

Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation

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on behalf of The Transplantation Society International CMV Consensus Group

Cytomegalovirus (CMV) continues to be one of the most common infections after solid-organ transplantation, resulting in significant morbidity, graft loss, and adverse outcomes. Management of CMV varies considerably among transplant centers but has become more standardized by publication of consensus guidelines by the Infectious Diseases Section of The Transplantation Society. An international panel of experts was reconvened in October 2012 to revise and expand evidence and expert opinion-based consensus guidelines on CMV management, including diagnostics, immunology, prevention, treatment, drug resistance, and pediatric issues. The following report summarizes the recommendations.

Keywords: Cytomegalovirus, CMV, Ganciclovir, Prevention, Prophylaxis, Resistance, Treatment, Valganciclovir.

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Cytomegalovirus (CMV) remains one of the most common complications affecting organ transplant recipients, with significant morbidity and occasional mortality. In addition to the direct effects of CMV infection and disease, there are “indirect effects,” both general and transplant specific, which can

significantly impact outcomes (Table 1). In December 2008, a panel of experts on CMV and solid-organ transplantation (SOT) was convened by the Infectious Diseases Section of The Transplantation Society to develop consensus guidelines on CMV management, subsequently published in 2010 (1). Topics included diagnostics, immunology, prevention, treatment, resistance, and pediatrics. Given numerous recent advances in the field, a second meeting of experts was convened in October 2012 to update the guidelines.

To rate the quality of evidence upon which recommendations are based, the expert panel followed a process used in the development of other guidelines, including those by the Infectious Diseases Society of America. This included a systematic weighting of the strength of recommendation and quality of evidence using the Grading of Recommendations Assessment, Development and Evaluation system (2–7), which includes a systematic weighting of the strength of recommendation (e.g., “high, moderate, low, very low”) and quality of evidence (e.g., “strong, weak”) (Table 2).

For clarity, the following definitions, which are consistent with the American Society of Transplantation recommendations for use in clinical trials (8), are used in this document:

- CMV infection: evidence of CMV replication regardless of symptoms (differs from latent CMV).
- CMV disease: evidence of CMV infection with attributable symptoms. CMV disease can be further categorized as a viral syndrome with fever, malaise, leukopenia, and/or thrombocytopenia or as tissue-invasive disease.

In addition, the term DNAemia will be used instead of viremia to reflect the detection of CMV DNA in blood or plasma (whether actively replicating virus or not). The phrases

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TABLE 1. Possible indirect effects of CMV**Transplant-specific indirect effects**

Chronic allograft nephropathy and/or allograft loss after renal transplantation (144, 246, 247)

Accelerated hepatitis C virus recurrence after liver transplantation (248)

Hepatic artery thrombosis after liver transplantation (249–251)

Allograft vasculopathy after cardiac transplantation (252, 253)

Bronchiolitis obliterans after lung transplantation (131, 254, 255)

General indirect effects—elevated risks

Bacterial infections (144, 256, 257)

Fungal infection (144, 148)

Viral infections (summarized in (258))

Posttransplantation lymphoproliferative disorder (259)

Cardiovascular events (260)

New-onset diabetes mellitus after transplantation (261, 262)

Immunosenescence (263)

Acute rejection (131, 257, 264)

Mortality (144, 251, 254–256, 265)

The association between CMV disease and these indirect effects has not been demonstrated in all studies. References listed here are examples supporting these statements and are not meant to include all references on this topic. Additional references can be found in the comprehensive review by Freeman; (266) table modified from Kotton (267).

“viral load” or “quantitative nucleic acid amplification testing (QNAT)” will replace the use of “polymerase chain reaction (PCR)” for enhanced accuracy. The definition of CMV syndrome has been less stringent in recent studies, perhaps resulting in higher rates of CMV syndrome than in previous trials.

DIAGNOSTICS

Pretransplantation Management

CMV serology should be performed before transplantation on both the organ donor and the recipient. A test measuring anti-CMV IgG should be used, as IgG serologic tests have better specificity compared with IgM or combination IgG and IgM tests; neither of which should be used for screening, because false-positive IgM reactions may significantly decrease screening specificity (9–11). Because donor and recipient serostatus (cited as D/R) are key predictors of infection risk and management, it is imperative that a test with high sensitivity and specificity be used. Not all serologic tests are equivalent; thus, it is important to understand the performance characteristics of the specific test used (12). A change in the serologic test requires evaluation of the test performance, including comparison with the previously used test. If the donor or recipient is seronegative during the pretransplantation evaluation and there is a significant time interval between screening and transplant, 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 blood products and in seropositive children younger than 12 months, as passive transfer of antibody can lead to transient false-positive serologic results; (13) a pretransfusion sample is preferable for testing if available.

There is some evidence that cell-mediated immunity assays may be useful in assisting in establishing true serostatus in both transfused patients and children younger than 12 months (14, 15). In infants and children younger than 12 months, culture or nucleic acid amplification tests (NAT) of urine or throat swabs may be helpful to identify infected patients, as children shed virus for long periods after primary infection. In adults, an equivocal serologic assay result in the donor should be assumed to be positive, whereas this result in the recipient should be interpreted to assign the recipient to the highest appropriate CMV risk group for posttransplantation management decisions.

Posttransplantation Role of Diagnostics

Serology has no role in the diagnosis of active CMV disease after transplantation. 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. Viral culture of blood for CMV has limited clinical utility for diagnosis of disease due to poor sensitivity. There is no role for CMV urine culture in the diagnosis of disease due to poor specificity (16). Viral load testing is the cornerstone for diagnosis and monitoring for CMV infection and disease; both QNAT and antigenemia testing are available for these purposes.

The CMV pp65 antigenemia test is a semiquantitative test that is useful for the diagnosis of clinical disease, initiating preemptive therapy and monitoring response to therapy (17–21). Studies have shown that higher numbers of positive staining cells correlate better with disease (17, 18), although tissue-invasive disease can occur with low or negative cell counts. The antigenemia test has advantages in some settings, because it does not require expensive equipment and the assay is relatively easy to perform. There are problems with a lack of assay standardization, including subjective result interpretation, and it is unlikely that better standardization of this assay will occur, because most laboratories have moved to molecular methods. The assay performance diminishes when the absolute neutrophil count is less than 1000/mm³. The test is labor intensive, and the blood specimen has limited stability and should be processed within 6 to 8 hr of collection to avoid a decrease in test sensitivity; thus, transplant centers managing patients at distant sites whose blood samples are mailed into the laboratory may prefer to use QNAT rather than antigenemia.

QNAT is the most widely used method for diagnosis, preemptive strategies, and monitoring response to therapy (22–28). Real-time QNAT methods are now the standard of care, because they have better precision, broader linear range, faster turnaround time, higher throughput, and less risk of contamination compared with conventional polymerase chain reaction tests (29). The testing requires expensive equipment and reagents, although testing is less complex with the availability of an increasing number of commercial reagents. Plasma and whole-blood specimens both provide prognostic and diagnostic information regarding CMV (30–34). CMV DNA is generally detected earlier and in greater quantitative amounts in whole blood compared with plasma; one specimen type should be used when serially monitoring patients. One study showed that persistent plasma DNAemia was a better predictor of relapse at day 21 of treatment compared

TABLE 2. Grading of Recommendations Assessment, Development and Evaluation strength of recommendations and quality of the evidence (2–6, 268, 269)

| Strength of recommendation and quality of evidence | Clarity of balance between desirable and undesirable effects | Methodologic 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, methodologic 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 one 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 one 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 one 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, methodologic 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 one 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 one critical outcome from unsystematic clinical observations or very indirect evidence | Other alternatives may be equally reasonable. Any estimate of effect, for at least one critical outcome, is very uncertain. |

RCTs, randomized controlled trials.

with persistent whole-blood DNAemia (34). CMV DNA is stable for 14 days at 4°C in both plasma and whole blood (35), which may be important when specimens are shipped.

There is poor interinstitutional correlation of QNAT results partly due to the historical lack of an international reference standard and variation in assay design (36). This has prevented the establishment of broadly applicable cutoffs for clinical decision-making, particularly for preemptive strategies. In October 2010, a World Health Organization (WHO) International Reference Standard became available from the National Institute of Biological Standards and Controls (United Kingdom). The standard was made from a clinical isolate (Merlin) and has a titer of 5×10^6 IU/mL. All commercial and laboratory developed tests should be recalibrated and show colinearity to the WHO International Standard and results should be reported as IU/mL. A recent study showed good reproducibility in viral load values across multiple laboratories when using a commercial test calibrated to the WHO standard (37). Additional sources of variability include the specific target, probe, and extraction method (38). It remains imperative that laboratories use an external quantitative standard material (independent of that provided by the manufacturer) to monitor quantification across different lots of reagents to ensure consistency of assay performance. If the laboratory changes QNAT or extraction method, there must be a comparison of the performance characteristics of the new versus old tests. Interinstitutional comparison of QNAT values requires cross-referencing via specimen exchange or common external reference material (39). Until test harmonization has been clearly demonstrated, a single test should be used for clinical trials and for monitoring patients over time.

Multiple and mixed viral genotypes are associated with higher rates of CMV infection and disease (40). There is currently no evidence that genotyping is needed for patient management other than to confirm antiviral drug resistance (see below).

There are few natural history studies available to help define trigger points for intervention therapy when using a preemptive approach. Two studies have shown that higher viral load values correlate with increased risk for disease (26, 27). One study (27) established a cutoff for predicting disease of 2000 to 5000 copies/mL in plasma in CMV seropositive liver transplant recipients, using a commercial QNAT (Amplicor Monitor), which is no longer available. This cutoff may not apply to different specimen types, other assays, or in different populations and risk groups. A recent study of low-risk CMV seropositive kidney, heart, and liver transplant recipients not receiving antilymphocyte globulins suggested a preemptive therapy trigger point of 3893 IU/mL plasma (41). 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 (i.e., more frequent testing will be more effective). Until viral load result harmonization is achieved, optimal trigger points for intervention cannot be determined.

Trends in viral loads over time may be more important in predicting disease than any absolute viral load value (26), especially with lower copy numbers. The limit of detection varies among the different viral load tests; a lower limit of detection of greater than 1000 IU/mL (using either whole blood or plasma) may be inadequate to detect disease (42),

because some patients with end-organ disease may have very low to undetectable viral load values. Conversely, a very sensitive test (limit of detection <10 IU/mL) may detect latent virus, particularly if whole-blood specimens are used, which limits the clinical utility of an extremely sensitive test. QNAT results should be linear throughout the important range of clinical values (up to ~1 million IU/mL). The precision of QNAT results is such that changes in values should be at least threefold ($0.5 \log_{10}$ copies/mL) to represent biologically important changes in viral replication (37, 39, 43). QNAT variability is greatest for low viral loads, where changes may need to be greater than fivefold ($0.7 \log_{10}$ copies/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.

QNAT is preferred for diagnosis and monitoring of CMV infection and disease, as the international reference standard will allow test harmonization. No such standard is available for the antigenemia test. The antigenemia test may still be used depending on available resources, technical expertise, patient population, required turnaround time, volume of samples tested, and cost. Ideally, CMV QNAT and antigenemia results should be available within 24 to 48 hr.

Diagnostics for Tissue-Invasive Disease

The definitive diagnosis of tissue-invasive disease relies on detection of CMV in the tissue specimen, with the exception of central nervous system disease and retinitis. Identification of inclusion bodies or viral antigens in biopsy material by immunohistochemistry (44, 45) is the preferred method for the diagnosis of tissue-invasive disease. Cultures (either more rapid shell vial or routine viral culture) should routinely be sent on gastrointestinal biopsies given the diagnostic challenges with potentially negative blood testing. Culture or QNAT results of a tissue specimen may be difficult to interpret, particularly in the setting of active viremia, as they could reflect shedding as well as active disease; however, if the tissue immunohistochemistry and blood DNAemia are negative, a positive tissue culture or QNAT can support the diagnosis of tissue-invasive disease. QNAT on tissue samples should be normalized using a housekeeping gene. The diagnosis of tissue-invasive CMV disease, such as hepatitis and gastrointestinal infection, should be confirmed by immunohistochemistry or *in situ* DNA hybridization (46–48). When performing histopathology of biopsy specimens, immunostaining should be routinely performed to maximize sensitivity. Not all antibodies have equal sensitivity and the performance may also differ between fresh and formalin-fixed, paraffin-embedded tissue (48). Gastrointestinal disease in all transplant types and pneumonitis in lung transplant recipients may have undetectable or low viral load values in peripheral blood samples (49, 50).

A positive culture or qualitative NAT from bronchoalveolar lavage (BAL) specimens in lung and nonlung transplant recipients may reflect viral shedding rather than pulmonary disease (51, 52). Immunocytochemistry of BAL cells may improve the predictive value of a positive culture. QNAT on BAL specimens has shown improved sensitivity without loss of specificity (50, 53, 54). High viral load values may be predictive of pneumonitis in lung transplant recipients; normalization of the dilution factor in BAL specimens (by comparison

of the plasma/bronchoalveolar urea concentration) may improve the predictive value (55, 56). Clinical trials to further evaluate QNAT on BAL specimens are required.

Central nervous system disease in SOT recipients is extremely rare. In the absence of extensive clinical studies, the presence of CMV DNA in the cerebrospinal fluid (CSF) likely represents CMV disease necessitating treatment. The diagnosis of retinitis is based on ophthalmologic examination; viral load in blood, plasma, or other laboratory tests are rarely useful as predictors of CMV retinitis, although they may be positive before and at the time of diagnosis. A positive viral load in vitreous fluid may be helpful in guiding the diagnosis of retinitis.

Consensus Recommendations

- Pretransplantation donor and recipient serology should be performed. If pretransplantation serology of the recipient is negative, retest at time of transplantation (strong, low). In adults, an equivocal serologic assay result in the donor should be assumed to be positive, whereas this result in the recipient should 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.)
- Viral culture of blood or urine has a very limited role for the diagnosis of disease. Histology/immunohistochemistry is the preferred method for diagnosis of tissue-invasive disease. Culture and QNAT of tissue specimens have a limited role in the diagnosis of invasive disease but may be helpful in gastrointestinal disease, where blood QNAT may not be positive. Positive culture of BAL samples may not always correlate with disease (strong, moderate).
- Histopathologic examination of tissue should routinely include immunostaining or in situ hybridization for CMV (strong, moderate).
- QNAT is preferred for diagnosis, decisions regarding preemptive therapy, and monitoring response to therapy due to the ability to harmonize and standardize these tests (strong, moderate). If QNAT is not available, antigenemia is an acceptable alternative.
- Either plasma or whole blood is an acceptable specimen for QNAT, with an appreciation of the differences in viral load values and viral kinetics. Specimen type should not be changed when monitoring patients (strong, moderate).
- Commercial and laboratory-developed tests must be calibrated and show colinearity to the WHO international standard; results should be reported as IU/mL (strong, moderate).
- Until harmonization of viral load tests is achieved, it is not possible to establish universal quantitative levels for trigger points of therapy or treatment endpoints. Establishment of trigger points will require standardization of preemptive protocols, including monitoring frequencies. In the interim, laboratories must establish their own cutoffs and audit clinical outcomes to verify the trigger points used (strong, moderate).

Future Directions

Numerous questions remain unanswered in this field. Future studies are needed to:

- Compare the performance characteristics of the different serologic tests and assess the utility of cell-mediated immunity assays and QNAT using a variety of sample types for the interpretation of passive immunity due to transfusion of blood products and maternal antibodies.
- Determine the commutability of the WHO International Standard (for whole blood, plasma, BAL specimens, CSF, and other sample types) and assess test harmonization after recalibration of tests with the WHO standard.
- Determine the viral form (virions, fragmented, or genomic CMV) and viral kinetics in cellular and acellular compartments in peripheral blood and other sampling sites.
- Directly compare QNAT monitoring in plasma, whole blood, and BAL specimens with respect to disease prediction and monitoring response to therapy.
- Assess the role of digital QNAT to eliminate the need for international standards.

Once viral load tests are harmonized, establish values for initiating preemptive therapy and end of treatment decisions.

IMMUNOLOGIC MONITORING FOR CMV AND CMV VACCINES

Immunologic Control of CMV

Immunologic control of CMV in the immunocompromised host is complex, involving both the innate and adaptive immune systems (57–59). In the innate immune system, polymorphisms of Toll-like receptors 2 and 4 are associated with an increased risk of CMV disease. Single nucleotide polymorphisms in genes for mannose binding lectin and ficolin-2 may be associated with increased risk of CMV disease (60–63). Natural killer cells play a critical role in the control of primary and recurrent CMV infection, typically increasing in frequency in response to viral replication (64, 65). Adaptive immune responses of B and T lymphocytes are critical in controlling CMV replication. B cells are important in the humoral response to CMV, producing neutralizing antibodies that primarily target glycoprotein B (gB) and glycoprotein H (57, 58) and the pentameric glycoprotein H/gL/UL128/UL130/UL131A complex (66–68). A significant number of posttransplantation patients develop hypogammaglobulinemia (26%–70%), and hypogammaglobulinemia was a risk factor for CMV infection in heart and lung transplant recipients but not in liver and kidney transplant recipients; (69–72) the link with CMV risk remains controversial.

T-cell responses, particularly CD4⁺ and CD8⁺ T cells, are critically important components of CMV immune control. There is increasing knowledge about $\gamma\delta$ T-cell expansion in CMV infection; patients who have late expansion of $\gamma\delta$ T cells in response to CMV appear to have longer duration of infection (73, 74). Regulatory T cells (Tregs) may also play a role in the control of CMV viremia. Studies show that low levels of Tregs favor CMV control (75, 76). Despite these advances in knowledge on $\gamma\delta$ T cells and Tregs, the majority of literature is directed toward the study of CD4⁺ and cytotoxic CD8⁺ T lymphocytes (57–59). T-cell reactivity is directed toward a wide range of CMV antigens such as pp65, pp50, IE-1, gB, and others (57, 77). Sufficient levels of polyfunctional T cells that express a variety of cytokines such as interferon (IFN)- γ , tumor necrosis factor- α , and interleukin (IL)-2 seem especially important in CMV control, whereas the loss of T-cell

polyfunctionality and/or up-regulation of anergy markers appears to promote increased CMV replication (78, 79). The key role of T cells in the control of CMV has been further demonstrated (primarily in hematopoietic stem cell transplantation [HSCT] recipients) through the use of adoptive immunotherapy for both prophylaxis and therapy of CMV infection. In seropositive SOT recipients, it is feasible to generate CMV-specific T cell lines, potentially for adoptive immunotherapy (80). A case report in a lung transplant recipient described how infusion of autologous T cells stimulated with CMV antigens *ex vivo* resulted in temporary control of CMV replication (81).

Immune Monitoring

Immune monitoring of CMV-specific T-cell responses can predict individuals at increased risk of CMV disease after transplantation and may be useful in guiding prophylaxis and preemptive therapies. There are a variety of T-cell assays for CMV. Some 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 (PBMCs) with CMV-specific antigens or peptides (57–59). In addition to IFN- γ , other markers, including IL-2, tumor necrosis factor- α , CD107, programmed death-1 (PD-1), and CD154, have been used to correlate CMV-specific T-cell responses with the risk of CMV. 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 have specific advantages and limitations and have been studied in various clinical applications to predict disease or infection (Table 3).

The QuantiFERON-CMV assay is an enzyme-linked immunosorbent assay–based IFN- γ release CD8⁺ assay, available commercially in some regions (CE marked in Europe); it has been clinically evaluated in transplant patients at high risk of CMV and shown to be predictive of disease (82–85). A negative test before transplantation may aid in predicting viremia after transplantation (86) and the dynamics of T-cell responses may be used as a monitoring tool in preemptive management (87). In a small cohort of transplant recipients with low-level CMV DNAemia, a positive assay was predictive of spontaneous clearance (88). Test interpretation is unclear if a posttransplantation patient does not respond to the mitogen control; this may be a marker for global immunosuppression and has been associated with a subsequent higher incidence of CMV disease (84). Test sensitivity decreases with lymphopenia because an adequate number of cells are required for IFN- γ production.

The ELISpot assay quantifies T cells producing IFN- γ in response to CMV. Various ELISpot assays have been shown to be predictive of disease and viremia (89–92). As with the QuantiFERON assay, a mitogen control may indicate general T-cell responsiveness. The ELISpot assay cannot differentiate CD4⁺ and CD8⁺ T cells. Studies have used cutoffs for defining positive responses ranging between 5 and 50 spot-forming cells per 200,000 PBMC. An ELISpot assay (T-Track CMV; Lophius Biosciences, Regensburg, Germany) has recently received CE marking in Europe.

Most studies that have analyzed CMV-specific T-cell responses have used intracellular cytokine staining (ICS) for IFN- γ using flow cytometry. Unlike ELISpot or QuantiFERON assays, ICS can 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 showed an increased risk of CMV disease with low levels of specific T-cell immunity (93–96). Similarly, the absence of anti-CMV T-cell response by this technique correlates with the inability to clear viremia (93, 96, 97). Stable levels of CMV specific CD4⁺ T cells were associated with lower risk of CMV replication (93, 96, 98). The development of T-cell immunity has been associated with freedom from CMV disease after lung transplantation (99). The predictive value for viremia may be improved when the analysis of IFN- γ is combined with other cytokines (IL-2) and markers (PD-1) (79).

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 (100, 101). Both ICS and MHC-multimer staining require a fluorescence-activated cell sorting facility, which limits widespread use.

The ImmuKnow (Cylex, Columbia, MD) assay is not specific for CMV. This assay, which is commercially available in the United States and some European countries, measures overall immune function by determining the amount of ATP produced in response to whole blood stimulation by phytohemagglutinin. A Cylex assay specific to CMV is available for research purposes and has been studied in a cohort of lung transplant recipients to monitor CMV-specific responses over time; (102) there are no studies indicating whether this assay is predictive of CMV viremia or disease.

Despite the lack of widely available assays, 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. Data are accumulating that suggest that immune monitoring may be valuable in combination with viral load monitoring. Clinical utility studies are needed that demonstrate that alteration of patient management based on the results of an immune-based assay is feasible, safe, and cost-effective. Potential areas of application for immune-based assays are summarized in Table 4.

Immune-based assays may have utility for risk stratification of patients before transplantation. In one study, determination of T-cell immunity before transplantation was able to predict CMV replication after transplantation (86). Preliminary studies suggest that detection of CMV-specific T cells may be an alternative to serology in accurately determining CMV immune status in adults and children with passively transfused or maternal antibodies (14, 15).

TABLE 3. Advantages and limitations of various assays for immune monitoring of CMV

| Assay | Advantages | Limitations | Comments | Predict viremia | Predict disease |
|---------------------------------|---|---|--|---|-----------------|
| Intracellular cytokine staining | Whole-blood assay with low blood volume (1 mL) or PBMCs; short incubation time; results available after 8 hr; 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 (Cellestis) | Whole-blood assay with low blood volume (3 mL); simple to perform; results available after 30–40 hr; 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 hr | Need for purified PBMCs from at least 10 mL blood; 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) | 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 |
| Cylex Immuknow | CD4 ⁺ response | Not specific for CMV | Commercially available in the United States | No | No |

CMV Vaccines

Several CMV vaccines are under development; none are currently available for routine clinical use. Types of vaccines includes live attenuated, DNA, subunit, and recombinant viral vaccines (59). 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 although CMV disease was attenuated, the vaccine failed to prevent infection (103, 104). A recombinant gB vaccine with MF59 adjuvant was shown to induce neutralizing antibodies (105) and prevent infection (106). In a recent study, this vaccine was administered in a three-dose schedule to both CMV seropositive and seronegative transplant candidates (107). 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 has been completed. This vaccine was administered as one pre-transplantation dose and five posttransplantation doses. It showed a significant reduction in viremia versus placebo as well as a reduction in the number of CMV episodes (108). A clinical trial with the plasmid vaccine in SOT is under development. 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 (109). Other vaccines include canary pox gB and pp65 vaccines that produce T-cell responses and neutralizing antibodies (110, 111). An adenoviral chimeric vaccine encoding gB and multiple CMV epitopes was able to produce a robust cellular response and neutralizing antibodies in mice (112). In general, CMV vaccines have reached human studies with clinical endpoints but are still in early stages of clinical development.

Consensus Recommendations

- Hypogammaglobulinemia may increase the risk of CMV disease after transplantation. Measurement of total immunoglobulins for the prevention of CMV is not routinely recommended, although it may be used in situations where CMV is difficult to control (weak, low).
- Immunologic monitoring can be used as an adjunct tool to predict risk of viremia and disease in the postprophylaxis and preemptive setting (strong, moderate).

- 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 (e.g., reduction in viral replication) can be used to evaluate vaccine efficacy (strong, moderate).

Future Research 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 improved for ease of use and standardization. An ideal immune monitoring assay should provide information on both CMV-specific CD4⁺ and CD8⁺ T-cell frequency and function. Optimally, the assay should measure IFN- γ and additional markers that indicate functionality and/or anergy. In addition, Tregs and $\gamma\delta$ 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. In addition, cutoff values for positivity need to be established for ELISpot and ICS assays.
- Immune-based assays could have potential to be used as an alternative or an adjunct to serology in children and adults where potential passive antibody immunity is a concern; further studies are needed especially in transplant candidates younger than 12 months.
- Adoptive T-cell therapy for CMV has been used in clinical studies of HSCT recipients. This is an area where formal clinical studies in organ transplantation are needed.
- 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 the burden of disease or impact the course of latent CMV infection in seropositive patients; vaccination trials

TABLE 4. Potential clinical scenarios for the use of immune-based assays

| Clinical scenarios | Assays studied | Potential clinical management ^a |
|---|------------------------------------|---|
| CMV D+/R- and R+ at the end of prophylaxis | QFT, ELISpot, ICS | For negative assay, prolong prophylaxis; for positive assay, no further prophylaxis |
| CMV D+/R- and R+ during preemptive strategy | QFT, ELISpot, ICS | Result may help guide frequency of viral load monitoring and thresholds for initiating antiviral therapy |
| Posttherapy for acute rejection | ICS (small number, not predictive) | For negative assay, restart prophylaxis or viral load monitoring; for positive assay, no further intervention |
| Recent completion of therapy for CMV disease or viremia (prediction of relapse) | No studies | For negative assay, secondary prophylaxis; for positive assay, no further therapy |
| Risk stratification in patients before transplantation | ICS, QFT | For positive assay, assume true positive CMV status |

^a No formal studies of clinical management have been published to date. QFT, QuantiFERON-CMV.

should therefore focus on this group also; and (iii) vaccine studies should include an evaluation of both humoral and cellular immunity where applicable as well as longevity of responses.

PREVENTION OF CMV

Strategies to prevent CMV have significantly reduced CMV disease and decreased the “indirect effects” of CMV infection. Two major strategies are commonly employed for the prevention of CMV: universal prophylaxis and preemptive therapy. There is significant variation in clinical application of these strategies among centers.

Universal Prophylaxis

Universal prophylaxis involves the administration of antiviral medication to all patients or a subset of “at-risk” patients. Antivirals are usually begun in the immediate or very early posttransplantation period and continued for a finite period of time, often in the range of 3 to 6 months. Several antivirals have been evaluated for universal prophylaxis, including acyclovir, valacyclovir, intravenous ganciclovir, oral ganciclovir, and valganciclovir. In early studies, acyclovir was determined to be inferior to ganciclovir for the prevention of CMV (113). A large study comparing oral ganciclovir to valganciclovir in D+/R- transplant patients (PV16000) demonstrated equivalent efficacy; concern was raised regarding an increased incidence of tissue-invasive disease in liver transplant patients who received valganciclovir (114). Late-onset CMV disease, defined as disease occurring after the discontinuation of prophylaxis, has been found in all studies evaluating universal prophylaxis. In the PV16000 study, late-onset CMV disease occurred in 18% at 12 months (closer to 30% when including investigator-treated disease) (114). In the Improved Protection Against Cytomegalovirus in Transplantation study in D+/R- kidney recipients, 36.8% of those on 100 days of prophylaxis developed confirmed CMV disease compared with 16.1% on 200 days of prophylaxis ($P<0.0001$) (mostly viral syndrome) (115) at 1 year of follow-up; by 2 years after transplantation, 63 of 163 (38.7%) patients in the 100-day group developed CMV disease compared with 33 of 155 (21.3%) patients in the 200-day group ($P<0.001$). There were similar rates of biopsy-proven acute rejection and graft loss (116). The determinants of late-onset CMV disease in patients receiving prophylaxis have not been fully elucidated but are likely related to ongoing immunosuppression accompanied by a lack of development of significant CMV-specific cell-mediated immunity. Risk factors for late-onset disease include D+/R- serostatus, shorter courses of prophylaxis, higher levels of immunosuppression, and allograft rejection (115, 117).

Preemptive Therapy

With preemptive therapy, laboratory monitoring is performed at regular intervals to detect early viral replication; once viral replication reaches a certain assay threshold, optimally before the development of symptoms, antiviral treatment is initiated to prevent the progression to clinical disease. Diagnostic improvements and better availability of assays have made this approach more feasible in the past decade. Given the variability among diagnostic assays (36), a threshold for starting therapy cannot be defined, although there was strong consensus that a very low threshold should be used especially with D+/R-, as higher thresholds may result in higher rates

of disease and antiviral resistance; (118) some recommend starting treatment with any detectable DNAemia, as viral kinetics are unpredictable and may increase very rapidly (119, 120). Preemptive therapy is more difficult to coordinate because it requires weekly laboratory monitoring and prompt results. In settings where viral doubling time is very rapid (especially with D+/R- (118, 119)), there may be insufficient time to begin treatment for CMV infection before the development of symptoms or tissue-invasive disease; one recent study demonstrated a median (range) doubling time of 1.54 (0.55–5.5) days in D+/R- compared with 2.67 (0.27–26.7) days in the D+/R+ recipients ($P<0.0001$) (119). High rates of CMV disease would be expected if therapy is not initiated in a timely manner. Coordinating the logistics of routine screening, reviewing results, initiating therapy rapidly after positive assays, and performing subsequent monitoring and management may be difficult or not possible for some centers. Only one assay and one specimen type, either whole blood or plasma, should be used for an individual patient to ensure comparability of results.

The advantages of preemptive therapy include lower rates of late CMV, more selective drug targeting, decreased drug cost, and associated toxicities. One of the major concerns with preemptive therapy is that it may not prevent the indirect effects of CMV infection, including effects on graft and patient survival; recent studies have demonstrated conflicting data (121–124). In addition, second episodes of replication are observed in about 30% of patients treated for CMV DNAemia, some of which require further therapeutic intervention (125). Patients who are D+/R-, certain transplant types (e.g., lung), and those on potent immunosuppression are more prone to recurrent episodes of DNAemia (discussed further in Treatment section).

Universal Prophylaxis Versus Preemptive Therapy

A comparison of universal prophylaxis with preemptive therapy is provided in Table 5. Preemptive therapy was directly compared with prophylaxis in renal transplant recipients in four randomized trials (121–124) with long-term results available in three studies (123, 126, 127). The results were somewhat contradictory and notably affected by the variation in frequency of CMV monitoring in preemptive therapy groups and perhaps by the fact that three different antiviral drugs were used for prophylaxis. The studies using weekly monitoring for 4 months after transplantation with high compliance rates showed similar reductions of CMV disease (121, 122), intra-graft CMV infection (128), and comparable (126) or better (127) long-term graft survival in patients managed by preemptive therapy approach. Long-term follow-up in one study showed that multiple posttransplantation outcomes, including acute rejection, graft loss, cardiovascular events, new-onset diabetes mellitus, allograft nephropathy, chronic rejection, and mortality, were similar between prophylaxis and preemptive therapy groups; (126) in another study, preemptive therapy improved 4-year graft survival (92% vs. 74%; $P=0.049$) as a result of worse outcomes in patients with late-onset CMV DNAemia (127). In contrast, with less frequent monitoring, preemptive therapy failed to prevent CMV disease (123, 124) and long-term graft survival was superior with prophylaxis (123). Although the overall

TABLE 5. Comparison of prophylaxis versus preemptive therapy

| | Prophylaxis | Preemptive therapy |
|--------------------------------|-------------------|---|
| Early CMV DNAemia | Rare | Common |
| Prevention of CMV disease | Good efficacy | Good efficacy (less optimal in high-risk populations) |
| Late CMV (infection/disease) | Common | Rare |
| Resistance | Uncommon | Uncommon |
| Ease of implementation | Relatively easy | More difficult |
| Other herpes viruses | Prevents HSV, VZV | Does not prevent |
| Other opportunistic infections | May prevent | Unknown |
| Cost | Drug costs | Monitoring costs |
| Safety | Drug side effects | Less drug toxicity |
| Prevention of rejection | May prevent | Unknown |
| Graft survival | May improve | May improve |

number of D+/R- patients was low (up to ~1/3) or not always included (124), long-term graft survival was similar in D+/R- patients in all studies (123, 126, 127).

There is no published randomized trial directly comparing preemptive therapy and prophylaxis in nonrenal SOT recipients. In a relatively large cohort study including D+/R- liver transplant patients, there were no differences in the incidence of both bacterial and fungal infections and patient and graft survival for up to 3 years after transplantation between the patients treated preemptively compared with those who never develop CMV viremia (129). In another retrospective trial comparing preemptive therapy and prophylaxis in liver transplant recipients, prophylaxis was more effective than preemptive therapy in the prevention of CMV disease in D+/R-, but no differences in acute allograft rejection, other opportunistic infections, or case fatality rates were seen (130). In summary, with careful and compliant monitoring, currently available data do not suggest a superiority of either prophylaxis or preemptive therapy in renal or liver transplant recipients in either R+ or D+/R- recipients.

Due to the lack of data and the high risk of disease (especially with D+/R-), prophylaxis may be preferred in nonliver and nonkidney SOT recipients. Individual transplant centers should weigh the risks and benefits of each strategy based on their frequency of CMV disease, ability to monitor recipients (i.e., logistics), cost of antiviral medications, frequency of late-onset CMV disease, and occurrence of indirect effects. Given the high rates of disease seen in D+/R-, centers may prefer to not use preemptive therapy in D+/R-; in one recent study of renal and liver transplant recipients monitored once to twice a week, the majority of D+/R- patients developed viremia and required treatment (viremia 58/74 [78%] and treatment 51/74 [69%]), with significant rates of multiple episodes of viremia especially in kidney recipients (119). CMV infection and disease within the lung allograft are associated with chronic lung allograft dysfunction (131, 132), such that universal prophylaxis is preferred over a preemptive approach in the majority of lung transplant centers (133). With high rates of CMV complications in vascularized composite allotransplantation, especially when active infection is managed concomitantly with acute graft rejection, prophylaxis is generally preferred; (134–138) consideration should be given to possible donor–recipient matching, because this type of transplant is “life-enhancing” and not considered “life-saving.”

Hybrid Approach

A number of transplant centers utilize a hybrid strategy in which preemptive monitoring is initiated after completing prophylaxis (often 90–100 days in studies). Use of a hybrid strategy is not supported by the available data (139–142) and cannot be routinely recommended at this time (Table 6). The majority of experts at the meeting reported using a hybrid approach at least occasionally at their center, however, especially for high-risk recipients.

Late-Onset Disease After Discontinuing Prophylaxis (“Late CMV”)

The occurrence of late-onset CMV disease after discontinuing prophylaxis is an important issue and is associated with higher rates of mortality (143) and graft loss (144). Transplant centers should monitor clinically for signs and symptoms of late-onset CMV disease and educate their patients about symptoms of CMV disease. Additional strategies to prevent late-onset disease may be considered in high-risk subgroups of patients. Monitoring after the end of prophylaxis is often used but has not been shown to be effective (see Hybrid section). Other strategies include prolongation of prophylaxis or use of immunodiagnosics to better define risk.

Consensus Recommendations

Use of Prophylaxis versus Preemptive Therapy

- Both universal prophylaxis and preemptive strategies are viable approaches for the prevention of CMV disease (strong, high).
- For D+/R-, the majority of consensus conference participants endorsed the use of either prophylaxis or preemptive therapy after kidney and liver transplantation (strong, high). For centers or patients unable to meet the stringent logistic requirements required with a preemptive therapy strategy, prophylaxis is preferred.
- For D+/R-, the majority of consensus conference participants endorsed the use of prophylaxis over preemptive therapy after heart and lung transplantation 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 (i.e., hand and face); prophylaxis may be preferable over preemptive therapy until more data are available (weak, very low).

TABLE 6. Summary of data assessing the hybrid strategy of preemptive monitoring after 90–100 days of antiviral prophylaxis in solid organ transplant recipients

| Study | Patients | Frequency of monitoring | Duration of monitoring after prophylaxis | Method of detection | CMV treatment trigger | CMV disease (D+/R-) | CMV disease (R+) |
|-----------------------------|--------------------------------|--|--|---------------------|-----------------------|----------------------|------------------|
| Lisboa et al. (139) | 71 D+/R- SOT | Weekly | 8 weeks | Plasma QNAT | 25,000 copies/mL | 41% (29/71) | N/A |
| Boillat Blanco et al. (141) | 86 (30 D+/R- and 56 R+) kidney | Every 2 weeks | 3 months | Whole-blood QNAT | 10,000 copies/mL | 23% (7/30) | 0% (0/56) |
| van der Beek et al. (142) | 29 D+/R- kidney | Weekly (most likely) | 3 months | Plasma QNAT | Detected | No end-organ disease | N/A |
| Montejo et al. (140) | 23 D+/R- liver | Every other week × 3 months; then monthly × 6 months | 9 months | Antigenemia | +Antigenemia | 9% (2/23) | N/A |

- For seropositive recipients after kidney, liver, and heart transplantation, either strategy is acceptable. Preemptive therapy has not been well studied in some seropositive populations, including lung, heart, vascularized composite, pancreas, islet, and intestinal transplantation; prophylaxis may be preferable (weak, low).
- Prophylaxis may be preferred in other high-risk patients, 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 (weak, moderate) (145).
- Transplant recipients on mammalian target of rapamycin (mTOR) inhibitors such as sirolimus and everolimus may have lower rates of CMV; (146–152) whether this should alter their prevention strategy is unknown.
- Routine viral load monitoring (without symptoms) in patients receiving antiviral prophylaxis or at the conclusion of antiviral prophylaxis has not been shown to be of benefit.
- To mitigate the risk of late CMV, some use a hybrid approach (i.e., prophylaxis followed by preemptive therapy), especially for those recipients felt to be at high risk for late CMV disease. With the limitations of the available data, the routine use of the hybrid strategy is not recommended at this time in any risk group (weak, low).

Prophylaxis Strategy D+/R-: Recommended Durations

- Where possible, 6 months may be preferable for D+/R- kidney recipients (strong, high).
- The duration of prophylaxis in D+/R- patients after liver, heart, and pancreas transplantation should generally be between 3 months (strong, moderate) and 6 months (strong, low).
- Three months is recommended after islet transplantation (weak, low).
- The decision to use 3 versus 6 months or longer of prophylaxis may depend on degree of immunosuppression, including the use of antilymphocyte antibodies for induction.
- Between 6 and 12 months prophylaxis is recommended for D+/R- lung transplant recipients (strong, moderate) (131, 153–156). Compared with extended prophylaxis, short duration (3 months) prophylaxis is associated with increased CMV infection and disease (155). In a recent study of 6 months of prophylaxis, almost 50% of D+/R- lung transplant patients developed late-onset CMV infection or disease: 29% developed CMV infection and 20% developed CMV disease (156), again suggesting longer prophylaxis may be warranted.
- A minimum of 6 months of prophylaxis is recommended for D+/R- vascularized composite (i.e., hand and face) and intestinal transplant recipients (weak, low).

Prophylaxis Strategy R+: Recommended Durations

- When a prophylaxis strategy is used for the 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 kidney, pancreas, liver, and heart transplant recipients (strong, high/moderate) and islet (weak, low).
- In those receiving 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 recommended (strong, moderate). Serostatus at the time of transplantation may help guide the duration of prophylaxis; after 6 months of prophylaxis after lung transplantation, 34% of D+/R+ and only 6% of D-/R+ developed infection or disease (156).

- Although D+/R+ patients are discussed here together with the D-/R+ group, the former group is typically at higher risk for developing CMV disease (124, 156).

Prophylaxis Strategy D-/R-

In general, this population is at low risk for CMV disease. The routine use of prophylaxis against CMV (i.e., valganciclovir or ganciclovir) is not recommended in most situations with D-/R-.

- The use of leukodepleted or CMV-seronegative blood products is recommended for these recipients to decrease the risk of transfusion transmitted CMV (strong, moderate). The incremental additional benefit of screening or prophylaxis for CMV when such blood products are being used is uncertain. Extensive transfusion of blood products increases the risk of CMV disease (especially if not CMV screened or leukodepleted), and transplant centers may wish to monitor such recipients with weekly viral load testing or give CMV prophylaxis (weak, very low).
- Antiviral prophylaxis against other herpes infections (varicella and herpes simplex) with acyclovir, famciclovir, or valacyclovir should be considered.

Medications Used in Prophylaxis Strategy

When used for prophylaxis, the usual dose of valganciclovir is 900 mg daily versus treatment dose, which is 900 mg every 12 hr; both should be adjusted for renal function. Dosing of antiviral medication should be based on standard recommended dosing algorithms and adjusted for renal function (Table 7). Although some centers have successfully used half the recommended dose of valganciclovir for prophylaxis (i.e., 450 mg daily in patients with normal renal function, sometimes called “mini-dosing”) based on pharmacokinetic equivalence to 3 g daily of oral ganciclovir and also to minimize toxicity and cost of prophylaxis, there are insufficient data to support the routine use of such dosing (weak, low). Such an approach may convey more risk with D+/R-, who are at higher risk for breakthrough disease (156) and development of resistance. Future prospective studies are required to determine the efficacy of lower dose valganciclovir.

At the time of this meeting, oral ganciclovir had very limited to no availability internationally; if it were to become available, it could be used for prophylaxis after kidney, liver, heart, pancreas, and islet transplantation (but would not be recommended after lung, intestinal, or vascularized composite transplants, due to high rates of CMV, and/or lack of data). Oral ganciclovir had been the gold standard for numerous prevention studies; whereas PV16000 demonstrated noninferiority to valganciclovir (114), other trials have sometimes shown variable outcomes.

In renal transplantation, high-dose valacyclovir prophylaxis is effective for CMV disease and CMV viremia prevention in both D+/R- and D±/R+ patients (157). The

TABLE 7. Dosage recommendations for ganciclovir, valganciclovir, and valacyclovir for adult transplant patients with impaired renal function (using Cockcroft-Gault formula).

| CrCl (mL/min) | Treatment dose | Maintenance/ prevention dose |
|--|---|---|
| Intravenous Ganciclovir (adapted from 270) | | |
| ≥70 | 5.0 mg/kg q12 hr | 5.0 mg/kg q24 hr |
| 50–69 | 2.5 mg/kg q12 hr | 2.5 mg/kg q24 hr |
| 25–49 | 2.5 mg/kg q24 hr | 1.25 mg/kg q24 hr |
| 10–24 | 1.25 mg/kg q24 hr | 0.625 mg/kg q24 hr |
| <10 | 1.25 mg/kg 3 times a week after hemodialysis | 0.625 mg/kg 3 times a week after hemodialysis |
| Valganciclovir (adapted from 271, 272) | | |
| ≥60 | 900 mg every 12 hr | 900 mg once daily |
| 40–59 | 450 mg every 12 hr | 450 mg once daily |
| 25–39 | 450 mg once daily | 450 mg every 2 days |
| 10–24 | 450 mg every 2 days | 450 mg twice weekly |
| <10 | 200 mg 3 times a week after hemodialysis ^a | 100 mg 3 times a week after hemodialysis ^a |
| CrCr (mL/min) | Prevention dose (kidney only) | |
| Valacyclovir (high dose) (157) | | |
| >75 | 2000 mg four times per day | |
| 51–75 | 1500 mg four times per day | |
| 26–50 | 1500 mg three 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 valganciclovir tablets cannot be split.

efficacy seems to be comparable with oral ganciclovir prophylaxis (158, 159). Major advantages of valganciclovir regimens include less bone marrow suppression and lower cost in some settings; (157, 159, 160) disadvantages include high pill burden and neuropsychiatric side effects mainly in patients with delayed graft function (157, 159).

There are limited data to support the use of CMV immunoglobulin (CMV Ig) for prophylaxis when appropriate antivirals are given. Some centers use these products in conjunction with antiviral prophylaxis, primarily for high-risk thoracic and intestinal transplant recipients. Not all recommended agents are approved in various jurisdictions.

Recommended Medications for Prophylaxis

- Kidney transplant: valganciclovir, intravenous ganciclovir, or high-dose valganciclovir.
- Pancreas transplant (including kidney/pancreas or islet): valganciclovir or intravenous ganciclovir.
- Liver transplant: intravenous ganciclovir or valganciclovir. In a subgroup analysis, valganciclovir was associated with a higher rate of tissue-invasive disease in liver transplant recipients (114). Multiple studies have shown noninferiority to oral ganciclovir; (143, 161–164) however, some do show less optimal outcomes (165). In one survey, it was the most commonly used drug for CMV prevention in liver transplant recipients (166).
- Heart, lung, intestinal, and vascularized composite (i.e., hand and face) transplants: valganciclovir, intravenous ganciclovir, +/- CMV Ig.

Preemptive Therapy Strategy

- When a preemptive therapy strategy is used, it is recommended that the center develop and validate their local protocol (167). Because preemptive therapy relies on laboratory monitoring, it is important that an appropriate threshold value be chosen for the specific assay that is used. A sufficiently low threshold for initiation of treatment during preemptive therapy is recommended to prevent the majority of CMV disease. There is insufficient evidence to recommend universal threshold values.
- For optimal preemptive therapy, there was strong consensus that transplant recipients should be monitored by viral load testing every week for 3 to 4 months after transplantation (strong, moderate). Meticulous weekly monitoring is needed for preemptive therapy to be effective.
- The same sample type (plasma, whole blood) and assay should be used throughout the monitoring period.
- Some studies of preemptive therapy have included secondary antiviral prophylaxis for 2 to 4 weeks after treatment (123, 124). Alternatively, once treatment of DNAemia is complete, weekly monitoring can be reinitiated as originally planned (i.e., for 3–4 months after transplantation) (weak, low).
- Once a certain positive threshold is reached, therapy with treatment dose (not prophylactic dose) valganciclovir or intravenous ganciclovir (strong, high) should be started as soon as possible and continued until one or two negative tests are obtained. If there is a delay in initiating treatment, the assay should be repeated upon initiation of therapy; if it returns negative, therapy should be ceased and monitoring

reinitiated. Weekly testing while on treatment is generally recommended.

- Some programs send patients home with valganciclovir starter packs so they can rapidly initiate therapy if their testing is positive (168).
- Further studies are needed for better refinement of the preemptive therapy approach.

Prevention during Treatment of Rejection

There was consensus 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); (169) a similar strategy may be considered during treatment of rejection with high-dose steroids (weak, very low).

Future Directions

Large prospective comparative trials of prophylaxis and preemptive approaches are required to assess the preferred method of prevention in specific SOT recipients. Optimal duration of prevention for all organs also requires additional investigation. Studies thus far do not support the use of a hybrid approach, although available data have been limited by short monitoring periods, long intervals between assays, and other methodologic issues. Future studies should assess potential ways to improve the efficacy of the hybrid strategy such as more stringent monitoring, lower thresholds for initiating antiviral therapy, and the adjunctive use of immunodiagnostic assays. Optimal dosing of valganciclovir and comparison of agents across different organ transplants also requires additional investigation. The risk of resistance with different approaches also bears further exploration. New potent oral drugs are needed. Further studies on the use of novel antiviral agents for the prevention may be helpful, including CMX001, higher-dose maribavir, AIC246 (letermovir), and others. The impact of certain new immunosuppressive medications for induction and maintenance (i.e., IL-2 inhibitors and mTOR inhibitors) may affect prevention. Immunodiagnostics may eventually result in individualized prevention strategies.

CMV TREATMENT

Valganciclovir and intravenous ganciclovir were both recommended in previous guidelines (1) for the treatment of nonsevere CMV disease, based on data from the VICTOR trial (1, 42), and the equivalency of plasma concentrations obtained with valganciclovir. In recent years, the international transplant community has gained more experience with the use of valganciclovir for the treatment of CMV disease. Orally administered therapy is convenient for both the caregiver and the patient, reduces hospital stays, and avoids the risks of intravenous therapy (i.e., line sepsis and damage to veins that may be later needed for dialysis). Although valganciclovir generally has good bioavailability (~60%), systemic bioavailability remains a potential source of variability with oral formulations, particularly in patients with intestinal disease. Therefore, when optimal drug exposure is required, such as in life-threatening CMV disease, intravenous ganciclovir is recommended. In addition, there are minimal pharmacokinetic data confirming adequate valganciclovir bioavailability

in patients with gastrointestinal disease and with cystic fibrosis; further clinical studies of the reliability of drug absorption in such high-risk patients are needed (170, 171).

Whether valganciclovir or intravenous ganciclovir is used, it is important that appropriate doses be administered (Table 7). Inadequate dosing may result in lack of clinical efficacy and the development of resistance (172), whereas supratherapeutic doses increase toxicity (173). The dose of ganciclovir or valganciclovir must be adjusted according to each patient's renal function. The pivotal trials with valganciclovir and ganciclovir for the prevention and treatment of CMV disease used the Cockcroft-Gault formula (174). Use of other methods to estimate renal function such as the Modified Diet in Renal Disease formula may lead to underdosing (175). In patients with normal renal function, twice daily dosing should be used for the treatment of disease and once daily dosing for secondary prophylaxis (see below).

The recommended length of treatment is determined by the monitoring of weekly CMV viral loads and continuing treatment until one or two consecutive negative samples are obtained with a minimum treatment course of 2 weeks, which minimizes the risk for development of resistance and disease recurrence (125, 176, 177). Concurrent clinical monitoring for response to therapy is recommended. More frequent monitoring of the viral load has not demonstrated any additional therapeutic value. Plasma or whole-blood viral loads do not necessarily reflect compartmentalized disease (particularly in sanctuary sites such as CSF and vitreous humor), and patients with such disease should be treated until clinical resolution (49). Patients with gastrointestinal tissue-invasive disease also may need longer treatment courses than what is reflected by viral load testing.

Secondary prophylaxis is defined as prolonged therapy with standard prophylaxis doses (e.g., once daily) after a successful treatment course as indicated above. The use of secondary prophylaxis is variable across transplant centers, but when used the duration often ranges from 1 to 3 months (42, 125). Use and duration should reflect the likelihood of recurrent CMV infection. In cases of serious disease and in tissue-invasive disease without viremia, a longer duration of secondary prophylaxis with clinical monitoring of the specific disease manifestation may be preferred. In cases of recurrent CMV disease, secondary prophylaxis after successful retreatment may need to be prolonged (and level of immunosuppression potentially decreased).

Risk factors for recurrence of CMV infection include primary CMV infection, deceased-donor transplantation, high initial viral load, slow reduction in viral load on treatment, persistent viremia when transferred to secondary prophylaxis, multiorgan disease, and treatment of rejection during treatment for CMV disease (125, 177–179). Additional factors that influence viral decay are a high net state of immunosuppression, thoracic organ transplantation, and gastrointestinal tissue-invasive CMV disease (49, 180, 181). Knowledge of these risk factors allows for some individualization of therapy but only as a supplement to clinical and virologic monitoring.

Consensus Recommendations

- For nonsevere CMV disease, valganciclovir (900 mg every 12 hr) or intravenous ganciclovir (5 mg/kg every 12 hr) are recommended as first-line treatment in adults (strong,

moderate) (42). Valganciclovir is preferred except in cases of life-threatening disease and in situations where poor oral drug bioavailability or medication nonadherence is likely (strong, low). Conversion between the two drugs (i.e., from intravenous ganciclovir to valganciclovir) may be performed without interrupting dosing (strong, low). Oral ganciclovir, acyclovir, or valacyclovir should not be used for the treatment of CMV disease (strong, moderate). Renal function should be monitored frequently during treatment and antiviral dose adjustment (Table 7) should be performed based on renal function estimated by Cockcroft-Gault (strong, high) (174, 182). Dose reduction of valganciclovir and ganciclovir due to side effects such as leukopenia should be avoided due to risk of resistance. Other potential causes of leukopenia should be evaluated and addressed, with dose reductions or modifications made where possible to any myelosuppressive therapies such as immunosuppressive drugs (e.g., mycophenolate mofetil) or antibiotic prophylaxis (e.g., sulfamethoxazole-trimethoprim). The addition of granulocyte colony-stimulating factor should also be considered before dose reduction or cessation of antiviral therapy (strong, low).

- Treatment with valganciclovir or intravenous ganciclovir every 12 hr should be continued until viral eradication is achieved on one or two assays after a minimum of 2 weeks (strong, moderate) (28, 42, 125). Risk factors indicating possible longer treatment duration are CMV IgG seronegativity at the onset of initial viremia (42), high initial viral load, high net state of immunosuppression, thoracic transplant recipients, and gastrointestinal tissue-invasive disease (42, 49, 125, 177, 180, 181). Secondary prophylaxis with valganciclovir 900 mg once daily (renally adjusted) for 1 to 3 months may be given, with the longer duration employed in high-risk patients as outlined above (weak, low).
- Laboratory monitoring of CMV should be performed weekly during the treatment phase to monitor response (strong, moderate) (28, 42). Trends of serial monitoring are easier to interpret than an individual test result. Two consecutive negative results (preferably 1 week apart) help ensure viral clearance (strong, moderate). Periodic viral load monitoring may sometimes be performed during secondary prophylaxis (weak, moderate); the correct time interval for monitoring is not known, but more frequent monitoring should be done in those at high risk for breakthrough disease.
- Dose reduction of immunosuppressive therapy should be considered in severe CMV disease, in nonresponding patients, in patients with high viral loads, and with leukopenia (strong, low) (180, 183). If the immunosuppressive therapy is reduced, clinicians may consider returning to prior immunosuppressive treatment when adequate clinical and viral response is obtained (strong, low).
- In the case of recurrent CMV disease after a disease and drug-free period, the same treatment options apply as with a first episode of CMV disease (strong, moderate). A general evaluation of the overall immunosuppressive status of the patient should be performed and immunosuppression should be adjusted when indicated (strong, moderate) (180). For recurrent CMV disease in thoracic organ transplant recipients, intravenous immunoglobulin (IVIG) or CMV Ig may be considered as adjunctive therapy in cases of hypogammaglobulinemia (weak, moderate) (184–186).

ANTIVIRAL DRUG RESISTANCE

Risk Factors, Frequency, and Clinical Consequences

Risk factors for drug resistance include prolonged antiviral drug exposure (median, 5 months) and ongoing active viral replication due to factors such as the lack of prior CMV immunity (D+/R-), high levels of immunosuppressive therapy, or inadequate antiviral drug delivery (187). Ganciclovir resistance occurs mainly in the D+/R- subset where the usual incidence of resistance after viremia is 5% to 12% and is higher in lung transplant recipients (187–190). Presentations of drug-resistant CMV infection range from asymptomatic (e.g., when drug resistance is monitored during antiviral prophylaxis clinical trials (191)) to severe or fatal end-organ disease (192). During CMV prophylaxis, the incidence of ganciclovir resistance is low, in the 0% to 3% range, and did not appear to be increased when the prophylaxis duration was increased from 100 to 200 days in D+/R- kidney recipients (191). Depending on the amount of active viral replication during antiviral therapy and valganciclovir dose, a higher incidence of ganciclovir resistance has sometimes been reported with preemptive therapy compared with prophylaxis (118, 190).

Diagnosis of Drug Resistance

Antiviral drug resistance should be suspected when there is no improvement (or with relapses) in CMV viremia or clinical disease during prolonged antiviral therapy especially in the presence of risk factors. Generally, prolonged therapy means 6 or more weeks of cumulative antiviral drug exposure, including more than 2 weeks of ongoing full dose therapy at the time of evaluation. Increases in viral loads in the first 2 weeks of treatment are not predictors of drug resistance (188). Although clinical risk factors for drug resistance are becoming better defined, diagnostic laboratory testing is needed to support decisions on switching therapies with potential adverse effects.

Genotypic assays for viral drug resistance mutations are available in reference and commercial laboratories, with a turnaround time of less than 1 week, and can be performed on viral sequences directly amplified from blood (whole blood, plasma, or leukocytes), fluids (CSF and BAL), or tissue specimens. The same blood specimens used for CMV QNAT are usually tested, although there are reports of discordant findings of resistance mutations in different body compartments. Testing is more reliable if the CMV load in the specimen is at least 1000 copies/mL. Quality-control concerns include false-

positive detection of mutations due to contamination or technical errors and false-negatives due to insensitivity of current sequencing methods in detecting mutant subpopulations comprising less than 20% to 30% of the total. Evolving sequencing technologies offer the potential of detecting far smaller mutant subpopulations.

At present, genotypic testing usually includes the UL97 kinase (codons 400–670) and the UL54 DNA polymerase (codons 300–1000) genes that contain known resistance mutations for current anti-CMV drugs. The relevant genes and codon ranges will evolve as new mutations and antiviral drugs are characterized. There is an increasing database of CMV sequence variants (187, 193). Occurrence of resistance mutations without prior antiviral drug exposure is very rare. In patients treated with ganciclovir, UL97 mutations appear first in about 90% of cases followed later by the addition of UL54 mutations that confer increased ganciclovir resistance. On this basis, it is reasonable to begin genotypic testing with UL97 alone, leaving the UL54 analysis for later follow-up. UL97 mutations conferring ganciclovir resistance are strongly clustered at codons 460, 520, or 590 to 607. The seven most common (“canonical”) mutations listed in Table 8 account for more than 80% of cases. Other UL97 sequence changes may confer varying degrees of ganciclovir resistance (Table 8). UL97 mutations do not affect foscarnet or cidofovir susceptibility. UL54 drug resistance mutations tend to occur in the conserved functional domains and may confer cross-resistance to other drugs (detailed in Fig. 1). In both UL97 and UL54, uncharacterized sequence variants cannot be presumed to be resistance-related without careful analysis of such factors as presence in serial specimens, treatment history, proximity to known gene mutations, and corroboration by recombinant phenotyping (187). Genotype assay reports from diagnostic laboratories vary in the degree of detail and accuracy of interpretive information provided.

The traditional plaque reduction (phenotypic) assay for susceptibility testing of viral isolates is impractical because of slow turnaround time and the lack of CMV culture isolates in current diagnostic practice (187). Contemporary recombinant phenotyping involves the targeted mutagenesis of laboratory CMV strains and is for research use only (1).

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 algorithm (Fig. 2) based on consensus expert

TABLE 8. Ganciclovir resistance levels associated with UL97 genotypes by Fold change in ganciclovir EC50^a

| Genotype frequency | 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 596del, L595F/W, K599T, C603R, C607Y, del(≥3) ^c | A594E/T, E596G, C603S, 600del2, ^b C607F | A591V, N597D, K599E/R, L600I, 600del, ^b T601M, D605E ^d |

^a Moderate resistance (5–15×), low-grade resistance (2–5×), or insignificant resistance (<2×).

^b del=in-frame deletion of single codon; del2=deletion of two codons.

^c In-frame deletion of ≥3 codons in the 590–607 range can be assumed to confer moderate ganciclovir resistance, although only a few examples have been phenotyped. Deletion of less than 3 codons may confer varying degrees of ganciclovir resistance.

^d D605E is a baseline sequence polymorphism common in east Asia, unrelated to drug resistance.

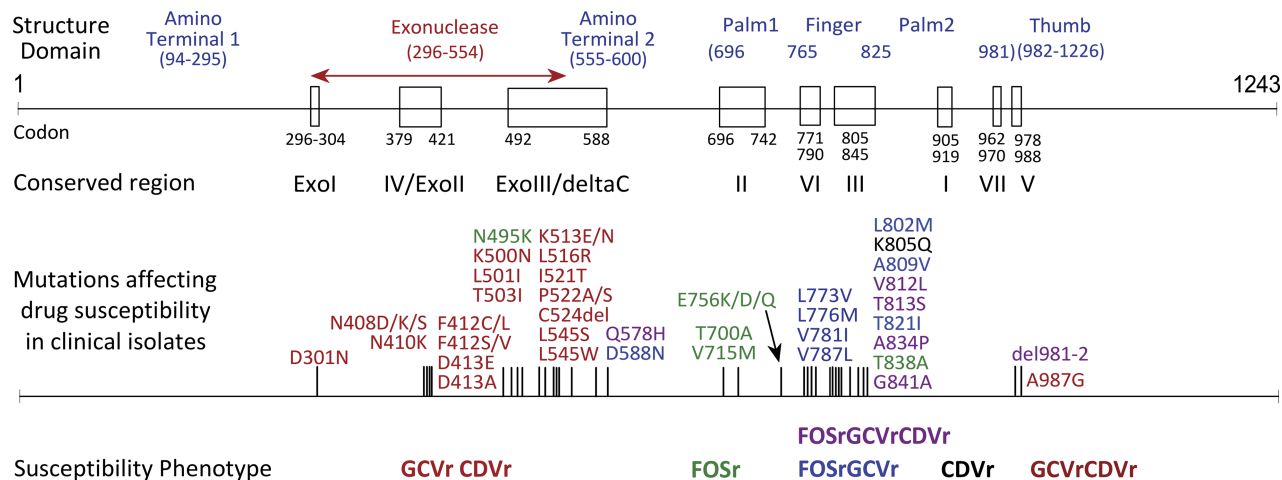


FIGURE 1. CMV UL54 DNA polymerase gene mutation map. Structure domains and regions of amino acid sequence conservation in herpes virus polymerases, where resistance mutations are clustered. Adapted and updated from prior publications (187).

opinion has been slightly modified from the prior version (1) to clarify decision-making criteria. 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. An effort should be made to deliver full therapeutic doses of all selected drugs to reduce the further emergence of resistance mutations. Only a fraction of cases with clinical suspicion of drug resistance will be genotypically confirmed (189). If laboratory testing returns no evidence supporting drug resistance, emphasis should be given to optimization of host factors rather than switching antiviral medications. Immunosuppressive therapy should be reduced to the lowest feasible amount, and adjunctive measures described in the next section can be considered. Therapeutic drug monitoring may potentially be helpful in adjusting doses to maintain effective drug levels in relation to viral inhibitory concentrations, although no optimum target levels have been established (194).

Some UL97 or UL54 mutations confer low levels of ganciclovir resistance by themselves (Table 8) (187) and may be amenable to ganciclovir dose escalation (up to 10 mg/kg every 12 hr) combined with optimization of host factors, if severe disease is not present. This is double the standard dose and needs monitoring for bone marrow suppression and dose adjustment for renal function. Switching to foscarnet is recommended if a mutation confers higher-level ganciclovir resistance, or UL97 and UL54 mutations combine to confer high-level ganciclovir resistance and usually cidofovir cross-resistance. There is little information on the efficacy of cidofovir as salvage therapy in SOT; its use in HSCT gave mixed results (195), and dose-limiting nephrotoxicity is frequent. Short-term use of cidofovir or available experimental treatments can be considered when both ganciclovir and foscarnet resistance mutations have been detected that do not confer cidofovir cross-resistance.

Adjunctive Therapy

Adjunctive treatments, defined as those without a specific CMV antiviral drug target, have not been adequately

evaluated. CMV Ig (or IVIG) and adoptive infusions of CMV-specific T cells 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 (147, 196, 197). Switching immunosuppressive therapy to an mTOR inhibitor may be worthwhile based on studies showing a lower incidence of CMV infection and disease (152). Leflunomide has been advocated but lacks controlled trial data to prove antiviral efficacy (196), and caution is advised when used for cases of severe disease or with high viral loads. Use of artesunate has been the subject of case reports (197, 198), with mixed outcomes suggesting a similar degree of caution as with leflunomide.

Experimental CMV Antiviral Agents

Hexadecyloxypropyl cidofovir conjugate (CMX001) is an orally bioavailable derivative of cidofovir with improved intracellular active drug delivery and in vitro antiviral potency while avoiding the high renal concentrations and frequent nephrotoxicity associated with intravenous cidofovir (199). It was effective in a phase II trial as CMV prophylaxis in HSCT recipients and has been used as salvage therapy for some cases of ganciclovir-resistant CMV disease with mixed results in an expanded access study (200). Optimal dosing has yet to be determined, and diarrhea is a dose-limiting adverse effect. Resistance to CMX001 is expected to involve similar mutations of UL54 as cidofovir (187).

Maribavir is an oral benzimidazole L-riboside inhibitor of the CMV UL97 kinase (201). After promising early-phase clinical trials, phase III trials in HSCT and liver transplant recipients demonstrated no antiviral efficacy of low-dose (100 mg twice daily) maribavir (202, 203). It has been used as salvage therapy at a higher dose (400 mg twice daily) for drug-resistant CMV infection, with mixed results including success in treating lower initial viral loads (204) and a case of viral rebound and proven maribavir resistance when treating a very high initial viral load (205). A new phase II trial of maribavir for salvage treatment of refractory and resistant CMV infection was launched in 2012. Resistance to maribavir involves mutations in the UL97 kinase distinct from those

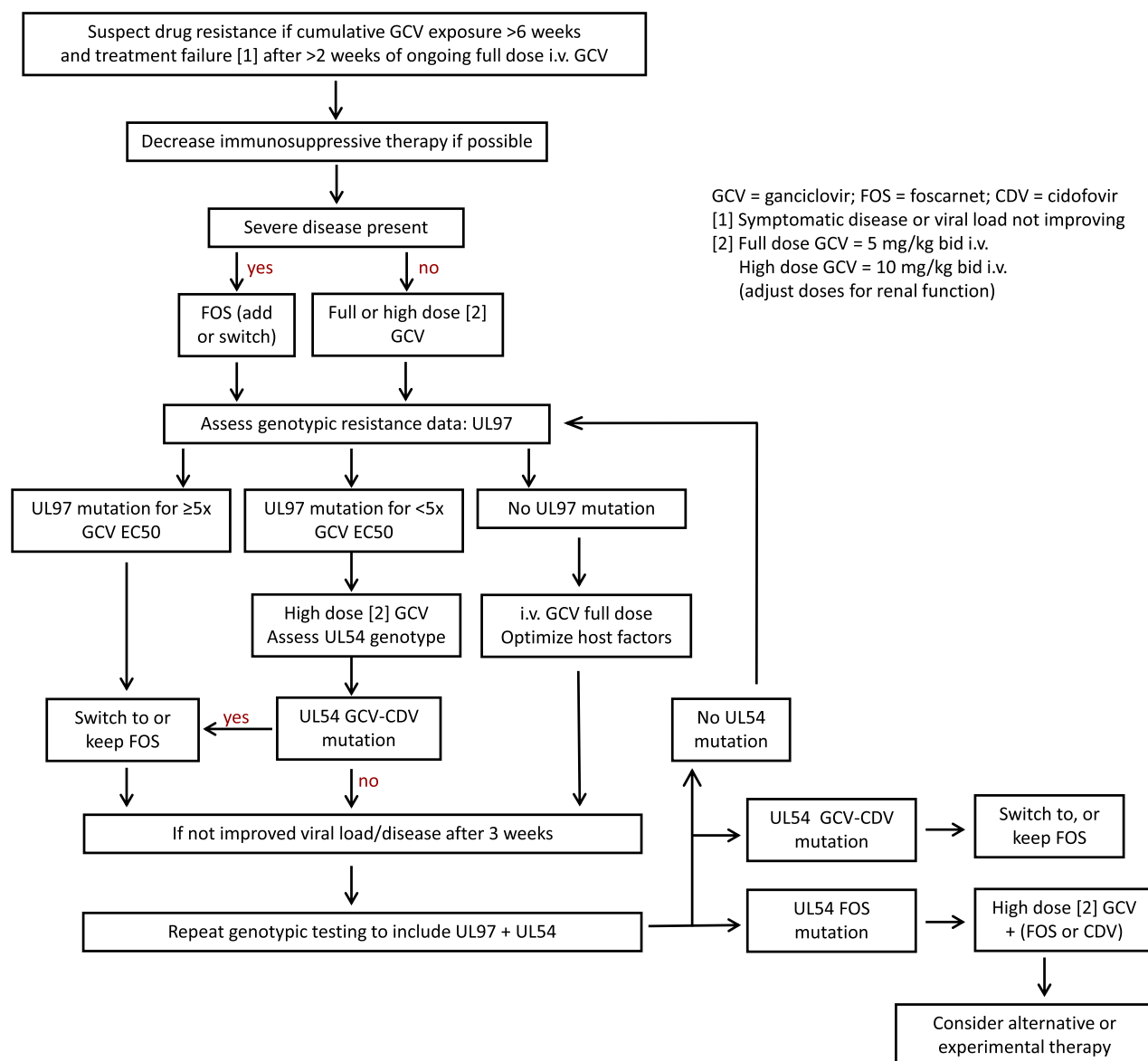


FIGURE 2. Proposed algorithm for management of suspected antiviral drug resistance based on consensus expert opinion. There are no controlled trials that define clinical outcomes according to genotypic diagnosis and selection of alternative therapy.

conferring ganciclovir resistance in clinical CMV isolates, and assorted mutations in the gene UL27 that confer low-level resistance (206).

Letemovir is a CMV UL56 terminase inhibitor with high in vitro potency against baseline CMV strains (207). Antiviral efficacy was reported in phase II prophylaxis studies in HSCT recipients (208), and a single case has been published of letemovir use to clear a decreasing viral load in conjunction with reduction of immunosuppression (209). High-level resistance to letemovir has been associated with some UL56 mutations; (210) no cross-resistance with current antivirals is expected.

Future Research and Clinical Practice Needs

Adequate prospective studies have not been performed to define the outcomes of drug-resistant CMV under various

management options. Genotypic resistance testing needs improved quality control and interpretation of the level of drug resistance and cross-resistance conferred by various mutations. The role of next-generation genotyping technology remains to be defined. New therapeutic options of adequate potency, bioavailability, and lack of toxicity and cross-resistance with current drugs are needed.

Consensus Recommendations

Interpretation of genotypic resistance testing is described above and summarized in Table 8 and Figure 1. Recognition and management of CMV drug resistance is presented in the algorithm (Fig. 2) and discussed in the text. 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 in the management algorithm should be considered “strong, low.”

PEDIATRIC ISSUES IN CMV MANAGEMENT

Prevention and treatment of CMV infection and disease in pediatric and adolescent SOT recipients present several unique issues described here, incorporating and expanding on previous guidelines (1, 211–213). The pediatric group was defined as younger than 12 years; additional factors, including body weight and developmental challenges, may influence clinical decisions.

Burden of CMV Disease in Children

There are limited data on the precise disease burden in pediatric SOT recipients. Nonuniform approaches to diagnosis, varying definitions of CMV, and inconsistent durations of monitoring hamper data interpretation. Epidemiologic 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 (214, 215). With prophylaxis, CMV disease decreased to 10% to 20% within the first 2 years after liver transplantation (216). Declines in CMV disease from 24% to 12% also have been documented in pediatric intestinal transplant recipients after the introduction of antiviral prophylaxis (217, 218). The incidence of CMV DNAemia after pediatric renal transplantation is approximately 20%, with disease in 3% to 10% (219, 220). CMV was detected in the blood in 29% to 32% of pediatric lung transplant recipients in the first year, with CMV pneumonitis in 20% (221, 222).

Primary Risk Factors for the Development of CMV Disease in Children

In general, adult and pediatric patients share similar risk factors for CMV disease after SOT (223). However, children have an increased likelihood of acquiring primary CMV infection because they are more often CMV naïve at transplant. In addition, CMV D-/R- pediatric SOT recipients have a greater risk of acquiring de novo CMV infection from community exposures. As many as 7% of pediatric CMV D-/R- recipients developed primary CMV infection in the first year after transplantation (222). Characterizing donor and recipient serostatus for children younger than 12 months is confounded by the potential presence of maternal CMV antibodies acquired transplacentally. Transplant recipients younger than 12 months receiving an organ from a seropositive donor are generally presumed to be seronegative unless CMV infection is confirmed by culture or NAT.

Indirect Effects of CMV in Pediatrics

The nature and definition of the indirect effects of CMV may be different in children. Unlike the adult SOT population where studies demonstrate significant indirect effects including increased risk of fungal and other opportunistic infections, coronary artery vasculopathy, and chronic allograft rejection (224), data in pediatric SOT recipients are limited. In pediatric lung transplant recipients, CMV is associated with increased mortality within the first year of transplantation; (222) however, an association with chronic allograft rejection and opportunistic infections has not been

demonstrated (225, 226). For kidney transplant recipients, CMV DNAemia was associated with an increased risk of histologic graft rejection (220). These results are potentially confounded by the presence of Epstein-Barr virus coinfection in half of the small sample size. In heart transplantation, CMV prophylaxis with either CMV Ig or antiviral agents was associated with decreased mortality (227). Others have reported association between CMV seropositivity and coronary artery vasculopathy (228), yet data from the multicenter Pediatric Heart Transplant Study did not demonstrate this association (229). The lack of evidence that CMV has substantial indirect deleterious effects in pediatric transplantation recipients coupled with the more limited pharmacokinetic studies and the potential toxicities associated with antiviral therapy in the developing child provide a less compelling rationale for prolonged antiviral prophylaxis in children.

Optimal Laboratory Methods for the Diagnosis of Pediatric CMV Infection/Disease

A few caveats exist for the diagnosis of CMV in children. The amount of blood obtained by venipuncture may be limited (thus, QNAT may be easier than antigenemia). Some invasive diagnostic procedures are more difficult (e.g., transbronchial biopsies in infants). Like adults, the level of CMV DNAemia that should trigger the initiation of preemptive therapy has not been determined.

The potential role of monitoring for general or CMV-specific immune reconstitution has only been explored in uncontrolled and small studies in pediatric SOT (230). Larger studies of T-cell responses and their potential role as a biomarker of risk for CMV disease are needed before introducing these assays into clinical practice (see Immunology section).

Prevention of Pediatric CMV Disease

Prevention strategies in pediatric SOT recipients include preemptive therapy, antiviral prophylaxis, or a hybrid strategy, consisting of antiviral prophylaxis for 2 to 12 weeks followed by viral load monitoring. The rationale for at least a 2-week course of antiviral therapy in the hybrid strategy is based on the presumption that it may reduce viral replication within the incoming CMV+ graft at a time when the recipient may have little or no immune response. This notion has never been tested, however, and the optimal duration of prophylaxis in hybrid prevention strategies is not known (231–233). Results of all three strategies to prevent CMV disease after pediatric transplantation have been reported with comparable efficacies, although the broadest collective experience includes some period of antiviral prophylaxis. Notably, there are limited data on the pharmacokinetics of valganciclovir in infants and young children. For these reasons, the recommendations for the prevention of CMV infection and disease in pediatric SOT differ from adult recommendations.

Antiviral prophylaxis may be administered either with intravenous ganciclovir or oral valganciclovir. Intravenous ganciclovir is usually dosed at 5 mg/kg per day, although some centers start with 10 mg/kg in two divided doses for the initial 2 weeks of the prophylaxis period based on the rationale that a higher (treatment) dose may reduce viral replication within the graft (231, 233). There are no data to suggest that the higher dose is superior. In contrast to adults, prophylaxis duration is more varied both between individual

centers and among different organs. Concerns for prolonged exposure to ganciclovir or valganciclovir in the very young recipient have been raised due to animal toxicity studies demonstrating carcinogenesis or an adverse effect on spermatogenesis, but these have not been observed in humans (234). Prolonged intravenous ganciclovir (12 weeks) has been used safely in pediatric transplant recipients (235).

Compared with adults, less data are available to define the role of valganciclovir in pediatric SOT recipients. Whereas recent studies have addressed pharmacokinetics in older children (236, 237), pharmacokinetic data in young children and infants are lacking but may be extrapolated from studies in infants treated for congenital CMV (238). Data evaluating the efficacy of valganciclovir for the prevention and treatment of CMV in pediatric SOT recipients are needed, particularly given concerns for lower than anticipated plasma (and presumably intracellular) ganciclovir levels and potential subsequent risk for ganciclovir resistance. Absorption issues might be of particular concern in small bowel transplant recipients. The efficacy and safety of prolonged valganciclovir prophylaxis has not been the subject of randomized studies in children. No statistically significant difference was found in the incidence of early- or late-onset CMV disease after pediatric liver transplantation in a study comparing valganciclovir to oral ganciclovir prophylaxis for 120 days (239).

The use of preemptive therapy alone (240) or with IVIG (241) has been reported in pediatric liver transplant recipients; CMV disease was documented in only 5% of patients receiving a preemptive strategy, but sample sizes were limited. A hybrid strategy with short courses of prophylaxis (14–28 days) followed by preemptive therapy also successfully prevented CMV disease in pediatric liver and heart transplant recipients, with CMV disease incidences of 8% to 10% (231, 232).

CMV Ig and IVIG are sometimes used in combination with antivirals to prevent CMV. 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 (241–243). 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 (227, 244). Krampe et al. 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 (241). 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 intravenous ganciclovir, 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 (243). In one prospective randomized pediatric study that primarily targeted Epstein-Barr virus, 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 (216). Finally, similar to adult recommendations, the use of leukodepleted or CMV-negative blood products should be considered for special populations (e.g., bowel, lung, and heart transplants) and in CMV D-/R- patients.

Treatment of Pediatric CMV Disease

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. Many principles that guide therapy in children are similar to those among adults.

Ganciclovir Resistance in Pediatric Organ Transplantation

Due to the high likelihood of CMV D+/R- status in pediatric SOT recipients, ganciclovir resistance is of significant concern (245). Few studies describing ganciclovir resistance among pediatric SOT recipients have been published. It is unclear if this is due to low resistance burden, lack of generated data, or underreporting. The currently available agents for the treatment of ganciclovir-resistant CMV in children are similar to those used in adults.

Consensus Recommendations

- Given the challenge of characterizing donor and recipient serostatus in those younger than 12 months, risk assessment in this age group should assume the highest risk level for purposes of CMV prevention (strong, moderate). Seropositive infant donors should be presumed CMV positive. Conversely, any CMV seropositive recipient who is younger than 12 months receiving an organ from a seropositive donor should be assumed to be seronegative, as passively acquired maternal antibody may account for this finding, unless CMV urine or saliva culture or NAT confirms there is prior CMV infection. It should be noted that the predictive value of a positive CMV assay is limited by intermittent CMV shedding.
- In general, the principles that guide the use of prophylaxis in adults are similar in children as defined by CMV donor and recipient serostatus. Table 9 provides suggested approaches to CMV prevention in children.
- Use of the valganciclovir dosing algorithm that adjusted for body surface area and renal function provides ganciclovir exposures similar to those established as safe and effective in adults and is recommended in children older than 3 months for prophylaxis (strong, moderate) (Table 10). Alternate dosing of valganciclovir for infants younger than 3 months can be extrapolated from studies in congenital CMV infection (16 mg/kg/dose every 12 hr) until additional data are available in infant transplant recipients (weak, low). A liquid formulation is now commercially available.
- No pediatric trials have evaluated the comparative efficacy of prophylaxis, preemptive therapy, or hybrid strategies; retrospective data provide equal support for these three prevention strategies, and as such, all three are recommended (strong, moderate).
- Monitoring is recommended by some experts during prophylaxis due to the risk of breakthrough DNAemia (weak, low). Frequency of monitoring should take into account prophylaxis (oral or intravenous), immunosuppressive regimen (including T-cell-depleting induction), and likelihood of compliance with the prophylactic regimen. Adherence can be a particular problem with adolescents.
- Monitoring for CMV DNAemia for patients being managed preemptively or with hybrid regimens should follow adult

TABLE 9. Recommended regimens for CMV prevention in children

| Organ | Serostatus ^a | Risk level | Recommended | Alternate |
|-----------------------------------|-------------------------|----------------------|--|---|
| All, except small bowel Kidney | D-/R- | Lowest ^b | Monitoring for clinical symptoms | Preemptive monitoring |
| | R+ | Low | 2–4 weeks IV GCV/VGC with sequential monitoring ^c | 3–6 months of VGCV as recommended in adults ^c |
| Liver | D+/R- | Intermediate to high | 2–4 weeks IV GCV/VGC with sequential monitoring OR 3–6 months of IV GCV/VGC as recommended in adults ^c | 3–4 months of VGCV OR Preemptive monitoring ^c |
| | R+ | Intermediate | 2 weeks of IV GCV/VGC with sequential monitoring ^c (VGCV not FDA approved in liver) | Preemptive monitoring ^c |
| Heart | D+/R- | Intermediate to high | 2 weeks of IV GCV/VGC with sequential monitoring ^c (VGCV not FDA approved in liver) OR 3–4 months of IV GCV/VGC | Preemptive monitoring ^c |
| | R+ | Intermediate to high | 2–4 weeks IV GCV/VGC with sequential monitoring OR 3 months of IV GCV/VGC ^c | Some experts add CMV Ig |
| Lung | D+/R- | High | 4 weeks IV GCV/VGC with sequential monitoring OR 3 months of IV GCV/VGC ^c | Some experts add CMV Ig |
| | R+ or D+/R- | High | 3–6 months of IV GCV/VGC | Shorter courses have been used with sequential monitoring |
| Small bowel ^d | D-/R- | Low | Preemptive monitoring OR 2 weeks IV ganciclovir with sequential monitoring | Some experts add CMV Ig |
| | R+ | High | 2 weeks IV GVC with sequential monitoring OR 3–12 months IV GCV/VGC +/- CMV Ig | |
| | D+/R- | High | 3–12 months IV GCV/VGC + CMV Ig | |

^a Refer to serostatus recommendation for infants <12 months.

^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.

Some experts recommend CMV Ig for intermediate- and higher-risk recipients, but there are no randomized studies indicating that CMV Ig is any better than ganciclovir or valganciclovir alone. The above regimens do not imply an exclusive course of action.

IV, intravenous; GCV, ganciclovir; PO, oral; VGCV, valganciclovir.

recommendations of weekly testing but continue for at least 4 to 6 months after transplantation (strong, moderate). Some continue monitoring at less frequent intervals beyond 6 months if risk persists.

- Given that the risk of DNAemia is greatest during the 4 to 6 weeks after completing prophylaxis in those using a

hybrid strategy, weekly monitoring should be performed for at least this period of time (strong, moderate), although, as noted above, monitoring is recommended for 4 to 6 months after transplantation, particularly with short courses of prophylaxis or for patients being managed with a preemptive strategy.

TABLE 10. Calculation of pediatric dosing for valganciclovir for prevention of CMV disease in kidney or heart transplant patients (4 months to 16 years)

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

^a Where k=0.45 for patients aged <1 year, 0.45 for patients aged 1 to <2 years, 0.55 for boys aged 2 to <13 years and girls aged 2 to 16 years, and 0.7 for boys aged 13 to 16 years.

^b Maximum dose is 900 mg per day.

Available at: http://www.valcyte.com/hcp/resources/professional/calculators/index.html?cid=val_PS_00000180&c=val_PS_00000180.

- The initial treatment of CMV disease (mild, moderate, or severe) in children younger than 12 years should be with intravenous ganciclovir at a dose of 5 mg/kg every 12 hr (strong, moderate) with appropriate adjustments for renal function. For children older than 12 years, pharmacokinetics and biological responses with valganciclovir may be similar to adults; issues with adherence need to be considered in the decision to use either valganciclovir or intravenous ganciclovir (strong, moderate). Some experts consider oral therapy for some older children and adolescents toward the end of their treatment courses (weak, low).
- The initial treatment of asymptomatic DNAemia for infants and children younger than 5 years should also be with intravenous ganciclovir (5 mg/kg every 12 hr), although some experts recommend valganciclovir (weak, low). In older children and adolescents, most experts would use valganciclovir (weak, 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.
- Evaluation of the immunosuppression regimen should be done with all CMV infection and disease, with reduction when indicated (strong, low).
- CMV Ig is recommended for the treatment of severe CMV disease (i.e., pneumonitis and enteritis) in children (weak, low) and for hypogammaglobulinemia during CMV infection or disease (weak, low).
- Antiviral prophylaxis with valganciclovir or ganciclovir should be strongly considered for children at risk for CMV due to significant immunosuppression intensification (e.g., antilymphocyte therapy) (weak, low).
- Children with recurrent CMV DNAemia or disease may benefit from secondary antiviral prophylaxis; the duration of prophylaxis should vary based on immunosuppression, age, presence of other opportunistic infections, and other risk factors (weak, low).

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. Further, additional investigation into the diagnostic and predictive utility of immunogenetic biomarkers and adjunctive immunologic monitoring to guide preventative and treatment strategies should be explored. Investigation into optimal CMV prevention strategies should include appreciation for impact on different age groups and potential consequences of antiviral side effects in pediatric-aged patients. Finally, pediatric data should be obtained for emerging antiviral agents to expand the opportunities to prevent and treat CMV.

Summary of Updates to Guidelines

Numerous updates have been added to these guidelines. In the diagnostics field, the advent of an international standard for CMV viral load testing and reporting of values in IU/mL will eventually allow for harmonization of viral load tests with subsequent development of thresholds for preemptive and diagnostic protocols. QNAT is increasingly used

and preferred for diagnosis, decisions regarding preemptive therapy, and monitoring response to therapy. There is substantially more evidence to support the use of immunodiagnosics as an adjunct tool to predict the risk of CMV disease. Vaccine development continues and holds increasing promise as a future prevention strategy. Updates in the prevention field include the effectiveness of prolonging prophylaxis in D+/R- kidney recipients from 100 to 200 days and from 3 to 12 months in lung transplant recipients. Trials with carefully executed preemptive therapy, using low viral load thresholds, demonstrate similar outcomes to universal prophylaxis including similar long-term graft survival. Some experts are using a hybrid approach (prophylaxis followed by preemptive therapy) with increased frequency. Valganciclovir is increasingly used as the preferred agent for treatment (except for life-threatening cases and situations with questionable drug bioavailability or noncompliance). Additional specific recommendations on the use of IVIG with CMV treatment are included. Differences between different algorithms for determining estimated glomerular filtration rate and the risk of overdosing have been highlighted. Diagnostic resistance mutations have been updated and the clinical management algorithm for ganciclovir-resistant CMV has been slightly modified to clarify decision-making criteria. Alternative therapy has been updated to reflect current experimental drugs. In the pediatrics section, valganciclovir is included in the prevention and treatment of CMV due to new data detailing the pharmacokinetics of valganciclovir in pediatrics. Prophylaxis, preemptive therapy, and hybrid regimens are all now recommended regimens for CMV prevention in children, as emerging data support each of these strategies, albeit without comparative efficacy studies.

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REFERENCES

1. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010; 89: 779.
2. Guyatt GH, Oxman AD, Kunz R, et al. Going from evidence to recommendations. *BMJ* 2008; 336: 1049.
3. 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.
4. 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.
5. Guyatt GH, Oxman AD, Kunz R, et al. Incorporating considerations of resources use into grading recommendations. *BMJ* 2008; 336: 1170.
6. 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.
7. Kish MA. Guide to development of practice guidelines. *Clin Infect Dis* 2001; 32: 851.
8. 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.
9. 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.
 10. 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.
 11. Lazzarotto T, Brojanac S, Maine GT, et al. Search for cytomegalovirus-specific immunoglobulin M: comparison between a new western blot, conventional western blot, and nine commercially available assays. *Clin Diagn Lab Immunol* 1997; 4: 483.
 12. Weber B, Berger A, Rabenau H. Human cytomegalovirus infection: diagnostic potential of recombinant antigens for cytomegalovirus antibody detection. *J Virol Methods* 2001; 96: 157.
 13. 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.
 14. 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.
 15. 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.
 16. 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.
 17. Rayes N, Seehofer D, Lullius SG, et al. Monitoring of human cytomegalovirus, HHV-6 and HHV-7 infection in kidney transplant recipients by molecular methods to predict HCMV disease after transplantation: a prospective study. *Ann Transplant* 2005; 10: 23.
 18. Schroeder R, Michelon T, Fagundes I, et al. Antigenemia for cytomegalovirus in renal transplantation: choosing a cutoff for the diagnosis criteria in cytomegalovirus disease. *Transplant Proc* 2005; 37: 2781.
 19. Baldanti F, Lillieri D, Gerna G. Monitoring human cytomegalovirus infection in transplant recipients. *J Clin Virol* 2008; 41: 237.
 20. Gerna G, Baldanti F, Lillieri D, et al. Human cytomegalovirus pp67 mRNAemia versus pp65 antigenemia for guiding preemptive therapy in heart and lung transplant recipients: a prospective, randomized, controlled, open-label trial. *Transplantation* 2003; 75: 1012.
 21. Villa M, Lage E, Ballesteros S, et al. Preemptive therapy for the prevention of cytomegalovirus disease following heart transplantation directed by pp65 antigenemia. *Transplant Proc* 2003; 35: 732.
 22. 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.
 23. Lao WC, Lee D, Burroughs A, et al. Use of polymerase chain reaction to provide prognostic information of human cytomegalovirus disease after liver transplantation. *J Med Virol* 1997; 51: 152.
 24. Razonable RR, van Crujnsen H, Brown RA, et al. Dynamics of cytomegalovirus replication during preemptive therapy with oral ganciclovir. *J Infect Dis* 2003; 187: 1801.
 25. 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.
 26. 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.
 27. 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.
 28. 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.
 29. Piiparinen H, Hockerstedt K, Gronhagen-Riska C, et al. Comparison of two quantitative CMV PCR tests, Cobas Amplicor CMV Monitor and TaqMan assay, and pp65-antigenemia assay in the determination of viral loads from peripheral blood of organ transplant patients. *J Clin Virol* 2004; 30: 258.
 30. 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.
 31. 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.
 32. 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.
 33. 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.
 34. Lisboa LF, Åsberg 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.
 35. Abdul-Ali D, Kraft CS, Ingersoll J, et al. Cytomegalovirus DNA stability in EDTA anti-coagulated whole blood and plasma samples. *J Clin Virol* 2011; 52: 222.
 36. Pang XL, Fox JD, Fenton JM, et al. Interlaboratory comparison of cytomegalovirus viral load assays. *Am J Transplant* 2009; 9: 258.
 37. Hirsch HH, Lautenschlager I, Pinsky BA, et al. An international multicenter performance analysis of cytomegalovirus load tests. *Clin Infect Dis* 2013; 56: 367.
 38. 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.
 39. Lillieri D, Lazzarotto T, Ghisetti V, et al. Multicenter quality control study for human cytomegalovirus DNAemia quantification. *New Microbiol* 2009; 32: 245.
 40. Manuel O, Åsberg A, Pang X, et al. Impact of genetic polymorphisms in cytomegalovirus glycoprotein B on outcomes in solid-organ transplant recipients with cytomegalovirus disease. *Clin Infect Dis* 2009; 49: 1160.
 41. Martín-Gandul C, Perez-Romero P, Sanchez 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.
 42. 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.
 43. 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.
 44. 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.
 45. 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.
 46. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis* 2002; 34: 1094.
 47. Lautenschlager I, Halme L, Hockerstedt K, et al. Cytomegalovirus infection of the liver transplant: virological, histological, immunological, and clinical observations. *Transpl Infect Dis* 2006; 8: 21.
 48. Halme L, Lempinen M, Arola J, et al. High frequency of gastrooduodenal cytomegalovirus infection in liver transplant patients. *Appl Immunol* 2008; 116: 99.
 49. 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.
 50. 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.
 51. 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.

52. 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.
53. Riise GC, Andersson R, Bergstrom T, et al. Quantification of cytomegalovirus DNA in BAL fluid: a longitudinal study in lung transplant recipients. *Chest* 2000; 118: 1653.
54. 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.
55. 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.
56. Puchhammer-Stockl E. Herpesviruses and the transplanted lung: looking at the air side. *J Clin Virol* 2008; 43: 415.
57. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. *Clin Microbiol Rev* 2009; 22: 76.
58. Sester M, Gärtner BC, Sester U. Monitoring of CMV specific T-cell levels after organ transplantation. *J Lab Med* 2008; 32: 121.
59. Gandhi MK, Khanna R. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. *Lancet Infect Dis* 2004; 4: 725.
60. Kijpittayarit S, Eid AJ, Brown RA, et al. Relationship between Toll-like receptor 2 polymorphism and cytomegalovirus disease after liver transplantation. *Clin Infect Dis* 2007; 44: 1315.
61. Cervera C, Lozano F, Saval N, et al. The influence of innate immunity gene receptors polymorphisms in renal transplant infections. *Transplantation* 2007; 83: 1493.
62. Manuel O, Pascual M, Trendelenburg M, et al. Association between mannose-binding lectin deficiency and cytomegalovirus infection after kidney transplantation. *Transplantation* 2007; 83: 359.
63. 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.
64. Hadaya K, de Rham C, Bandelier C, et al. Natural killer cell receptor repertoire and their ligands, and the risk of CMV infection after kidney transplantation. *Am J Transplant* 2008; 8: 2674.
65. Stern M, Elsasser H, Honger G, et al. The number of activating KIR genes inversely correlates with the rate of CMV infection/reactivation in kidney transplant recipients. *Am J Transplant* 2008; 8: 1312.
66. 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.
67. 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.
68. 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.
69. Corales R, Chua J, Mawhorter S, et al. Significant post-transplant hypogammaglobulinemia in six heart transplant recipients: an emerging clinical phenomenon? *Transpl Infect Dis* 2000; 2: 133.
70. Goldfarb NS, Avery RK, Goormastic M, et al. Hypogammaglobulinemia in lung transplant recipients. *Transplantation* 2001; 71: 242.
71. Doron S, Ruthazer R, Werner BG, et al. Hypogammaglobulinemia in liver transplant recipients: incidence, timing, risk factors, and outcomes. *Transplantation* 2006; 81: 697.
72. 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.
73. Couzi L, Pitard V, Netzer S, et al. Common features of gammadelta T cells and CD8(+) alpha beta T cells responding to human cytomegalovirus infection in kidney transplant recipients. *J Infect Dis* 2009; 200: 1415.
74. Couzi L, Pitard V, Sicard X, et al. Antibody-dependent anti-cytomegalovirus activity of human gammadelta T cells expressing CD16 (Fc gamma RIIIa). *Blood* 2012; 119: 1418.
75. Presser D, Sester U, Mohrbach J, et al. Differential kinetics of effector and regulatory T cells in patients on calcineurin inhibitor-based drug regimens. *Kidney Int* 2009; 76: 557.
76. Egli A, Silva M Jr, O'Shea D, et al. An analysis of regulatory T-cell and Th-17 cell dynamics during cytomegalovirus replication in solid organ transplant recipients. *PLoS One* 2012; 7: e43937.
77. Sylwester AW, Mitchell BL, Edgar JB, et al. Broadly targeted human cytomegalovirus-specific CD4⁺ and CD8⁺ T cells dominate the memory compartments of exposed subjects. *J Exp Med* 2005; 202: 673.
78. 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.
79. 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.
80. Brestrich G, Zwinger S, Roemhild A, et al. Generation of HCMV-specific T-cell lines from seropositive solid-organ-transplant recipients for adoptive T-cell therapy. *J Immunother* 2009; 32: 932.
81. 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.
82. 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.
83. 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.
84. 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.
85. 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.
86. Cantisan S, Lara R, Montejo M, et al. Pretransplant interferon-gamma secretion by CMV-specific CD8⁺ T cells informs the risk of CMV replication after transplantation. *Am J Transplant* 2013; 13: 738.
87. 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.
88. 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.
89. 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.
90. Abate D, Fison 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.
91. Abate D, Saldan A, Fison 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.
92. 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.
93. 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.
94. 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.
95. 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.
96. 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.
97. Radha R, Jordan S, Puliyaanda D, et al. Cellular immune responses to cytomegalovirus in renal transplant recipients. *Am J Transplant* 2005; 5: 110.
 98. 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.
 99. Shlobin OA, West EE, Lechtzin N, et al. Persistent cytomegalovirus-specific memory responses in the lung allograft and blood following primary infection in lung transplant recipients. *J Immunol* 2006; 176: 2625.
 100. 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.
 101. 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.
 102. Zeevi A, Husain S, Spichty KJ, et al. Recovery of functional memory T cells in lung transplant recipients following induction therapy with alemtuzumab. *Am J Transplant* 2007; 7: 471.
 103. 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.
 104. Balfour HH Jr. Options for prevention of cytomegalovirus disease. *Ann Intern Med* 1991; 114: 598.
 105. 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.
 106. Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med* 2009; 360: 1191.
 107. 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.
 108. 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.
 109. Reap EA, Morris J, Dryga SA, et al. Development and preclinical evaluation of an alphavirus replicon particle vaccine for cytomegalovirus. *Vaccine* 2007; 25: 7441.
 110. 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.
 111. 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.
 112. 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.
 113. 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.
 114. 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.
 115. 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.
 116. 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.
 117. 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.
 118. 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.
 119. 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.
 120. 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.
 121. 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.
 122. 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.
 123. 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.
 124. 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.
 125. Åsberg 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.
 126. Spinner ML, Saab G, Casabar E, et al. Impact of prophylactic versus preemptive valganciclovir on long-term renal allograft outcomes. *Transplantation* 2010; 90: 412.
 127. 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.
 128. Reischig T, Nemcova J, Vanecek T, et al. Intra-graft cytomegalovirus infection: a randomized trial of valacyclovir prophylaxis versus pre-emptive therapy in renal transplant recipients. *Antivir Ther* 2010; 15: 23.
 129. Singh N, Wannstedt C, Keyes L, et al. Indirect outcomes associated with cytomegalovirus (opportunistic infections, hepatitis C virus sequelae, and mortality) in liver-transplant recipients with the use of preemptive therapy for 13 years. *Transplantation* 2005; 79: 1428.
 130. Bodro M, Sabe N, Llado L, et al. Prophylaxis versus preemptive therapy for cytomegalovirus disease in high-risk liver transplant recipients. *Liver Transpl* 2012; 18: 1093.
 131. 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.
 132. 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.
 133. Zuk DM, Humar A, Weinkauff JG, et al. An international survey of cytomegalovirus management practices in lung transplantation. *Transplantation* 2010; 90: 672.
 134. BenMarzouk-Hidalgo OJ, Cordero E, Gomez-Cia T, et al. First face composite-tissue transplant recipient successfully treated for cytomegalovirus infection with preemptive valganciclovir treatment. *Antimicrobial Agents Chemother* 2011; 55: 5949.
 135. Bonatti H, Brandacher G, Margreiter R, et al. Infectious complications in three double hand recipients: experience from a single center. *Transplant Proc* 2009; 41: 517.
 136. Schneeberger S, Lucchina S, Lanzetta M, et al. Cytomegalovirus-related complications in human hand transplantation. *Transplantation* 2005; 80: 441.
 137. Gordon CR, Abouhassan W, Avery RK. What is the true significance of donor-related cytomegalovirus transmission in the setting of facial composite tissue allotransplantation? *Transplant Proc* 2011; 43: 3516.
 138. Gordon CR, Avery RK, Abouhassan W, et al. Cytomegalovirus and other infectious issues related to face transplantation: specific considerations, lessons learned, and future recommendations. *Plast Reconstr Surg* 2011; 127: 1515.
 139. 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.

140. Montejo M, Montejo E, Gastaca M, et al. Prophylactic therapy with valganciclovir in high-risk (cytomegalovirus D+/R-) liver transplant recipients: a single-center experience. *Transplant Proc* 2009; 41: 2189.
141. 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.
142. 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.
143. Limaye AP, Bakthavatsalam R, Kim HW, et al. Impact of cytomegalovirus in organ transplant recipients in the era of antiviral prophylaxis. *Transplantation* 2006; 81: 1645.
144. Arthurs SK, Eid AJ, Pedersen RA, et al. Delayed-onset primary cytomegalovirus disease and the risk of allograft failure and mortality after kidney transplantation. *Clin Infect Dis* 2008; 46: 840.
145. Moreno A, Cervera C, Fortun 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.
146. San Juan R, Aguado JM, Lumberras C, et al. Impact of current transplantation management on the development of cytomegalovirus disease after renal transplantation. *Clin Infect Dis* 2008; 47: 875.
147. 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.
148. Fortun J, Martin-Davila P, Pascual J, et al. Immunosuppressive therapy and infection after kidney transplantation. *Transpl Infect Dis* 2010; 12: 397.
149. Cervera C, Fernandez-Ruiz M, Villedor A, et al. Epidemiology and risk factors for late infection in solid organ transplant recipients. *Transpl Infect Dis* 2011; 13: 598.
150. Ekberg H, Bernasconi C, Noldeke J, et al. Cyclosporine, tacrolimus and sirolimus retain their distinct toxicity profiles despite low doses in the Symphony study. *Nephrol Dial Transplant* 2010; 25: 2004.
151. Brennan DC, Legendre C, Patel D, et al. Cytomegalovirus incidence between everolimus versus mycophenolate in de novo renal transplants: pooled analysis of three clinical trials. *Am J Transplant* 2011; 11: 2453.
152. 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.
153. 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.
154. 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.
155. 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.
156. 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.
157. 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.
158. 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.
159. Reischig T, Jindra P, Mares J, et al. Valacyclovir for cytomegalovirus prophylaxis reduces the risk of acute renal allograft rejection. *Transplantation* 2005; 79: 317.
160. Kielberger L, Bouda M, Jindra P, et al. Pharmacoeconomic impact of different regimens to prevent cytomegalovirus infection in renal transplant recipients. *Kidney Blood Press Res* 2012; 35: 407.
161. Park JM, Lake KD, Arenas JD, et al. Efficacy and safety of low-dose valganciclovir in the prevention of cytomegalovirus disease in adult liver transplant recipients. *Liver Transpl* 2006; 12: 112.
162. Arthurs SK, Eid AJ, Pedersen RA, et al. Delayed-onset primary cytomegalovirus disease after liver transplantation. *Liver Transpl* 2007; 13: 1703.
163. Brady RL, Green K, Frei C, et al. Oral ganciclovir versus valganciclovir for cytomegalovirus prophylaxis in high-risk liver transplant recipients. *Transpl Infect Dis* 2009; 11: 106.
164. Fayek SA, Mantipititkul W, Rasetto F, et al. Valganciclovir is an effective prophylaxis for cytomegalovirus disease in liver transplant recipients. *HPB (Oxford)* 2010; 12: 657.
165. Shiley KT, Gasink LB, Barton TD, et al. Increased incidence of cytomegalovirus infection in high-risk liver transplant recipients receiving valganciclovir prophylaxis versus ganciclovir prophylaxis. *Liver Transpl* 2009; 15: 963.
166. Levitsky J, Singh N, Wagener MM, et al. A survey of CMV prevention strategies after liver transplantation. *Am J Transplant* 2008; 8: 158.
167. Greiner M, Cusini A, Ruesch M, et al. A stringent preemptive protocol reduces cytomegalovirus disease in the first 6 months after kidney transplantation. *Infection* 2012; 40: 669.
168. Atabani SF, Emery VC, Smith C, et al. Response to letter regarding "cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy". *Am J Transplant* 2012; 12: 2859.
169. 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.
170. Lefevre S, Chevalier P, Charpentier C, et al. Valganciclovir prophylaxis for cytomegalovirus infection in thoracic transplant patients: retrospective study of efficacy, safety, and drug exposure. *Transpl Infect Dis* 2010; 12: 213.
171. Radeva G, Buyse M, Hindlet P, et al. Regulation of the oligopeptide transporter, PEPT-1, in DSS-induced rat colitis. *Dig Dis Sci* 2007; 52: 1653.
172. Emery VC, Griffiths PD. Prediction of cytomegalovirus load and resistance patterns after antiviral chemotherapy. *Proc Natl Acad Sci U S A* 2000; 97: 8039.
173. 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.
174. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31.
175. Levey AS, Coresh J, Greene T, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; 145: 247.
176. Chou SW. Cytomegalovirus drug resistance and clinical implications. *Transpl Infect Dis* 2001; 3: 20.
177. Dia IG, Wilson JA, Groettum CM, et al. Cytomegalovirus (CMV) DNA load predicts relapsing CMV infection after solid organ transplantation. *J Infect Dis* 2000; 181: 717.
178. Falagas ME, Snyderman DR, Griffith J, et al. Clinical and epidemiological predictors of recurrent cytomegalovirus disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG Study Group. *Clin Infect Dis* 1997; 25: 314.
179. Humar A, Uknis M, Carlone-Jambor C, et al. Cytomegalovirus disease recurrence after ganciclovir treatment in kidney and kidney-pancreas transplant recipients. *Transplantation* 1999; 67: 94.
180. Åsberg 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.
181. Shanahan A, Malani PN, Kaul DR. Relapsing cytomegalovirus infection in solid organ transplant recipients. *Transpl Infect Dis* 2009; 11: 513.
182. Sommadossi JP, Bevan R, Ling T, et al. Clinical pharmacokinetics of ganciclovir in patients with normal and impaired renal function. *Rev Infect Dis* 1988; 10: S507.
183. Boeckh M, Nichols WG. Immunosuppressive effects of beta-herpesviruses. *Herpes* 2003; 10: 12.
184. Sarmiento E, Fernandez-Yanez J, Munoz P, et al. Hypogammaglobulinemia after heart transplantation: use of intravenous

- immunoglobulin replacement therapy in relapsing CMV disease. *Int Immunopharmacol* 2005; 5: 97.
185. Sarmiento E, Rodriguez-Molina J, Munoz P, et al. Decreased levels of serum immunoglobulins as a risk factor for infection after heart transplantation. *Transpl Proc* 2005; 37: 4046.
 186. Sarmiento E, Rodriguez-Molina JJ, Fernandez-Yanez J, et al. IgG monitoring to identify the risk for development of infection in heart transplant recipients. *Transpl Infect Dis* 2006; 8: 49.
 187. Lurain NS, Chou S. Antiviral drug resistance of human cytomegalovirus. *Clin Microbiol Rev* 2010; 23: 689.
 188. 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.
 189. 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.
 190. 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.
 191. 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.
 192. 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.
 193. 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.
 194. 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.
 195. Ljungman P, Deliliers GL, Platzbecker U, et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Blood* 2001; 97: 388.
 196. Avery RK, Mossad SB, Poggio E, et al. Utility of leflunomide in the treatment of complex cytomegalovirus syndromes. *Transplantation* 2010; 90: 419.
 197. 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.
 198. Wolf DG, Shimoni A, Resnick IB, et al. Human cytomegalovirus kinetics following institution of artesunate after hematopoietic stem cell transplantation. *Antiviral Res* 2011; 90: 183.
 199. Painter W, Robertson A, Trost LC, et al. First pharmacokinetic and safety study in humans of the novel lipid antiviral conjugate CMX001, a broad-spectrum oral drug active against double-stranded DNA viruses. *Antimicrob Agents Chemother* 2012; 56: 2726.
 200. Papanicolaou G, Mommeja-Marin H, Anderson M, et al. *CMX001 Is an Effective Treatment Option for Refractory Cytomegalovirus Infection or Disease in Hematopoietic Cell Transplant Recipients: A Single Center Experience*. San Francisco, CA: 52nd Intersci Conf Antimicrob Agents Chemother; 2012: Abstract T.
 201. Biron KK, Harvey RJ, Chamberlain SC, et al. Potent and selective inhibition of human cytomegalovirus replication by 1263W94, a benzimidazole L-riboside with a unique mode of action. *Antimicrob Agents Chemother* 2002; 46: 2365.
 202. 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.
 203. 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.
 204. 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.
 205. Strasfeld L, Lee I, Villano S, et al. Virologic characterization of multidrug-resistant cytomegalovirus infection in two transplant recipients treated with maribavir. *J Infect Dis* 2010; 202: 104.
 206. 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.
 207. Lischka P, Hewlett G, Wunberg T, et al. In vitro and in vivo activities of the novel anticytomegalovirus compound AIC246. *Antimicrob Agents Chemother* 2010; 54: 1290.
 208. Chemaly RF, Ehninger G, Champlin R, et al. *Letermovir (AIC246) for the Prevention of CMV Infections Meets Primary Endpoint in Phase 2b Trial in Human Blood Precursor Cell Transplant Recipients*. San Francisco, CA: 52nd Intersci Conf Antimicrob Agents Chemother; 2012: Abstract T.
 209. 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.
 210. Goldner T, Hewlett G, Ettischer N, et al. The novel anticytomegalovirus compound AIC246 (Letermovir) inhibits human cytomegalovirus replication through a specific antiviral mechanism that involves the viral terminase. *J Virol* 2011; 85: 10884.
 211. Cytomegalovirus. Infectious Diseases Community of Practice, American Society of Transplantation. Guidelines for the prevention and management of infectious complications of solid organ transplantation. *Am J Transplant* 2004; 4: 51.
 212. Humar A, Snyderman D. Cytomegalovirus in solid organ transplant recipients. *Am J Transplant* 2009; 9: S78.
 213. Preiksaitis JK, Brennan DC, Fishman J, et al. Canadian society of transplantation consensus workshop on cytomegalovirus management in solid organ transplantation final report. *Am J Transplant* 2005; 5: 218.
 214. Bowman JS, Green M, Scantlebury VP, et al. OKT3 and viral disease in pediatric liver transplant recipients. *Clin Transplant* 1991; 5: 294.
 215. Iragorri S, Pillay D, Scrine M, et al. Prospective cytomegalovirus surveillance in paediatric renal transplant patients. *Pediatr Nephrol* 1993; 7: 55.
 216. 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.
 217. Bueno J, Green M, Kocoshis S, et al. Cytomegalovirus infection after intestinal transplantation in children. *Clin Infect Dis* 1997; 25: 1078.
 218. Nayyar N, Mazariegos G, Ranganathan S, et al. Pediatric small bowel transplantation. *Semin Pediatr Surg* 2010; 19: 68.
 219. Kranz B, Vester U, Wingen AM, et al. Acute rejection episodes in pediatric renal transplant recipients with cytomegalovirus infection. *Pediatr Transplant* 2008; 12: 474.
 220. 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.
 221. Danziger-Isakov LA, DelaMorena M, Hayashi RJ, et al. Cytomegalovirus viremia associated with death or retransplantation in pediatric lung-transplant recipients. *Transplantation* 2003; 75: 1538.
 222. Danziger-Isakov LA, Worley S, Michaels MG, et al. The risk, prevention, and outcome of cytomegalovirus after pediatric lung transplantation. *Transplantation* 2009; 87: 1541.
 223. Bueno J, Ramil C, Green M. Current management strategies for the prevention and treatment of cytomegalovirus infection in pediatric transplant recipients. *Paediatr Drugs* 2002; 4: 279.
 224. Paya CV. Indirect effects of CMV in the solid organ transplant patient. *Transpl Infect Dis* 1999; 1: 8.
 225. 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.
 226. Liu M, Worley S, Arrigain S, et al. Respiratory viral infections within one year after pediatric lung transplant. *Transpl Infect Dis* 2009; 11: 304.
 227. Snyderman 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.
 228. 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.
 229. Mahle WT, Fourshee MT, Naftel DM, et al. Does cytomegalovirus serology impact outcome after pediatric heart transplantation? *J Heart Lung Transplant* 2009; 28: 1299.

230. 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.
231. 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.
232. Lin A, Worley S, Brubaker J, et al. Assessment of cytomegalovirus hybrid preventative strategy in pediatric heart transplant patients. *J Pediatr Infect Dis Soc* 2012. doi: 10.1093/jpids/pis056.
233. 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.
234. Physician's Desk Reference. PDR Network, LLC. <http://www.pdr.net/druggages/concisemonograph.aspx?concise=1242>. Accessed November 6, 2012.
235. 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.
236. Pescovitz MD, Jain A, Robson R, et al. Establishing pharmacokinetic bioequivalence of valganciclovir oral solution versus the tablet formulation. *Transplant Proc* 2007; 39: 3111.
237. 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.
238. Kimberlin DW, Acosta EP, Sanchez PJ, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. *J Infect Dis* 2008; 197: 836.
239. Bedel AN, Hemmelgarn TS, Kohli R. Retrospective review of the incidence of cytomegalovirus infection and disease after liver transplantation in pediatric patients: comparison of prophylactic oral ganciclovir and/or oral valganciclovir. *Liver Transpl* 2012; 18: 347.
240. 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.
241. 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 immunosuppressive regimen. *Pediatr Transplant* 2010; 14: 67.
242. 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.
243. 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.
244. Snyderman 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.
245. Gilbert C, Boivin G. Human cytomegalovirus resistance to antiviral drugs. *Antimicrob Agents Chemother* 2005; 49: 873.
246. Freeman RB Jr. The 'indirect' effects of cytomegalovirus infection. *Am J Transplant* 2009; 9: 2453.
247. Reischig T, Jindra P, Hes O, et al. Effect of cytomegalovirus viremia on subclinical rejection or interstitial fibrosis and tubular atrophy in protocol biopsy at 3 months in renal allograft recipients managed by preemptive therapy or antiviral prophylaxis. *Transplantation* 2009; 87: 436.
248. Bosch W, Heckman MG, Pungpapong S, et al. Association of cytomegalovirus infection and disease with recurrent hepatitis C after liver transplantation. *Transplantation* 2012; 93: 723.
249. Gunsar F, Rolando N, Pastacaldi S, et al. Late hepatic artery thrombosis after orthotopic liver transplantation. *Liver Transpl* 2003; 9: 605.
250. Silva MA, Jambulingam PS, Gunson BK, et al. Hepatic artery thrombosis following orthotopic liver transplantation: a 10-year experience from a single centre in the United Kingdom. *Liver Transpl* 2006; 12: 146.
251. Seehofer D, Rayes N, Tullius SG, et al. CMV hepatitis after liver transplantation: incidence, clinical course, and long-term follow-up. *Liver Transpl* 2002; 8: 1138.
252. Potena L, Grigioni F, Magnani G, et al. Prophylaxis versus preemptive anti-cytomegalovirus approach for prevention of allograft vasculopathy in heart transplant recipients. *J Heart Lung Transplant* 2009; 28: 461.
253. Petrakopoulou P, Kubrich M, Pehlivanli S, et al. Cytomegalovirus infection in heart transplant recipients is associated with impaired endothelial function. *Circulation* 2004; 110: II207.
254. Johansson I, Martensson G, Andersson R. Cytomegalovirus and long-term outcome after lung transplantation in Gothenburg, Sweden. *Scand J Infect Dis* 2010; 42: 129.
255. Chmiel C, Speich R, Hofer M, et al. Ganciclovir/valganciclovir prophylaxis decreases cytomegalovirus-related events and bronchiolitis obliterans syndrome after lung transplantation. *Clin Infect Dis* 2008; 46: 831.
256. Linares L, Sanclemente G, Cervera C, et al. Influence of cytomegalovirus disease in outcome of solid organ transplant patients. *Transplant Proc* 2011; 43: 2145.
257. Munoz-Price LS, Slifkin M, Ruthazer R, et al. The clinical impact of ganciclovir prophylaxis on the occurrence of bacteremia in orthotopic liver transplant recipients. *Clin Infect Dis* 2004; 39: 1293.
258. Fishman JA. Infection in solid-organ transplant recipients. *N Engl J Med* 2007; 357: 2601.
259. Walker RC, Marshall WF, Strickler JG, et al. Pretransplantation assessment of the risk of lymphoproliferative disorder. *Clin Infect Dis* 1995; 20: 1346.
260. Gomez E, Lares A, Baltar JM, et al. Cytomegalovirus replication and "herpesvirus burden" as risk factor of cardiovascular events in the first year after renal transplantation. *Transplant Proc* 2005; 37: 3760.
261. Van Laecke S, Desideri F, Geerts A, et al. Hypomagnesemia and the risk of new-onset diabetes after liver transplantation. *Liver Transpl* 2010; 16: 1278.
262. Leung Ki EL, Venetz JP, Meylan P, et al. Cytomegalovirus infection and new-onset post-transplant diabetes mellitus. *Clin Transplant* 2008; 22: 245.
263. Koch S, Larbi A, Ozelik D, et al. Cytomegalovirus infection: a driving force in human T cell immunosenescence. *Ann N Y Acad Sci* 2007; 1114: 23.
264. Monforte V, Lopez C, Santos F, et al. A multicenter study of valganciclovir prophylaxis up to day 120 in CMV-seropositive lung transplant recipients. *Am J Transplant* 2009; 9: 1134.
265. Sagedal S, Rollag H, Hartmann A. Cytomegalovirus infection in renal transplant recipients is associated with impaired survival irrespective of expected mortality risk. *Clin Transplant* 2007; 21: 309.
266. Kotton CN. CMV: prevention, diagnosis and therapy. *Am J Transplant* 2013; 13: 24.
267. Dzabic M, Rahbar A, Yaiw KC, et al. Intra-graft cytomegalovirus protein expression is associated with reduced renal allograft survival. *Clin Infect Dis* 2011; 53: 969.
268. GRADE Strength of Recommendations and Quality of the Evidence. Infectious Disease Society of America. http://www.idsociety.org/uploadedFiles/IDSA/Guidelines-Patient_Care/PDF_Library/GRADE%20Table%20and%20Reference.pdf. Accessed January 20, 2013.
269. Guyatt GH, Oxman AD, Kunz R, et al. What is "quality of evidence" and why is it important to clinicians? *BMJ* 2008; 336: 995.
270. Product Monograph Cytovene® Hoffmann-La Roche Ltd./CYTOVENE®-IV (ganciclovir sodium for injection). Hoffmann-La Roche Ltd., 2010. <http://www.gene.com/gene/products/information/cytovene/pdf/pi.pdf>. Accessed March 13, 2013.
271. Product Monograph Valcyte® Hoffmann-La Roche Ltd., Hoffmann-La Roche Ltd., 2010. <http://www.gene.com/gene/products/information/valcyte/pdf/pi.pdf>. Accessed March 13, 2013.
272. Product Monograph Valcyte® Hoffmann-La Roche Ltd., Valganciclovir Film Coated Tablets, Valganciclovir Powder for Oral Solution. Hoffmann-La Roche Ltd., 2010. <http://rochecanada.com/fmfiles/re7234008/Research/ClinicalTrialsForms/Products/ConsumerInformation/MonographsandPublicAdvisories/Valcyte/valcyteHPE.pdf>. Accessed March 13, 2013.

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