Cytomegalovirus Infection in Transplantation

Internal Medicine Grand Rounds

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This is to acknowledge that Brendan De Marco, MD, MPH, has disclosed that he does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. De Marco will be discussing off-label uses in his presentation.

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Purpose and Overview:

Cytomegalovirus (CMV) is a significant pathogen causing problems post-transplantation due to the lifelong latency of this virus as well as new acquisition or reactivation as a result of immunosuppression after transplantation. Despite significant efforts to prevent CMV infection post-transplantation we continue to see patients with complications from CMV. Newer strategies and agents have been implemented in order to prevent complications related to cytomegalovirus including prophylactic strategies, prolonging of antiviral prophylaxis, and monitoring immune responses prior to discontinuation of antiviral prophylaxis. Upcoming options include the potential availability of a CMV vaccine. Despite these measures, CMV remains a significant presence among transplanted patients, and treatment options in the past have been limited. This talk will review currently available as well as new and upcoming treatment options. Although rare, CMV resistance makes treatment even more complicated, and this talk will also discuss issues related to the diagnosis and treatment of resistant CMV infection.

Educational Objectives:

1. To review and discuss the background and pathogenesis of cytomegalovirus infection after solid organ transplantation including the direct and indirect effects.

2. To review and discuss prophylactic strategies and other preventative measures to prevent cytomegalovirus infection post-transplantation.

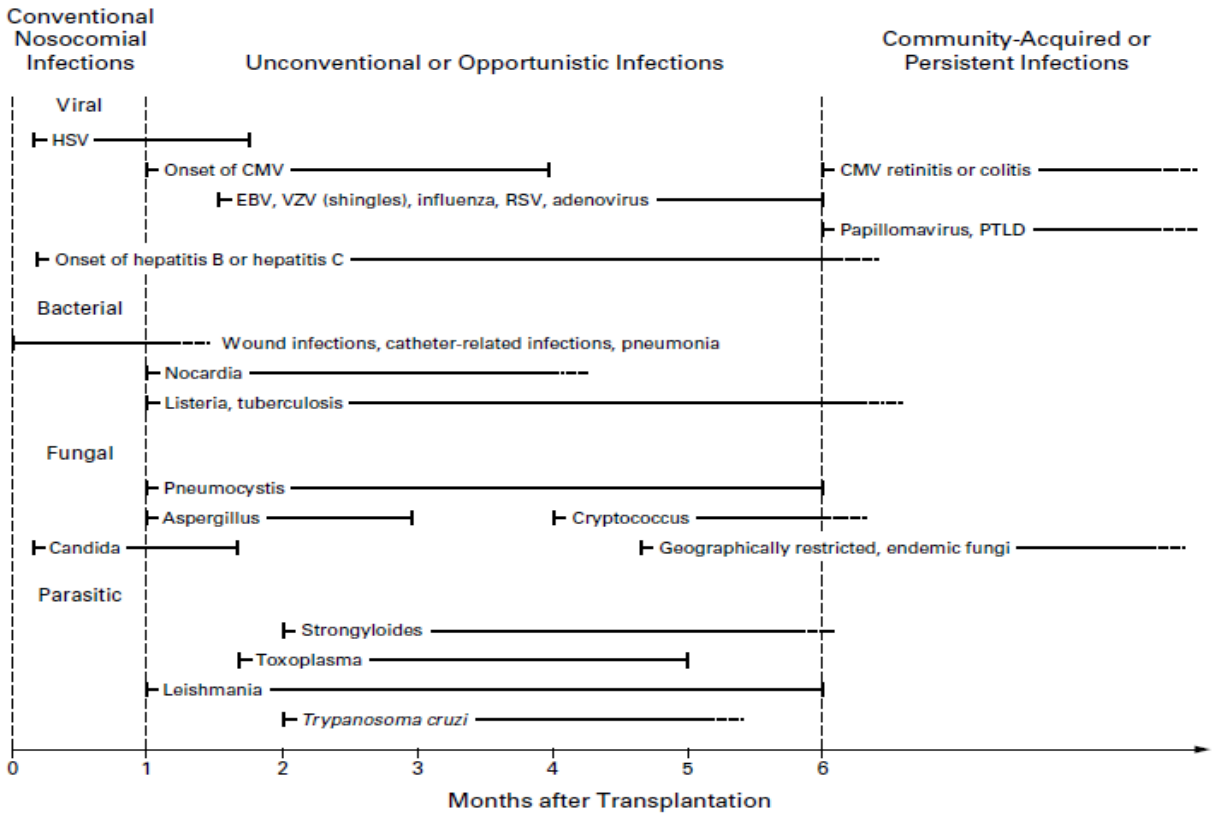
3. To review and discuss the available treatment options for cytomegalovirus infection including new and upcoming treatments.

4. To review and discuss the problem of resistant cytomegalovirus infection including new and upcoming treatment options.

Introduction to Transplant Infectious Diseases

Certain infections occur at expected periods after solid organ transplantation. This was summarized in a timeline **(Figure 1)** published by Drs. Jay Fishman and Robert Rubin(1). They described that in the early post-transplant period, within the first month post-transplantation, the majority of infections were nosocomial or due to surgical complications. Within the first month after transplantation the immunosuppressant/anti-rejection medications have not fully taken effect. Common infections during this period include post-operative wound infections, central line infections, bacteremias, nosocomial pneumonias, and *Clostridium difficile* colitis, among others. This is also a period when early donor-derived infections can occur, as was demonstrated in the cases of rabies that were transmitted via organ transplantation in 2004(2). The intermediate period from one to six months post-transplantation is when the immunosuppressant/anti-rejection medications take their full effect. In general, antibiotic, antiviral, and sometimes antifungal prophylaxis is used during this period. For example, Bactrim is commonly given to prevent *Pneumocystis jirovecii* pneumonia (PJP) and other opportunistic infections. It is also during this period when herpesvirus infections such as herpes simplex virus (HSV), varicella zoster virus (VZV), and cytomegalovirus (CMV) commonly reactivate. Finally, the late post-transplantation period occurs after six months when the immunosuppressants are usually tapered. Patients are still at risk for infections, but these are more commonly community-acquired pathogens such as respiratory viruses, because at this point, most patients are living back at home in their communities being exposed to such infections(3).

Figure 1: Usual sequence of infections after organ transplant (Source: Fishman & Rubin, *NEJM* 1998)



Another concept that is important in terms of transplant infectious diseases is the "net state of immunosuppression." This is the balance that occurs between immunosuppression being given to prevent rejection of the organ and the epidemiologic exposure of the patients that puts them at risk for infection. Immunosuppression can not only be from anti-rejection medications, but can also be due to underlying immunodeficiencies (e.g., hypogammaglobulinemia), disruption of the mucocutaneous barrier (e.g., central lines/dialysis catheters), neutropenia due to underlying medical issues or as a side effect from medications, viral infections, and metabolic conditions such as uremia, cirrhosis, malnutrition, and diabetes mellitus (3).

With regard to immunosuppressant medications, solid organ transplants usually receive a combination of calcineurin inhibitor such as Cyclosporine A or Tacrolimus, corticosteroids, and either Mycophenolate mofetil or Azathioprine. Overuse of immunosuppression will decrease the risk of infection, but will increase the risk of infections such as CMV, as well as malignancy, and papillomatosis. After the early and intermediate post-transplant period the risk of rejection is decreased and usually immunosuppressants can be decreased over time with low dose maintenance after the first six months to one year. Calcineurin inhibitors inhibit the cytokine calcineurin which is important for helper T-cell function. They also dampen the response of viral, fungal, and mycobacterial pathogens. Corticosteroids broadly inhibit the immune response, but are associated with multiple metabolic toxicities as well as increased risk for PJP. Azathioprine is an inhibitor of T-lymphocytes, and Mycophenolate mofetil has an anti-proliferative effect on B- and T-lymphocytes and is associated with more invasive CMV infection. A less commonly used immunosuppressant, Sirolimus, is an mTOR or proliferation signal inhibitor. This immunosuppressant is actually associated with a lower risk for CMV disease and can sometimes be used as an adjunctive agent for prevention and treatment of recurrent or resistant CMV. Finally the anti-lymphocyte antibodies (e.g., Antithymocyte globulin, Thymoglobulin, and OKT3) are used for induction of immunosuppression or rejection and can have a profound and prolonged inhibitory effect on T-cells as well as alter their function. These antibody preparations are highly associated with herpesvirus infections (e.g., HSV, VZV, and CMV), PJP, and EBV-related post-transplant lymphoproliferative disorder (PTLD)(4).

Cytomegalovirus (CMV)

CMV is a member of the human herpesvirus beta subfamily **(Table 1)**. In population studies, 40-90% of healthy individuals are CMV seropositive. A common means of transmission is via saliva, but it can also be acquired *in utero*, transmitted at birth during delivery, through blood and other body fluids, and through transplanted organs and stem cells. Most infection is acquired in childhood, and this infection establishes a lifelong latency in mononuclear cells such as monocytes, macrophages, and lymphocytes (5). The spectrum of CMV infection is diverse and is dependent on the host. Infection in immunocompetent patients is generally asymptomatic or may involve a mononucleosis-like syndrome. Infection in pregnant women is associated with congenital CMV in newborns. Infections are more severe in immunocompromised patients, and are commonly seen in HIV/AIDS patients with CD4 counts less than 50, as well as patient with inflammatory bowel disease, lupus, and other autoimmune diseases which require immunosuppressant therapy. In the population of AIDS patients, CMV most commonly

manifests as chorioretinitis, but it can also involve the GI tract causing esophagitis and colitis, as well as the lungs causing pneumonitis(6).

Following solid organ transplantation, CMV can be a major cause of morbidity and mortality. As a result, donor and recipient CMV IgG antibody status or serostatus is important. Transplant recipients who are CMV seronegative and receive an organ from a seropositive donor can develop primary CMV infection which is transmitted via the organ. If the recipient is already seropositive, then reactivation of latent CMV infection can occur post-transplantation. In recipients who are CMV seropositive and receive an organ from a seropositive donor, they can either get reactivation of latent CMV infection or a superinfection with a new strain of CMV. Without some form of prevention, CMV reactivation or primary/superinfection commonly occurs within the first three to six months following transplant. Up to 75% of all transplant patients will experience a new infection or reactivation of latent CMV. If both the donor and the recipient are CMV seronegative, they can still acquire primary CMV infection via the receipt of blood products that are not CMV-negative or leukoreduced, or they can acquire CMV via a new exogenous exposure after transplantation (7).

Table 1: The human herpesviruses (HHV) (Source: Fishman, *Amer J Transpl* 2013)

Type	Common name	Major syndromes	Site of latency	Means of spread
α (Alpha) herpesviruses: rapid reproduction and cell lysis *in vitro*, rapid cell lysis and spread *in vivo*, primary target mucoepithelial cells, latency in sensory ganglia				
HHV–1	Herpes simplex virus-1 (HSV-1)	Oral herpes, genital herpes (predominantly orofacial), as well as other herpes simplex infections	Sensory and cranial nerve ganglia	Close contact (sexually transmitted disease)
HHV-2	Herpes simplex virus-2 (HSV-2)	Oral and/or genital herpes (predominantly genital), as well as other herpes simplex infections	Sensory and cranial nerve ganglia	Close contact (sexually transmitted disease)
HHV-3	Varicella zoster virus (VZV)	Chickenpox and shingles	Sensory and cranial nerve ganglia	Respiratory and close contact (including sexually transmitted disease)
γ(Gamma): replication in lymphoblastoid cells, lytic cycle in some fibroblasts and epithelial cells				
HHV-4	Epstein–Barr virus (EBV), lymphocryptovirus (gamma-1-herpesvirus)	Infectious mononucleosis, Burkitt's lymphoma, CNS lymphoma, posttransplant lymphoproliferative syndrome (PTLD), nasopharyngeal carcinoma, HIV-associated hairy leukoplakia	Memory B cells	Close contact, transfusions, tissue transplant and congenital
HHV-8	Kaposi's sarcoma-associated herpesvirus (KSHV), human rhadinovirus (gamma-2-herpesvirus)	Kaposi's sarcoma, primary effusion lymphoma, some types of multicentric Castleman's disease	B cells	Close contact (sexual), saliva?
β (Beta): long replication cycle *in vivo* and *in vitro*, limited host range, large infected cells, latency in mononuclear cells, secretory cells, some epithelial cells, others				
HHV-5	Cytomegalovirus (CMV) Monocyte, lymphocyte and epithelial cells	Infectious mononucleosis-like syndrome,[10] retinitis, etc.	Monocytes, macrophages, lymphocytes, others	Saliva
HHV-6A and HHV-6B	Roseolovirus, Herpes lymphotropic virus T cells, other cells	*Sixth disease* (roseola infantum or *exanthem subitum*)	T, B, NK cells, monocytes, macrophages, liver, salivary endothelial, neuronal cells	Respiratory and close contact?
HHV-7	Roseolovirus T cells, other cells	*Sixth disease* (roseola infantum or *exanthem subitum*)	CD4+ T cells, salivary epithelial, lung, skin cells	?

Impact of CMV Infection and Disease in Transplantation

It is important to distinguish the difference between CMV infection and CMV disease. Replication of CMV virus is commonly referred to as CMV infection, whereas replication of CMV virus with symptoms is referred to as CMV disease(7). These symptoms can be wide and varied and include not only a viral syndrome, but also tissue invasive disease. CMV infection is a balance between viral and host factors. The viral factors include replication dynamics, immune evasion, viral heterogeneity, and viral co-infections. Host factors include donor and recipient serostatus, CD4- and CD8-positive T-cells, NK cells, B-cells, and also are determined by exogenous immunosuppression. Reactivation of CMV in solid organ transplantation is a complex process with factors including immunosuppression, co-infection with other herpesviruses, acute rejection, sepsis, and even the surgical procedure itself(8).

CMV is the most important pathogen post-transplantation with multiple consequences involving both direct and indirect effects **(Figure 2)**. The direct effects are commonly referred to as the CMV syndrome which is a flulike, mononucleosis-like syndrome with neutropenia that can also include myelosuppression, pneumonia, gastrointestinal invasion, hepatitis, pancreatitis, and chorioretinitis. The indirect effects have been the subject of more study in recent years and have shown that CMV has many other effects including increased risk of secondary infections with bacteria, fungi, and other viruses, increased incidence of both acute and chronic graft rejection, many metabolic effects, and increased overall mortality(8).

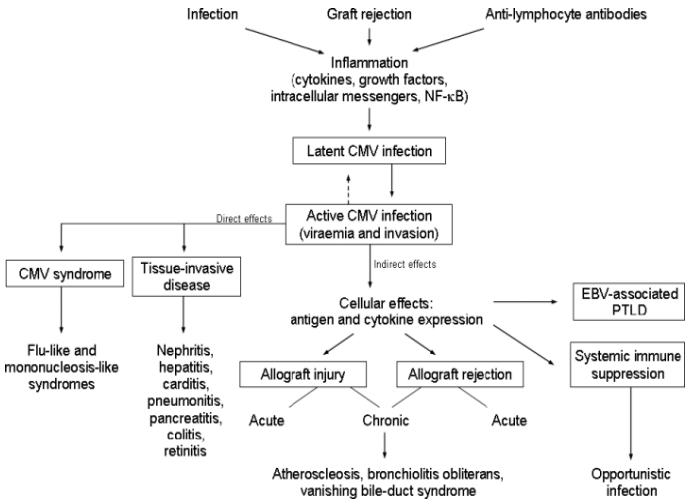


Figure 2: Role of cytomegalovirus infection in transplant recipients (Source: Fishman, *Clin Transplant* 2007)

CMV Direct Effects

Among the direct effects of CMV disease after solid organ transplantation, CMV syndrome is the most common. CMV syndrome is defined as the presence of CMV in the blood with fever and one or more of the following: malaise, leukopenia, atypical lymphocytosis, thrombocytopenia, and elevated hepatic enzymes. Tissue invasive disease can also occur in almost any organ and is defined as evidence of CMV on histology in a biopsy specimen with compatible signs and symptoms. Hepatitis, colitis, and pneumonitis are the most common presentations of tissue invasive disease with encephalitis and retinitis being rare. Of note, CMV has a predilection to involve the allograft(8)**.** CMV pneumonitis is

diagnosed when a patient has compatible signs and symptoms including fever, dyspnea, and hypoxemia with consistent imaging findings showing diffuse interstitial infiltrates with evidence of CMV replication in the blood, or a bronchoscopy and lung biopsy with pathognomonic CMV intranuclear inclusions on histology(9).

CMV colitis is diagnosed by the triad of cardinal symptoms including fever, abdominal pain, and diarrhea, and visualization of mucosal ulcers or erosions on endoscopy, and histological evidence of tissue destruction and presence of viral inclusion bodies. The presentation of CMV colitis can vary endoscopically anywhere from punctate and superficial erosions to deep ulcerations and necrotizing colitis. Viral cultures of mucosal biopsies do not establish the diagnosis of CMV disease as immunosuppressed patients may have viral shedding in the absence of clinical disease. Pathology is characterized by mucosal inflammation, tissue necrosis, and vascular endothelial involvement. Characteristically, cytomegalic cells are present in mucosal biopsies and are described as large cells containing eosinophilic intranuclear and frequently intracytoplasmic inclusions when mucosal biopsies are stained with hematoxylin and eosin stain(10)**.**

CMV chorioretinitis causes characteristic retinal findings which may cause vision changes. It is more common in HIV/AIDS patients and is rare in transplant recipients. However, when it occurs it can become apparent several months after transplant even in patients without evidence of symptomatic infection. It is important to note that there may be no evidence of viremia, or replication of virus in the blood, in patients with GI disease and retinitis, likely due to compartmentalization of the virus. In such cases it is important to consult GI for endoscopy and ophthalmology for retinal exam urgently in order to establish a diagnosis and initiate prompt treatment(7)**.**

CMV Indirect Effects

The mechanism for the indirect effects of CMV infection are various and include an increase in the adhesion molecules VCAM, ICAM, LFA-1, and VLA-4, increases in IL-10 homologue with decreased MHC expression and decreased lymphocyte proliferation, increase in IL-8 like chemokine and neutrophil chemotaxis, increase in HLA-DR and MHC class 1 mimic, immune evasion mechanisms, decrease in altered mobilization by inflammatory cells, and altered antigen presentation(11). There are multiple indirect associations that have been found with CMV infection. The most significant of which are the increased risks for rejection and allograft loss. The indirect effects of CMV infection include acute allograft rejection, chronic allograft rejection, and allograft loss. In a study of kidney transplant patients, CMV infection and disease was associated with acute allograft rejection, and CMV disease was found to predict subsequent allograft loss(12). The etiology for this appears to be related to CMV infection facilitating an inflammatory process that leads to endothelial damage by alloreactive T-cells and chronic allograft rejection. Each transplant type has its own name for this process. For example, in kidney transplants it is referred to as chronic allograft nephropathy; in liver transplants this is the vanishing bile duct syndrome; in lung transplants this is referred to as bronchiolitis obliterans; and in heart transplants this is called accelerated coronary atherosclerosis(13)(14). The influence of donor seropositivity in CMV seronegative recipients is independently associated with a significant increase in bacteremias , aspergillosis, and other invasive mold infections, as well as all-cause mortality(15)**,** (16)**,**(17)**.**

Another association thought to be related to the indirect effects of CMV is the increased incidence of EBV-related post-transplant lymphoproliferative disorder (PTLD). In patients at high risk for developing PTLD (i.e., EBV donor seropositive/recipient seronegative), CMV disease has been shown to be an independent predictor of this outcome(18). The risk of developing PTLD is increased seven-fold in those with CMV disease. CMV also appears to be associated with infection or reactivation of human herpesvirus 6 and 7 (HHV-6 and HHV-7)(19)(20)(21). CMV has also been associated with worse outcomes when occurring in patients with hepatitis C virus (HCV) infections. In liver transplant patients who were infected with HCV, CMV reactivation was independently associated with allograft failure and mortality(22).

Other metabolic complications and overall mortality risk have been noted to be associated with CMV as well. The incidence of new onset of diabetes after transplantation may be associated with CMV infection and disease. In a study of 160 consecutive non-diabetic renal transplants, a higher incidence of new onset diabetes was seen in patients who developed CMV infection compared to the control group who did not develop CMV infection (26% vs. 6%; p=0.03)(23). In addition, CMV has also been associated with atherosclerosis and other vascular injuries such as transplant glomerulopathy and hemolytic uremic syndrome(23)(24). Post-transplant diabetes mellitus is a potential risk factor for these cardiovascular events. Finally, CMV reduces survival among renal transplant recipients beyond their expected mortality risk(25).

Diagnosis of CMV

Characteristic histopathologic findings are confirmation of CMV disease; however, the incidence of making a diagnosis is declining due to the availability of less invasive testing methods. Nonetheless, a biopsy with histopathology is still recommended in cases where a predominant pathogen or co-pathogen is suspected, and also if a patient is not responding to CMV treatment. Another method of diagnosis is viral culture, either viral tissue culture or cell vial centrifugation assay. Culture is highly specific for the diagnosis of CMV infection, but its use is limited by modest sensitivity rates and a slow turnaround time. Viral culture is less sensitive than newer more available molecular assays, but it is still used in isolating CMV in non-blood clinical specimens. However, caution should be used when interpreting viral culture results since immunosuppressed patients commonly have asymptomatic viremia or viral shedding. Serology (CMV IgM and IgG) is used commonly in diagnosis of immunocompetent patients for diagnosing acute CMV infection; however, it is also used prior to transplantation to establish CMV IgG serostatus in the donor (designated “D”) and recipient (designated “R”). The CMV IgG serostatus is designated as either positive (+) or negative (-). The possible combinations are as follows: D+/R-, D+/R+, D-/R+, or D-/R-. Serology is of limited utility for diagnosis of CMV in immunocompromised patients especially after transplantation.

The CMV antigenemia assay (i.e., CMV pp65) is a semi-quantitative assay that detects the pp65 antigen in CMV-infected peripheral blood leukocytes. This test has a higher sensitivity than culture and is comparable to molecular assays. As a result, it is useful for diagnosis and for monitoring in the setting of preemptive prophylaxis, monitoring treatment response, and for monitoring for recurrence after completion of treatment. The main disadvantage is due to the need for quick processing within a few

hours to avoid a decrease in sensitivity and the need for an adequate neutrophil count since the test is based on CMV staining of infected leukocytes.

Molecular assays such as the CMV PCR have become the preferred method for diagnosis of CMV after solid organ transplantation. These assays are highly sensitive but have low specificity, especially if using a qualitative PCR assay, a CMV DNA PCR, or when assaying whole blood for CMV PCR. Detection of CMV DNA may or may not reflect active CMV replication since highly sensitive nucleic acid testing may amplify latent viral DNA. Several different methods have been used to increase the specificity of the molecular tests. Looking at CMV DNA in plasma alone and excluding whole blood, using quantitative assays (such as quantitative nucleic acid testing or QNAT), and detecting mRNA (which is an indication of active CMV replication) are all associated with increased specificity. Therefore, quantitative assays or QNAT have been developed to differentiate between active viral replication and latent virus. In addition, the amount of CMV in the blood or the so-called "CMV viral load" is directly proportional to the likelihood for developing tissue invasive disease. For example, a higher CMV viral load is more associated with tissue invasive disease while lower CMV viral load values are seen more with asymptomatic CMV infection or the CMV syndrome. In addition, the rate of rise of the viral load is also an important marker of CMV disease with a faster rise in viral load being more associated with a higher risk of CMV disease. As mentioned previously, sometimes patients with CMV disease, especially GI CMV disease and retinitis, have only a very low or even undetectable CMV viral load. This may be due to CMV disease compartmentalization or because of the use of less sensitive assays(7).

The optimal cutoff for predicting CMV disease is unclear, but studies have attempted to define appropriate viral load cutoffs. In an evaluation of 97 liver transplant patients, the optimal cutoff for detecting CMV disease was in the range of 2000-5000 copies/mL of plasma, and patients with a viral load over 20,000 copies/mL all developed CMV disease(26). These cutoffs are not generalizable among different laboratories/institutions, and establishing an appropriate viral load cutoff depends on which CMV assay is used, whether plasma, whole blood, or leukocytes are assayed, the type of organ transplanted, the serostatus of the donor and recipient, the type of immunosuppression, and the presence of other immunomodulating viruses such as HHV-6. There is, in fact, a wide variation in results when specimens are sent to different labs, indicating the high variability of the various assays available. This is a major drawback of nucleic acid testing. One study found that the viral load results of one assay could not be directly extrapolated to another assay, and up to a 3-$\log_{10}$ variation was found among 33 reference samples sent to various laboratories in the United States, Canada, and Europe(27). This variability is concerning and could make the difference between a diagnosis of invasive CMV disease and completely missing the diagnosis. This lack of standardization has limited the generation and implementation of widely accepted threshold values which could be used for guiding diagnosis as well as prophylactic and therapeutic monitoring. Currently, each transplant center has been advised to work with their own clinical laboratory to define relative viral load thresholds, and it is not recommended to send samples to various labs given this inter-laboratory variability(28).

As a result, in 2011 the World Health Organization revealed the first international reference standard for the quantification of CMV nucleic acid, and all laboratory and commercially developed molecular assays should now be calibrated to this standard. This reference reagent is an important

advance in CMV testing since it allows for the standardization of viral load values among different laboratories. The results are given in International Units per mL (IU/ml)(29). This reference standard is not currently available, but when it is implemented it may ensure uniformity in viral load reporting allowing defined viral thresholds for various clinical applications which can be useful for preemptive therapy, disease prognostication, and therapeutic monitoring.

Risk Factors for CMV Infection and Disease

Certain risk factors are associated increased risk of CMV disease in solid organ transplant recipients. Primarily, the use of donor and recipient serologies has been associated with increased risk of primary, reactivation, or superinfection. The highest risk is seen in donor seropositive and recipient seronegative patients (D+/R-). Without prophylaxis, this high risk population has an 80 to 100 percent risk of CMV infection. In the intermediate risk group lies donor seropositive, recipient seropositive (D+/R+) and donor seronegative, recipient seropositive (D-/R+) patients. Without prophylaxis, this group has about a 16-21% risk of CMV infection. Finally, in the lowest risk group, those with donor and recipient seronegative CMV status (D-/R-), CMV infections are rare but not impossible. For example, if a patient receives multiple blood products that are not CMV negative or leukoreduced, there is the potential for new acquisition of CMV. In addition, patients can acquire new CMV infection after transplantation. In addition, the type of transplant is an important risk factor for the development of CMV infection and disease. Those organs that have more lymphoid tissue transplanted with them are at higher risk for developing CMV infection and disease. Lung, small intestine, and now vascularized composite allograft tissue such as hand and face transplants are all at the highest risk, with heart and pancreas transplants at a medium risk, and kidney and liver transplants in the lowest risk group. Also, acute graft rejection and the use of anti-lymphocyte antibody preparations are associated with increased risk of CMV. The degree of viremia is important, since higher viral loads found on the CMV pp65 or PCR assays are more highly associated with CMV. Common viral infections such as EBV, HCV, and HHV-6 and -7, may also play a role in the risk for CMV infection. Other risk factors include donor and recipient age, with high risk donors or extended criteria donors having an increased risk of developing CMV. Lack of or an inadequate duration of CMV prophylaxis as well as lower dosing of antiviral prophylaxis is associated with CMV. Lymphocyte depleting antibody use such as Thymoglobulin, OKT3, and Alemtuzumab, as well as intense immunosuppression with commonly used antirejection medications increase the risk of CMV. Overall, the net state of immunosuppression is the most important risk factor for CMV. The greater the net state of immunosuppression the higher the risk of CMV infection and disease which by itself contributes to an increased risk of immunosuppression indicating a bi-direction association(8).

Prevention of CMV Infection and Disease

There are various strategies for preventing CMV infection in solid organ transplant patients and various strategies are used among the different transplant centers. One commonly used strategy and the one used here at UT Southwestern is known as Universal Prophylaxis. This entails treatment of all patients during the highest risk period of CMV infection which is usually within the first 6 months after transplantation. Another strategy is known as the Preemptive Strategy. This is more labor intensive and

requires frequent monitoring for CMV viremia with early detection prompting the initiation of treatment prior to the onset of symptoms(8).

Preemptive Strategy

The preemptive practice involves serial monitoring of pp65 antigenemia or PCR testing done on a weekly basis, and once this is above a certain threshold designated by the transplant center, then preemptive antiviral therapy is given prior to the onset of symptoms associated with CMV disease. The preemptive strategy has its advantages and disadvantages. The advantages include minimization of drug exposure since patients who remain CMV negative do not require any prophylaxis or treatment. This may potentially decrease the associated toxicity and cost of these medications. They also theoretically lower the risk of CMV drug resistance since there is less exposure to the drug. In addition, there is a theoretical advantage of preventing late onset CMV disease (i.e. CMV infection and disease that occurs after prophylaxis is discontinued). Since low level viremia may occur in these patients, this may also allow for the development of a cell mediated immune response by the host, which may in turn help control the viremia and prevent CMV disease.

The main disadvantage of preemptive therapy is that it is logistically more difficult to coordinate since it requires a very well organized strategy of monitoring, checking the results, and acting when CMV virus is detected, and goes above a certain threshold. This may be difficult to do in large transplant centers with many patients who live remotely from the transplant center and are difficult to contact. It also may be unsuccessful in preventing the progression to active disease in high-risk patients due to the rapid doubling time of CMV virus in these patients. For example, even within one week, the increasing rate of CMV viremia may be high enough to cause patients to have CMV active disease. In addition, this strategy may not eliminate the indirect effects of CMV infection, since even low level viremia may be contributing to some of these indirect effects. Several meta-analyses have been published with regard to preemptive therapy. Overall, the risk of CMV disease is prevented, but all-cause mortality does not seem to be effected by the preemptive therapy(8).

Prophylactic Strategy

The most commonly used prophylactic strategy, and the one used here at UT Southwestern, involves giving all at risk patients antiviral prophylaxis. Antiviral preventive therapy is prescribed from the time of transplant; to all patients in a universal approach or targeted only to high risk patients such as those with high or intermediate risk CMV serostatus, those who receive multiple blood products, and/or those who are being treated for rejection with intensified immunosuppression. The advantages of this strategy include the fact that it has proven efficacy in preventing CMV infection and disease. It also decreases the indirect effects of CMV. It is less labor intensive in that it is easier to administer universal prophylaxis to all at-risk patients. However, the disadvantages include the high cost of CMV prophylaxis as well as toxicities from these medications. In addition, exposure to these antiviral medications can lead to an increased risk of drug resistant CMV later on. Late onset CMV disease can also occur(8).

A meta-analysis of multiple studies of CMV prophylaxis showed a reduced relative risk of CMV infection, CMV disease, and CMV-associated mortality vs. placebo. In addition, related to the indirect effects of CMV infection, there are other prophylactic benefits for concomitant infections. For example, these antivirals will also prevent HSV and VZV infections, will decrease the risk of bacterial super-infections, and interestingly, also decrease the risk of protozoal infections(30).

There are very few randomized trials comparing preemptive vs. prophylaxis strategies. In a study by Khoury, *et al*, in 2006, 98 kidney transplant patients were randomized to either preemptive or prophylactic therapy with oral Valganciclovir for 100 days. Both strategies were equally effective in preventing CMV disease(31). Another randomized trial by KIiem, et al, from 2008, randomized 148 kidney transplant patients to preemptive therapy with IV Ganciclovir vs. prophylaxis with 3 months of oral Ganciclovir(32). Long-term graft survival at 4 years post-transplantation was significantly improved in the prophylaxis group. It is difficult to extrapolate these results, however, as oral Ganciclovir is a less preferred agent for prophylaxis since oral Valganciclovir with its higher bioavailability is the preferred prophylactic antiviral agent. In the 2006 study by Khoury, *et al*, they did a cost analysis of the two strategies. The mean overall cost was about $7130 +/- 3748 per patient in the preemptive arm, and $7678 +/- 6486 per patient in the prophylaxis arm when accounting for lab monitoring and drug costs(31).

The overall comparison of preemptive versus prophylaxis strategies is outlined below **(Table 2)**, and each strategy has its own advantages and disadvantages. The choice of which strategy to implement depends on various factors. Based on a recent international CMV consensus, both the prophylaxis and preemptive strategies were reasonable alternatives for R+ patients. However, the majority of the participants in this consensus paper favored prophylaxis over preemptive in the high-risk D+/R- population and certain high-risk groups, based on studies showing better graft survival and overall clinical outcomes. However, to mitigate risk, some may choose to implement a hybrid approach between these two strategies(28).

Table 2: Characteristics of antiviral prophylaxis and preemptive therapy (Source: Razonable, *Amer J Transpl* 2013)

	Prophylaxis	Preemptive therapy
Efficacy	Yes: large randomized trials	Yes: smaller trials; fewer D+/R–
Ease	Relatively easy to coordinate	More difficult to coordinate Viral load thresholds not standardized
Late-onset CMV disease	Occurs commonly in CMV D+/R– transplant recipients	Occurs much less commonly
Cost	Higher drug costs	Higher laboratory costs
Toxicity	Greater drug toxicity (myelosuppression)	Potential for less drug toxicity with shorter courses of antivirals
Indirect effects (graft loss, mortality and opportunistic infections)	Positive impact based on meta-analyses and limited comparative trials	Very limited data that preemptive therapy affects indirect effects
Drug resistance	Yes	Yes

Prophylactic Agents

The medications used for prophylaxis are similar to those used for CMV treatment, which will be discussed later. However, in overview, Ganciclovir, Valganciclovir, Valacyclovir and CMV hyperimmune globulin are used. Ganciclovir is a synthetic drug of 2-deoxyguanosine which inhibits viral DNA

polymerase and is available in both oral and IV preparations. Predominant adverse effects include hematologic effects such as bone marrow suppression, but it can also cause gastrointestinal and neurologic and renal dysfunction as well. Oral Ganciclovir prophylaxis is typically dosed at 1 gram three times per day, but it is not used very often given its poor oral bioavaibility (around 5% fasting and around 8% with food). If Ganciclovir is used, it is typically given as an IV preparation, either as 5mg per kg every 12 hours, or once per day. This dose should be adjusted for renal function. Valganciclovir is an oral prodrug of Ganciclovir with improved oral bioavailability (50-60%). The adverse effects are similar to Ganciclovir with myelosuppression being the main side effect that limits its use. Dosing for prophylaxis is usually 900mg once a day, adjusted for renal function.

Valacyclovir is a hydrochloride salt of the L-valylester of Acyclovir. It is predominantly used for the prevention and treatment of HSV and VZV; however, it has been shown to be somewhat effective in prevention of CMV infection, predominantly in renal transplant patients. The adverse effects of Valacyclovir include thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, which is a rare idiosyncratic reaction. It can also have central nervous system effects such as agitation, confusion, delirium, and seizures in the elderly, or those with underlying renal disease. It can also cause acute renal failure at increased risk, especially the elderly, and those with underlying renal dysfunction, or those taking other nephrotoxic medications, and with dehydration. Dosing for CMV prophylaxis in renal transplantation is very high dose: 2 grams four times a day, adjusted for renal function.

Finally, immunoglobulin preparations such as CMV hyperimmune globulin have been used for prophylaxis. They have been studied in relatively few randomized, non-blinded trials. Further research is needed to delineate the benefit of adding these preparations to current CMV prophylaxis strategies. However, they are used especially in high-risk heart and lung transplant patients, and they are also used as adjunctive therapies for the treatment of CMV infection(28).

Prophylaxis strategies by type of organ transplantation are slightly different **(Table 3)**. For example, kidney transplants can be give Valganciclovir, Ganciclovir (oral or IV), or Valacyclovir. Pancreas transplants including kidney/pancreas transplants can receive Valganciclovir or Ganciclovir (oral or IV). Liver transplant patients can receive oral Ganciclovir or Valganciclovir; however, the FDA notes caution in the use of Valganciclovir due to the increased risk of CMV invasive tissue disease seen in studies. In heart transplants, Valganciclovir, Ganciclovir (oral or IV), +/- CMV hyperimmune globulin (for high-risk patients) can be used, and the same is true for lung transplants or heart/lung transplants. Intestinal transplants can receive Valganciclovir, Ganciclovir (oral or IV), +/- CMV immunoglobulin (for high-risk patients).

The duration of CMV prophylaxis is generally for that high risk period between zero and six months. Generally it is recommended to continue CMV prophylaxis between three and six months post transplantation, but the duration depends on donor and recipient CMV serostatus, the degree of immunosuppression (especially if patients receive anti-lymphocyte globulin), and the type of transplantation. For example, in lung and small intestine recipients, a minimum of six months is recommended, but many favor twelve months or longer for lung transplant patients given the high risk of late-onset CMV disease(7).

Table 3: Recommendations for CMV prevention in SOT recipients (Source: Razonable, Amer J Transpl 2013)

Organ	Risk category	Recommendation/options (see Table 4 for dose and text for special pediatric issues)	Evidence
Kidney	D+/R-	*Antiviral prophylaxis* is preferred	I
		Drugs: valganciclovir, oral ganciclovir, intravenous ganciclovir or valacyclovir	
		Duration: 6 months	
		Preemptive therapy is an option	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	*Antiviral prophylaxis*	I
		Drugs: Valganciclovir, oral ganciclovir, intravenous ganciclovir or valacyclovir	
		Duration: 3 months	
		Preemptive therapy	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
Pancreas and kidney/pancreas	D+/R-	Antiviral prophylaxis is preferred	I (3-month prophylaxis)
		Drugs: valganciclovir, oral ganciclovir or intravenous ganciclovir	III (6-month prophylaxis)
		Duration: 3–6 months	
		Preemptive therapy	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	Antiviral prophylaxis	II-2
		Drugs: Valganciclovir, oral ganciclovir or intravenous ganciclovir	
		Duration: 3 months	
		Preemptive therapy	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
Liver	D+/R-	*Antiviral prophylaxis* is preferred:	I (3-month prophylaxis)
		Drugs: valganciclovir (note FDA caution), oral ganciclovir or intravenous ganciclovir	III (6-month prophylaxis)
		Duration: 3–6 months	
		Preemptive therapy is an option	I
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	*Antiviral prophylaxis*	I
		Drugs: Valganciclovir (note FDA caution), oral ganciclovir or intravenous ganciclovir	
		Duration: 3 months	
		Preemptive therapy	
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
Heart	D+/R-	*Antiviral prophylaxis* is preferred	I (3-month prophylaxis)
		Drugs: valganciclovir, oral ganciclovir or intravenous ganciclovir. Some centers add adjunctive CMV immune globulin.	III (6-month prophylaxis)
		Duration: 3–6 months	II-2 (immune globulin)
		Preemptive therapy is an option	
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
	R+	Antiviral prophylaxis	II-2
		Drugs: Valganciclovir, oral ganciclovir or intravenous ganciclovir. Some centers add adjunctive CMV immune globulin.	
		Duration: 3 months	
		Preemptive therapy	
		Weekly CMV PCR or pp65 antigenemia for 12 weeks after transplantation, and if a positive CMV threshold is reached, treat with (1) valganciclovir 900-mg p.o. BID, or (2) IV ganciclovir 5-mg/kg IV every 12 h until negative test	
Lung, heart–lung	D+/R-	*Antiviral prophylaxis*	I (12-month prophylaxis)
		Drugs: valganciclovir or intravenous ganciclovir	
		Duration: 12 months.	II-2 (>12 months)
		Some centers prolong prophylaxis beyond 12 months	
		Some centers add CMV immune globulin	II-2 (immune globulin)
	R+	Antiviral prophylaxis	II-2
		Drugs: valganciclovir or intravenous ganciclovir	
		Duration: 6–12 months	
Intestinal	D+/R-, R+	*Antiviral prophylaxis*	III
		Drugs: Valganciclovir or intravenous ganciclovir	
		Duration: 3–6 months.	
Composite tissue allograft	D+/R-, R+	*Antiviral prophylaxis*	III
		Drugs: valganciclovir or intravenous ganciclovir	
		Duration: 3–6 months	

Late Onset CMV

Late onset CMV is defined as CMV disease occurring greater than three months post transplantation. This may be either primary infection in the D+/R- patient or reactivation/super-infection in R+ patients. In epidemiologic studies, late onset CMV disease is associated with significant morbidity and graft dysfunction, and occasional mortality due to these indirect effects of CMV. The incidence of late onset CMV is anywhere between 3% and 37%, depending on various risk factors.

Late Onset CMV Disease has similar risk factors to those that are associated with CMV infection and disease in the first place. For example, high-risk CMV serostatus, such as CMV D+/R- individuals, or CMV R+ on potent immunosuppression or anti-lymphocyte antibody preparations, treatment for acute rejection, and higher risk organ transplant such as lung transplant(33).

There are various options for dealing with late onset CMV infection and disease. One option is to do nothing and accept the risks of the disease as infections arise. Another option is to prolong prophylaxis beyond the 3 to 6 month window, and this was studied in the recent IMPACT trial, which will be discussed shortly. A third option is to use better prophylaxis if any are available. It is also an option to do careful virology monitoring of high risk patients after completing prophylaxis. Finally, it is also possible to monitor cellular host immune response after prophylaxis is complete to delineate which patients are at the highest risk of late onset CMV disease.

In terms of prolonging prophylaxis, the benefits include decreased incidence of CMV infection and disease as well as improved graft outcomes. However, there are potential pitfalls of pushing the incidence of CMV disease out past whatever duration of prophylaxis is used, and also the cost associated with prolonging prophylaxis as well as the toxicities of these prophylactic antivirals.

The IMPACT (IMproved Protection Against Cytomegalovirus in Transplant) trial was designed to answer the question: Is longer prophylaxis better? This was a trial of 316 Kidney transplant patients, all high risk (D+/R-), who received 3 months of Valganciclovir followed by 3 months of placebo vs. 6 months of Valganciclovir. Patients all received the normal prophylactic dose of Valganciclovir which is 900mg once per day adjusted for renal function. The incidence of CMV disease at 12 and 24 months post-transplantation was assessed. There was an overall benefit in the incidence of confirmed CMV disease with only 16% of patients developing CMV disease who received 6 months of prophylactics versus 37% of patients who received only 3 months of Valganciclovir Prophylaxis. This incidence appeared to taper off after the first year such that no further benefit was seen by prolonging prophylaxis past six months(34).

Because of the high risk nature of lung transplant patients, some centers prolong prophylaxis for 12 months, or even lifelong. Longer periods of prophylaxis have been studied; notably, 12 months of Valganciclovir prophylaxis vs. 3 months in lung transplant recipients. Longer term prophylaxis demonstrated short-term efficacy and safety compared to shorter course, and during a mean follow up of 3.9 years provided a sustained benefit with a lifetime incidence of CMV of 12% for the 12 month arm vs. 55% in the 3 month arm(35)(36). Another study by Wiita, et al, from 2012, looked at 128 patients who were receiving indefinite CMV valganciclovir prophylaxis. There was a high incidence of

discontinuation or medication reduction due to side effects; however, a low incidence of Valganciclovir resistance and CMV infection was noted(37). Thus, longer term and even indefinite antiviral prophylaxis is a reasonable option to consider for high-risk lung transplant patients.

Monitoring post immune response is another important factor for determining who should receive prolonged prophylaxis or monitoring after prophylaxis is complete. Although CMV serostatus is important prior to transplantation, studies looking at seroconversion of CMV antibody status after transplantation did not appear to be predictive of developing CMV infection or disease. Instead, cell mediated immunity (CD4 and CD8 T-cell responses) are critical for the prevention and control of CMV. There are a number of methods that are used to assess this including ELISPOT, HLA tetramers, cytokine flow cytometry, intracellular cytokine staining, ATP release assays, and also the QuantiFERON-CMV assay(28).

Most of these methods are experimental and only available in research settings; however, the QuantiFERON-CMV assay is available overseas and should be available in the United States soon. This assay is a whole blood assay that looks at CD8 T-cell response to CMV antigens and measures its production of interferon gamma. The assay is simple to perform, requiring only a small volume of blood, but the results are available in 20 to 30 hours. The test requires whole blood incubated overnight with CMV and CD8 epitopes are assayed for interferon gamma production. Positive and negative controls are used as well. This test has limited utility in patients who are lymphopenic and in rare patients whose HLA types are not covered in the assay. There is no data on the predictive value for CMV viremia; however, it does seem to predict CMV disease. This assay may be useful for defining risk, guiding prophylaxis, and possibly for guiding when to stop treatment. In a prospective study of organ transplant recipients, this assay was used to measure cell mediated immunity shortly after the onset of CMV viremia. Among 37 evaluable patients, viremia occurred at a median of 76 days post-transplant with the median viral load of 1140 copies per ml. Using a cutoff value of 0.2 IU per mL, 92.3% of patients with a positive test had spontaneous clearance of virus without the need for antivirals compared with only 45.5% of patients with a negative test(38). Another study by Kumar, *et al*, looked at the predictive value of interferon gamma production by CD8 cells in terms of development of CMV disease. In patients who had detectable interferon gamma response, they were less likely to develop CMV disease after prophylaxis was discontinued compared to those who had no detectable interferon gamma response(39).

A vaccine for CMV is thought to be a good mechanism for CMV prevention. However, none have previously been available despite multiple vaccines being studied in the past. There are different methods for development of a CMV vaccine, and several are currently in various stages of development, including a live, attenuated DNA subunit vaccine, as well as recombinant viral vaccines. In a study by Plotkin, *et al*, in 1991, the administration of a live, attenuated vaccine derived from the Towne strain of CMV resulted in suboptimal antibody response during clinical studies of renal transplant patients. CMV disease was attenuated, but the vaccine failed to prevent infection(40). Another study by Pass, *et al*, in 2009, looked at a CMV glycoprotein B (gB) vaccine given with an MF59 adjuvant and showed decreased incidence cases of *de novo* maternal and congenital CMV infection(41). Giving 2-3 doses of this same vaccine to liver and kidney transplant patients in a phase II randomized, placebo-controlled trial showed

that these patients had improved gB titers in both the seropositive and CMV seronegative patients pre-transplantation. Post-transplant, patients who received the vaccine that developed CMV viremia had antibody titers that correlated with the duration of viremia. In the high risk (D+/R-) patients, the duration of viremia and the number of days on antiviral therapy was reduced in the vaccine recipients group; however, no significant response was seen in low (D-/R-) or intermediate (D+/R+) risk patients(42).

CMV Treatment

The mainstay of treatment for CMV should always include a reduction of immunosuppression as these infections usually indicate that the patient is over-immunosuppressed. In addition, antiviral agents and certain antibody preparations are used **(Table 4)**. The antiviral drugs that are available include Ganciclovir and Valganciclovir, which are the current treatments of choice for CMV. Foscarnet and Cidofovir are available for treatment for CMV, but they are rarely used because of high levels of nephrotoxicity and other toxicities. As a result, they are usually only used in cases of Ganciclovir-resistant CMV, which will be discussed in a later section. CMV hyperimmune globulin can also be used as an adjunctive therapeutic option for severe cases of CMV infection and disease. The mainstay of treatment has been using full dose Ganciclovir or oral Valganciclovir. Oral Valganciclovir therapy was shown to as efficacious in most cases of CMV disease in the VICTOR trial, which will be discussed shortly. It is important to monitor the CMV viral load or antigenemia while on therapy, and in general, treatment should be continued until clinical signs and symptoms resolve and viremia or antigenemia becomes undetectable. In addition, many experts consider a 1- to 3-month course of secondary prophylaxis after the completion of induction therapy(7)**.**

The VICTOR trial was a study of oral Valganciclovir compared to IV Ganciclovir in solid organ transplant recipients with CMV disease. This was a multi-center non inferiority study that included 42 centers from around the world. Patients were enrolled into either Valganciclovir 900 mg po twice daily or Ganciclovir 5mg per kg IV twice daily for 20 days followed by a maintenance arm from 21 to 50 days of Valganciclovir 900 mg once daily. These patients were followed up for 3 to 12 months. Based on the CMV clearance kinetics, there was no difference in terms of clearance of CMV viremia with either oral Valganciclovir or IV Ganciclovir, showing that oral Valganciclovir can be used for most cases of CMV infection(43). There are certain indications when IV Ganciclovir would still be preferred over oral Valganciclovir: for example, life threatening or severe CMV disease, GI disease, if there are questions of absorption of oral medications, for resistant CMV infection, and for pediatric patients.

It is important to monitor patients after completion of treatment because of the high incidence of relapse. Relapse can occur in 6% to 35% of solid organ transplant recipients. More severe CMV disease, especially multi-organ disease, is independently associated with occurrence of relapse. High-risk CMV serostatus is an independent predictor, as well as high-dose immunosuppression. Antirejection treatment may also increase the incidence of CMV relapse, and because of the increased risk of relapse with an antimetabolite, some centers may choose to discontinue their use. Recurrence is more indicative of an incomplete viral suppression during therapy rather than development of drug resistance underscoring the importance of treating with induction dosing until CMV viremia is undetectable. Other

risk factors for relapse after treatment is complete include high baseline viral load and deceased donor transplantation(44).

Table 4: Antiviral drugs for CMV prevention and treatment in solid organ transplant recipients
(Source: Razonable, *Amer J Transpl* 2013)

Drug	Treatment[1]	Prophylaxis	Comments on use and toxicity
Valganciclovir	900-mg[2] p.o. twice daily	900 mg[2] p.o. once daily	Ease of administration Leukopenia is major toxicity
Oral Ganciclovir	NOT recommended	1 g p.o. three times daily	Low oral bioavailability High pill burden Leukopenia and risk of resistance development NOT recommended for preemptive therapy
IV Ganciclovir	5-mg/kg IV every 12 h	5 mg/kg IV once daily	Intravenous access and complications Leukopenia is major toxicity
Valacyclovir	NOT recommended	2 g p.o. four times daily	Use in kidney transplant recipients only NOT recommended for heart, liver, pancreas, lung, intestinal and composite tissue transplant recipients High pill burden High risk for neurologic adverse effects NOT recommended for preemptive therapy
Foscarnet	60 mg/kg IV every 8 h (or 90 mg/kg every 12 h)	NOT recommended	Second-line agent for treatment Highly nephrotoxic Used for UL97-mutant ganciclovir-resistant CMV disease NOT recommended for preemptive therapy
Cidofovir	5 mg/kg once weekly × 2 then every 2 weeks thereafter	NOT recommended	Third-line agent Highly nephrotoxic Used for UL97-mutant ganciclovir-resistant CMV disease NOT recommended for preemptive therapy

CMV-immune globulin has been used by some centers as an adjunct to antiviral prophylaxis, especially in heart and lung transplant recipients. The efficacy of this approach is debatable.
The doses of the antiviral drugs are for adults and should be adjusted based on renal function.
[1]These treatment doses are also recommended for preemptive therapy of asymptomatic CMV replication. Foscarnet, valacyclovir, oral ganciclovir and cidofovir are not recommended for preemptive therapy.
[2]Pediatric valganciclovir dose is mg = 7 × BSA × Creatinine clearance.

CMV Drug Resistance

Drug resistant CMV is uncommon overall, but is very difficult to treat, and is associated with increased complications and higher mortality. The incidence of drug resistant CMV depends on the type of transplant with the highest risk being seen in lung transplantation. Similar risk factors are seen that influence the incidence of drug resistant CMV with high risk D+/R- serostatus, high viral load, and highly potent immunosuppression (especially with the use of anti-lymphocyte antibodies) all being independently associated with drug resistant CMV. In addition, prolonged or suboptimal Ganciclovir exposure, especially with oral Ganciclovir, which has poor oral bioavailability, is associated with CMV disease. This is one of the arguments for using a more preemptive approach for CMV prophylaxis since it avoids unnecessary and prolonged administration of Ganciclovir. However, given the benefits of universal prophylaxis, this is the strategy that is more widely used. It is important to keep in mind that dosing should always be adjusted appropriately to avoid suboptimal antiviral exposure which leads to drug resistance. An increase in the viral load is a surrogate marker for resistance. Because of viral kinetics, viral loads can initially increase in the first two weeks despite being on effective therapy. Therefore, it is important to avoid checking a repeat CMV viral load within the first two weeks of therapy, especially if the patient is clinically stable and/or improving. In drug naïve subjects, early during treatment and in a low risk setting, drug resistance is unlikely, and early increases in viral load are more

likely due to underlying over-immunosuppression. After a significant exposure, of low-dose Ganciclovir or Valganciclovir over a median of 5-6 months, and in the high-risk setting, drug resistance is more likely, especially if the viral load increases at least 0.5 $\log_{10}$ or >3 times baseline levels(28)**.**

There are several methods for diagnosing drug resistant CMV. One method, similar to antibacterial resistance, is a plaque reduction assay or a phenotypic approach. This is the traditional approach that allows for a result of an inhibitor concentration of 50% (IC50). It is important for research purposes; however, it is technically complex, poorly standardized, and has a slow turnaround time. Nonetheless, it is required for reference standards. More commonly, a genotypic or molecular assay is performed to detect CMV drug resistance. This method involves looking at mutations in either the UL97 gene, which predicts Ganciclovir resistance, or the UL54 *pol* gene, which can be predictive of Foscarnet, Cidofovir, and Ganciclovir resistance. Various mutations in either of these genes are associated with different levels of resistance, or IC50 values. The UL97 is a kinase responsible for the initial phosphorylation of Ganciclovir, and is essential for antiviral activity. It can confer resistance to Ganciclovir and to a newer agent that is not yet available, Maribavir. UL54, the DNA polymerase or *pol* gene, can confer resistance to all of the currently available drugs, Ganciclovir, Foscarnet, and Cidofovir. Mutations in the UL97 gene are either point mutations or deletions that lead to decreased levels of Ganciclovir triphosphate in CMV infected cells. They do not generally confer cross-resistance to either Foscarnet or Cidofovir. Currently, there is a large and evolving database that showing that >90% of Ganciclovir-resistant isolates contain the UL97 mutations at certain codons: 460, 520, and 590-607.Knowing which mutation is important since these confer various IC50s or levels of resistance. For example, M460V/I, C592G, A594V, L595S, and C603W are the most common mutations and confer a 5 to 10-fold increase in the IC50. The exception to this is the C592G mutation, which only confers a 2.5-fold increase in IC50. Sequence changes at codons 590-607 are less common and confer various degrees of Ganciclovir resistance. UL97 mutations usually appear first followed later by the addition of UL54 *pol* mutations. These mutations confer resistance to any or all of the current drugs, and point mutations tend to occur in conserved functional domains. Mutations that confer Ganciclovir and Cidofovir resistance are clustered in exonuclease domains and region V. Mutations conferring Foscarnet resistance are often located in or between regions and II, III, and VI. Some Foscarnet resistance mutations in region III confer low-grade Ganciclovir cross-resistance(45)(46)

A consensus guideline paper from 2010 put forth an algorithm which is helpful for treating patients with known or suspected resistance **(Figure 2)**. It is important to suspect CMV resistance in patients with a high or an increasing CMV viral load with the previously mentioned risk factors (i.e., high-dose immunosuppression, D+/R- serostatus, lung transplantation, and prolonged, low-dose oral prophylaxis). UL97 mutations confer different IC50 values, and some mutations can be overcome with high-dose Ganciclovir. However, if the patient has severe disease or has an increasing viral load despite 2 weeks of adequate treatment, it is important not to wait for the genotype result, but instead to empirically change to or add Foscarnet while waiting for resistance mutation results. A large part of treating drug-resistant CMV depends on the CMV genotype and the particular mutation that is found (28).

Traditional therapy for Ganciclovir-resistant CMV is Foscarnet; however, it carries with it a high rate of nephrotoxicity and other toxicities including electrolyte abnormalities, genitourinary ulcers, and cardiac issues. Cidofovir has also been used but is also nephro- and oculotoxic causing uveitis and loss of intraocular pressure. The combination of Ganciclovir and Foscarnet in reduced dosing has been used with some success(47), and CMV hyperimmune globulin has been used as an adjunctive therapy. The addition of IVIG or CMV hyperimmune globulin is of unknown benefit but many opt to add as an adjunctive agent. However, it is not felt to be a primary treatment option by itself. Another option that has some supportive data is adoptive infusions of CMV-specific T-cells, but this is usually only available in specialized research settings. Other potential adjunctive treatments include Leflunomide, Sirolimus, and Artesunate; however, these are largely unproven. There are also experimental CMV antivirals that are coming, including Maribavir, CMX001, and AIC246 (Letermovir). Regarding these newer options for Ganciclovir-resistant CMV, Leflunomide is currently available for off-label use, CMX001, Maribavir, and AIC246 (Letermovir) have been available in investigational or compassionate use settings, and in specialized research centers, adoptive immunotherapy can be used(7).

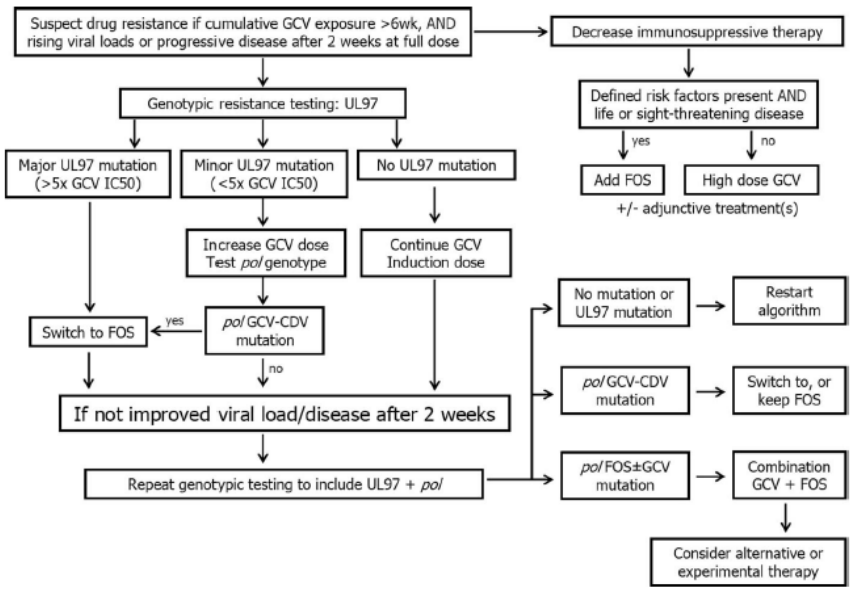


Figure 2: Suspected algorithm for management of suspected CMV drug resistance
(Source: Kotton, T*ransplantation* 2010)

The mTOR inhibitors (Sirolimus and Everolimus) have been shown to have antiviral effects and may decrease the risk of CMV. Findings from two analyses suggest that the use of these agents in transplant recipients was associated with a lower risk of CMV infection(48)(49). Kaplan-Meier analyses of 3 randomized trials revealed longer freedom from CMV viremia and infection/syndrome in Everolimus-treated heart and renal transplant recipients. Among 1398 renal transplant patients on maintenance treatment with Sirolimus, there was an independently associated lower risk for CMV infection (OR 0.16) but a higher rate of surgical site infections (OR 3.21).

Leflunomide is an approved agent for rheumatoid arthritis and is a DMARD (disease modifying anti-rheumatic drug). It is an inhibitor of protein kinase activity and pyrimidine synthesis, and it has activity against CMV *in vitro* including wild type and resistant strains. Viral load reduction is noted in

animal models and the mechanism of action is thought to be related to inhibition of virion assembly. Toxicities include hepatotoxicity, teratogenicity, and neurotoxicity. There are case reports of small non-randomized series for CMV treatment of resistant disease. In a study by Avery in 2010, 9 patients achieved long-term viral suppression with Leflunomide(50). It appears to work best when the viral load has already been reduced by another agent, such as Foscarnet.

A newer drug soon to be available is CMX001, which is lipid conjugated Cidofovir. It is highly active *in vitro* including against Ganciclovir-resistant strains of CMV. Because of its pharmacokinetic properties it allows for oral dosing once every 3 days. An abstract presented at the American Transplant Congress in 2010 described 3 complex cases of CMV that were successfully treated with CMX001(51)**.** Of interest, it also has broad spectrum antiviral activity including BK virus, adenovirus, HPV, and other herpesviruses. It was previously only available through compassionate and clinical trials(52).

Another drug that may be coming soon is oral Maribavir. This is a benzimidazole L-riboside compound that is a potent inhibitor of CMV UL97 kinase. It prevents viral encapsidation and nuclear egress, which is a novel mechanism of action, and has no known cross-resistance with currently available antiviral agents. It should be noted that Maribavir only has activity against CMV and EBV but does not inhibit HSV or VZV. It has no renal, hepatic, and hematologic toxicities; however, the major side effect is dysgeusia. This drug showed much promise until several trials in liver and stem cell transplant patients showed no benefit for prophylaxis of CMV over placebo. Analyses of these trials indicate that possibly the dose used for prophylaxis was too low to show any benefit; and the drug has also been used as salvage therapy for patients with multi-drug resistant CMV infection with success(53). Further studies on this agent are ongoing.

AIC236 (Letermovir) is a non-nucleosidic CMV inhibitor, or a terminase inhibitor. It is an oral agent that has a novel mechanism of action, and also has no cross-resistance with other antiviral agents. In a Phase II study, 27 patients (26 kidney and 1 stem cell transplant) were randomized to receive two different doses of AIC246 per day compared to Valganciclovir for preemptive treatment of CMV viremia. It was well-tolerated and had similar efficacy to Valganciclovir, and one patient even had resistance to Ganciclovir(54). In a case report of a lung transplant patient with disseminated multi-drug resistant CMV in the lungs, GI tract, and retina, this agent was given under emergency use investigational new drug program with complete viral, clinical, and radiological resolution(55).

Another agent under investigational use is Artesunate which, interestingly, is an anti-malarial agent that has anti-CMV activity. The mechanism of action involves interference with host cell kinase cascades. It has clinical utility in stem cell transplant recipient with Ganciclovir and Foscarnet-resistant CMV, and has resulted in a reduction in the viral load between 1- and 2-$\log_{10}$ in 7 days(56). A study of preemptive therapy of 6 stem cell transplant patients noted variable reductions in viral load(57). It can also be used in combination with other antivirals and has been found to be synergistic(58)**,**(59)**.**

A final option for resistant CMV infection/disease is adoptive immunotherapy. In a study by Walter, *et al,* from 1995, 14 stem cell transplant patients received CMV-specific CD8+ lymphocytes from their donors, and 4 had infusions starting 30 days post-transplant. Clones of CD8+ lymphocytes persisted

for at least 12 weeks, and there was noted to be increased cellular immunity (CTL activity) to CMV. No CMV viremia or disease was noted in these patients(60). Currently, this is an option only available at certain research institutions where it can be performed, and it also requires the use of obtaining donor CMV-specific CD8+ cytotoxic lymphocytes which may not be available in many cases.

Future Directions

In general, the future of CMV in transplantation is exciting in terms of using translational research to establish better predictive tools such as host response, looking at viral factors, and understanding the impact of herpesvirus infections. In addition, novel targets for prevention and treatment are being developed including tailored drug therapy and selective immunosuppression with medications that have coexistent antiviral activity. There are also novel preventative strategies such as vaccine strategies as well as cell mediated therapeutic modalities.

Conclusions

In summary, CMV remains a significant pathogen post-transplantation. Despite advantages in preventative strategies and new agents for prevention, CMV has not been eliminated, and CMV-infected patients are some of the most complicated patients seen after transplantation due to the direct and indirect effects of CMV infection. In general, prophylaxis strategies prevent most cases of CMV; however, there are issues with late onset CMV which occurs after prophylaxis is complete. There are new advances forthcoming with regard to monitoring patients after the completion of CMV prophylaxis, and one of these is the QuantiFERON-CMV assay. There is also the possibility of a CMV vaccine for prevention, which is showing promise. Diagnosis of CMV infection and disease has been made easier with the widespread availability of molecular assay testing such as QNAT, but there are issues with standardization and reproducibility which will hopefully be addressed by a reference standard developed by the World Health Organization. Treatment options are becoming more available, with oral options that appear to be as good as IV in most cases. Despite intensive efforts at prevention and treatment, drug resistant CMV can be a problem. However, new treatment modalities for resistant CMV are forthcoming.

References:

1. *Infection in organ-transplant recipients.* **Fishman, JA and Rubin, RH.** 1998, New England Journal of Medicine, pp. 1741-1751.

2. *Transmission of rabies virus from an organ donor to four transplant recipients.* **Srinivasan, A, et al.** 2005, New England Journal of Medicine, pp. 1103-1111.

3. *Infection in solid-organ transplant recipients.* **Fishman, JA.** 2007, New England Journal of Medicine, pp. 2601-2614.

4. *Immunosuppressive drugs for kidney transplantation.* **Halloran, PF.** 2004, New England Journal of Medicine, pp. 2715-2729.

5. *Overview: cytomegalovirus and the herpesviruses in transplantation.* **Fishman, JA.** 2013, American Journal of Transplantation, pp. 1-8.

6. *Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.* **Kaplan, JE, et al.** 2009, MMWR Recommendations & Reports, pp. 1-207.

7. *Cytomegalovirus in solid organ transplantation.* **Razonable, RR and Humar, A.** 2012, Amer J Transpl, pp. 93-106.

8. *Cytomegalovirus in transplantation - challenging the status quo.* **Fishman, JA, et al.** 2007, Clin Transplant, pp. 149-158.

9. *Cytomegalovirus and lung transplantation.* **Zamora, MR.** 2004, Am J Transplant, pp. 1219-1226.

10. *The role of early colonoscopy in CMV colitis of transplant recipients.* **Korkmaz, M, et al.** 2005, Transplant Proc, pp. 3059-3060.

11. *Immune response to CMV in solid organ transplant recipients: current concepts and future directions.* **Watkins, RR, Lemonovich, TL and Razonable, RR.** 2012, Expert Rev Clin Immunol, pp. 383-393.

12. *Etiology and impact of cytomegalovirus disease on solid organ transplant recipients.* **McDevitt, LM.** 2006, Am J Health Syst Pharm, pp. S3-9.

13. *Risk factors for invasive fungal infections complicating orthotopic liver transplantation.* **Collins, LA, et al.** 1994, J Infect Dis, pp. 644-652.

14. *The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland.* **George, MJ, et al.** 1997, Am J Med, pp. 106-113.

15. *Risk factors of invasive aspergillosis after heart transplantation: protective role of oral itraconazole prophylaxis.* **Muñoz, P, et al.** 2004, Am J Transplant, pp. 636-643.

16. *Cytomegalovirus infection is a risk factor for invasive aspergillosis in lung transplant recipients.* **Husni, RN, et al.** 1998, Clin Infect Dis, pp. 753-755.

17. *Risk factors for invasive aspergillosis in liver transplant recipients.* **Fortún, J, et al.** 2002, Liver Transpl, pp. 1065-1070.

18. *Coincidence of Epstein-Barr virus reactivation, cytomegalovirus infection, and rejection episodes in renal transplant recipients.* **Hornef, MW, et al.** 1995, Transplantation, pp. 474-480.

19. *Human beta-herpesvirus interactions in solid organ transplant recipients.* **Mendez, JC, et al.** 2001, J Infect Dis, pp. 179-184.

20. *Prospective study of human betaherpesviruses after renal transplantation: association of human herpesvirus 7 and cytomegalovirus co-infection with cytomegalovirus disease and increased rejection.* **Kidd, IM, et al.** 2000, Transplantation, pp. 2400-2404.

21. *Betaherpesviruses in transplant recipients.* **Griffiths, PD, Clark, DA and Emery, VC.** 2000, J Antimicrob Chemother, pp. 29-34.

22. *The pathogenesis of hepatitis C virus is influenced by cytomegalovirus.* **Razonable, RR, et al.** 2002, Clin Infect Dis, pp. 974-981.

23. *Asymptomatic cytomegalovirus infection is associated with increased risk of new-onset diabetes mellitus and impaired insulin release after renal transplantation.* **Hjelmesaeth, J, et al.** 2004, Diabetologia, pp. 1550-1556.

24. *The association between cytomegalovirus infection and atherosclerotic events in renal transplant recipients.* **Ozdemir, FN, et al.** 2007, Transplant Proc, pp. 990-992.

25. *Cytomegalovirus infection in renal transplant recipients is associated with impaired survival irrespective of expected mortality risk.* **Sagedal, S, Rollag, H and Hartmann, A.** 2007, Clin Transplant, pp. 309-313.

26. *Clinical utility of quantitative cytomegalovirus viral load determination for predicting cytomegalovirus disease in liver transplant recipients.* **Humar, A, et al.** 1999, Transplantation, pp. 1305-1311.

27. *Interlaboratory comparison of cytomegalovirus.* **Pang, XL, et al.** 2009, American Journal of Transplantation, pp. 258–268.

28. *International consensus guidelines on the management of cytomegalovirus in solid organ transplantation.* **Kotton, CN, et al.** 2010, Transplantation, pp. 779–795.

29. *The long road toward standardization of viral load testing for cytomegalovirus.* **Caliendo, AM.** 2013, Clin Infect Dis, pp. 374-375.

30. *Antiviral medications to prevent cytomegalovirus disease and early death in recipients of solid-organ transplants: a systematic review of randomised controlled trials.* **Hodson, EM, et al.** 2005, Lancet, pp. 2105-2115.

31. *Prophylactic versus preemptive oral valganciclovir for the management of cytomegalovirus infection in adult renal transplant recipients.* **Khoury, JA, et al.** 2006, Am J Transplant, pp. 2134-2143.

32. *Improvement in long-term renal graft survival due to CMV prophylaxis with oral ganciclovir: results of a randomized clinical trial.* **Kliem, V, et al.** 2008, Am J Transplant, pp. 975-983.

33. *Late-onset cytomegalovirus disease in liver transplant recipients despite antiviral prophylaxis.* **Limaye, AP, et al.** 2004, Transplantation, pp. 1390-1396.

34. *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.* **Humar, A, et al.** 2010, Transplantation, pp. 1427-1431.

35. *Extended valganciclovir prophylaxis to prevent cytomegalovirus after lung transplantation: a randomized, controlled trial.* **Palmer, SM, et al.** 2010, Ann Intern Med, pp. 761-769.

36. *Long-term efficacy and safety of 12 months of valganciclovir prophylaxis compared with 3 months after lung transplantation: a single-center, long-term follow-up analysis from a randomized, controlled cytomegalovirus prevention trial.* **Finlen Copeland, CA, et al.** 2011, J Heart Lung Transplant, pp. 990-996.

37. *Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis.* **Wiita, AP, et al.** 2012, Transpl Infect Dis, pp. 248-258.

38. *Clinical utility of cytomegalovirus cell-mediated immunity in transplant recipients with cytomegalovirus viremia.* **Lisboa, LF, et al.** 2012, Transplantation, pp. 195-200.

39. *Cell-mediated immunity to predict cytomegalovirus disease in high-risk solid organ transplant recipients.* **Kumar, D, et al.** 2009, Am J Transplant, pp. 1214-1222.

40. *Effect of Towne live virus vaccine on cytomegalovirus disease after renal transplant. A controlled trial.* **Plotkin, SA, et al.** 1991, Ann Intern Med, pp. 525-531.

41. *Vaccine prevention of maternal cytomegalovirus infection.* **Pass, RF, et al.** 2009, N Engl J Med, pp. 1191-119.

42. *Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial.* **Griffiths, PD, et al.** 2011, Lancet, pp. 1256-1263.

43. *Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients.* **Asberg, A, et al.** 2007, Am J Transplant, pp. 2106-2113.

44. *Canadian society of transplantation consensus workshop on cytomegalovirus management in solid organ transplantation final report.* **Preiksaitis, JK, et al.** 2005, Am J Transplant, pp. 218-227.

45. *Cytomegalovirus UL97 phosphotransferase mutations that affect susceptibility to ganciclovir.* **Chou, S, et al.** 2002, J Infect Dis, pp. 162-169.

46. *Viral DNA polymerase mutations associated with drug resistance in human cytomegalovirus.* **Chou, S, et al.** 2003, J Infect Dis, pp. 32-39.

47. *Combination antiviral therapy for ganciclovir-resistant cytomegalovirus infection in solid-organ transplant recipients.* **Mylonakis, E, Kallas, WM and Fishman, JA.** 2002, Clin Infect Dis, pp. 1337-1341.

48. *Cytomegalovirus incidence between everolimus versus mycophenolate in de novo renal transplants: pooled analysis of three clinical trials.* **Brennan, DC, et al.** 2011, Am J Transplant, pp. 2453-2462.

49. *Immunosuppressive therapy and infection after kidney transplantation.* **Fortun, J, et al.** 2010, Transpl Infect Dis, pp. 397-405.

50. *Utility of leflunomide in the treatment of complex cytomegalovirus syndromes.* **Avery, RK, et al.** 2010, Transplantation, pp. 419-426.

51. **Painter, W.** American Transplant Congress, 2010, Abstract [LB10].

52. *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.* **Painter, W, et al.** 2012, Antimicrob Agents Chemother, pp. 2726-2734.

53. *Oral maribavir for treatment of refractory or resistant cytomegalovirus infections in transplant recipients.* **Avery, RK, et al.** 2010, Transpl Infect Dis, pp. 489-496.

54. *In vitro and in vivo activities of the novel anticytomegalovirus compound AIC246.* **Lischka, P, et al.** 2010, Antimicrob Agents Chemother, pp. 1290-1297.

55. *First report of successful treatment of multidrug-resistant cytomegalovirus disease with the novel anti-CMV compound AIC246.* **Kaul, DR, et al.** 2011, Am J Transplant, pp. 1079-1084.

56. *Artesunate as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation.* **Shapira, MY, et al.** 2008, Clin Infect Dis, pp. 1455-1457.

57. *Human cytomegalovirus kinetics following institution of artesunate after hematopoietic stem cell transplantation.* **Wolf, DG, et al.** 2011, Antiviral Res, pp. 183-186.

58. *Artemisinin-derived dimer diphenyl phosphate is an irreversible inhibitor of human cytomegalovirus replication.* **He, R, et al.** 2012, Antimicrob Agents Chemother, pp. 3508-3515.

59. *The unique antiviral activity of artesunate is broadly effective against human cytomegaloviruses including therapy-resistant mutants.* **Chou, S, et al.** 2011, Antiviral Res, pp. 364-368.

60. *Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor.* **Walter, EA, et al.** 1995, N Engl J Med, pp. 1038-1044.