

Mini-review

Antiviral drugs for cytomegalovirus diseases

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Dedicated to Prof. Erik De Clercq on the occasion of reaching the status of Emeritus-Professor at the Katholieke Universiteit Leuven in September 2006

Abstract

Cytomegalovirus infections are associated with severe morbidity and mortality in patients at risk for disease because of immune system disabilities; in particular, recipients of stem cell (HSCT) or solid organ (SOT) transplants. There are three systemic drugs approved for CMV treatment: ganciclovir, or its prodrug valganciclovir, foscarnet, and cidofovir. An anti-sense therapeutic, ISIS 2922, is also approved specifically as an intravitreal treatment for CMV retinitis. Ganciclovir, and more recently, valganciclovir, have been useful in proactive approaches of CMV disease management; in both prophylactic and preemptive regimens in HSCT and SOT populations. The major anti-herpes agent valacyclovir has also been approved for prophylaxis of renal transplant recipients, or SOTs outside of the US. These drugs have provided major advances in CMV disease management, although they are limited by intolerable toxicities, oral bioavailability and efficacy, and risk of drug resistance with extended use. Several drugs are in early clinical development which may address these limitations; this review will provide an overview of our current arsenal of available drugs, and of those in the early clinical development pipeline.

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Human cytomegalovirus (CMV) is an opportunistic pathogen associated with significant morbidity and mortality in susceptible populations; i.e. those with immature or immunocompromised immune systems. Numerous antiviral agents with in vitro activity against the various human herpesviruses have been described over the past three decades, yet only a few have been approved for the treatment or prophylaxis of CMV diseases. This article will provide an overview of the diseases caused by this ubiquitous virus and a description of approved drug products, and will briefly describe several drug candidates that are in early stages of clinical development.

1. CMV infection and CMV disease

CMV is a double-stranded DNA virus of approximately 220 kb and is a member of the beta class of human herpesviruses. Cytomegalovirus is easily transmitted, usually through contact with bodily fluids or by placental transfer. Seroprevalence rates vary by socioeconomic class and geographic location, but the overall seroprevalence in developed countries is estimated to be in the range of 30–70% (Pass, 1985). Primary infection in immunocompetent individuals is usually benign, with minimal or no clinical manifestations (although approximately 10% of mononucleosis syndromes are a result of CMV infection). Following primary infection, the virus establishes latency, and viremia is mainly controlled by cell-mediated immunity. Virus reactivation occurs when this protective immune surveillance fails; e.g. as a result of chemotherapy or in patients who have AIDS or who are immunosuppressed for transplantation purposes. Such reactivation or primary infection in the context of a disabled immune system can lead to overt disease. In the case of vertical transmission of CMV to the developing fetus, adverse outcomes are most commonly associated with primary infection of the mother, although significant morbidity has also been associated with secondary infection.

2. Congenital CMV infection

In developed countries, congenital CMV infection occurs in approximately 1% of live births. The majority of the cases are asymptomatic, but approximately 5–10% of infants with congenital CMV will have symptomatic disease, associated with profoundly deleterious effects on the central nervous system (CNS), including microcephaly, intracranial calcifications, and ventriculomegaly. Prognosis for neonates with symptomatic disease is poor, with a high likelihood of mental deficits, hearing loss and psychomotor and perceptual handicaps (reviewed in Ross and Boppana, 2004; Griffiths and Walter, 2005).

It is now recognized that even asymptomatic congenital CMV is associated with increased risk of sensorineural hearing loss (SNHL) (Ross and Boppana, 2004), an observation that highlights the importance of identifying infants with congenital CMV infection and conducting periodic auditory assessments. The morbidity and mortality associated with congenital CMV infection underscores the need for a vaccine to prevent CMV infection. CMV vaccines currently in preclinical and clinical development are reviewed in Schleiss and Heineman (2005).

3. CMV-associated disease in transplant recipients

CMV infection is the leading viral cause of morbidity and mortality facing patients who receive hematopoietic stem cell transplant (HSCT) or solid organ transplant (SOT), with both direct adverse effects resulting from viral invasion of organ systems and indirect effects on the immune systems that increase the risk of other infections and promote acute graft rejection (reviewed in Gandhi and Khanna, 2004). CMV viremia is a significant predictor for organ involvement and progression to CMV disease (Cope et al., 1997).

Risk of CMV-associated complications is increased with more potent immunosuppressive regimens, such as many of those required for HSCT, and transplant patients are at greatest risk for CMV-associated disease within the first 100 days post-transplant. For recipients of SOTs, the most vulnerable patients (“high-risk patients”) are CMV-seronegative recipients who receive an organ from a CMV-seropositive donor (D+/R–). CMV-seropositive recipients of allogeneic stem cell transplants are at risk for reactivation of latent CMV infection.

In high-risk patients without symptomatic CMV disease, two common strategies of disease management are prophylactic and preemptive therapy, both of which are designed to prevent CMV disease. In the prophylactic approach, therapy is usually initiated at the time of stem cell engraftment or solid organ transplant. The suppressive doses used for prophylaxis are generally lower than those instituted for induction treatment of active disease, and the suppression of CMV reactivation in specific transplant populations can be successfully accomplished with a less potent antiviral agent than would be used for treatment. In the preemptive approach, therapy is initiated in asymptomatic high-risk patients based on diagnostic test results indicating primary CMV infection or reactivation of latent virus to a threshold level that signals the potential for disease escalation (blood CMV DNA load by PCR or pp65 antigenemia). This latter strategy often involves intermittent therapy, creating conditions thought to pose a greater risk of selection of resistant virus. However, this risk may be balanced by the protective effect of restoration of T-cell responses to CMV afforded by the delay in treatment with potent antivirals, particularly with myelosuppressive agents. On the other hand, the longer duration of drug exposure in the prophylactic approach also poses risk of resistance emergence.

4. CMV retinitis in AIDS patients

Although CMV retinitis is a relatively rare manifestation of CMV disease in other immunocompromised populations, it is the primary manifestation of CMV infection in patients with AIDS, usually resulting from reactivation of latent virus. CMV retinitis is a disease characterized by progressive, necrotizing retinitis that can lead to retinal detachment and blindness. Initial symptoms are non-specific, but may include blurred or distorted vision, floaters, light flashes, and loss of peripheral vision.

CMV retinitis and other manifestations of CMV disease in individuals with HIV-1 infection are opportunistic infections, occurring when CD4+ cell counts are profoundly suppressed (e.g. <50 cells/ μ l). Since the advent of highly active antiretro-

viral therapy for treatment of HIV-1 infection, CMV retinitis is a condition rarely seen in developed countries, although asymptomatic CMV viremia remains a significant risk factor for death (Deayton et al., 2004)

5. Antiviral therapies for CMV

The nucleoside analog class of compounds has historically provided the richest source of antiviral agents, originating from basic cancer research programs into purine and pyrimidine metabolic pathways. These nucleoside analogs have been highly successful due to the potential for chemical diversity within the class, and the differentiation of target viral DNA polymerases or reverse transcriptases from host enzymes. The herpesviral-encoded nucleoside kinases (HSV, VZV and EBV thymidine kinases, and the CMV protein kinase) provided added selectivity in the initial phosphorylation of the various nucleoside analogs. The triphosphorylated forms ultimately served as competitive inhibitors of, and substrates for, the viral DNA polymerases, thus reducing the amount of viral DNA synthesized in infected cells.

Nucleoside analogs with variable anti-tumor cell activity, notably adenosine arabinoside (ara A), cytosine arabinoside (ara C), and trifluorothymidine (TFT), were among those first described to have anti-HSV and anti-VZV activity. Acyclovir (9-[(2-hydroxyethoxy)methyl]guanine) was the first really selective nucleoside analog (Elion et al., 1977) with potent activity against HSV 1 and 2, VZV and EBV, and moderate activity against CMV in vitro. Acyclovir (ACV) and its prodrug, the L-valyl ester valacyclovir, have become the standard of care for prophylaxis and treatment of the most common diseases caused by HSV and VZV, and both have provided benefit in CMV diseases in certain transplant populations.

The discovery of ACV was quickly followed by discovery of related purine analogs ganciclovir (GCV) and penciclovir

(PCV). Ganciclovir has become the gold standard for management of CMV diseases in the majority of patient settings. A series of nucleotide analogs with broad activity across viruses was discovered by DeClercq and colleagues; from this class, cidofovir (CDV) was evaluated for anti-CMV activity. Other antiviral agents designed to exploit the unusual characteristics of the herpesviral DNA polymerases are the pyrophosphate analog phosphonoacetic acid (PAA) and its analog foscarnet (PFA), which have broad inhibitory activity across the herpesviruses. These agents have facilitated the management of CMV infections; the next section of this review will highlight their therapeutic applications and advantages based on key pivotal studies and experience in broader clinical practice.

6. Currently marketed antiviral agents

Three of the antiviral agents mentioned earlier, GCV, CDV, and FOS (Fig. 1), have received marketing approval for the systemic treatment of CMV infection. ACV has also received marketing approval in various European countries for prophylaxis of CMV disease in solid organ transplant (SOT) recipients, but lacks sufficient potency to be used for treatment of active CMV disease. An anti-sense RNA (fomivirsen) is approved for local treatment of CMV retinitis by intraocular injection.

6.1. Ganciclovir

GCV was the first antiviral agent approved for treatment of CMV disease, and remains the first-line treatment for CMV infection and CMV disease in transplant recipients (Razonable and Emery, 2004). GCV is an acyclic nucleoside analogue of 2'-deoxyguanosine (Fig. 1). In a multi-step process dependent on both viral and cellular enzymes, ganciclovir is converted to ganciclovir triphosphate, the chemical form that is active

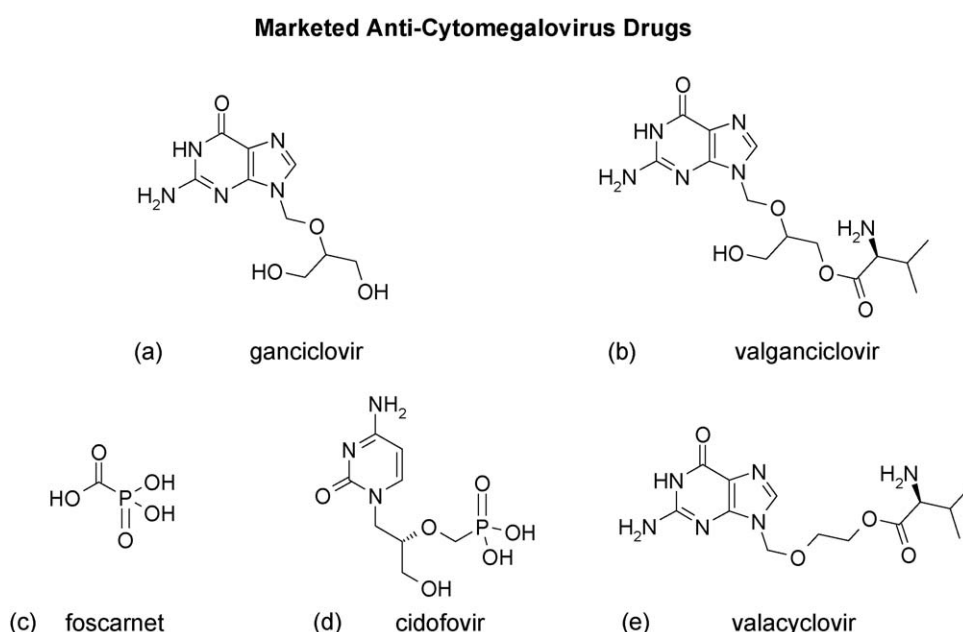


Fig. 1. Approved anti-CMV drugs. (a) Ganciclovir; (b) valganciclovir; (c) foscarnet; (d) cidofovir; (e) valacyclovir. Not shown is fomivirsen.

against CMV. The initial phosphorylation is catalyzed by an unusual protein kinase homolog encoded by the CMV UL97 open reading frame (Sullivan et al., 1992). Cellular enzymes generate the triphosphate form. Ganciclovir triphosphate competitively inhibits DNA synthesis catalyzed by the viral DNA polymerase (encoded by the UL54 gene), with slower chain elongation resulting from incorporation of ganciclovir triphosphate in place of dGTP into the growing viral DNA chain.

Resistance to GCV arises from mutations in either the UL97 or the UL54 genes. The point mutations or small deletions in the UL97 protein kinase gene lead to changes at codons 460 or 520, or changes clustered in codons 590–607 (regions attributed to ATP-binding and substrate recognition, respectively) that apparently do not prevent the protein kinase function (Gilbert and Boivin, 2005). The resistance mutations associated with GCV in the pol gene UL54 generally occur in specific conserved subdomains, and may confer cross-resistance to CDV or less commonly, to FOS (Gilbert and Boivin, 2005).

The side effects of GCV include hematologic abnormalities (primarily neutropenia, anemia, and thrombocytopenia) and, based on preclinical toxicologic studies, probable long-term reproductive toxicity (Cytovene-IV package insert, 2000). In animal studies GCV was both carcinogenic and teratogenic and caused aspermatogenesis.

GCV as an intravenous (IV) formulation (Cytovene-IV®, Roche) was approved in 1989 for treatment of CMV retinitis in AIDS patients. The IV formulation was later approved for prevention of CMV disease in SOT recipients and in individuals with advanced HIV infection at risk for CMV disease. The pivotal clinical trials for the transplant indication included two double-blind, placebo-controlled trials evaluating the incidence of CMV disease in D+/R– or D+/R+ heart transplant recipients (Merigan et al., 1992) or in allogeneic bone marrow transplant recipients with positive CMV cultures (Goodrich et al., 1993).

In the heart transplant study, patients were given study drug for 28 days post-transplant. The incidence of CMV illness at post-transplant day 120 was assessed, with the results stratified based on the recipient's serostatus. For seropositive recipients, significantly fewer patients given GCV had CMV illness during the first 120 days post-transplant compared to patients given placebo (5/56 patients, 9% versus 26/56 patients, 46%; $p < 0.001$). For D+/R– transplant recipients there was no significant difference among treatment groups in the incidence of CMV disease during the first 120 days.

In the bone marrow transplant study, patients were given study drug from the time of engraftment until 100 days post-transplant. The incidence of CMV disease occurring within the first 100 days post-transplant was significantly less in the GCV arm (1/37 patients, 3%) compared to the placebo arm (15/35 patients, 43%; $p < 0.00001$). In addition, patients receiving GCV had significantly greater overall survival than the placebo group at both 100 and 180 days post-transplantation ($p = 0.041$ and 0.027 , respectively).

To circumvent the risks and inconvenience associated with the need for an indwelling catheter for intravenous administration, an oral formulation was developed. Oral GCV (250 and 500 mg GCV capsules; Cytovene®, Roche) was approved in

1994 for treatment of CMV retinitis, but only as maintenance therapy, as the low bioavailability (approximately 5%) of the oral formulation was considered insufficient for induction therapy.

A sustained-release GCV intraocular implant (Vitrasert®; developed by Chiron, now marketed by Bausch and Lomb) for treatment of CMV retinitis in AIDS patients was approved in 1996. In a clinical study comparing the implant to IV GCV in HIV-1-infected patients with CMV retinitis, the implant was significantly more efficacious in treating CMV retinitis in the affected eye, but patients treated with the implant alone were at significantly greater risk for developing CMV disease in the contralateral eye or in other organ systems than were patients who received systemic GCV (Musch et al., 1997). The addition of oral ganciclovir to the implant controlled the systemic CMV disease (Martin et al., 1999).

Oral GCV represented a major advance in treatment options for maintenance therapy and prophylaxis. However, the low bioavailability and the high pill burden from the t.i.d. regimen were limitations. In addition, there were concerns that inadequate viral suppression resulting from the lower systemic exposure from oral GCV could lead to emergence of drug resistance. Development of prodrugs has been one valuable strategy in circumventing problems of poor solubility or low bioavailability (reviewed in De Clercq and Field, 2006). Based on the model exemplified by valacyclovir (see below), a prodrug was developed to improve the bioavailability of oral GCV.

Valganciclovir (Valcyte®, Roche) is the L-valyl ester of ganciclovir (Fig. 1). After oral administration, valganciclovir is rapidly metabolized to the active form (ganciclovir) in the intestinal wall and liver. Valganciclovir has an oral bioavailability of around 60% (Valcyte package insert, 2003). Once-daily administration of valganciclovir 900 mg produces systemic exposure to GCV that is equivalent to that produced with once-daily administration of IV GCV 5 mg/kg, and 1.7-fold greater than the systemic exposure produced by oral GCV 1000 mg given t.i.d. (Cvetkovic and Wellington, 2005). In principle, the valganciclovir formulation could be used to deliver the exposures demonstrated to be efficacious in various transplant populations treated with IV or high-dose oral GCV, barring absorption deficiencies associated with GI disruption, as in graft-versus-host-disease. Indeed, registrational studies and multiple comparative trials have been reported (Hodson et al., 2005).

Valganciclovir was approved in 2000 for treatment of CMV retinitis in AIDS patients (reviewed in Cvetkovic and Wellington, 2005) and was later approved for prophylactic treatment of CMV in certain SOT recipients. The first study of valganciclovir for CMV prophylaxis in SOT recipients compared valganciclovir to oral GCV in a randomized, double-blind, double-dummy study of 364 D+/R– SOT recipients (Paya et al., 2004). Patients were randomized 2:1 to receive valganciclovir 900 mg once-daily or oral GCV 1000 mg t.i.d. for 100 days post-transplant. The study was designed to show equivalence, rather than superiority of valganciclovir compared to oral GCV. The incidence of CMV disease by 12 months were comparable in the two treatment groups, and the safety profiles were similar (Paya et al., 2004). Valganciclovir has now replaced oral ganciclovir in clinical practice.

Debate continues over the most appropriate antiviral treatment for prevention of CMV disease in high-risk SOT recipients (D+/R–) and whether prophylactic therapy or preemptive therapy should be used for asymptomatic high-risk patients (Kalil et al., 2005). In one study late-onset CMV disease was more prevalent in D+/R– liver transplant recipients who had received prophylaxis versus preemptive therapy, suggesting that perhaps prophylactic treatment with a potent antiviral agent interfered with development of cell-mediated immune response (Singh, 2006). Similar outcomes have been reported in HSCT populations (Li et al., 1994; Boeckh et al., 2003). The International Herpes Management Forum issued a set of guidelines in 2004 for the use of ganciclovir and valganciclovir as either prophylactic or preemptive therapy in SOT and HSCT patients (Razonable and Emery, 2004). This consensus opinion provided recommendations for quantitative monitoring of CMV load to optimize the timing, duration and intensity of therapy. The guidelines also compare the efficacy of several regimens for first- and second-line viral control, as well as for treatment of established disease.

To date (2006), no anti-CMV agent has been approved for treatment of congenital CMV disease, although the Collaborative Antiviral Study Group (CASG) of the National Institute of Allergy and Infectious Diseases has conducted Phase II and III trials evaluating twice-daily dosing of 6 mg/kg IV GCV for treatment of infants with symptomatic congenital CMV involving the CNS. Study results showed clear benefits that for neonates with severe CMV disease outweighed the risks of acute toxicities and long-term reproductive toxicities associated with intravenous ganciclovir (Whitley et al., 1997; Kimberlin et al., 2003; Bradford et al., 2005). A large multicenter trial is underway to assess the safety and efficacy of valganciclovir syrup on infants with symptomatic congenital CMV disease. However, while the oral formulation would avoid the considerable risks and disadvantages associated with IV administration, the hematologic and reproductive toxicities would remain, limiting the usefulness of the therapy for all but the most severely affected infants.

6.2. Foscarnet

In 1991 foscarnet (Foscavir®, AstraZeneca) became the second drug approved for treatment of CMV retinitis in AIDS patients. Foscavir®, or foscarnet sodium, is the trisodium salt of phosphormorphonic acid, a pyrophosphonate analogue. Foscarnet (FOS) inhibits activity of the viral DNA polymerase by binding to the pyrophosphate binding site and blocking cleavage of pyrophosphate from the terminal nucleoside triphosphate added to the growing DNA chain. Resistance to FOS in the laboratory or clinical setting has been mapped to point mutations in the pol gene UL54. Cross-resistance has been observed between GCV and FOS in several laboratory and clinical isolates with both phenotypic and genotypic resistance.

The major dose-limiting toxicity of FOS is renal impairment, underscoring the importance of adequate hydration and frequent monitoring of serum creatinine levels in patients receiving FOS. Mineral and electrolyte abnormalities resulting from renal impairment can lead to a number of cardiac or neurologic

disorders, including seizures, that in several cases resulted in death.

FOS is considered second-line therapy, but is the preferred drug for patients who are failing GCV therapy due to viral resistance, or those who cannot be treated with GCV due to dose-limiting neutropenia or leucopenia (Razonable and Emery, 2004). In one study, FOS was compared to IV GCV as a preemptive therapy in a large, prospective, randomized, open-label study in HSCT patients. FOS and IV GCV were equally effective in prevention of CMV disease and mortality within 180 days of HSCT (Reusser et al., 2002). FOS has also been used in combination with IV GCV, each at half dose, and the combination was compared to IV GCV alone in SOT patients. The outcome was unfavorable for the combination in terms of virologic response and toxicities (Mattes et al., 2004).

6.3. Cidofovir

Cidofovir (Vistide®, Gilead) is an acyclic nucleoside phosphonate, with the chemical name 1-[(S)-3-hydroxy-2-(phosphonomethoxy)propyl]cytosine dihydrate (HPMPC, Fig. 1). CDV is a broad-spectrum antiviral agent with potency against both herpesviruses and other DNA viruses, such as smallpox virus (De Clercq and Holy, 2005). Host kinases convert CDV to the active diphosphoryl form, and cidofovir diphosphate then acts as a competitive inhibitor of the viral DNA polymerase, causing premature chain termination in viral DNA synthesis. Resistance to CDV has been difficult to select in the laboratory (Cihlar et al., 1998). Resistant isolates have not been reported in the clinic, although this may reflect the shorter treatment regimens and the limited use of this agent. Several GCV-resistant CMV clinical isolates or laboratory-selected strains with specific pol gene mutations are clearly cross-resistant to CDV (Gilbert and Boivin, 2005).

CDV received US marketing approval in 1996 for treatment of CMV retinitis in AIDS patients. CDV is available only as an IV formulation; its oral bioavailability is less than 5%. One of the distinguishing features of CDV, and others in this nucleotide analog class, is the stability of the active form in cells. The intracellular half-life of CDV-DP is reported to be >24 h, and efficacy in both animal models and in humans can be achieved with infrequent dosing (De Clercq and Holy, 2005). Recommended treatment for CMV retinitis in AIDS patients consists of 5 mg/kg administered over a 1-h period once a week for two consecutive weeks (induction phase), followed by 5 mg/kg once every 2 weeks (maintenance phase).

The major limitation of CDV as an antiviral agent is severe renal toxicity (Vistide package insert, 1996). An anion transporter located in the convoluted proximal tubules binds to CDV with high affinity, leading to the accumulation of CDV in the renal cortex (Ho et al., 2000). The major route of drug elimination occurs through the kidney. Patients receiving IV CDV must be given oral probenecid to protect against kidney failure, and must be prehydrated before infusion. Neutropenia is another toxicity associated with CDV, and CDV was shown to be both carcinogenic and teratogenic in preclinical toxicological studies (Vistide package insert, 1996).

Due to the risks of renal damage, CDV remains a second-line therapy. In the transplant setting, CDV has been used primarily in preemptive strategies to rescue allogeneic HSCT patients following therapy with GCV, FOS, or both drugs, with successes ranking from 62% to 66% (Ljungman et al., 2001). In this same retrospective analysis, patients with established CMV disease were also treated, and 50% (of a total of 20 subjects) responded clinically and virologically. However, 25% of these patients experienced renal toxicity, which was irreversible in approximately half of the affected patients. More recently, similar efficacy was seen following preemptive administration of CDV in pediatric HSCT patients failing GCV or FOS, with reasonable success achieved in the management of renal function in these patients (Cesaro et al., 2005).

The usual treatment regimen in these studies involves an induction dosage of 1–5 mg/kg/week, followed by a maintenance dose every other week. The toxicities of CDV or the adjunct probenecid limit the utility of this interesting and potent antiviral drug. The potential of CDV prodrugs to avoid renal tubular uptake and concentration is under evaluation.

6.4. Acyclovir

Acyclovir is an analogue of 2'-deoxyguanosine. Like GCV, acyclovir must be phosphorylated in a multi-step process in the host cell to the active triphosphate form. The CMV-encoded protein kinase pUL97 catalyzes the initial phosphorylation step of this purine analog, which, like GCV monophosphate, is subsequently di- and tri-phosphorylated by host kinases. ACV is a less efficient substrate than GCV, which in part explains the lower in vitro potency of ACV compared to GCV in CMV-infected cells. Another factor that clearly differentiates ACV and GCV is the four- to five-fold shorter half-life of ACV-TP compared to GCV-TP in infected cells, resulting in the lower intracellular levels of the active ACV-TP. As with GCV, drug resistance to ACV results from mutations in the viral DNA polymerase or UL97 genes.

Oral acyclovir has approximately 6–10% bioavailability, which increases to approximately 55% with administration of valacyclovir (Valtrex®, GlaxoSmithKline), the L-valyl ester of acyclovir (Valtrex package insert, 2005). The primary use of high-dose oral ACV or its prodrug valacyclovir in transplant patients has been for suppression of HSV reactivation. However, prophylactic treatment can also significantly reduce the incidence of CMV infection and CMV disease in SOT patients. In a meta-analysis of 12 randomized trials enrolling 1574 SOT patients, Fiddian et al. (2002) found a 56% decrease in the risk of CMV infection ($p < 0.001$), a 59% reduction in CMV disease ($p < 0.001$), and a 30% reduction in opportunistic infections ($p < 0.009$) in patients receiving prophylactic therapy with high-dose oral acyclovir or valacyclovir, compared to patients receiving placebo or no prophylactic therapy.

The safety and efficacy of valacyclovir for prevention of CMV disease was evaluated in 408 R+ and 208 R–/D+ renal transplant patients, randomly assigned to treatment with either 2000 g valacyclovir or placebo q.i.d. for 90 days post-transplant

(Lowance et al., 1999). Valacyclovir prophylaxis significantly reduced the incidence of CMV disease among transplant recipients at 6 months post-transplant, at 1% versus 6% for seropositive patients and 16% versus 45% for seronegative patients randomized to valacyclovir versus placebo, respectively. Similar results were seen in incidence of active CMV infection and graft rejection. Although not potent enough for treatment of established CMV disease, valacyclovir has been approved in several countries for prophylaxis of CMV infection and CMV disease in renal or heart transplant recipients or SOT recipients.

6.5. Fomivirsen

Fomivirsen (Vitravene®; developed by Isis Pharmaceuticals, licensed to Novartis Ophthalmics) is a 21-nucleotide anti-sense RNA (5'-GCG TTT GCT CTT CTT CTT GCG-3'), specifically targeted against the mRNA from the major immediately transcriptional unit of CMV. Fomivirsen is administered by intraocular injection. Fomivirsen was approved in 1998 as a second-line therapy for local treatment of CMV retinitis in AIDS patients. Recommended treatment consists of a 4-week induction phase, with a single injection every other week (i.e. two doses), followed by a maintenance phase, in which a single injection is administered every 4 weeks. The most frequent adverse effect is ocular inflammation (uveitis), which can be managed by treatment with topic corticosteroids and by delaying additional injections.

7. Anti-CMV drugs in clinical development

New drugs, preferably in oral formulations, are needed for treatment of CMV disease, especially congenital disease in neonates. The currently approved systemic drugs have an unfavorable safety profile, with severe acute and long-term toxicities. Of special concern for a pediatric population is long-term reproductive toxicity and carcinogenicity. Moreover, the approved systemic drugs share a similar mechanism of action, targeting the viral DNA polymerase. As a consequence, viral cross-resistance is a potential problem with the current drug arsenal.

Despite the medical need for new drugs, relatively few research programs currently focus on anti-CMV drug development. One reason may be the reduction in CMV retinitis in AIDS patients following the introduction of HAART.

7.1. Maribavir

One of the most promising anti-CMV drugs in clinical development is maribavir (1-(β-L-ribofuranosyl)-2-isopropylamino-5,6-dichlorobenzimidazole), also known as GW1263W94 (Fig. 2). Although this drug is a riboside analog, it does not act as such: it is not anabolized in infected cells, nor do its phosphorylated forms directly inhibit the viral DNA polymerase (Biron et al., 2002). Maribavir is a potent and selective, orally bioavailable drug with a novel mechanism of action against only two of the human herpesviruses: CMV and EBV. Maribavir inhibits

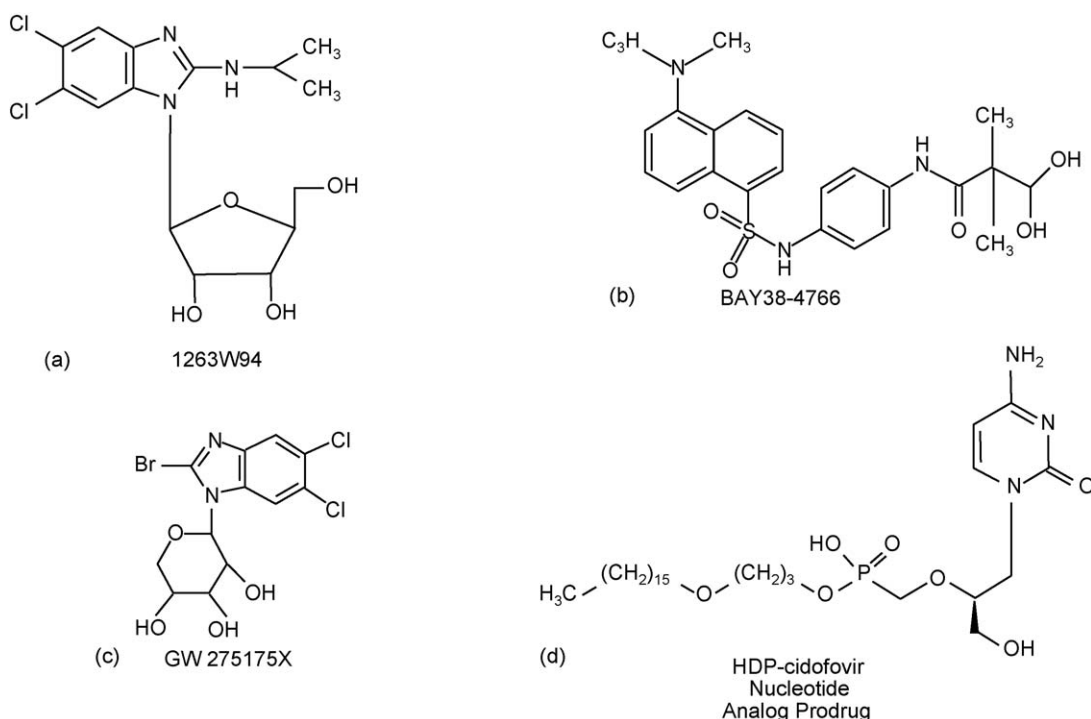


Fig. 2. Anti-CMV drugs in clinical development. (a) Maribavir; (b) BAY 38-4766; (c) GW275175X; (d) CMX001.

the replication of both CMV and EBV in cell culture by interfering with viral DNA synthesis (Zacny et al., 1999; Biron et al., 2002). In CMV-infected cells, maribavir has also been shown to interfere with viral nucleocapsid egress from the nucleus, thereby reducing the yield of infectious CMV (Krosky et al., 2003a). A key target for maribavir's action in the CMV life cycle is the viral-encoded protein kinase, pUL97, a finding that was based on the genetics of resistance, direct protein kinase inhibition studies (Biron et al., 2002), and on phenotypic similarity of maribavir-treated, CMV-infected cells and cells infected with the pUL97-deleted virus (Wolf et al., 2001; Krosky et al., 2003a). Inhibition of viral DNA synthesis is likely a consequence of a block in the phosphorylation of the polymerase accessory protein, pUL44, by the pUL97 protein kinase (Krosky et al., 2003b; Marschall et al., 2003).

Clearly the mechanism of action of maribavir against CMV has not been fully elucidated, since the role of the pUL97 protein kinase in CMV replication or disease pathogenesis is still under study. Moreover, laboratory-generated resistant mutations also map to the CMV UL27 gene, a protein of unknown function (Komazin et al., 2003; Chou et al., 2004).

Maribavir preclinically shows advantages over existing anti-CMV drugs in its in vitro potency, bioavailability, safety profile in acute, chronic and genetic toxicology testing, and the lack of cross-resistance inherent in its novel mechanism of action. The drug has completed several Phase 1 clinical studies (Wang et al., 2003; ViroPharma Inc., 2005a), and its potential for efficacy was demonstrated in a 28-day study in HIV-infected subjects that showed a reduction in viral shedding in the semen and urine (Lalezari et al., 2002). This drug is currently in a prophylaxis study in allogeneic stem cell transplants, with results expected in 2006 (ViroPharma Inc., 2005b).

7.2. BAY 38-4766

BAY 38-4766 (Bayer Pharmaceuticals), or 3-hydroxy-2,2-dimethyl-*N*-[4-[[[5-(dimethylamino)-1-naphthyl]sulfonyl]amino]-phenyl]propanamide, represents a novel class of non-nucleoside antiviral agents. BAY 38-4766 is a highly selective inhibitor of CMV in vitro (Reefschlaeger et al., 2001) (Fig. 2). In an immunodeficient mouse model the compound shows anti-CMV activity against human CMV similar to that of GCV (Weber et al., 2001). BAY 38-4766 had a favorable safety and efficacy profile in a guinea pig model of CMV, and measurable amounts of drug were detected in fetal blood, indicating that the compound crosses the placenta in pregnant guinea pigs (Schleiss et al., 2005).

BAY 38-4766 was active against strains resistant to currently approved anti-CMV agents (McSharry et al., 2001), and no cross-resistance to these agents was seen in virus selected for resistance to BAY 38-4766 (Reefschlaeger et al., 2001). Antiviral activity of BAY 38-4766 results from inhibition of DNA maturation, and mutations conferring drug resistance map in the UL89 and UL56 genes, which encode subunits of the viral terminase (Buerger et al., 2001). This mechanism of action is similar to that of the original benzimidazole riboside leads, BDCRB and TCRB (Underwood et al., 1998; Krosky et al., 1998), an observation that is interesting in view of the differences in structures between these unrelated chemical series and the complexity of the drug target.

BAY 38-4766 entered clinical development and showed a favorable safety profile in healthy male volunteers at single oral doses up to 2000 mg. However, no recent reports have revealed the current status of clinical development of this compound or related compounds in the series.

7.3. GW275175X

Another interesting clinical candidate to emerge from the novel benzimidazole riboside class of CMV inhibitors is the β -D-pyranosyl sugar analog of the original leads BDCRB and TCRB (Townsend et al., 1995; Underwood et al., 2004). Known as GW275175X (2-bromo-5,6-dichloro-1- β -D-ribofuranosyl-1*H*-benzimidazole), this molecule addressed the *in vivo* lability of the glycosidic linkage of BDCRB by substitution of the six-membered sugar ring for the β -D-ribose moiety. GW275175X retains the mechanism of action of the parent compound BDCRB; that of blocking the maturational cleavage of high molecular weight CMV DNA by interaction with pUL56 and pUL89, the two subunits of the viral terminase complex (Underwood et al., 2004).

GW275175X was advanced through a Phase 1 single-escalating dose trial of safety, tolerability and pharmacokinetics, but was then shelved in favor of the advancement of maribavir. The clinical potential of this early candidate is yet to be determined.

7.4. Cidofovir esters

Renal toxicity associated with CDV treatment limits the usefulness of the drug, despite its efficacy as an anti-CMV agent. However, recent reports describe a promising series of CDV derivatives that overcome this limitation. Alkoxyalkyl esters of CDV have been developed that retain the efficacy of the parent compound (Beadle et al., 2002), without the associated renal toxicity (Ciesla et al., 2003). Moreover, the derivatives showed improved uptake and absorption, and had oral bioavailabilities in mice in the range of 88–97%, compared to less than 5% for CDV (Ciesla et al., 2003).

CMX001, or hexadecyloxypropyl-cidofovir (HDP-CDV), is currently under development by Chimerix as an oral drug for the treatment of smallpox infection (Painter and Hostetler, 2004). The drug is also active against CMV and other herpesviruses. The evaluation of HDP-CDV in symptomatic congenital CMV infections is under consideration.

8. Summary

Cytomegalovirus has been referred to as the “troll of transplantation” (Balfour, 1979), an apt description for an opportunistic pathogen that can produce such direct and indirect damage in the transplant setting. Significant progress has been made in the control of CMV infections in transplant patients using the current arsenal of antiviral therapies, notably ganciclovir and its prodrug, valganciclovir. Numerous clinical studies have explored the optimal use of the five approved agents, and strategies for intervention have been shaped through a better definition of viral replication dynamics (Cope et al., 1997), and the correlation of viral load with disease progression. Broader use of prophylactic regimens has led to a greater occurrence of late onset CMV disease, and the longer duration of drug treatment in the face of potent immunosuppression has allowed resistant virus to emerge (Limaye et al., 2000; Boivin et al., 2004 JID). On the other hand, prophylaxis with ganciclovir or its pro-

drug has provided a reduction in the risk of diseases caused by other herpesviruses, as well as bacterial and fungal infections, which could reflect the direct activity of the broad spectrum anti-herpetic, as well as the indirect benefits of suppressing CMV (Hodson et al., 2005; Razonable et al., 2005).

Additional progress in reducing the consequences of CMV infection in all the susceptible populations will be made with the introduction of new drugs with greater efficacy and safety, and long term management of infection will be facilitated by drugs with non-overlapping mechanisms of action.

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