Overview of congenitally and perinatally acquired cytomegalovirus infections: recent advances in antiviral therapy

Mark R Schleiss and Michael A McVoy

Congenital and perinatal infection with human cytomegalovirus (CMV) are commonly encountered in newborns. In recent years there has been increased awareness of the disabilities that result from congenital CMV infection, which in turn has prompted interest in examining the potential efficacy of antiviral agents to prevent or ameliorate neurodevelopmental injury. Currently, there are three licensed systemic antivirals for the treatment of CMV: ganciclovir (Cytovene®, Roche) and its prodrug valganciclovir (Valcyte®, Roche); foscarnet (Foscavir®/ Zosyn®, AstaZenecon); and cidofovir (Vistide®, Pharmacia). A CMV-specific immunoglobulin is also available. Experience with these agents in the setting of congenital and perinatal CMV infection is very limited, but there are encouraging data from a controlled clinical trial indicating that ganciclovir therapy may be of value in limiting one form of neurodevelopmental injury caused by congenital infection, that of sensorineural hearing loss. Licensed antivirals for the treatment of CMV all share the common mechanism of targeting the viral DNA polymerase, but novel therapies that employ alternative modes of action are in development. Ultimately, the problem of perinatal CMV infection may be best controlled by the development of CMV vaccines, which could be administered to young women of childbearing age to help control this important public health problem.


Cytomegalovirus: overview of the pathogen

Primary infection with cytomegalovirus (CMV) is generally asymptomatic in healthy adults but several high-risk groups, including immunocompromised organ transplant recipients and individuals infected with HIV, are at risk of developing life- and sight-threatening CMV disease. In addition, CMV has emerged in recent years as the most important cause of congenital viral infection in the developed world, commonly leading to mental retardation and developmental disability. The syndrome associated with congenital CMV infection was first reported in 1904 by Ribbert, who identified previously unrecognized histological lesions in tissues from a congenitally infected infant. It was mistakenly assumed at that time that the large inclusion-bearing cells observed at autopsy were caused by a protozoan species (dubbed Entamoeba morinutudum). In 1920, Goodpasture correctly postulated the viral etiology of these inclusions. He used the term 'cytomegala' to refer to the enlarged swollen nature of the infected cells. Human CMV was first isolated in tissue culture in the late 1950s, and the propensity of this organism to infect the salivary gland led to its initial designation as salivary gland virus. In 1960, Weller designated the virus cytomegalovirus and during the 1970s and 1980s, knowledge of the role of CMV as an important pathogen with diverse clinical manifestations increased steadily.

CMV is a member of the family of eight human herpesviruses (HHVs), designated as HHV-5. Taxonomically, CMV is a member...
of the Betaherpesvirinae subfamily of the HHVs, with molecular and phylogenetic similarity to HHV-6 and -7, the etiologic agents of roseola. CMV is the largest member of the herpesvirus family, with a double-stranded DNA genome of approximately 235 kbp. The viral genome is divided into a unique long (U₃) region and a unique short (U₂) region, with the nomenclature of viral gene designation being based on where in the genome the gene maps to. An understanding of the biology of viral replication provides insights into the molecular mechanisms of antiviral therapy. The replication cycle of CMV is divided temporally into the following three regulated classes:

• Immediate early
• Early
• Late

Immediate early gene transcription occurs in the first 4 h following viral infection, and key regulatory proteins are made which allow the virus to take control of cellular machinery. Following the synthesis of immediate early genes, the early genes are transcribed. Early gene products include DNA replication proteins and some structural proteins. These proteins are of considerable importance in the understanding of mechanisms of activity of antiviral drugs for CMV. The U₃S₄ (DNA polymerase) is the major target of licensed antivirals for CMV and the U₃G₅ (phosphotransferase protein) is required for phosphorylation of the viral agent, ganciclovir (Cytovene® Roche), to its active metabolite in vivo. The late genes are transcribed approximately 24 h after infection and these proteins are chiefly structural, and are involved in virion assembly and egress. Licensed antivirals for CMV largely interfere with the early gene products involved in DNA replication, although any of these points in the viral life cycle could, in principle, be targets for drug development.

Immunity to CMV is complex and involves both humoral and cell-mediated responses. Several CMV gene products are of particular importance in CMV immunity. The outer envelope of the virus, which is derived from the host cell nuclear membrane, contains multiple virally encoded glycoproteins. Glycoprotein (g)B and gH appear to be the major determinants of protective humoral immunity. Antibodies to these proteins are capable of neutralizing the virus, and therefore, gB and gH are potential targets for investigational CMV subunit vaccine and therapeutic monoclonal antibody development [9-11].

Recent investigations into the molecular biology of CMV have revealed the presence of many viral gene products that appear to modulate host inflammatory and immune responses [12-15]. Several CMV genes interfere with normal antigen processing and generation of cell-mediated immune responses. To date, four viral gene products have been identified that inhibit major histocompatibility (MHC) class I antigen presentation. The U₃S and U₃G₁1 gene products interfere with immune responses to CMV by exporting the class I heavy chain from the endoplasmic reticulum (ER) to the cytosol (rendering it nonfunctional). The U₃G₁3 gene product retains MHC molecules in the ER, preventing them from trafficking to the plasma membrane. Finally, the U₃G₁6 protein inhibits peptide translocation by transporters associated with antigen processing (TAP). Other viral gene products, of the U₃S₃S, U₃G₂7 and U₃G₂8 genes, are functional homologs of cellular G-protein-coupled receptors that may, via molecular mimicry, subvert normal inflammatory responses and, in the process, promote tissue dissemination of the virus and interfere with the host immune response. The CMV genome also encodes a homolog of the cellular MHC class I gene, which appears to contribute to the ability of CMV to evade the host defense. The U₃1L open reading frame found in clinical isolates of CMV encodes a structural homolog of the tumor necrosis factor receptor superfamily, which may contribute to the ability of human CMV to escape immune clearance. This plethora of putative viral immune-evasion genes complicates the clearance of virus following natural infection, and may retard the development of protective long-term immunity, underscoring the need for advances in antiviral therapy to effect control of CMV disease in high-risk patients.

Overview of congenital & perinatal cytomegalovirus infections
In the developed world, CMV is the most common viral pathogen encountered in newborns. The natal acquisition of CMV may occur by either prenatal infection in utero, resulting in congenital CMV infection, or by perinatal acquisition – either during the labor and delivery process, via blood transfusion or by breastfeeding. Infants infected with CMV in utero are at risk for short- and long-term systemic and neurodevelopmental sequelae, and CMV can produce life-threatening disease in premature infants who acquire the virus during the perinatal period. Both groups of infants are potential candidates for antiviral therapy.

Congenitally acquired CMV infection
Congenital CMV infection is present in 0.5 to 2% of all deliveries in the developed world [16]. In the USA, this corresponds to approximately 40,000 infants infected annually. The clinical manifestations are quite variable. Approximately 10% of congenitally infected infants have clinically evident disease at birth. Newborns with symptomatic congenital CMV infection often present with visceral organomegaly, microcephaly with intracranial calcifications, chorioretinitis and dramatic skin manifestations, including petechiae and purpura. This complex of findings, characteristic of the syndrome of cytomegalic inclusion disease (CID) of the newborn, predicts a poor prognosis, with rates of audiologic and neurodevelopmental sequelae of up to 90% [17-19]. A high percentage of these babies have profound neurodevelopmental handicaps, including mental retardation and sensorineural deafness. In contrast to this subset of symptomatic CMV-infected newborns, approximately 90%
of congenitally infected infants have asymptomatic infection at birth. Although most of these infants appear normal, up to 10 to 15% of these clinically silent congenital infections may be associated with hearing loss and possibly other forms of neurodevelopmental injury [28]. Sensorineural hearing impairment may be present at birth or may be progressive in nature during early childhood, suggesting an ongoing postnatal injury mediated by the virus [29].

It has generally been believed that preconceptual maternal immunity to CMV provides some degree of protection against the most devastating sequelae of congenital infection, including CID. In a comparison of outcomes of CMV-infected infants born to mothers who acquired primary CMV infection during pregnancy (primary infection group) with those of CMV-infected infants born to mothers with preconceptual immunity (recurrent infection group), only infants born in the primary infection group had symptomatic disease at birth and these infants were at substantially higher risk for sequelae [22]. More recent studies, however, have questioned the degree of protection conferred by preconceptual immunity. In a recently reported study of mothers with preconceptual immunity to CMV who gave birth to congenitally infected infants, there was evidence for acquisition of new strains of CMV, as evidenced by the development of new antibody specificities against an envelope glycoprotein [23]. This observation suggests that some women who are seropositive for CMV may become infected with new CMV strains and that reinfection may lead to symptomatic disease in the neonate. Other studies have similarly identified severe symptomatic disease in newborns born to women with preconceptual immunity [24,25]. If preconceptual immunity to CMV provides only partial protection against intraterine transmission as well as the attendant neurodevelopmental sequelae, then future disease control efforts may require an increased emphasis on the use of antiviral agents, until there is a better understanding of the immune correlates involved in the protection of the fetus.

Perinatally acquired CMV Infection

Perinatal acquisition of CMV may occur by one of three different routes:

• Exposure to CMV in the birth canal during labor and delivery
• Transmission of CMV by blood transfusion
• Transmission by breast-feeding

The problem of transfusion-associated infection has largely been eliminated by the use of safer blood products [26-28]. Historically, breast milk-acquired CMV infections in term infants have been felt to be of little clinical significance, even being referred to by one author as a form of 'natural immunization' [29]. More recent evidence, however, indicates that, in contrast to full-term babies, the acquisition of CMV by the premature infant can produce severe disease. In a prospective study in premature infants receiving breast milk containing CMV, transmission was observed in 33 out of 87 exposed infants - 16 of these babies (48%) developed disease, including hepatitis, neutropenia, thrombocytopenia and sepsis-like state [30]. Other studies have suggested lower transmission rates, particularly if breast milk is routinely frozen prior to being fed to the infant [31]. It is uncertain if CMV infection of low-birth-weight premature infants by this route carries any risk of long-term neurodevelopmental sequelae. More information is needed on the consequences of breast milk-acquired CMV infections, as well as the potential ameliorative role of antiviral therapy, for high-risk premature infants.

Monitoring for CMV in pregnant patients: a target population for antiviral agents?

In light of the public health importance of congenital CMV infection, better education of the risks of CMV infection during pregnancy will be required. In this context, it is important to keep in perspective that most CMV infections in pregnant women do not lead to adverse fetal outcomes. The available data indicate that CMV is transmitted to the fetus in approximately 40% of primary maternal infections and that only 5 to 10% of the fetuses are adversely affected by the virus. Prenatal CMV antibody screening is one option for increasing public awareness and improving prenatal counseling. Although the finding of preconceptual immunity to CMV cannot provide complete assurance that a newborn will be uninfected and more importantly, unaffected, documentation of serologic status may nonetheless be very useful in some situations for anticipatory monitoring of the pregnancy [32]. Although there are no specific guidelines for prenatal virologic or serologic screening of women of childbearing age, situations that, in principle, require monitoring could include CMV-seronegative women with significant occupational exposure to CMV, including those who work in child-care environments, or women with young children enrolled in day-care centers [33-35]. In the circumstance where a woman was found to convert her CMV antibody titer while pregnant, options for monitoring could include serial ultrasonography and, conceivably, amniocentesis for the identification of CMV genome and quantitative determination of viral load by polymerase chain reaction (PCR) [36,37]. For high-risk pregnancies, antiviral therapy during pregnancy could theoretically be contemplated, with the goal of minimizing the risk of viral transmission, analogous to the administration of antiretroviral therapy to HIV-positive women. However, there are many potential pitfalls with this approach, including the toxicity of licensed antivirals to the fetus, an inability to predict which pregnancies will be complicated by in utero infection and which infected infants will have disabilities related to CMV. Among women at high risk for acquisition of primary infection, at present, behavioral modification (i.e., avoidance of infectious saliva and urine and careful hygienic practices) may be the only strategy likely to minimize the risk of infection during pregnancy [38].

www.future-drugs.com
Currently licensed antivirals for CMV

The use of nucleoside antivirals for CMV infection was first contemplated in the early 1980s, when acyclovir (Zovirax®, GlaxoSmithKline) was used in bone marrow transplant patients at high risk for CMV disease [39]. Since that time, considerable experience has accrued with anti-CMV therapies in immunosuppressed patients, particularly solid organ and bone marrow transplant patients and patients with HIV infection. The use of anti-CMV antivirals in these populations has been the subject of a number of recent reviews [40-43]. The currently licensed antivirals for CMV therapy share the feature of inhibition of the viral DNA polymerase, although they differ in their pharmacology and are summarized in Table 1.

**Ganciclovir**

Ganciclovir was the first compound licensed specifically for treatment of CMV infections. Ganciclovir is a synthetic acyclic nucleoside analog, structurally similar to guanine. Its structure is also similar to that of acyclovir and, like acyclovir, it requires phosphorylation for antiviral activity. The

<table>
<thead>
<tr>
<th>Table 1. Selected agents licensed for antiviral therapy or prophylaxis against systemic CMV disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>Ganciclovir</td>
</tr>
<tr>
<td>Foscarnet</td>
</tr>
<tr>
<td>Cidofovir</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
</tr>
</tbody>
</table>

CMV: Cytomegalovirus; GCSF: Granulocyte colony stimulating factor; GM-CSF: Granulocyte-monocyte colony stimulating factor; Ig: Immunoglobulin; iv: Intravenous; po: Orally; q: Every
enzyme responsible for phosphorylation of ganciclovir is the product of the CMV \( U_{97} \) gene, a protein kinase and phosphotransferase (44-46). Following phosphorylation by \( U_{97} \), cellular enzymes phosphorylate the monophosphate form to di- and triphosphate metabolites; the ganciclovir triphosphate metabolite exerts the antiviral effect in the CMV-infected cell.

Ganciclovir is the treatment of choice for severe CMV disease. For bone marrow transplant or solid organ transplant patients, ganciclovir is indicated both for active CMV disease (e.g., CMV pneumonia) and as preemptive therapy when there is an indication of active viral replication, such as a positive PCR assay or pp65 antigenemia test [47]. Therapy in immunocompromised patients is typically administered in a staged fashion, with an induction dose of 10 mg/kg/day divided every 12 h for 14 to 21 days, followed by a single daily maintenance dose of 5 mg/kg/day. For congenital and perinatal CMV infections, the optimal dose is less certain, although a total daily dose of 10 to 12 mg/kg/day appears to be appropriate. Duration of therapy depends upon the clinical setting; to date, the best data regarding dose and duration of ganciclovir therapy in neonates come from clinical trials which have examined a total of 6 weeks of treatment at a dose of 12 mg/kg/day.

Ganciclovir is associated with a number of drug toxicities. Myelosuppression (e.g., granulocytopenia, anemia and thrombocytopenia) is often a dose-limiting toxicity in immunocompromised patients, particularly those who are often on other myelosuppressive agents. Concomitant administration of granulocyte colony stimulating factor (GCSF) or granulocyte-monocyte colony stimulating factor (GMCSF) can be considered, if the perceived benefits of therapy warrant completion of a full 6-week course. In animal models, ganciclovir is carcinogenic and gonadotoxic. Clearance of ganciclovir occurs by renal mechanisms, with dose reduction being recommended in the setting of renal insufficiency. Ganciclovir penetrates well into the CNS [42], an observation of potential importance for treatment strategies in newborns designed to provide protection against CMV-induced neurodevelopmental injury. However, it is important to keep in perspective, outstanding CNS penetration notwithstanding, that postnatal ganciclovir is unlikely to be useful for severe forms of CNS injury associated with early \textit{in utero} acquisition of infection. Resistance to ganciclovir may emerge on therapy (46). This resistance may occur either by mutations in the viral \( U_{97} \) gene, which encodes the phosphotransferase required for phosphorylation of ganciclovir to its active metabolites, or less commonly, mutations in the viral DNA polymerase, \( U_{54} \), the target enzyme of ganciclovir-triphosphate. Approximately 95% of ganciclovir-resistant CMV isolates have mutations in the viral \( U_{97} \) phosphotransferase; 5% have mutations in the viral DNA polymerase (46). PCR-based assays are available to screen for ganciclovir resistance; for \( U_{97} \)-based resistance, alternative therapies (foscarnet [Foscavir\textsuperscript{®}, AstraZeneca], and cidoflovir [Vistide\textsuperscript{®}, Pharmacia]) may be considered.

In addition to consideration of the use of ganciclovir in an effort to improve neurodevelopmental and hearing outcomes in symptomatic infants with congenital CMV involving the CNS, therapy should also be considered in any severely ill infant. This could include any symptomatic infant with congenital or postnatally acquired CMV infection and evidence of end-organ disease (pneumonia, viremia, hepatitis, sight-threatening retinopathy and refractory thrombocytopenia).

**Valganciclovir**

Valganciclovir (Valcyte\textsuperscript{®}, Roche) is the valine ester of ganciclovir. In contrast to oral formulations of ganciclovir, valganciclovir is very well absorbed following oral administration. It is rapidly metabolized into ganciclovir following oral dosing. Valganciclovir is indicated for therapy of CMV retinitis in HIV-positive patients and for the prevention of CMV disease in kidney, heart and kidney-pancreas transplant patients at high risk for CMV disease (donor CMV seropositive/recipient CMV seronegative [D+/R-]). The side-effect profile is similar to that of ganciclovir. The commercially available formulation is produced as a tablet; suspension formulations are investigational [42]. There is currently little experience with this agent in infants and children but the convenience of oral dosage makes this agent attractive for future clinical trial evaluation in the setting of congenitally or perinatally acquired CMV infection.

**Foscarnet**

Foscarnet, an inorganic pyrophosphate analog, is a second-line treatment for CMV infection. The mechanism of action of foscarnet, similar to ganciclovir's, is inhibition of the CMV DNA polymerase. However, in contrast to ganciclovir, foscarnet does not require phosphorylation for antiviral activity. The drug directly blocks the pyrophosphate binding site of the DNA polymerase. Resistance to foscarnet may emerge on therapy. Mutations which confer resistance to foscarnet are described in the CMV \( U_{54} \) (DNA polymerase) gene (48). Foscarnet has a significant risk of producing nephrotoxicity, and prehydration with saline is often recommended. Foscarnet has an important toxicity relevant to pediatric practice, which is its ability to affect bone and tooth development, necessitating caution when used in children or pregnant women [42]. The recommended dosage in immunocompromised patients is 180 mg/kg/day administered intravenously in two or three divided doses for 14 to 21 days (induction therapy), followed by 90 to 120 mg/kg once daily (maintenance therapy). In contrast to ganciclovir, the risks of myelosuppression with foscarnet are minimal, and this agent is the recommended CMV therapy for patients with bone marrow failure. Although consideration should be given to the use of foscarnet in a setting of known or suspected ganciclovir resistance, there are very few data on the use of this agent in newborns or young infants, making definitive dose recommendations in this setting problematic.
Cidofovir

Another second-line therapy for CMV disease, cidofovir, is, like foscamet and ganciclovir, an inhibitor of the CMV DNA polymerase. Cidofovir is an acyclic phosphonate nucleotide analog, which already has a single phosphate group attached in the formulation used for clinical administration. Hence, it does not require or utilize an initial viral (ULT-mediated) phosphorylation for antiviral activity but does undergo additional phosphorylation by cellular kinases to its active form. In its fully phosphorylated form, cidofovir is selectively incorporated into the viral DNA chain, inhibiting viral DNA synthesis. Cidofovir is eliminated by renal clearance, although the drug persists in cells for prolonged periods and its metabolites have very long intracellular half-lives, which allows for an intermittent dosage schedule. Unfortunately, cidofovir has a wide range of significant toxicities, including nephrotoxicity, neutropenia, ocular toxicity and metabolic acidosis. In animal studies, cidofovir is carcinogenic and teratogenic and may be associated with gonadal injury in males. Prehydration with saline and treatment with probenecid prior to the infusion are recommended to prevent the problem of nephrotoxicity. Dose adjustment is required for patients with renal insufficiency and the drug should be avoided in patients with azotemia, or on those other nephrotoxic drugs.

The recommended dose of cidofovir in immunocompromised patients with CMV disease is 5 mg/kg once weekly for 2 weeks, followed by maintenance dosing administered on an 2-weekly basis. There is no information available about the use of cidofovir in neonates with CMV infection, making dose recommendations in this setting uncertain.

Cytomegalovirus immunoglobulin

Cytomegalovirus immunoglobulin (CytGam®; MedImmune Inc.) is a pooled, high-titer intravenous immunoglobulin preparation prepared from donors with high titers of CMV antibodies. The presumed mechanism is neutralization of virus infectivity, via interactions with viral envelope glycoproteins. Cytomegalovirus immunoglobulin is indicated, either alone or in combination with nucleoside analogs, for prophylaxis against CMV disease in solid organ transplant patients. It may also represent a useful adjunctive therapy for patients with severe CMV end-organ disease already being treated with antiviral agents. The dose most commonly employed is 100 to 150 mg/kg, intravenously, initiated within 3 days post-transplant, then repeated every 2 to 4 weeks for 4 months. Since it is a hyperimmunoglobulin preparation, administration of cytomegalovirus immunoglobulin may interfere with responses to live virus vaccines. There are no data regarding the use of CMV immunoglobulin in newborns and its potential effects on congenital or perinatal CMV infections.

New antivirals in development for CMV

Currently licensed antivirals for CMV all share the same fundamental mechanism of action, namely, the inhibition of the viral DNA polymerase. Emergence of drug-resistant viruses is a major problem associated with long-term therapy [46,49]. Each of the available antivirals has the potential to be associated with significant toxicities. These concerns, coupled with the clinical need for new anti-CMV therapies, should have driven the development of additional therapies; however, despite this, the field has advanced at a disappointingly slow pace. A modest number of new treatments for CMV infection are nonetheless in development. Strategies to improve delivery or potency of existing nucleoside analogs show promise, and several new anti-CMV compounds that target novel processes are in various stages of development. Some of the more promising compounds are discussed below, and summarized in Table 2. Currently, there are no safety or efficacy data regarding the use of the following drugs in the treatment of congenital or perinatal CMV infections.

<table>
<thead>
<tr>
<th>Table 2: New antivirals for CMV currently in preclinical development.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>HDP-CDV</td>
</tr>
<tr>
<td>ODE-CDV</td>
</tr>
<tr>
<td>Mycophenolate mofetil (Cicept®)</td>
</tr>
<tr>
<td>GW1263W94 (Maribavir®)</td>
</tr>
<tr>
<td>GW275175X</td>
</tr>
<tr>
<td>BAV 38-4766 (Tomoglyco®)</td>
</tr>
</tbody>
</table>

CDV: Cidofovir; dGTP: Deoxyguanosine triphosphate; HDP-CDV: Hexadehydropropyl-CDV; ODE-CDV: Octadehydroethyl-CDV.
Antiviral therapy for perinatally acquired cytomegalovirus

Mycophenylate mofetil
Mycophenolic acid (CellCept®, Roche), the active metabolite of mycophenylate mofetil, is an inhibitor of guanine monophosphate synthesis and has the effect of reducing levels of intracellular deoxyguanine triphosphate (dGTP). While having no antiviral activity on its own, mycophenylate mofetil significantly increases the antiviral activities of guanine-based nucleoside analogs, including the anti-CMV activity of ganciclovir [59-62]. This effect is believed to result from the reduction in dGTP levels as the inhibitory analogs must compete with dGTP for polymerase binding. While additional in vitro and in vivo studies are needed, mycophenylate mofetil has the significant advantage of being approved and in common use as an immunosuppressive agent in transplant patients. As these patients frequently receive concomitant antiviral treatments, clinical experience with coadministration of mycophenylate mofetil and antivirals, such as ganciclovir, already exists. Combination therapy may prove valuable, both in avoiding ganciclovir toxicity and in overcoming ganciclovir-resistance.

Hexadecylhexyl-propyl- & octadecylhydroxtyl-cidofovir
The prodrug derivatives of cidofovir, hexadecylhexylpropyl-cidofovir (HDP-CDV) and octadecylhydroxtyl-cidofovir (ODE-CDV) were anticipated to have improved oral absorption but somewhat surprisingly, both exhibited several logs improvement in anti-CMV activity in vitro [53]. Initial in vivo studies measuring expression of CMV replication in severe combined immune deficient (SCID-hu) mice observed a four- to eightfold enhancement, on a molar basis, over cidofovir and demonstrated that levels sufficient to inhibit CMV replication in target organs, including the eye, were achievable by oral delivery [54]. These prodrug derivatives have several advantages making them attractive for future development. In the SCID-hu retinal model, the efficacy of these compounds suggested that in addition to systemic activity, HDP-CDV and ODE-CDV can also cross the blood-ocular barrier and inhibit CMV infection of ocular tissue. The produg formulations may overcome cidofovir toxicity, as improved activity may allow lower dosing and the greater uniformity afforded by oral delivery could ameliorate the toxicities which arise as serum levels peak following intravenous administration.

GW275175X
In 1998, several compounds in the halogenated benzimidazole family were found to inhibit CMV replication by a completely unique mode of action – they inhibit the cleavage of viral genomes from replicative intermediate DNA and interfere with packaging of DNA within capsids [55,56]. These compounds target a viral enzyme referred to as the 'terminase', that both packages the DNA into capsids and cleaves the DNA to form mature genomes (recently reviewed in [57]). GW275175X, a derivative in which the sugar is in the pyranosyl (rather than furanosyl) form, has encouraging oral bioavailability and stability in vivo. However, its development is on hold in favor of the related compound GW1263W94 (Maribavir®, ViroPharma).

GW1263W94
The terminase-inhibiting halogenated benzimidazoles described above are D-ribose isomers. Curiously, the L-ribose stereoisomer GW1263W94 was found to have significant anti-CMV activity but not through inhibition of DNA cleavage and packaging. Although this compound has an effect on DNA synthesis [58], the significant mode of action appears to be inhibition of capsid egress across the nuclear envelope [59]. Its targets, identified by mapping drug resistance mutations, are a protein kinase encoded by the CMV U1 gene [58] and a protein of unknown function encoded by CMV U1.27 [60]. It is not known whether any of these proteins function in DNA synthesis or capsid egress.

Interestingly, U1.27 is the viral kinase responsible for phosphorylating ganciclovir to ganciclovir-monophosphate, a step critical to its subsequent phosphorylation by cellular enzymes to its active ganciclovir-triphosphate form [61,62]. Indeed, most ganciclovir-resistance mutations map to the U1.27 gene, rather than the DNA polymerase [45]. Although this might raise concerns that GW1263W94 could antagonize ganciclovir by blocking its phosphorylation, this appears not to be the case. In vitro, GW1263W94 is synergistic with cidofovir and foscarnet and additive (but not antagonistic) with ganciclovir [61,62]. Importantly for the HIV patient population, GW1263W94 is not antagonized by any of the commonly used anti-HIV agents [62]. Although mutations conferring resistance to both GW1263W94 and ganciclovir lie within U1.97, they do not confer cross-resistance: U1.97 mutants resistant to ganciclovir retain sensitivity to GW1263W94 [58,63] and conversely, mutants resistant to GW1263W94 are sensitive to ganciclovir [64]. Thus, this compound is an attractive anti-CMV candidate for use on its own or in combination with existing therapies.

Of the novel CMV antivirals, GW1263W94 is perhaps the furthest along in clinical development. In Phase I trials of HIV-infected men with asymptomatic CMV shedding, GW1263W94 exhibited promising safety and pharmacokinetic properties and affected reductions of several logs in CMV semen titers [64-67]. Both GW275175X and GW1263W94 were recently licensed to ViroPharma, which has plans to initiate clinical trials and a Phase II study in stem cell transplant patients in the near future [MCKINLAY M, FERS. COMMUN]. The observation that both GW275175X and GW1263W94 have activity against Epstein-Barr virus may provide an additional impetus for clinical development [68].

BAY 38-4766
Despite structural dissimilarity with the halogenated benzimidazoles, the non-nucleoside compound BAY 38-4766 (Tome- glovir®, Bayer AG) has a very similar mode of anti-CMV activity – that of inhibiting DNA cleavage and packaging
through the viral terminase [98, 70]. However, the sites of interaction with terminase appear to be distinct, as mutations within terminase that confer benzimidazole-resistance are not cross-resistant to BAY 38-4766 [41]. Surprisingly, drug interaction studies reveal that BAY 38-4766 antagonizes ganciclovir [69]. Although this may counter indicate combination therapy with ganciclovir, the fact that viruses resistant to ganciclovir, foscamet and cidoviro are susceptible to BAY 38-4766 [72, 73] suggests this drug may be of value in treating patients who have failed conventional therapies. In vivo, BAY 38-4766 has encouraging safety and pharmacokinetic properties. In mice it protects against pathogenic murine CMV infection [71, 72], and in healthy male human volunteers, single oral doses of up to 2000 mg were safe and well-tolerated [73].

Leflunomide

Leflunomide (Arava®; Aventis Pharma) is a licensed and approved therapy for rheumatoid arthritis. The agent is immunosuppressive and functions as an inhibitor of protein kinase activity and pyrimidine synthesis. Interestingly, the agent has activity against CMV both in vitro and in vivo, and has been shown to reduce viral load in a rat model of disseminated CMV infection [74]. The mechanism of action is unclear but the drug appears to interfere with virus capsid assembly, probably related to its inhibition of protein kinase activity. Unfortunately, the agent is teratogenic in animal models and this, coupled with its immunosuppressive effect, may limit its further development as a CMV antiviral.

Antiviral therapy for congenital & perinatal CMV infections

Ganciclovir therapy

Plotkin and Steilner first proposed the utilization of antiviral therapy for infants with congenital CMV infection in 1969 [75]. With the advent of ganciclovir, an effective antiviral drug for CMV, reports of therapy for congenital CMV infection began to be published in the late 1980s [76, 77]. In numerous uncontrolled reports [78-86], ganciclovir has been shown to be generally safe and well-tolerated when used in newborns and has appeared to be useful in ameliorating the severity of focal, end-organ disease (for example, pneumonitis and hepatitis) in infants. Based on these reports, the use of ganciclovir is worth considering and in general is probably indicated in the short-term management of any infant with severe or symptomatic CMV disease, including symptomatic viremia, pneumonitis or refractory thrombocytopenia. It is important to note that no sustained effect on CMV shedding at mucosal sites can be expected; once therapy is completed, infants resume excretion of CMV in urine and saliva cultures.

Although ganciclovir appears valuable for short-term management of CMV infection in infants in some settings, it is less clear if use of ganciclovir provides any long-term benefit for congenital or perinatally-acquired CMV infection. Case reports describing the use of ganciclovir for perinatal CMV infections, though encouraging, are very difficult to put into perspective because of their uncontrolled nature. Recently, however, a series of studies of ganciclovir therapy in congenitally infected infants has shed light on the potential long-term benefits of this intervention. These studies, conducted by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (CASG), have been led by investigators from the University of Alabama, AL, USA. Ganciclovir trials in these infants have been designed to address the impact of antiviral therapy on symptomatic congenital CMV infection with CNS involvement. The first data reported by the CASG concerned the safety and pharmacokinetics of ganciclovir in this population [87, 88]. Subsequent reports from this Phase II study evaluated toxicity, virologic response and clinical outcomes in newborns with symptomatic congenital CMV infection receiving 6 weeks of parenteral ganciclovir therapy [89]. Given the prolonged duration of parenteral therapy, it was not surprising to note that most of these infants had thrombocytopenia and neutropenia attributed to the ganciclovir. Encouragingly, however, hearing improvement or stabilization was noted in 16% of 30 infants at follow-up, an observation lending credence to the concept of performing a larger efficacy study in CMV-affected newborns.

Recently, the results of a Phase III randomized controlled study of parenteral ganciclovir in neonates with symptomatic congenital CMV infection involving the CNS have been reported [90]. This study was not a placebo-controlled trial because of the ethical concerns regarding the prolonged intravenous administration of a placebo through a central venous catheter. Rather, patients were randomized either to ganciclovir therapy (6 mg/kg dose administered intravenously every 12 h for 6 weeks) or to no treatment. Enrollment took place at participating CASG sites over a period of nearly 10 years. The primary study end point was improved brainstem-evoked response (BSER) between baseline and 6-month follow-up, or, for those infants with normal hearing at enrollment, maintenance of normal hearing between baseline and 6-month follow-up. Of the 100 patients enrolled in the study, 42 patients had both a baseline and 6-month follow-up BSER audiometric examination, and could be considered evaluable for the primary study end point. The demographics of the study population are summarized in Table 1. Of the ganciclovir recipients, 21 (84%) out of 25 either had improved hearing or maintained normal hearing between baseline and 6 months. In contrast, only ten (59%) out of 17 control patients had improved or stable hearing (p = 0.06; Table 1). Results were even more encouraging when the study and control groups were compared for subsequent deterioration in hearing from baseline, an important end point in light of the well-recognized potential for congenitally infected infants to suffer ongoing deterioration in hearing postnatally. Remarkably, none (0%) of 25 ganciclovir recipients showed a decline in hearing between baseline and 6-month follow-up, compared with seven (41%) out of 17 control patients (p < 0.01). Notably, 63% of 46 ganciclovir-treated patients had significant
Table 3. Comparison of baseline demographic and clinical characteristics in infants with evaluable hearing outcomes at 6 months and at more than 1 year.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BSER change evaluable at 6 months</th>
<th>BSER change evaluable at more than 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ganciclovir (n = 25)</td>
<td>No treatment (n = 17)</td>
</tr>
<tr>
<td></td>
<td>Median age (days)</td>
<td>Median age (days)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10 (40%)</td>
<td>9 (53%)</td>
</tr>
<tr>
<td>Male</td>
<td>15 (60%)</td>
<td>8 (47%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>18 (72%)</td>
<td>10 (59%)</td>
</tr>
<tr>
<td>Black</td>
<td>4 (16%)</td>
<td>4 (24%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3 (12%)</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Prematurity (≤37 weeks)</td>
<td>10/25 (40%)</td>
<td>8/17 (47%)</td>
</tr>
<tr>
<td>Median gestational age (weeks)</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Median weight (kg)</td>
<td>2.55</td>
<td>2.3</td>
</tr>
<tr>
<td>Abnormal CT</td>
<td>21/24 (88%)</td>
<td>14/16 (88%)</td>
</tr>
<tr>
<td>(calcifications)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal CSF indices</td>
<td>9/21 (43%)</td>
<td>9/15 (60%)</td>
</tr>
<tr>
<td>ALT ≥100 IU/L</td>
<td>5/23 (22%)</td>
<td>3/17 (18%)</td>
</tr>
<tr>
<td>Platelet count ≤100,000/mm³</td>
<td>10/24 (42%)</td>
<td>7/17 (41%)</td>
</tr>
<tr>
<td>Abnormal bilirubin</td>
<td>3/24 (13%)</td>
<td>4/16 (25%)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>15 (60%)</td>
<td>13 (76%)</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>16 (64%)</td>
<td>13 (76%)</td>
</tr>
<tr>
<td>Baseline BSER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(best ear)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>15 (60%)</td>
<td>10 (59%)</td>
</tr>
<tr>
<td>Mild</td>
<td>5 (20%)</td>
<td>5 (29%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Severe</td>
<td>5 (20%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Baseline BSER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(total ears)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>23/49 (47%)</td>
<td>18/36 (50%)</td>
</tr>
<tr>
<td>Mild</td>
<td>8/49 (16%)</td>
<td>11/36 (31%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>3/49 (6%)</td>
<td>3/36 (8%)</td>
</tr>
<tr>
<td>Severe</td>
<td>15/49 (31%)</td>
<td>4/36 (11%)</td>
</tr>
</tbody>
</table>

*The denominator represents the number of total evaluable ears; p-value was obtained through a logistic regression analysis using generalized estimating equations.

ALT: Alanine aminotransferase; BSER: Brainstem-evoked response; CSF: Cerebrospinal fluid; CT: Computed tomography; IU: International unit.


Neuropenia during therapy. The study further examined whether a therapeutic benefit was noted after 12 months of follow-up. Among 45 patients who had a BSER at both baseline and at 1 year or beyond, five (21%) of 24 ganciclovir recipients had worsening of hearing, versus 13 (68%) of 19 control patients (p < 0.01; Table 4). Several important limitations were noted in this study. As already stated, only 42 of the 100 subjects enrolled could be evaluated for the primary end point — hearing function — at 6 months. This large proportion of unevaluable patients raises concerns of follow-up bias. Nonetheless, this study represents a vitally important contribution to the field. A
Table 4. Unadjusted analyses of change in BSER.

<table>
<thead>
<tr>
<th>Change between baseline and 6 months</th>
<th>Change between baseline and more than 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ganciclovir</strong> (n = 25)</td>
<td><strong>No treatment</strong> (n = 17)</td>
</tr>
<tr>
<td>Improved hearing between baseline and follow-up</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>No change - normal hearing at baseline and follow-up</td>
<td>15 (60%)</td>
</tr>
<tr>
<td>No change - same degree of hearing loss at both baseline and follow-up</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Worsening hearing between baseline and follow-up</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Improved and no change (normal to normal)</th>
<th>Improved and no change (normal to normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved or worsening hearing indicates changes in decibel, which result in movement to a different category of hearing (e.g., normal-to-mild hearing, moderate-to-severe, moderate-to-mild). BSER: Brainstem-evoked response.</td>
<td></td>
</tr>
<tr>
<td><em>p = 0.002</em></td>
<td><em>p = 0.002</em></td>
</tr>
</tbody>
</table>

number of important questions can be raised. Given that the study focused on infants with CNS involvement, will the unfavorable neurodevelopment outcomes noted in this patient population mean that any beneficial effects on hearing are unlikely to be clinically significant? Will the side effect of neutropenia associated with ganciclovir limit the application of this therapy, or can this be offset by the use of granulocyte colony-stimulating factor? Will the orally bioavailable valine ester of ganciclovir, valganciclovir, be equally effective, greatly simplifying therapy? Perhaps of greatest interest, will antiviral therapy in newborns without obvious CNS involvement be beneficial, particularly in preventing progression to hearing loss? Hopefully, future CAGS studies will address these and other questions. Until more answers are available, based on the available data, ganciclovir therapy should probably be offered as an option to families of infants with symptomatic congenital CMV infection involving the CNS. Since the putative benefits of ganciclovir have only been studied in the setting of commencement of therapy within the first month of life, any efficacy of antiviral drug intervention beyond 1 month of age is unproven.

**Clinical experience with other therapies for neonatal CMV infection**

Data on the use of antiviral agents other than ganciclovir for CMV infection in the neonatal period are very limited. The concurrent use of ganciclovir and foscarnet treatment for cytomegalovirus encephalitis and retinitis was described in an infant with AIDS [91]. Cidofovir has been used in young children with disseminated adenovirus disease [92], and in an infant with SCIDS who acquired CMV perinatally, probably by breast feeding [93]. CMV immunoglobulin infusion appeared to be ineffective in preventing CMV infection in a placebo-controlled study in premature infants whose risk factors for CMV disease were either being a recipient of a CMV-positive blood product, or being born to a CMV-seropositive woman [94]. Whether CMV-immunoglobulin has any therapeutic benefit in neonates with CMV disease is unknown.

**Could antepartum antiviral therapy prevent or modify CMV disease in newborns?**

One of the most important observations made in the era of antiviral therapy was the demonstration that administration of antiretroviral therapy to HIV-positive women dramatically modified the risk of vertical transmission to the newborn [95]. In light of the success of the Pediatric AIDS Clinical Trials Group 076 trial (a trial which demonstrated the benefits of antepartum anti-HIV therapy in reducing transmission to the newborn infant), it is reasonable to consider whether congenital CMV transmission could be modified by the use of antiviral therapy. In *in vitro* models, ganciclovir has been shown to initially concentrate at the maternal placental surface and then cross by passive diffusion into the fetal compartment [96,97]. Concerns have been raised about the potential for teratogenicity of ganciclovir and for the potential for reproductive toxicity in animal models [98]. However, use of oral ganciclovir in a pregnant, liver transplant patient was not associated with any evidence of teratogenicity [99]. This observation may provide reassurance in the use of ganciclovir in future pregnant, liver transplant patients, who appear to be at very high risk for
transmission of CMV to the fetus, with adverse outcome [100]. In another report, parenteral ganciclovir was well-tolerated in an immunocompetent pregnant woman with severe CMV hepatitis [101]. There is little information about the potential efficacy of ganciclovir administered during pregnancy for prevention of fetal CMV infection. In one report, administration of ganciclovir for approximately 2 weeks at 29 weeks gestational age for a fetus known to be infected in utero appeared to lower amniotic fluid viral load but stillbirth occurred at 32 weeks gestation and evidence of CMV was present in fetal organs at autopsy [102]. In another report, administration of ganciclovir was performed by intra-amniotic injection; however, at birth the infant had stigmata of CID, including sensorineural hearing loss [103]. Brady and colleagues recently observed that antepartum administration of ganciclovir to a pregnant HIV-positive patient was well-tolerated and although the newborn had congenital CMV infection, the baby had a normal physical examination and no clinical evidence of neurodevelopmental sequelae; furthermore, ganciclovir was present in the newborn circulation, confirming transplacental passage of the antiviral agent [104]. Clinical trial evaluation of antivirals during pregnancy for women at the highest risk of giving birth to infected, damaged infants is warranted: the challenge is in identifying which pregnancies might benefit from antepartum therapy. The use of quantitative PCR on amniotic fluid, coupled with maternal serological assessment during pregnancy, could, in principle, aid in identifying the highest risk pregnancies [34,105]. However, it is important to keep in perspective the limitations of such a strategy. Although screening studies may be useful in identifying a fetus infected in utero with CMV, no screening and intervention strategy has been shown to prevent CMV transmission and a management strategy employing antiviral therapy during pregnancy for prevention of infection and attendant neurodevelopmental injury to the fetus is speculative. Until more data are available, treatment of the pregnant patient with anti-CMV antivirals is not recommended. Ultimately, the control of CMV in newborns may depend upon the development of an effective vaccine, which at the present time remains a major but unmet, public health priority [106].

Expert opinion

- The long term morbidity associated with congenital CMV infection is substantial and underappreciated.
- Recent evidence indicates that preconception immunity to CMV may not prevent reinfection during pregnancy.
- Reinfection with new strains of CMV during pregnancy in previously CMV-immune women is not benign: such infections may lead to symptomatic infections in the fetus with neurodevelopmental sequelae.
- Post-natal infection of newborns with CMV can cause significant short-term morbidity. The possibility of long term sequelae associated with such infections requires further study.
- Ganciclovir is the treatment of choice for CMV infection, although it is associated with significant toxicity.
- Ganciclovir therapy in newborns with documented congenital CMV infection and evidence of CNS involvement may protect against the development or progression of sensorineural deafness and the potential risks and benefits of antiviral therapy should be discussed with families of such infants.
- Ganciclovir should be considered for any ill, symptomatic infant with a postnatally acquired CMV infection and evidence of end-organ disease (pneumonia, viemiza, hepatitis, sight-threatening retinitis and refractory thrombocytopenia).

Five-year view

- Newborn CMV screening programs will be developed and coupled to hearing screening programs, in an effort to better define the epidemiology of CMV-related disability.
- Strategies to minimize the risk of postnatal CMV transmission, particularly through breast milk, will be devised for high-risk premature infants.
- New therapies for CMV will be developed and tested in clinical trials, including in newborns with congenital infection.
- Novel antivirals which target steps in the viral life cycle other than viral DNA synthesis are likely to be licensed for CMV therapy in immunocompromised patients and may provide new and perhaps less toxic alternatives for treatment of CMV infections in infants and children.

Key issues

- Congenital cytomegalovirus infection is the most common congenital viral infection in the developed world and is associated with severe neurodevelopmental morbidity in affected newborns.
- Congenital cytomegalovirus infection is one of the most common causes of sensorineural hearing loss in infancy and childhood, even in infants who have no other stigmata of infection.
- Among the licensed systemic antivirals active against cytomegalovirus, ganciclovir is the best studied.
- Although it is commonly associated with neutropenia in infants, ganciclovir is generally well-tolerated and has been shown in a controlled clinical trial to be of potential benefit in preventing or ameliorating sensorineural hearing loss in congenitally infected infants with CNS involvement with cytomegalovirus.
- There is a need for the development of new antivirals active against cytomegalovirus. Several new compounds show promise in preclinical testing and warrant investigation in clinical trials.

www.future-drugs.com
Clinical trials in all newborns will be designed to examine the efficacy and tolerability of long term oral therapy for CMV, in recognition of the fact that CMV-mediated injury to the CNS is an ongoing phenomena (a Phase I/II study of oral valganciclovir is currently in progress).

CMV vaccines will continue to be evaluated in clinical trials and will represent a high-level public health priority.

Acknowledgements
Supported by NIH AI-65289 and HD044864-01.

References
Papers of special note have been highlighted as:
• of interest
** of considerable interest
** A landmark article outlining the wide range of clinical manifestations associated with cytomegalovirus infection.
** Outstanding overview of the rationale for a cytomegalovirus vaccine, potential strategies for vaccination and the types of vaccines currently in development.
** Excellent overview of a remarkable aspect of cytomegalovirus biology, that of viral immune evasion genes and functional mechanisms.
** Detailed and comprehensive overview of congenital cytomegalovirus infection.
** Novel insights into the natural history and hearing prognosis for infants with congenital cytomegalovirus infection.
** Landmark paper examining the role of maternal immunity in congenital cytomegalovirus infection.
** Another landmark paper examining the role of maternal immunity in congenital cytomegalovirus infection and disease.
Antiviral therapy for perinatally acquired cytomegalovirus


- Outstanding clinical research identifying previously underappreciated morbidity in low birth-weight premature infants due to cytomegalovirus infections acquired via breast milk.


- Extremely valuable review of all aspects of cytomegalovirus infection of maternal–placental–fetal unit: virology, pathogenesis, immunity, therapy and prognosis.


**An extraordinary accomplishment and a testimony to perseverance: results of a multicenter, controlled trial of ganciclovir in affected newborns shows that antiviral therapy is of value in protecting against sensorineural hearing loss.**


Antiviral therapy for perinatally acquired cytomegalovirus


- The Institute of Medicine has identified cytomegalovirus vaccine as the highest level area for new vaccine development in the new millennium. The costs to society associated with congenital cytomegalovirus infection are staggering. Vaccines are sorely needed.

Website

201 Collaborative Antiviral Study Group
http://www.casc.us.htm
Accessed May 2004

Affiliations

- Mark R Schlein, MD
Associate Professor of Pediatrics and Molecular and Developmental Biology. Children's Hospital Research Foundation. Cincinnati, Ohio 45229, USA
Tel.: 1 513 636 4578
Fax: 1 513 636 7055
Mark.Schlein@force.com

- Michael A McVey, PhD
Associate Professor of Pediatrics, Virginia Commonwealth University, Richmond, Virginia, USA
Tel.: 1 804 828 0132

www.future-drugs.com