Graft

CMV in Solid Organ Transplantation
Alan Koffron and Michael Abecassis

The online version of this article can be found at:

Additional services and information for *Graft* can be found at:

Email Alerts: http://gft.sagepub.com/cgi/alerts

Subscriptions: http://gft.sagepub.com/subscriptions

Reprints: http://www.sagepub.com/journalsReprints.nav

Permissions: http://www.sagepub.com/journalsPermissions.nav

CMV in Solid Organ Transplantation

Alan Koffron and Michael Abecassis

Michael Abecassis, M.D.
Division of Organ Transplantation
Northwestern University Medical School
Galter Pavilion, Suite 17-200
675 North St. Clair Street
Chicago, Illinois, USA 60611
Tel.: 312.695.0359
Fax: 312.695.5998
email: mabecass@nmh.org

Cytomegalovirus (CMV) is the most common viral pathogen in transplant recipients. The advent of sensitive diagnostic tests that result in earlier detection of CMV infection, combined with effective antiviral agents, has led to many trials of proactive CMV therapy in the forms of prophylaxis and preemptive therapy. Despite the advantages of increased sensitivity and earlier diagnosis, the lack of specificity and positive predictive value for CMV disease associated with these diagnostic tests has rendered preemptive therapy difficult to apply. This review summarizes the current knowledge of the pathogenesis, diagnosis, and treatment of CMV infection following solid organ transplantation (SOT).

CMV in the Normal Host

CMV is a ubiquitous β-herpesvirus that produces cytopathogenesis and direct immunosuppression. It typically infects individuals during the early decades of life, after which, the viral genome remains latent and asymptomatic in immunocompetent hosts. Between 25% and 80% of North Americans are infected with CMV, with variable prevalence by age, socioeconomic status, and geographical location.

CMV in the Immunocompromised Host

Immunocompromised patients who have not developed antibodies to CMV are labeled *seronegative*. These individuals are most vulnerable to primary infection and symptomatic disease upon exposure to CMV. *Seropositive* patients, however, may also become ill due to reactivation of the latent virus, or because of a superinfection with a different strain of CMV. Similarly, latent virus transplanted into either a seronegative or a seropositive recipient can reactivate and cause infection in the recipient, resulting in systemic and invasive disease.[1-3] The exact sites of CMV latency and mechanisms of reactivation remain difficult to elucidate and have been investigated primarily in animal models of CMV.[4,5]

Significance of CMV Infection in SOT Recipients

CMV infection generally occurs between 1 and 6 months following transplantation. In patients who receive treatment for acute and chronic rejection, CMV infection and disease can occur several months, or even years, after transplantation. CMV disease usually occurs within 2 weeks after the onset of viral shedding. The diverse symptoms of CMV disease vary from mild to life-threatening. In patients receiving no prophylaxis, symptomatic CMV infection occurs in 20% to 60%, 39% to 65%,[6] 29%, and 25%[7] of kidney, heart-lung, liver, and heart transplant recipients, respectively, depending on the serologic status of donor and recipient. As the most common cause of infection in SOT, CMV significantly increases mortality and attendant cost. Among orthotopic liver transplant recipients, CMV disease has been shown to be independently associated with increased cost and longer 1st posttransplant year hospital length of stay.[8]

CMV causes direct cytopathic effects that lead to well-described, clinical infectious disease syndromes unique to the target organ, whether kidney (nephritis), liver (hepatitis), heart (myocarditis), lung (pneumonia), or pancreas (pancreatitis).

In addition to the cytopathic effects directly attributable to CMV, indirect sequelae may include chronic rejection in the liver (vanishing bile duct syndrome), heart (accelerated atherosclerosis), lung (bronchiolitis obliterans), and kidney (glomerulopathy).[8] Furthermore, although the heightened immunosuppression required to manage allograft rejection increases susceptibility to CMV infection, the immunosuppressive properties of CMV infection have been shown to be an independent risk factor for infection with bacterial and fungal

pathogens, including *Pneumocystis carinii* pneumonia.[6] The virus has been shown to cause leukopenia, inversion of the ratio of helper to suppressor T-lymphocytes, dysregulation of the cytokine network, a reduction in the response of the lymphocytes to antigens, and the suppression of monocyte, macrophage, and natural killer cell functions.[9]

Several studies have shown that the most important risk factor for symptomatic CMV infection is the serologic status of donor and recipients, with the highest risk assigned to seronegative recipients who receive transplants from seropositve donors (D+/R–). Whether as induction therapy or for allograft rejection, antilymphocyte immunoglobulins enhance the rate of infection, especially in CMV seropositive individuals. In addition, viral load may play an important role in identifying patients at higher risk. Because viral load varies among transplant organs,[10] prophylactic measures found to be effective for renal transplantation, for example, may not apply to lung or gastrointestinal transplantation. Likewise, lessons learned from CMV infection in bone marrow transplantation can be only partially extrapolated to the nonhomogenous field of SOT because of differences in pathogenesis.[11]

Thus, CMV poses a serious health and financial burden in transplantation medicine. It is clear that patient risk identification, prompt diagnosis, and intervention are pivotal in diminishing the impact CMV plays in contemporary transplantation.

Diagnosis of CMV Infection

The diagnosis of CMV can be clinically made in most instances, although laboratory confirmation is helpful in the management of SOT recipients. More important, the early diagnosis and treatment of CMV may abrogate the sequelae of CMV infection.

Clinical Signs and Symptoms

The diverse symptoms of CMV disease vary from mild to life-threatening. Fever occurs in two-thirds of patients with CMV infection. Patients can present with symptoms similar to those of mononucleosis, including arthralgias, myalgias, malaise, and anorexia. Abnormal laboratory findings may include leukopenia, thrombocytopenia, and atypical lymphocytosis. The transplanted organ can be the most affected by the infection. CMV pneumonia,

for example, is most common among lung and heart-lung transplant recipients; it usually presents with fever, dyspnea, nonproductive cough, and hypoxemia. Radiologically, the disease is typically symmetrical with bilateral interstitial involvement, although atypical radiologic findings have been described. CMV pneumonia can also be seen as disseminated CMV disease in patients receiving other SOT and is associated with significant mortality.

Although it occurs infrequently in recipients of other solid organs, hepatitis is a common event in liver transplant recipients. Biochemical evidence of CMV hepatitis consists of a nonspecific elevation of aminotransferase levels, or a cholestatic pattern with elevations in serum bilirubin and alkaline phosphatase values. This diagnosis requires a tissue biopsy.

Involvement of the gastrointestinal tract usually presents with gastric, duodenal, or colonic ulcerations leading to nausea, vomiting, early satiety, hemorrhage, and, rarely, viscous perforation.[12] Retinitis is a rare and late complication of CMV infection posttransplant and implies chronic infection.

Diagnostic Tests

The diagnosis of CMV infection is predicated on the demonstration of replicating virus in tissues or the bloodstream.

The presence of IgG antibodies against CMV is a clear indication of past infection. Although the seropositive individual is immune in the immunologic sense, the individual is latently infected with a virus that is capable of reactivation, especially when the immune system is ablated. Infection with a different strain of CMV (e.g., from a donated organ) remains possible. Serologic tests are valuable for determining the clinical risk from CMV at the time of transplantation but are of little diagnostic value thereafter.

Classically, CMV infection in tissue is based on the identification of cytomegalic inclusion bodies in cells. CMV may also be detected in tissue specimens by immunohistochemistry or DNA hybridization. The two cell culture methods available are tube cell culture and shell vial culture. The shell vial culture is far more efficient and can detect CMV after 16 h of incubation. Because it detects the immediate-early or early gene products of

CMV replication, there is no need to wait for full replication before obtaining a diagnosis. Although the specificity of the shell vial assay is excellent (88%–95%), the sensitivity is less than optimal (<55%–63%). The conventional tube cell culture necessitates at least 7 to 14 days of incubation for CMV detection.

CMV antigenemia and other molecular methods, such as quantitative polymerase chain reaction (PCR), represent more sensitive techniques for demonstrating viremia. Demonstration of virus in urine or respiratory secretions, though expedient, is not predictive of clinically important infection.[13] In contrast, detection of CMV in the blood is considered clinically important, although the positive predictive value remains unclear.[14]

As noted, DNA amplification by PCR is a highly sensitive assay for CMV detection. Quantitative PCR is able to measure CMV load. The sensitivity of this molecular technique is of great value in detecting viral replication earlier in the posttransplant period, before the advent of symptomatic disease. Therefore, it can be used to facilitate preemptive therapy in patients at risk of initial CMV disease or recurrence. PCR demonstrates high negative predictive value, whereas its positive predictive value (i.e., number of patients with a positive PCR result who develop symptomatic CMV infection) in all patients except those at high risk of CMV is lower.[14,15]

Treatment of CMV Infection

Effective antiviral agents for the treatment of CMV include immunoglobulin, foscarnet, acyclovir (ACV), ganciclovir (GCV), valacyclovir, and valganciclovir. In addition, vaccines have been investigated as more definitive preventative agents.

CMV IgG

Cytomegalovirus hyperimmune globulin (CMV-IG) is an immunoglobulin containing a standardized amount of antibody to CMV. The high concentration of antibodies in the globulin has been shown to attenuate or reduce the incidence of serious CMV disease in studies of renal transplant patients. At present, it is indicated for use only in renal transplantation and has no impact on overall CMV infection rate. CMV-IG is not indicated for individuals with severe reactions to immunoglobulin preparations.[9]

Randomized trials in SOT recipients indicate that Ig confers some degree of efficacy in preventing CMV disease as outlined above. Results are more consistent with CMV-IG preparations and attenuated by the use of antilymphocyte antibody therapy. Advantages include the relatively infrequent administration and the diminished need for continuous intravenous (IV) access. The main disadvantages appear to be cost, inconvenience,[11] and the fact that IgG is not proven effective in either prophylaxis or preemptive treatments,[16] particularly for high-risk patients.

Foscarnet

The use of foscarnet (trisodium phosphonoformate hexahydrate) for the treatment of CMV disease in SOT recipients has been limited by its toxicity.[17] Until more data are available, foscarnet should be reserved for patients who are intolerant of ganciclovir or who have failed ganciclovir therapy. Foscarnet inhibits viral DNA polymerase. The main side effects are nephrotoxicity, anemia, electrolyte abnormalities, nausea, vomiting, and seizures. It has been suggested that combination therapy with ganciclovir provides synergy and that this combination may provide effective therapy for CMV infection and reduce the side effects experienced with foscarnet therapy alone.[18-20]

Acyclovir

As a cost-effective measure, many physicians rely on acyclovir as a prophylaxis for CMV. Such usage is off-label since acyclovir is not indicated for CMV prophylaxis. Appearing to have reduced susceptibility in vitro, acyclovir is not efficiently activated in CMV-infected cells. Acyclovir requires a difficult regimen and remains unproven in most transplant settings.

Ganciclovir

Ganciclovir is considered to be the 1st choice for prevention and treatment of CMV disease posttransplantation and for retinitis in HIV-positive patients. A nucleoside analogue, ganciclovir inhibits viral replication by competing with deoxyguanosine triphosphate (dGTP) as a substrate for viral DNA polymerase. Ganciclovir is approximately 26 times more potent than acyclovir against human CMV in vitro. Available in IV and oral

formulation, ganciclovir is potentially cost-effective. In addition, ganciclovir may be used in the context of renal insufficiency if dosing is adjusted appropriately.

Valacyclovir

Valacyclovir hydrochloride, the hydrochloride salt of L-valyl ester of acyclovir, is rapidly and almost completely converted to acyclovir after oral administration. It is currently undergoing clinical trials. The higher concentration of ganciclovir triphosphate in CMV-infected cells, and its prolonged intracellular half-life, make ganciclovir far superior to acyclovir in inhibiting the replication of CMV.[21] The role of valacyclovir in CMV prophylaxis and preemptive treatment is yet to be determined.

Valganciclovir

Valganciclovir is an orally administered valine ester of ganciclovir. This new anti-CMV agent offers high oral bioavailability equivalent compared with oral and IV ganciclovir blood levels,[22,23] and may avoid the complications associated with the requirement for long-term IV access.[24]

Vaccines

Renewed efforts by several biotechnology companies are under way to evaluate subunit vaccines containing recombinant CMV glycoproteins (B, H). Active immunization with the live attenuated CMV Towne vaccine has been shown to decrease the severity of CMV disease in R– renal transplant patients who received D+ organs. This vaccine is not currently licensed or available. When compared with placebo, it had no effect on the rate of CMV infection or disease, and it failed to prevent superinfection with other human CMV strains.[9] Although a vaccine may, at best, afford only partial protection against CMV, such an approach might be combined with the use of other current regimens.[11]

Therapeutic Strategies

There are 3 basic approaches to the management of CMV in the solid organ transplant population.

1. Standard therapy is treatment based on the detection of active CMV infection with signs and symptoms attributable to CMV.

2. Prophylactic therapy is the universal treatment of transplant patients to prevent CMV infection.

3. Preemptive therapy is often considered to be a subset of prophylaxis, where antiviral treatment is administered to patients who are thought to be at high risk to develop CMV disease.

Risk assessment can be based on either laboratory markers of CMV or patient characteristics, such as donor/recipient serologic status.[13,25] Although the treatment of CMV disease is well established, the use of several available agents in the context of prophylactic and preemptive therapy is not as clear and deserves special review.

Prophylaxis

Prophylactic therapy is the treatment of all transplant patients to prevent CMV infection. Arguments in favor of prophylaxis include

1. the fact that the deleterious effects of CMV may antedate clinical disease;[9]

2. the preference of prevention over treatment, given the increased morbidity and mortality of CMV in certain SOT recipients;[30]

3. the simplicity of patient compliance with a regimen.[11]

To date, a number of prophylactic measures have been evaluated in an attempt to prevent the occurrence of CMV infection and disease in SOT recipients. These include the use of CMV-seronegative blood products, active immunization with a vaccine, passive immunization with immune globulins, and prophylaxis with antiviral agents.[26]

Passive immunization was the 1st attempt at prophylaxis. Anti-CMV immunoglobulin was found to be of benefit in high-risk kidney transplant recipients.[26,27] This agent was also found to provide prophylaxis in seropositive liver transplant recipients; however, there was no impact on the incidence of CMV disease in liver recipients in the high-risk category (D+/R–).[16,28,29]

Investigation into the prophylactic use of high dose oral acyclovir (ACV) was shown to be beneficial in the prevention of CMV disease in kidney recipients during the 1st posttransplant year.[30] How-

ever, most trials of ACV prophylaxis provided no benefit to liver transplant recipients.[29,31] More recently, prolonged, high-dose oral ACV was found to reduce the incidence of CMV disease and delay the onset of CMV infection in liver transplant recipients. CMV prevention, however, was not significant.[32]

IV GCV used in short course (2 weeks) prophylaxis in liver transplant recipients, followed by longer course ACV, demonstrated a significant beneficial effect when compared with ACV.[33] However, long course prophylaxis with IV GCV prevented CMV disease in high-risk liver transplant recipients. This prolonged regimen (100 days posttransplant) virtually eradicated CMV disease.[34] However, long-term IV GCV treatment is expensive, requires maintenance of IV access for a considerable time, and may expose some patients to the deleterious side effects of IV GCV.

Long-term oral GCV has been used as prolonged prophylaxis, eliminating the need for long-term IV therapy. The initial experience with oral GCV demonstrated a beneficial effect in seropositive and high-risk liver transplant recipients.[33] This pivotal, multicenter randomized trial of 304 liver transplant recipients (R+ or D+) who received 100 days of oral GCV demonstrated the efficacy of oral GCV in prevention of CMV disease in SOT. In a recent retrospective analysis of 100 simultaneous kidney/pancreas patients, combined prophylaxis with long-term oral GCV and anti-CMV immunoglobulin in high-risk recipients delayed the onset of CMV disease but had minimal effects on severity.[28]

Current prophylactic approaches vary widely among different transplant programs. Reasons for discrepancies include the absence of large, multicenter randomized trials; the variety of agents used; and the more recent interest in preemptive therapy.

Preemptive Therapy

Considered a form of prophylaxis, *preemptive treatment* is administered to patients at high risk of developing clinical CMV disease. It may lessen toxicity risk, lower cost, and preclude the emergence of resistant viral strains. Preemptive treatment requires the ability to detect CMV infection when it is still subclinical, so that antiviral agents can be given to prevent symptomatic infection or CMV disease. Current laboratory methods include PCR and antigenemia.[25]

PCR for CMV detection, although highly sensitive, has been found to have a poor positive predictive value for the development of CMV disease in SOT recipients.[14] In contrast, quantitative PCR (QPCR) combines the sensitivity of PCR with the ability to identify the number of viral genomic copies per number of leuokocytes. In a cohort of 97 consecutive liver transplant recipients, plasma viral load by QPCR was as useful as antigenemia in predicting CMV disease.[35] These strategies allow initiation of treatment when a patient is found to have crossed a diagnostic threshold associated with the development of CMV disease. The lack of a standardized, commercially available QPCR assay for CMV has hindered further advances in preemptive therapy and has made available data difficult to interpret.

Antigenemia remains the most applicable assay for preemptive treatment of CMV in SOT. A recent, randomized controlled study of oral versus IV GCV in a CMV antigenemia-directed preemptive trial demonstrated that both oral and IV GCV prevent tissue invasive disease when used preemptively in 72 consecutive liver transplant recipients. As a result, 69% of patients never received anti-CMV therapy and did not develop CMV. These results suggest that preemptive administration of oral GCV is an effective prophylaxis for CMV disease after liver transplantation.[36] In another cohort of liver transplant recipients, preemptive therapy guided by CMV antigenemia was found to be useful and cost-effective.[37]

In a study of 77 consecutive liver transplant recipients, CMV antigenemia of less than 50 positive cells with low and intermediate risk for CMV disease were not treated unless they were symptomatic. These data suggest that mandatory therapy should be restricted to patients with either >50 positive cells or <50, only if symptomatic.[38]

In a lung transplant population, preemptive therapy with GCV using CMV antigenemia was found to be as effective as universal prophylaxis in preventing CMV disease.[39]

Thus, preemptive therapy is a promising approach to the prevention of CMV disease in transplant recipients. It may be applied to those patients at high risk for developing CMV disease. However, when based on the early detection of CMV, preemptive therapy is still undefined in terms of clinically sig-

nificant thresholds for initiation of treatment. Further investigation is needed to elucidate completely the diagnostic indicators for preemptive therapy (viral antigen or DNA load), in combination with the various antiviral regimens currently available.

CMV Resistance

Resistance to antiviral agents may occur, especially in the context of widespread prophylaxis and preemptive therapy. CMV resistance has been reported in HIV patients, bone marrow transplant recipients, and SOT recipients.[40-43] One study quotes a 7.6% incidence of CMV in AIDS patients treated with ganciclovir for more than 3 months, who then suffered progressive CMV disease.[44] Although the data in SOT does not allow an accurate estimate of frequency, it is observed that transplant patients who develop resistance to antiviral drugs are usually seronegative recipients receiving seropositive organs, and who have received several courses of antiviral agents.[41] These observations suggest that indiscriminate use of antiviral agents may increase the risk of developing resistant CMV strains and make it difficult to treat. Furthermore, genetic mutations may make the demonstration of resistance difficult by conventional virological assays,[41,43] requiring discriminative PCR testing to identify mutant viral strains.[42,43] Because of the difficulty in the diagnosis of antiviral drug resistance, clinicians must be able to recognize those patients at high risk for resistant CMV infection. Risk factors include

1. viral burden where higher viral load correlates with higher incidence of genetic mutation events;[45]

2. inadequate antiviral therapy, augmented immunosupression;[2]

3. and persistent viral replication during therapy.[46,47]

It is clear that viral resistance is an elusive threat that should be considered when formulating antiviral regimens.

Summary

In summary, the advent of both effective antiviral agents and sensitive diagnostic modalities has allowed for significant advancements in the diagnosis and treatment of CMV infection in SOT recipients. The treatment of active CMV infection and disease with ganciclovir is well established. However, optimal preventive measures need to be determined. Ideally, universal prophylaxis should be considered if the agent were inexpensive, orally formulated, and associated with minimal side effects. Alternatively, preemptive therapy combining effective antiviral agents with highly sensitive yet specific, diagnostic tests will result in the treatment of only those patients otherwise predicted to develop CMV disease. Continued emphasis should be placed on the identification of risk factors for CMV infection and the standardization of molecular diagnostic modalities, with high positive predictive values used to guide preemptive therapy. These therapeutic strategies will culminate in the cost-effective eradication of CMV disease in SOT recipients.

REFERENCES

1. Reinke P, Prösch S, Kern F, et al. Mechanisms of human cytomegalovirus (HCMV) (re)activation and its impact on organ transplant patients. **Transpl Infect Dis 1999;1:157-64**.

2. Rubin RH. Infection in the organ transplant recipient. In: Rubin RH, Young LS, editors. Clinical approach to infection in the immunocompromised host. 3rd ed. **New York: Plenum Medical Book Co; 1994. p. 629-705**.

3. Ho M. Observations from transplantation contributing to the understanding of pathogenesis of CMV infection. **Transplant Proc 1991;23(Suppl 3): 104-9**.

4. Koffron AJ, Hummel M, Patterson BK, et al. Cellular localization of latent murine cytomegalovirus. **J Virol 1998;72:95-103**.

5. Hummel M, Zhang J, Yan S, et al. Allogeneic transplantation induces expression of CMV immediate early genes in vivo: a model for reactivation from latency. **J Virol 2001;75:4814-22**.

6. Soghikian MV, Valentine VG, Berry GJ, et al. Impact of ganciclovir prophylaxis on heart-lung and lung transplant recipients. **J Heart Lung Transplant 1996;15:881-7**.

7. Patel R, Smith TF, Espy M, et al. Detection of cytomegalovirus DNA in sera of liver transplant recipients. **J Clin Microbiol 1994;32:1431-4**.

8. Falagas ME, Arbo M, Ruthazer R, et al. Cytomegalovirus disease is associated with increased cost and hospital length of stay among orthotopic liver transplant recipients. **Transplantation 1997;63:1595-601**.

9. Kanj S, Hamilton J. The 10 most common questions about cytomegalovirus infection in solid organ transplant recipients. **Infect Dis Clin Practice 1997;6:1-4**.

10. Koffron AJ, Patterson BK, Yan S, et al. Latent human cytomegalovirus: a functional study. **Transplant Proc 1997;29:793-5**.

11. Patel R, Snydman DR, Rubin RH, et al. Cytomegalovirus prophylaxis in solid organ transplant recipients. **Transplantation 1996;61:1279-89**.

12. Muldoon J, O'Riordan K, Rao S, et al. Ischemic colitis secondary to venous thrombosis: a rare presentation of cytomegalovirus vasculitis following renal transplantation. **Transplantation 1996;61:1651-53**.

13. Fishman JA, Rubin RH. Infection in organ-transplant recipients. **N Engl J Med 1998;338:1741-51**.

14. Abecassis MM, Koffron AJ, Kaplan B, et al. The role of PCR in the diagnosis and management of CMV in solid organ recipients: what is the predictive value for the development of disease and should PCR be used to guide antiviral therapy? **Transplantation 1997;63:275-9**.

15. Hebart H, Kanz L, Jahn G, et al. Management of cytomegalovirus infections after solid-organ or stem-cell transplantation. Current guidelines and future prospects. **Drugs 1998;55:59–72**.

16. Snydman DR, Werner BG, Heinze-Lacey B, et al. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. **N Engl J Med 1987;317:1049–54**.

17. Ringden O, Lonnqvist B, Paulin T, et al. Pharmacokinetics, safety and preliminary clinical experiences using foscarnet in the treatment of cytomegalovirus infections in bone marrow and renal transplant recipients. **J Antimicrob Chemother 1986;17:373–87**.

18. Manischewitz JF, Quinnan GF Jr, Lane HC, et al. Synergistic effect of ganciclovir and foscarnet on cytomegalovirus replication in vitro. **Antimicrob Agents Chemother 1990;34:373–5**.

19. Bacigalupo A, Bregante S, Tedone E, et al. Combined foscarnet-ganciclovir treatment for cytomegalovirus infections after allogeneic hemopoietic stem cell transplantation (Hsct). **Bone Marrow Transplant 1996;18(Suppl 2): 110–4**.

20. Bacigalupo A, Bregante S, Tedone E, et al. Combined foscarnet-ganciclovir treatment for cytomegalovirus infections after allogeneic hemopoietic stem cell transplantation. **Transplantation 1996;62:376–80**.

21. Crumpacker CS. Ganciclovir. **N Engl J Med 1996;335:721–9**.

22. Pescovitz MD, Rabkin J, Merion RM, et al. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. **Antimicrob Agents Chemother 2000;44:2811–5**.

23. Brown F, Banken L, Saywell K, et al. Pharmacokinetics of valganciclovir and ganciclovir following multiple oral dosages of valganciclovir in HIV- and CMV-seropositive volunteers. **Clin Pharmacokinet 1999;37:167–76**.

24. Hoffman VF, Skiest DJ. Therapeutic developments in cytomegalovirus retinitis. **Expert Opin Investig Drugs 2000;9:207–20**.

25. Rubin RH, Ikonen T, Gummert JF, et al. The therapeutic prescription for the organ transplant recipient: the linkage of immunosuppression and antimicrobial strategies. **Transpl Infect Dis 1999;1:29–39**.

26. Snydman DR, Werner BG, Dougherty NN, et al. Cytomegalovirus immune globulin prophylaxis in liver transplantation: a randomized, double-blind, placebo-controlled trial. **Ann Intern Med 1993;119:984–91**.

27. Conti DJ, Freed BM, Gruber SA, et al. Prophylaxis of primary cytomegalovirus disease in renal transplant recipients. A trial of ganciclovir vs. immunoglobulin. **Arch Surg 1994;12:443–7**.

28. Kaufman DB, Leventhal JR, Gallon LG, et al. Risk factors and impact of cytomegalovirus disease in simultaneous pancreas-kidney transplantation. **Transplant J 2000:in press**.

29. Martin M, Manez R, Linden P, et al. A prospective randomized trial comparing sequential ganciclovir-high dose acyclovir to high dose acyclovir for prevention of cytomegalovirus disease in adult liver transplant recipients. **Transplantation 1994;58:779–85**.

30. Balfour HH, Chace BA, Stapleton JT, et al. A randomized, placebo-controlled trial of oral acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. **N Engl J Med 1989;321:1268–9**.

31. 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;25:1344–9**.

32. Barkholt L, Lewensohn-Fuchs I, Ericzon B-G, et al. High-dose acyclovir prophylaxis reduces cytomegalovirus disease in liver transplant patients. **Transpl Infect Dis 1999;1:89–97**.

33. Seu P, Winston DJ, Holt CD, et al. Long-term ganciclovir prophylaxis for successful prevention of primary cytomegalovirus (CMV) disease in CMV-seronegative liver transplant recipients with CMV-seropositive donors. **Transplantation 1997;64:1614–7**.

34. Gane E, Saliba F, Valdecasas GJC, et al. Randomized trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver-transplant recipients. **Lancet 1997;350:1729–33**.

35. 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;68:1305–11**.

36. Vivarelli M, DeRuvo N, Lazzarotto T, et al. Abstension from treatment of low-level pp65 cytomegalovirus antigenemia after liver transplantation: a prospective study. **Transplantation 2000;70:1183–7**.

37. Kusne S, Grossi P, Irish W, et al. Cytomegalovirus pp65 antigenemia monitoring as a guide for preemptive therapy: a cost effective strategy for prevention of cytomegalovirus disease in adult liver transplant recipients. **Transplantation 1999;68:1125–31**.

38. Singh N, Paterson DL, Gayowski T, et al. Cytomegalovirus antigenemia directed pre-emptive prophylaxis with oral versus I.V. ganciclovir for the prevention of cytomegalovirus disease in liver transplant recipients: a randomized, controlled trial. **Transplantation 2000;70:717–22**.

39. Kelly J, Hurley D, Raghu G. Comparison of the efficacy and cost effectiveness of pre-emptive therapy as directed by CMV antigenemia and prophylaxis with ganciclovir in lung transplant recipients. **J Heart Lung Transplant 2000;19:355–9**.

40. Drobyski WR, Knox KK, Carrigan DR, et al. Foscarnet therapy of ganciclovir-resistant cytomegalovirus in marrow transplantation. **Transplantation 1991;52:155–7**.

41. Rosen HR, Benner KG, Flora KD, et al. Development of ganciclovir resistance during treatment of primary cytomegalovirus infection after liver transplantation. **Transplantation 1997;63:476–8**.

42. Alain S, Honderlick P, Grenet D, et al. Failure of ganciclovir treatment associated with selection of a ganciclovir-resistant cytomegalovirus strain in a lung transplant recipient. **Transplantation 1997;63:1533–6**.

43. Baldanti F, Simoncini L, Sarasini A, et al. Ganciclovir resistance as a result of oral ganciclovir in a heart transplant recipient with multiple human cytomegalovirus strains in blood. **Transplantation 1998;66:3249**.

44. Drew WL, Miner RC, Busch DF, et al. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. **J Infect Dis 1991;163:716–9**.

45. Marschner IC, Collier AC, Coombs RW, et al. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. **J Infect Dis 1998;177:40–7**.

46. Biron KK, Fyfe JA, Stanat SC, et al. A human cytomegalovirus mutant resistant to the nucleoside analong 9-([2-hydroxy-1-(hydroxymethyl) ethoxy] guanine (BW B759U) induces reduced levels of BW B759U triphosphate. **Proc Natl Acad Sci U S A 1986;83:8769–73**.

47. Shinkai M, Bozzette SA, Powderly W, et al. Utility of urine and leukocyte cultures and plasma DNA polymerase chain reaction for identification of AIDS patients at risk for developing human cytomegalovirus disease. **J Infect Dis 1997;175:302–8**.