplant patients who received valganciclovir prophylaxis. *J Clin Virol*. 2010;47:321–324.
387. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009;20:629–637.

APPENDIX

*Consensus Contributors (in alphabetical order).
Leaders: Camille N. Kotton (USA) and Atul Humar (Canada).
Diagnostics: Angela M. Caliendo (leader, USA), Randall Hayden (USA), Hans Hirsch (Switzerland), Tiziana Lazzarotto (Italy), Jutta Preiksaitis (Canada).
Immunology: Deepali Kumar (leader, Canada), Davide Abate (Italy), Oriol Bestard (Spain), Javier Carbone (Spain), Hannah Kaminski (France), Rajiv Khanna (Australia), Martina Sester (Germany).
Prevention: Atul Humar (coleader, Canada) and Camille N. Kotton (coleader, USA), Laura Barcan (Argentina), Emily Blumberg (USA), Jennifer Harrison (Canada), Nassim Kamar (France), Nicolas Mueller (Switzerland), Tomas Reischig (Czech Republic), Nina Singh (USA), David Snydman (USA), Helio Tedesco-Silva (Brazil), David Thomson (South Africa), Marty Zamora (USA).
Treatment: Shirish Huprikar (leader, USA), Jay Fishman (USA), Shahid Husain (Canada), Michele Morris (USA), Claudia Nagel (Argentina), William Rawlinson (Australia).
Resistance: Sunwen Chou (leader, USA), Sophie Alain (France), Guy Boivin (Canada), Klaus Hamprecht (Germany), Ajit P. Limaye (USA).
Pediatrics: Lara Danziger-Isakov (leader, USA), Upton Allen (Canada), Michael Green (USA), Britta Hoecker (Germany), Rebecca Madan (USA), Gustavo Varela-Fascinetto (Mexico).

Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.