

# Novel Therapies for Cytomegalovirus Disease

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**Abstract:** Cytomegalovirus (CMV) infection is one of the most important infectious complications of solid-organ transplantation, a serious, life-threatening, opportunistic pathogen in HIV-infected patients, and may cause hearing defects and irreversible central nervous system disease in infants infected during gestation. Four drugs are currently licensed for prophylaxis, pre-emptive therapy, and treatment of CMV infection - ganciclovir, and its oral prodrug valganciclovir, foscarnet, cidofovir, and fomivirsen. All four drugs are effective against CMV infection. Toxicities, drug-drug interactions, poor bioavailability, and the development of drug resistance, however, are clinically relevant and common limitations of these drugs. Novel compounds are on the horizon that possibly will become useful alternatives to currently licensed drugs. Maribavir, a benzimidazol, is the most promising novel drug and closest to clinical application. Several phase II clinical trials proved its good tolerability and effectivity. Other compounds are currently evaluated in pre-clinical and phase I trials with promising preliminary data. In addition, analogs of cidofovir possess significantly improved pharmacological and virological characteristics allowing their oral administration. This review summarizes the current status in drug development and will introduce the most recent patents on this line of research.

**Keywords:** Cytomegalovirus, herpesviruses, therapy.

## INTRODUCTION

### CMV DISEASE IN THE HUMAN HOST

CMV infection is one of the most important infectious complications of solid-organ transplantation [1] and a serious, life-threatening, opportunistic pathogen in HIV-infected patients [2]. CMV infection, defined as a significant rise in the titer of CMV-specific antibodies, occurs in 44% to 85% of kidney, heart, and liver transplant recipients - primarily in the first 3 months posttransplantation, when immunosuppression is most intense [3,4]. CMV disease manifests in transplant recipients primarily in the organ transplanted with the risk of consecutive dissemination and affection of other organs such as central-nervous system, eye, and urogenital- or gastrointestinal tract (Fig. 1). Symptomatic CMV disease occurs in 8, 29, 25, 50, 22, and 39% of kidney, liver, heart, pancreas/kidney-pancreas, human small bowel, and heart-lung transplantation recipients, respectively [5,6]. Particularly patients with primary CMV infection and recipients being treated with antibodies to lymphocyte antigens are at highest risk of symptomatic CMV disease [1,7].

Primary CMV infection during pregnancy carries a significant risk for the child, particularly when infection occurs during the first trimester. Infants infected during gestation but asymptomatic at birth will develop hearing defects or neurodevelopmental sequelae in 10-17% of cases. More importantly, 5-10% of congenitally infected neonates have symptoms of irreversible CNS involvement including microcephaly, encephalitis, seizures, upper motor neuron disorders, and psychomotor retardation [8].

In addition to causing symptomatic disease, CMV employs multiple mechanisms to evade the host's innate and

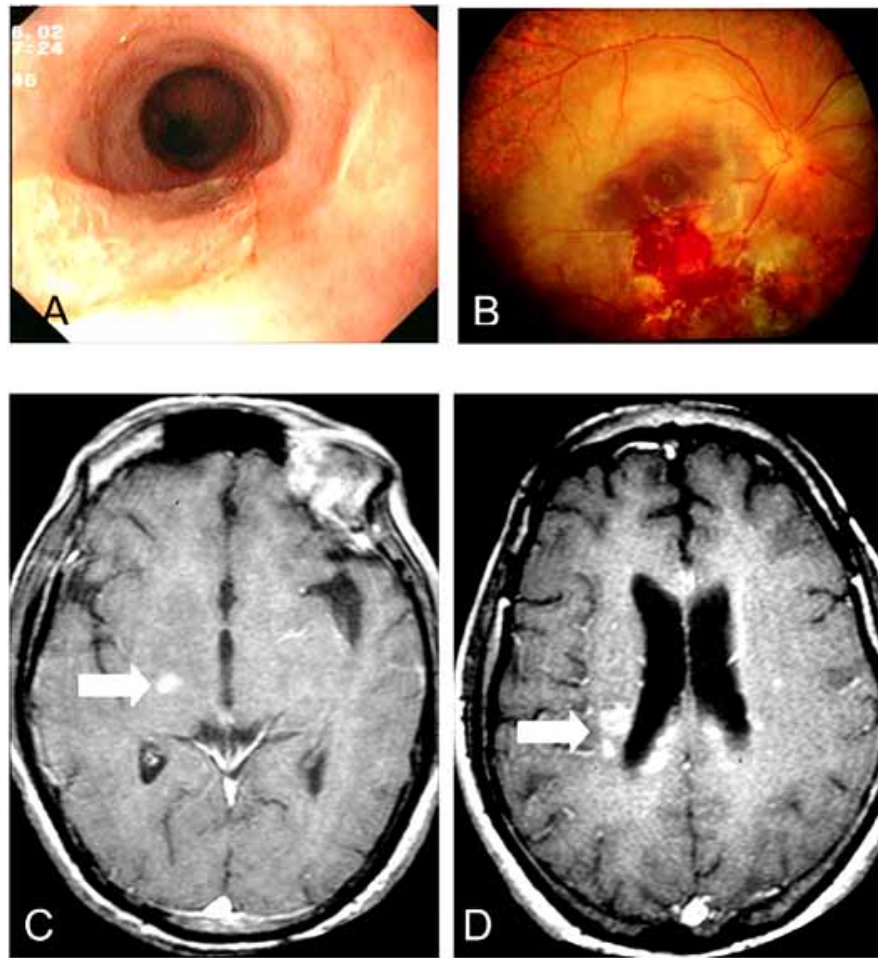
specific immunity [9]. As a result, the net level of therapeutic immunosuppression is augmented in transplant patients and the susceptibility to a variety of other opportunistic infections increased [1,10]. The clinical significance of these *in vitro* findings is demonstrated by CMV-associated acute rejection of transplanted organs [11], reduced longterm graft function [12,13], and 2.5 times more rapid progression to AIDS and death in CMV-seropositive HIV-infected patients than in those who are CMV-seronegative [14-16]. In the case of primary CMV infection, even HIV-infected patients with relatively high CD4 cell count (>100/mm<sup>3</sup>) are at a significantly increased risk for progression to AIDS [17].

### THE VIRUS

CMV is a member of the  $\beta$ -herpesvirus group and characterised by its strict species specificity, long life cycle, and persistence within the host for lifetime [18]. It is an enveloped DNA virus and the largest known human herpesvirus [19]. The double-stranded linear CMV genome of about 235-kbp [20] exhibits a pattern of terminal and inverted repeats that vary in size depending on the virus strain and passage history. Purified virus particles of CMV have been estimated to contain 30 to 40 polypeptides with molecular weights ranging in size from 20 to over 300 kd [21-24]. The virion of CMV contains two classes of RNA in addition to the DNA, unlike that of other DNA viruses. One type of RNA forms hybrid structures within the origin of replication (*oriLyt*) in packaged genomes [25], and the other type of RNA appears to be within the tegument and to be expressed after entry into cells [26]. The CMV genome also carries a large number of ORFs with characteristics of integral membrane glycoproteins that have not been studied in any detail but may encode minor envelope constituents that play roles in attachment and entry.

An icosahedral protein capsid encases the genome (Fig. 2). The capsid is composed of seven proteins, all of which

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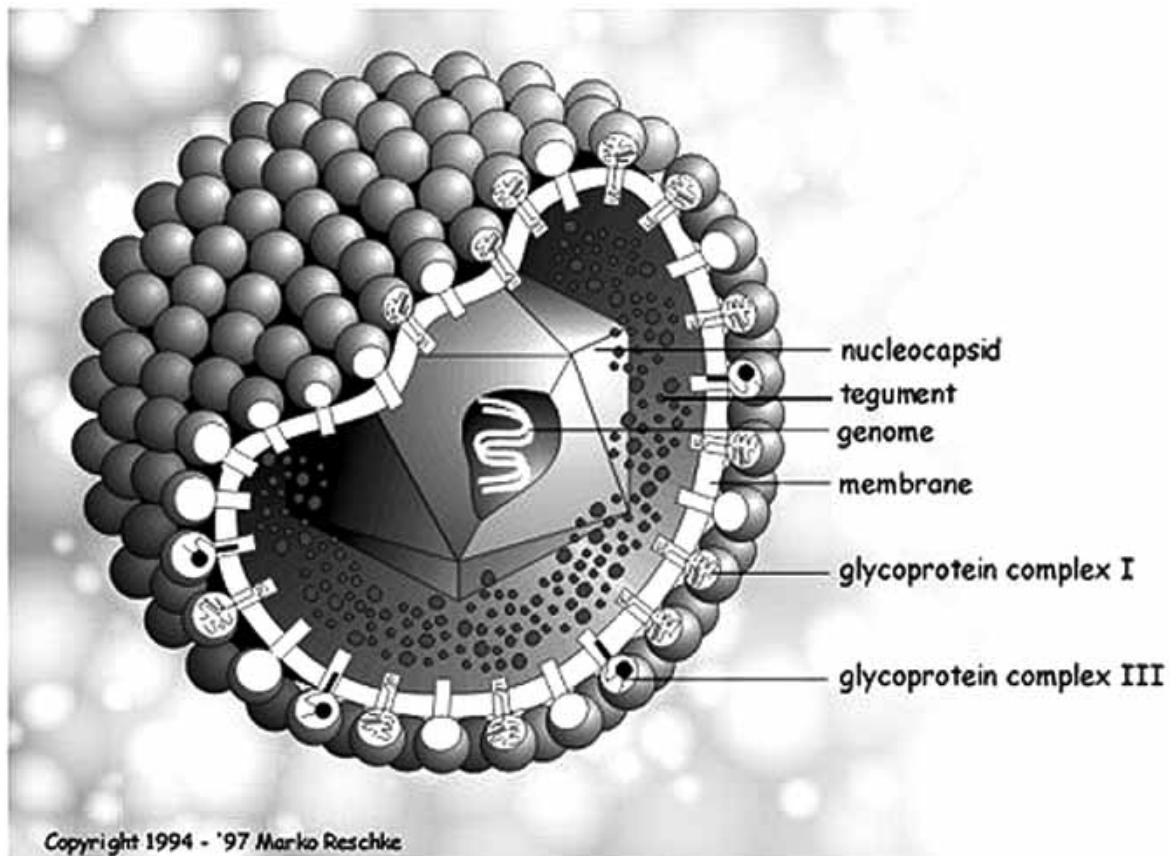
**Fig. (1).** CMV disease. A, CMV esophagitis presenting as large, shallow ulcer with punched out border. CMV was demonstrated immunohistochemically from a representative biopsy of the ulcerated area (image was kindly provided by Dr. Michael Häfner, Medical University Vienna, Austria). B, CMV retinitis in a patient with AIDS appears as an arcuate zone of retinitis with extensive hemorrhages and optic disc swelling (image was kindly provided by Dr. Dejaco-Ruhswurm, Medical University Vienna, Austria). C and D, CMV ventriculoencephalitis. Multiple small, periventricular lesions of the brain noted on brain magnetic resonance imaging studies (arrows) (image was kindly provided by Dr. Majda Thurnher, Medical University Vienna, Austria).

are homologous to those identified in human herpes simplex virus (HSV). Between the capsid shell and envelope is a tegument that contains as many as 25 proteins, many of which are phosphorylated as denoted by the prefix pp. The products of 11 ORFs (UL25, UL26, UL32, UL47, UL48, UL48.5, UL82, UL83, UL85, UL88, UL99) have been detected when virion/dense body polypeptides are electrophoretically separated [21,27], and all appear to be both phosphorylated [22,28] and highly immunogenic. Additional viral proteins have been localized to the tegument including viral DNA polymerase [29] and the UL97 kinase [30]. The most prominent are UL83 (pp65), UL32 (pp150), UL99 (pp28), UL82 (pp71), and UL48 (huge tegument pro-tein) [22,27,31]. Although several transcriptional transactivators (UL82, UL69, pTRS1, pIRS1) have been localized to the tegument [32,33], the functions of most tegument proteins remain uncharacterized. Tegument proteins are conserved among  $\beta$ -herpesviruses but are not herpesvirus-common.

The lipid bilayer envelope carries two prominent herpesvirus-conserved glycoprotein complexes [18,34]. One

is composed of covalently linked, proteolytically processed dimers of glycoprotein B (gB) encoded by UL55 [35-37], and the other is composed of the products of the UL75, UL115, and UL74 genes - gH, gL, and gO, respectively [38, 39]. These glycoprotein complexes play critical, but as yet incompletely understood roles in viral replication and infection that may be common to all herpesviruses. CMV envelope glycoproteins are presumed to be involved in virus adsorption to cellular receptors; fusion with the plasma membrane and penetration into the cytoplasm, virion assembly, and egress of progeny virus from the infected cell [40]. In addition, glycoprotein B is highly immunogenic [41-43].

Viral polymerases are classified according to their sequences and functional homologies. All herpesvirus polymerases belong to the family B DNA polymerases. Several eukaryotic polymerases, including human  $\alpha$ - and  $\gamma$ -polymerases, also belong to this family of DNA polymerases with human  $\gamma$ -polymerase sharing the highest degree of homology with the herpesvirus polymerases [44]. These family B DNA polymerases share six to seven highly



**Fig. (2).** Virtual three-dimensional model of human cytomegalovirus, showing various components of the virus (reproduced with permission from Marko Reschke and Markus Eickmann, Institut für Virologie, Marburg, Germany).

conserved domains labeled I through VII, in decreasing order of conservation. In addition to these family B conserved domains, herpesvirus DNA polymerases share an additional conserved domain referred to as the  $\alpha$ - or  $\beta$ -domain. Hence, broad inhibition of herpesvirus polymerases may be possible if compounds target conserved domains shared among the herpesvirus polymerases but not shared among other eukaryotic polymerases such as human  $\alpha$ - and  $\beta$ -DNA polymerases.

#### NATURAL COURSE OF CMV INFECTION

Human CMV can be transmitted via saliva, sexual contact, placental transfer, breastfeeding, blood transfusion, or solid-organ or haematopoietic stem-cell transplantation. After entry into the host, the virus disseminates within the host facilitated apparently by leukocytes and vascular endothelial cells. Productive (lytic) infection results in a coordinated sequence of events that leads to the consecutive synthesis of immediate-early, early, and late viral proteins. Productive infection resolves in the normal host spontaneously and CMV establishes lifelong latency or persistence within the infected person. In the developed world, acquisition of Cytomegalovirus (CMV) arises progressively from an early age, and in developed countries, the overall seroprevalence is approximately 60% [45]. Homosexual men, poor socioeconomic groups, and residents of developing countries have clearly higher seroprevalence rates

exceeding 90%. Thus, a large proportion of the adult population remains susceptible to primary infection in developed countries.

Cells of the myeloid lineage constitute an important reservoir in latency. Permissiveness for cytomegalovirus replication is cell-type specific-i.e. the virus can enter a cell, but because of transcriptional repression of the major immediate-early promoter there is no production of new virions. The permissiveness of myeloid cells for viral replication is related to their state of differentiation; monocytes are non-permissive, whereas differentiated macrophages and immature dendritic cells are permissive for productive infection [46]. During latency, viral gene expression is limited to early genes [47-49]. Only tissue macrophages (a differentiated form of circulating monocytes) express early and late CMV antigens [50]. In healthy carriers, viral DNA is also present in a small proportion of CD14<sup>+</sup> monocytes and in dendritic cells and megakaryocytes [46]. In contrast, presence of the virus in a subset of CD34<sup>+</sup> myeloid progenitor cells could be established only in immunosuppressed bone marrow recipients [51].

CMV is shed periodically in multiple body fluids, including saliva, urine, tears, semen, cervicovaginal fluid, and breast milk. Reactivation from latency may be observed in 13% of adults and periods of viral shedding in urine become less common with increasing age of the individual. The factors leading to reactivation of CMV from latency are

incompletely understood. Particularly the production of the stress hormones cortisol, ACTH, epinephrine, and norepinephrine has been implicated in the reactivation and shedding of CMV in urine [52].

### DRUGS CURRENTLY LICENSED FOR THE TREATMENT OF CMV DISEASE (TABLE 1)

The use of currently licensed drugs has decreased the burden of CMV disease in transplant patients and significantly improved survival and quality of life in AIDS patients. Antiviral prophylaxis or pre-emptive therapy is used commonly to decrease the risk of severe sequelae from cytomegalovirus infection in transplant recipients. Prophylactic regimens were found to be safe and effective in the prevention of CMV disease after solid-organ transplantation [53,54] and bone-marrow transplantation [55]. Antiviral prophylaxis for CMV disease was also shown to reduce the risk of acute allograft rejection [56].

There are currently four drugs licensed in the US for the treatment of cytomegalovirus infection and disease - ganciclovir, and its oral prodrug valganciclovir, foscarnet, cidofovir [57-61], and fomivirsen, with the latter being licensed for intra-ocular use only. All CMV inhibitors that are currently approved for the treatment of CMV infections target the viral DNA polymerase and thereby block elongation of the viral DNA chain; with the exception of fomivirsen, which is an antisense oligonucleotide targeted at the immediate early gene locus.

#### Ganciclovir and Valganciclovir

Ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl)guanine, Cymevene® or Cytovene®, Roche Pharmaceuticals], is a deoxyguanosine analogue that must be phosphorylated in three steps to ganciclovir triphosphate to exert its antiviral activity [59,62,63]. Of crucial importance in this phosphorylation process is the first phosphorylation step which is ensured by a specific virus-encoded (UL97) protein kinase

**Table 1. Drugs licensed for prophylaxis, pre-emptive Therapy, and Treatment of CMV Infection**

Compound name (brand name, manufacturer)	Drug class	Mechanism of action	Year of approval	Regimen	Major elimination pathway	Oral bioavailability, %	Main toxicity
Ganciclovir (Cymevene or Cytovene, Roche)	Purin nucleoside	competitive inhibitor of viral DNA polymerase	1989	Induction: 5 mg/kg IV q12h for 7-14 days Maintenance: 5 mg/kg IV q24h Intravitreally (Vitrasert®): 4.5-mg intraocular implant	renal	6-9	Myelosuppression
Valganciclovir (Valcyte, Roche)	Purin nucleoside	competitive inhibitor of viral DNA polymerase	2001	Induction: 900 mg PO q12h for 21 days Maintenance: 900 mg PO q24h	renal	60	Myelosuppression
Foscarnet (Foscavir, AstraZeneca)	Phosphono-formates	non-competitive inhibitor of viral DNA polymerase	1991	Induction: 90 mg/kg IV q12h or 60 mg/kg IV q8h for 14 days Maintenance: 90 mg/kg IV q24h Intravitreally: 2400 mg intravitreally	renal	0	Nephrotoxicity
Cidofovir (Vistide, Gilead)	Pyrimidine nucleosides	competitive inhibitor of viral DNA polymerase	1996	Induction: 5 mg/kg every week for 2 weeks <sup>†</sup> Maintenance: 5 mg/kg every 2 weeks <sup>†</sup>	renal	<5	Nephrotoxicity
Fomivirsen (Vitravene, Isis Pharmaceuticals, Inc.)	Antisense drugs	Inhibits translation of the CMV major immediate early proteins	1998 <sup>†</sup>	Induction : 330 µg intravitreally every 2 weeks Maintenance : 330 µg intravitreally every month	renal	n.a.	Uveitis

n.a., not applicable; ED<sub>50</sub>, median effective inhibitory dose; IV, intravenous; PO, per os; q8h, every 8 hours; q12h, every 12 hours; q24h, every 24 hours

<sup>†</sup>with sufficient hydration and under cover of probenecid to prevent nephrotoxicity; <sup>†</sup>fomivirsen was voluntarily withdrawn from the European market in 2002

(PK). The cellular kinase will afford the further phosphorylation to the di- and triphosphate stages as soon as the compound has been phosphorylated to the monophosphate. Nucleoside analogues are effective only to viruses that produce the specific kinase phosphorylating the compounds. Viruses that either do not induce a specific PK or have developed resistance to the compounds through mutations in these enzymes render the compounds inactive [64]. In their triphosphate form, the compounds then interact as competitive inhibitors or alternate substrates with the normal substrates [2'-deoxynucleoside 5'-triphosphates (dNTPs)], and if they are incorporated into the DNA chain, they may act as chain terminators, thus preventing further chain elongation. Ganciclovir triphosphate may be incorporated itself into the interior of the viral DNA chain causing a slowing and subsequent cessation of CMV DNA chain elongation [59,62,63].

Intravenous ganciclovir displays linear absorption kinetics, which approximate to a 2-compartment open model. Patients with normal renal function are unlikely to experience accumulation of ganciclovir during repeated administration. The drug is eliminated virtually unchanged by the kidneys, with a terminal phase half-life of up to 4.5 hours after oral or intravenous administration. Renal dysfunction reduces elimination of ganciclovir and dosage reductions are therefore required in these patients. Ganciclovir shows minimal binding to plasma proteins. The volume of distribution at steady state after intravenous administration is 0.74 L/kg. Ganciclovir is ~26 times more potent than aciclovir (acyclovir) against CMV *in vitro*, according to the mean concentration required to achieve 50% viral inhibition (IC<sub>50</sub>). Oral administration of ganciclovir generally provides plasma drug concentrations which are within the IC<sub>50</sub> range for most susceptible CMV strains, whereas those seen after intravenous administration are generally above the IC<sub>50</sub> range.

Valganciclovir (valine ester of ganciclovir, Valcyte®, Roche Pharmaceuticals) is the oral prodrug of ganciclovir. Equivalent systemic ganciclovir levels as intravenous ganciclovir 5 mg/kg twice daily are achieved after oral administration of valganciclovir (two 450 mg tablets twice daily). The oral bioavailability of valganciclovir is approximately 60%.

Multiple clinical trials have demonstrated the efficacy of ganciclovir in the treatment of patients with CMV disease. Ganciclovir improves markedly lesions in CMV esophagitis in 70% - 85% of patients [65,66]. In patients with CMV retinitis, ganciclovir prevents progression to bilateral disease [67] and facilitates healing in 85% [68]. In contrast, in HIV-infected patients with CMV encephalitis the use of ganciclovir is disappointing - the rate of survival approximated 0% in most studies [69,70]. Treatment of CMV pneumonia with ganciclovir was also of limited success in bone-marrow transplant recipients [71].

Prophylaxis or preemptive therapy (i.e. administration of chemotherapy against a virus at a time of particularly high risk for disease but before symptomatic disease) are used in transplant patients at significant risk of CMV disease. Prophylaxis with ganciclovir reduces significantly the risk of CMV disease and associated mortality in recipients of solid-

organ transplants [72]. The organ transplanted and the associated regimens of immunosuppressive drugs appear to have no effect on the efficacy of ganciclovir prophylaxis [72]. In accordance with *in vitro* studies, ganciclovir is more effective in preventing CMV disease than acyclovir. Valganciclovir has been established as the long-term prophylaxis of choice in transplant patients.

### Foscarnet

Foscarnet [trisodium phosphonoformate, phosphonoformic acid, Foscavir®, AstraZeneca], is a pyrophosphate analogue that reversibly and noncompetitively inhibits the activity of the CMV DNA polymerase [57,58]. Unlike ganciclovir, foscarnet doesn't require thymidine kinase for activation to exert its antiviral activity and is not incorporated into the growing viral DNA chain. Foscarnet reversibly blocks the pyrophosphate binding site of the viral DNA polymerase and inhibits the cleavage of pyrophosphate from deoxynucleoside triphosphates and elongation of the viral DNA chains [57,58].

The volume of distribution at steady state after intravenous administration is 0.52 L/kg. The foscarnet terminal half-life determined by urinary excretion is  $87.5 \pm 41.8$  hours. Deposition in bone matrix and consecutive release of foscarnet from bone is responsible for a triphasic elimination. Foscarnet shows minimal binding to plasma proteins (14%-17%). Approximately 80-87% of the drug is eliminated virtually unchanged by the kidneys, with a plasma half-life of 3-4 hours. Renal dysfunction reduces elimination of foscarnet and dosage reductions are therefore required in these patients.

Published clinical studies suggest that the efficacy of foscarnet is similar to that of ganciclovir in AIDS-related CMV disease [73,74] and in patients with bone-marrow transplantation [75]. Synergistic activity against CMV was evident when ganciclovir was coadministered with foscarnet *in vitro* [76,77]. Successful early treatment of CMV infection after bone-marrow transplantation or stem cell transplantation has been reported in small numbers of patients receiving ganciclovir plus foscarnet [78]. However, in bone-marrow, liver, and kidney transplant recipients, combined foscarnet and ganciclovir (both at half dose) versus full-dose ganciclovir monotherapy did not support a synergistic effect, with increased toxicity seen with the two-drug therapy [79].

### Cidofovir

Cidofovir [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC), Vistide®, Pharmacia Inc.], is an acyclic nucleoside phosphonate that must be phosphorylated in two steps to its diphosphoryl derivative to exert its antiviral activity [60,61]. These phosphorylations are carried out by host cellular enzymes and thus are independent of virus-induced phosphorylating enzymes [80]. Cidofovir diphosphate is a competitive inhibitor of the CMV DNA polymerase. Similarly to ganciclovir, the incorporation of cidofovir diphosphate into viral DNA causes a slowing and subsequent cessation of CMV DNA chain elongation.

The selectivity for CMV replication is also reflected at the viral DNA synthesis level, since cidofovir inhibits CMV

DNA synthesis at a concentration ( $IC_{50}$ , 0.1  $\mu\text{g/ml}$ ) that is 1,000-fold lower than the concentration ( $IC_{50}$ , 100  $\mu\text{g/ml}$ ) required to inhibit cellular DNA synthesis [81,82]. In contrast to ganciclovir, which provides only a weak and transient inhibition of viral DNA synthesis and viral replication, cidofovir was found to confer a pronounced and prolonged inhibition of viral DNA synthesis and viral replication, lasting for at least 7 days after an exposure time as short as 6 hours postinfection [83]. Cidofovir is characterized further by an anti-CMV activity *in vitro* 10 to 100 times greater than that of other available anti-CMV drugs [82] and a long intracellular half-life [84]. This long-lasting antiviral action of cidofovir allows infrequent dosing with the drug (i.e., only once a week or every other week), which clearly distinguishes cidofovir from ganciclovir, which has to be administered several times daily to sustain an antiviral response. The long-lasting antiviral action of cidofovir can be attributed to the long half-life of the HPMPC metabolites (HPMPCp, HPMPCpp, and HPMPCp-choline) that are formed intracellularly following uptake of HPMPC by the cells (presumably by endocytosis) [85]. Cidofovir accumulates in the renal proximal tubules; this accumulation is mediated by a specific organic anion transporter [80] and can be counteracted by probenecid [86]. Cidofovir is poorly bound to plasma proteins and is excreted almost entirely as unchanged drug in the urine.

The clinical experience with cidofovir is more limited than for other anti-CMV drugs. Clinical studies have shown that it is beneficial in the treatment of AIDS patients with CMV retinitis [60,61]. Cidofovir has proved efficacious in delaying the progression of CMV retinitis in patients with AIDS [60,61], was used successfully as a preemptive treatment for CMV disease after bone-marrow transplantation [87-89], and is an effective second-line therapy in patients with CMV disease failing to respond to ganciclovir or foscarnet [87,90]. *in vitro* Studies of antiviral activity using different drug combinations have suggested a synergistic inhibition of CMV replication when ganciclovir was combined with cidofovir [76]. To date, *in vivo* efficacy and tolerability was evaluated only in a phase I clinical trial (Jacobson MA, Wilson S, Stanley H, Holtzer C, Cherrington J, Safrin S. Phase I Study of Combination Therapy With Intravenous Cidofovir and Oral Ganciclovir for Cytomegalovirus Retinitis in Patients With AIDS. *Clin Infect Dis* 1999; 28: 528-33).

### Fomivirsen

Antisense oligonucleotides are synthetic, short, single-stranded sequences of DNA or RNA that are designed to target and bind to mRNA, thus disrupting gene expression and inhibiting protein synthesis [91]. Fomivirsen (ISIS 2922, Vitravene®, Novartis Ophthalmics) was the first drug of this class to be approved for treatment of patients with AIDS-related CMV retinitis. It is an antisense oligodeoxynucleotide composed of 21 phosphorothioate-linked nucleosides which bind to complementary sense sequences on mRNA transcribed from the major immediate-early transcriptional unit of CMV [92, 93]. Thereby, fomivirsen inhibits translation of CMV immediate-early proteins without interfering with the functioning of human genes [92, 93].

Fomivirsen is active *in vitro* against clinical isolates of CMV and drug-resistant mutants of the virus [93]. In cell culture, the mean 50% effective concentration ( $EC_{50}$ ) of fomivirsen against the AD169 strain of CMV is 0.37 mmol/L - 30 to 90 times lower than that of ganciclovir [92]. Elimination of the drug from vitreous humour is slow, appears to follow first-order kinetics, and metabolism is the primary route of elimination. The elimination half-life of fomivirsen in vitreous humour is 62 hours [94]. Although, selection of resistant point mutants in CMV has also been observed following treatment [95], this problem can be tackled relatively simply by the systematic evaluation and application of multiple effective sequences.

Treatment with fomivirsen significantly delayed progression of cytomegalovirus retinitis in 18 patients with AIDS and newly diagnosed unilateral peripheral cytomegalovirus retinitis compared with 10 similar patients in whom treatment with the drug was deferred until disease progression [91]. The time to progression among patients treated with fomivirsen was similar to that seen with systemically administered agents, and the time to progression among the deferral group was similar to that reported in other studies of this type [67,96]. However, fomivirsen was voluntarily withdrawn from the European market in 2002 for commercial reasons. The demand for fomivirsen was less than 100 units/year [EMA/12382/02 and Novartis Ophthalmics, personal communication]. In the US, fomivirsen is still available for the treatment of CMV retinitis in HIV-infected patients.

A series of candidate target sequences in CMV for the treatment with RNAi agents were subject of a more recent patent [97]. These sequences were selected from highly conserved regions of the CMV genome coding for immediate early proteins. Before marketing, these sequences first must overcome several hurdles; particularly they must be able to be manufactured at reasonable cost and administered safely and conveniently as demonstrated at the example of fomivirsen.

### LIMITATIONS OF CURRENTLY LICENSED DRUGS

Currently licensed anti-CMV drugs were shown to be highly effective in the prevention and treatment of CMV disease, either alone or in combination. Development of viral resistance to these drugs, toxicities, drug-drug interactions, and inhibition of the host's immune response to CMV may limit significantly the usefulness of these drugs in the clinical setting. Particularly, in immunocompromised patients, who are at highest risk of CMV disease, these limitations are most relevant because of the underlying disease that for instance may increase the risk of drug accumulation in patients with renal impairment or leukopenia in AIDS patients. The following chapter will elaborate on some of the clinically more relevant limitations of presently licensed anti-CMV drugs.

### Resistance

Chronic administration of antiviral drugs in immunocompromised patients is associated with the appearance of mutations in CMV genes for the UL97 phosphotransferase or protein kinase and UL54 DNA polymerase that confer phenotypic resistance to antiviral drugs [98-101]. Genetic

mutations associated with resistance are uncommon in patients prior to antiviral treatment, but they are quite common after several months of antiviral therapy [102]. The functional consequence of the UL97 mutations is an impaired phosphorylation of ganciclovir in virus-infected cells, with the consequent lack of synthesis of ganciclovir triphosphate, the active form of the drug [99,100,103].

CMV isolates resistant to antiviral agents have been recovered from immunocompromised patients treated with these drugs [104-108]. Abundant data in the literature suggest that resistance of CMV to ganciclovir is associated with lack of therapeutic response and progression of CMV disease. Isolation of ganciclovir-resistant CMV or detection of ganciclovir resistance UL97 and/or UL54 mutations in viral isolates or directly in clinical samples from patients with AIDS has been associated with recurrent or progressive CMV retinitis despite therapy [109-113], progressive extraocular CMV disease, development of CMV disease of the central nervous system while on therapy for CMV retinitis [114,115], persistent isolation or detection of CMV in blood leukocyte fractions, persistent detection of CMV DNA in plasma fractions [113,116], and increase in the CMV DNA burden in blood leukocyte and plasma fractions [116-118].

Mutations in the UL97 gene, that confer resistance to ganciclovir, do not lead to resistance to other anti-CMV drugs such as foscarnet and cidofovir, as they do not require phosphorylation by this kinase. However, cross-resistance to cidofovir may occur in patients with high-level ganciclovir resistance due to point mutations in the DNA polymerase gene of the virus. Patients with multiply resistant viruses have been reported also [110,119,120]. Characterization of laboratory and clinical CMV strains resistant to foscarnet and cidofovir has demonstrated that resistance to these compounds is associated with amino acid substitutions in conserved regions of the viral DNA polymerase [98,99,110, 110,112,119-121]. Some of these DNA polymerase mutant isolates are cross-resistant to ganciclovir, foscarnet, and cidofovir.

### Toxicities

Choices for a drug regimen are often based on the differential toxicity of the agents available, the need for long term intravenous catheters, infusion times, cost, reimbursement, and other quality of life issues. Consideration must be given to the unique characteristics of CMV retinitis, patient preference, the presence or risk of extraocular cytomegalovirus infection, concomitant therapy, underlying medical conditions and the patient's lifestyle and living conditions.

Retinitis is the single most common manifestation of CMV disease in HIV-infected patients [2,122]. Intraocular implantation of ganciclovir or injection of foscarnet, cidofovir, or fomivirsen proved beneficial due to high local concentrations and a longer intraocular half-life [123]. Nevertheless, most patients experience an immediate but temporary reduction in functional visual acuity secondary to the implant procedure or injection, with return to baseline by 28 days [96, 124]. In randomised trials, a vision compromi-

sing event occurred in approximately 10% of the patients who received implants [96,124]. Nowadays, intraocular application of anti-CMV drugs is only rarely used.

Foscarnet and cidofovir must be given intravenously; in the case of foscarnet 2-3 times daily. Beside the inconvenience and risk of infection at the site of the indwelling catheter, oral forms of drugs are cost-saving. The major cost savings are attributable to the decrease in home care expenditures [125].

Ganciclovir, cidofovir, and foscarnet are eliminated for the most part unchanged by the kidneys. Accumulation of the drugs in renal insufficiency is a clinically relevant issue and requires adaptation of drug dosage or avoidance (cidofovir). Valganciclovir, cidofovir, and foscarnet are metabolized to varying extent by the cytochrome P450 (CYP) isoenzyme CYP3A and should be avoided in combination with other drugs metabolized by this enzyme, such as carboplatin, cisplatin, clofarabine, clozapine, gallium nitrate and antimicrobials such as imipenem/cilastin or aminoglycosides. Some side-effect profiles are specific for certain drugs and have to be considered specifically; particularly in patients with the risk of accumulating drug levels due to impaired renal function.

### Ganciclovir

Granulocytopenia, anaemia and thrombocytopenia are the most common dose-limiting adverse effects of ganciclovir therapy and observed in 24 - 43% of patients within six months of the initiation of treatment [67,73,126-128]. Central nervous system adverse events, including confusion, hallucinations, seizures, psychosis, abnormal thought patterns, change in mental status, nightmares, anxiety, tremors, ataxia and headaches have been reported in 3 to 5% of patients [126]. Gastrointestinal adverse effects such as anorexia, nausea, vomiting and diarrhoea are reported in up to 6% of patients. Elevations in liver transaminase levels have been reported in 2% of recipients. Other reported symptoms include fever (2 to 5%), oedema (<1%), and myalgias (2 to 6%). The incidence and type of adverse reactions are similar between the oral and intravenous forms of ganciclovir.

### Foscarnet

Nephrotoxicity is the most frequently reported major adverse effect associated with foscarnet therapy [129,130]. Increases in serum creatinine level 2-3 times above baseline have been reported in 29-60% and dose-limiting nephrotoxicity in 17-23% of patients. Electrolyte abnormalities are common and are potentially serious. Paraesthesias involving the extremities and perioral areas are reported in 4-20% of patients. Other CNS effects associated with foscarnet include tremor, anxiety, fatigue, irritability, hallucinations and headaches. Nausea, vomiting, anorexia, and malaise are frequently described adverse reactions related to infusion of foscarnet and have been reported in 28 to 56% of patients [131-133]. Painful genital ulcerations or erosions on the glans of the penis or the vulval area have been reported in 5-28% of patients on foscarnet [126,134,135]. Overall, adverse effects appear to be more frequent with foscarnet than with ganciclovir [126].

### Cidofovir

Dose-related nephrotoxicity is the most important and potentially irreversible toxicity of this compound and is reported to occur in up to 50% of patients [60]. Iritis is a common adverse effect following intravitreal injection of cidofovir. Hypotony has been reported in 3-12% of patients, often in association with iritis. Uveitis has also been reported in 4-7% of patients. Neutropenia (absolute neutrophil count  $<750/\text{mm}^3$ ) has been reported in 15-30% of patients. In animal studies, cidofovir is carcinogenic, teratogenic and causes hypospermia and thus should be considered a potential carcinogen in humans.

### Fomivirsen

Increased intraocular pressure and mild to moderate intraocular inflammation of the anterior and posterior chambers were the most common adverse events reported in clinical trials of fomivirsen conducted in patients with AIDS-related cytomegalovirus retinitis [91]. The rates of ocular complications among patients treated with fomivirsen are nontrivial and are dose dependent [136]. The rates ranged between 4.06 events/person-year to 8.35 events/person-year.

### Anti-CMV Drug Associated Immunocompromise

Ganciclovir was suggested to inhibit the immune responses to CMV infection [137]. Long-term ganciclovir treatment apparently impaired CMV-specific cytotoxic T lymphocytes (CTLs) reconstitution, causing an increase in late CMV infections [138]. We found recently that prolonged ganciclovir prophylaxis in transplant patients was associated with delayed CMV-seroconversion and antibody-maturation [139,140]. In addition, immunoglobulin class-switching from IgM- to IgG-antibodies was impaired and antibody-maturation inhibited in selected transplant patients who received ganciclovir prophylaxis [140].

### DRUGS WITH ACTIVITY AGAINST CMV BUT LICENSED FOR OTHER INDICATIONS THAN CMV DISEASE

#### Mycophenolate Mofetil (MMF)

MMF (Cellcept®, Gilead Sciences), the morpholinoethyl ester of mycophenolic acid (MPA), is currently used as an immunosuppressant in kidney transplant recipients. After oral administration, MMF is hydrolyzed to MPA, the active immunosuppressive agent, which is a potent inhibitor of inosine 5'-monophosphate dehydrogenase. Inhibition of this enzyme results in a depletion of the intracellular GTP and dGTP pools [141,142]. MMF does not inhibit replication of HSV-1, -2, or VZV, but has some inhibitory effect on CMV [143]. MMF also potentiates the activity of ganciclovir *in vitro* and *in vivo* [143]. Depletion of endogenous dGTP pools favors the inhibitory effect of the triphosphate of ganciclovir on the viral DNA polymerase. In addition, MPA might enhance the intracellular phosphorylation of ganciclovir.

Still, transplant recipients under MMF treatment have a slightly increased risk of acquiring CMV viremia when compared to those receiving azathioprine [144]. This effect is most likely due to the profound immunosuppressive action of MMF. From a toxicological viewpoint it may be of

interest to monitor the toxicity of ganciclovir in patients under MMF treatment since the latter has the potential to increase the side effects of ganciclovir.

### Leflunomide

Leflunomide [N-(4'-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide, Arava®, Aventis Pharmaceuticals] is a licensed immunosuppressive drug for use in rheumatoid arthritis and rejection in solid organ transplantation. The antiviral activity of leflunomide against CMV was first described by Waldman and colleagues [145,146]. Plaque assays showed dramatic dose dependent attenuation of multiple clinical CMV isolates in leflunomide treated human fibroblasts and endothelial cells [146]. Leflunomide was shown to be also effective *in vitro* against drug resistant strains of CMV [146]. Subsequently, there have been a case series and several case reports describing the clinical use and benefit of leflunomide for CMV infection in kidney transplant recipients [147-149]. The patients received a loading dose of 100 mg of leflunomide once daily on days 1-3 and then 20 mg once daily for three months.

Leflunomide is metabolized to its active form, A77 1726 [N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide] by the cytochrome P450 system [150]. This metabolite exhibits two known mechanisms of action - inhibition of protein tyrosine kinase activity [151], and inhibition of dihydroorotate dehydrogenase, a key enzyme in the *de novo* biosynthesis of pyrimidine nucleotide triphosphates [152]. Contrary to expectation, leflunomide apparently does not inhibit viral DNA synthesis, but rather interferes with virion assembly by its protein kinase activity [146]. Recently, FK778, a drug structurally similar to the active metabolite of leflunomide, was evaluated for its antiviral activity and cellular target [153]. In contrast to leflunomide, FK778 predominantly inhibits virus replication by the specific inhibition of dihydroorotate dehydrogenase. The exact mode of action, however, has to be elucidated further for both compounds.

The low cost of this drug in comparison to ganciclovir makes it an attractive alternative in the resource poor setting. Recently, leflunomid was evaluated in a larger cohort of kidney transplant recipients (n=17) with a response rate of 88% to therapy [154]. Extensive clinical experience with leflunomide at drug dosage comparable to that used in the treatment of CMV disease indicates good tolerability and low toxicity. The most common side effects are headache, gastrointestinal symptoms (nausea, diarrhea, abdominal pain), and nasopharyngeal symptoms [155]. Leflunomid is a promising drug for the treatment of CMV disease, particularly in the case of ganciclovir-resistance or in the resource-poor setting.

### Artesunate

Artemisinins were found to be highly effective antimalarial drugs shortly after the isolation of the parent artemisinin in 1971 in China. These compounds combine potent, rapid antimalarial activity with a wide therapeutic index and an absence of clinically important resistance. Artesunate was found to possess also anti-CMV activity *in vitro* [156]. A concentration-dependent inhibition of the

replication of cytomegaloviruses with wild-type phenotype was demonstrated in several cell lines and IC<sub>50</sub> values ranged between 5.8 and 6.9  $\mu$ M. Further characterization of the antiviral properties and clinical trials, however, have to clarify the value of artesunate in the treatment of CMV disease. Recently, a phase III clinical trial started to evaluate the usefulness of artesunate in the preemptive treatment of CMV infection in stem cell transplant recipients (clinicaltrials.gov NCT00284687).

## NOVEL DRUGS IN THE TREATMENT OF CMV DISEASE (TABLE 2)

### Alkoxyalkyl and -Propyl Esters of Cidofovir

The poor oral bioavailability of cidofovir has been recognized as a significant disadvantage that limits its clinical usefulness. The oral uptake can be greatly enhanced if the phosphonate group is esterified [157]. Alkoxyalkyl or -propyl esters of cidofovir such as HDP-cidofovir (hexadecyloxypropyl-HPMPC, Chimerix Inc.) were developed to overcome this limitation by greater oral bioavailability. Cidofovir analogs protect *in vivo* against CMV disease after oral administration [158]. Oral administration of HDP-cidofovir results in significantly higher plasma levels than oral administration of cidofovir. The bioavailability of HDP-cidofovir is estimated to be 88-97% compared to <5% for cidofovir [159]. In addition, exposure of the kidney to either the conjugate or the parent drug, is five times lower with administration of HDP-cidofovir [159]. Consequently, nephrotoxicity of HDP-cidofovir may be significantly lower in comparison to cidofovir. In toxicological studies, the main dose-limiting effects were enteritis and gastropathy [159].

Surprisingly, the oral lipid prodrug of cidofovir also shows up to 1,000-fold enhanced inhibition of virus replication *in vitro* compared with that of the parental cidofovir [159-162]. Orally administered treatment with HDP-cidofovir is 4-8-fold more active in a rodent model than is intraperitoneally administered cidofovir [163]. Very recently, a phase I clinical trial of HDP-cidofovir has commenced [Chimerix Inc., personal communication].

### Benzimidazoles

Benzimidazole ribosides represent a promising new class of anti-CMV compounds, that do not inhibit the viral DNA polymerase [164] but CMV terminase. Terminases are responsible for translocation of viral genome into preformed procapsids by cleavage of the DNA and powering the insertion by its ATPase activity [165]. The CMV terminase is composed of two subunits, the large one encoding pUL56 and the small pUL89 [166]. The process of viral DNA packaging is multifunctional and determined by specific interactions of protein-DNA and protein-protein. An important role during this process is played by portal proteins. Portals are large macromolecules found throughout all herpesviruses. Portal proteins provide on one hand the channel for entry of the DNA during packaging and on the other the exit for releasing DNA during infection. Portal proteins function like a DNA pump as the docking site for the terminase-DNA complex and interact with proteins that seal the portal for preventing DNA loss and reopen it for ejection of the DNA into the nucleus of the host [167].

Benzimidazoles inhibit specifically the interaction between the large terminase subunit pUL56 and pUL104, an putative portal protein, and thereby very likely block the insertion of the DNA into the capsid [168]. Consequently, CMV maturation is inhibited by these compounds [169] at a late stage of CMV replication [164]. This novel mode of mechanism in the action of benzimidazoles, may be confer efficacy to this drug also in CMV strains resistant to ganciclovir, cidofovir, or foscarnet.

### BDCRB and TCRB

BDCRB (2-bromo-5,6-dichloro-1-*D*-ribofuranosyl benzimidazole) and TCRB (the 2-chloro analogue of BDCRB) are benzimidazole ribonucleosides that are analogues of 5,6-dichloro-1-*D*-ribofuranosyl benzimidazole (DCRB) with the addition of bromine to the 2-position of the core. *in vitro*, These compounds are highly active against CMV replication with sub-micromolar IC<sub>50</sub> values. However, clinical development was not pursued after preclinical pharmacokinetic studies demonstrated that both, BDCRB and TCRB, are cleaved *in vivo* to produce the less active but more cytotoxic aglycones [170]. Although BDCRB is not being developed further, other drugs that inhibit this stage of the replicative cycle are in development, including Maribavir and GW275175X.

### Maribavir

Modifications of BDCRB led to the synthesis of maribavir® [5,6-dichloro-2-isopropylamino-1-(*L*-ribofuranosyl)-1*H*-benzimidazole; also known as benzimidavir and 1263W94, ViroPharma][170]. The mode of action is somewhat different for maribavir in comparison to other compounds from the group of benzimidazoles. The main target of action of maribavir was postulated to be pUL97 [171]. The inhibition of the UL97 protein kinase activity by maribavir results in inhibition of CMV DNA synthesis [171] and nuclear egress [172,173]. The UL97 protein kinase is also involved in the monophosphorylation of ganciclovir (see above [174]). Unexpectedly however, UL97 mutants of CMV that are compromised in their ability to phosphorylate ganciclovir are fully susceptible to maribavir. Maribavir-resistance of CMV variants mapped to a mutation in a gene of unknown function (UL27), but lacked a mutation in UL97 [175,176]. This points to an even more complex mode of action of maribavir.

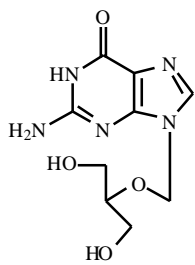
Maribavir shows excellent activity against both laboratory and clinical strains of CMV, including those that are resistant to ganciclovir [177]. In a murine model, maribavir was effective in inhibiting CMV replication by approximately 30- to 3,000-fold in comparison to the negative control [178]. Maribavir inhibits CMV synergistically with ganciclovir, acyclovir, and foscarnet [179]. Plasma maribavir concentrations exceed the IC<sub>50</sub> despite the fact that the drug is highly bound to plasma proteins (>97%) such as serum albumin [180].

Maribavir has been the subject of preclinical pharmacokinetic and toxicological studies in mouse, rat and monkey [181]. These studies demonstrated a favourable safety profile, good to excellent oral bioavailability and lower toxicity than currently available anti-CMV agents [180].

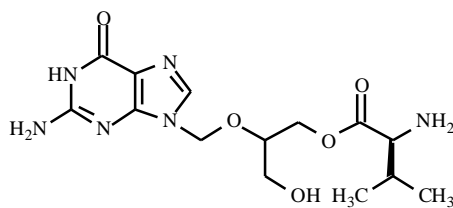
**Table 2. Novel Drugs for Prophylaxis, Pre-Emptive Therapy, and Treatment of CMV Disease**

Compound name (manufacturer)	Drug class	Drug target (mechanism of action)	Development or approval status	Comments
HDP-cidofovir (Chimerix Inc.)	Pyrimidine nucleosides	Viral DNA polymerase (competitive inhibitor of viral DNA polymerase)	Phase I clinical trial	Good oral bioavailability combined with high potency and potentially reduced nephrotoxicity
BDCRB and TCRB (ViroPharma & Glaxo Wellcome Inc.)	Benzimidazoles	CMV terminase (DNA maturation)	Preclinical evaluation	<i>In vivo</i> low activity and high toxicity
1263W94 (Maribavir®, ViroPharma)	Benzimidazoles	pUL97? (CMV DNA synthesis and nuclear egress)	Phase II clinical trials	most promising compound for treatment of CMV infection
GW275175X (ViroPharma)	Benzimidazoles	CMV terminase (DNA maturation)	Preclinical evaluation	<i>In vitro</i> efficacy and toxicity comparable to ganciclovir
4-Hydroxyquinoline carboxamides (Pharmacia)	Carboxamides	Viral DNA polymerase	Preclinical evaluation	Resembles non-nucleosid reverse transcriptase inhibitors
CMV423 (Pharmasset)	Indolizines	??? (possibly interference with immediate early antigen expression)	Preclinical evaluation	Highly synergistic with ganciclovir, foscarnet, and cidofovir
1,6-Naphthyridine derivatives (Biochem Pharma)	Naphthyridines	??? (possibly early, i.e. post-adsorption, event of the CMV replication cycle)	Pre-clinical evaluation	<i>In vivo</i> unstable
Isoquinoline-6-carboxamides (Biochem Pharma)	Isoquinolones	??? (possibly early, i.e. post-adsorption, event of the CMV replication cycle)	Pre-clinical evaluation	<i>In vivo</i> unstable
7,8-Dihydroisoquinoline (Biochem Pharma)	Isoquinolones	??? (possibly early, i.e. post-adsorption, event of the CMV replication cycle)	Pre-clinical evaluation	No data on stability <i>in vivo</i>
PD0084430	Phenols (tricyclic inhibitors)	??? (inhibition of CMV at or before viral DNA replication)	Pre-clinical evaluation	Rapidly metabolized to ineffective metabolites
Acyclonucleoside	Benzathiadiazine derivatives	??? (inhibition at first stages of the viral replicative cycle)	Pre-clinical evaluation	Series of compounds with potential as future anti-CMV drugs
BAY 38-4766 (tomoglovir®, Bayer AG)	Naphthalene derivatives	CMV terminase (cleavage and packaging of viral DNA)	Phase I clinical trial	Promising drug, particularly for treatment of patients with renal impairment
Gö 6976 (Biochem Pharma)	Indolocarbazoles	UL97 protein kinase (???)	Pre-clinical evaluation	poor pharmacokinetics and bioavailability
Ax7376, Ax7396, Ax7543	Quinazolines	UL97 protein kinase (inhibition during early-late phas of CMV replication)	Pre-clinical evaluation	Limited data
5-(4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile (Chemocentryx, Inc.)	US28-Agonist	US28 (agonist of chemokine receptor homolog)	Pre-clinical evaluation	Role of US28 and interaction with human chemokines uncertain

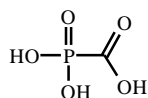
A



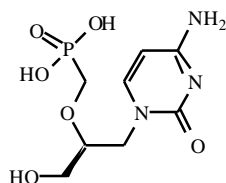
Ganciclovir



Valganciclovir

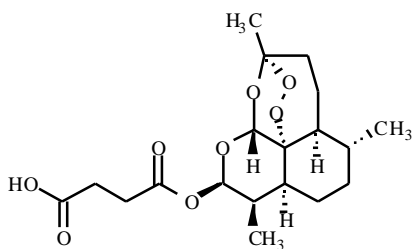


Foscamet

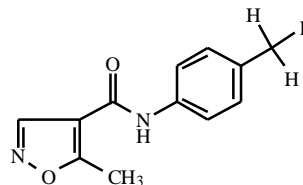


Cidofovir

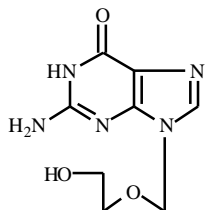
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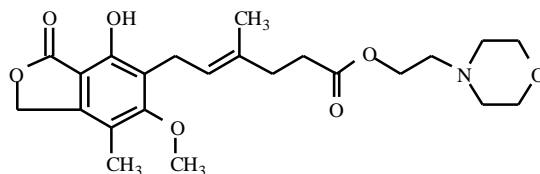
Artesunate



Leflunomide

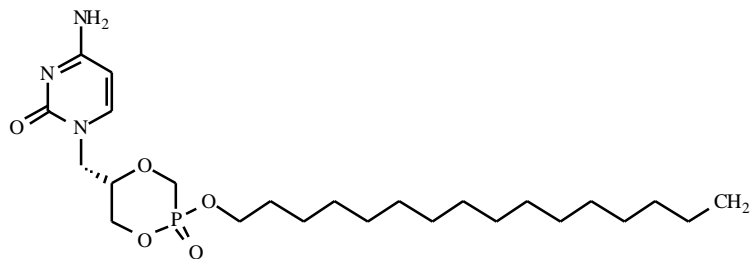


Acyclovir



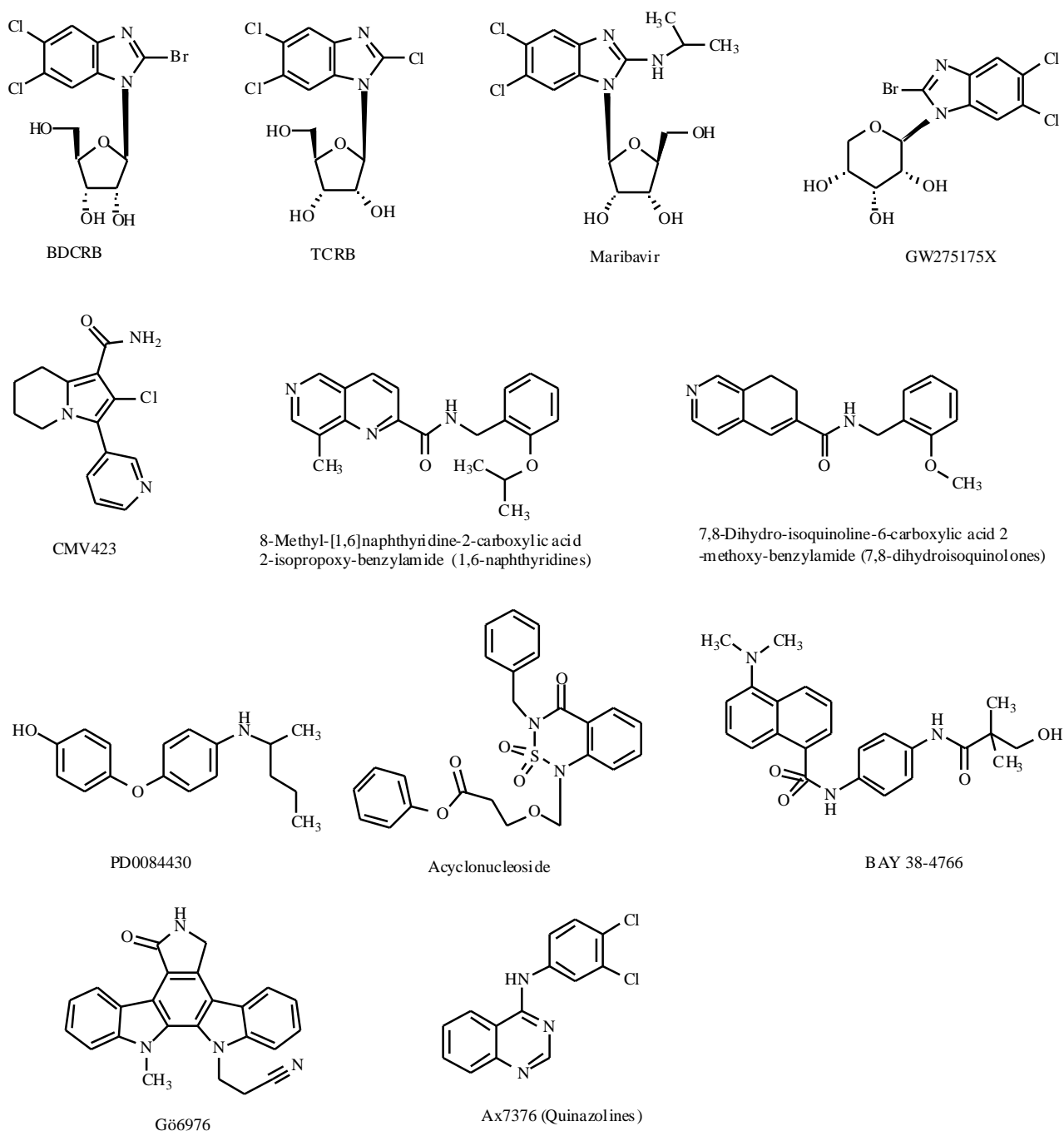
Mycophenolate mofetil

C



HDP-Cidofovir

(Fig. 3c) Contd....



**Fig. (3).** Chemical structures of anti-CMV drugs. A, drugs licensed for prophylaxis, pre-emptive therapy, and treatment of CMV infection. B, drugs with activity against CMV but not licenced for the treatment or prevention of CMV disease. C, novel drugs for the treatment of CMV disease. Source: Therapeutics Database, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, USA.

Maribavir was less toxic than ganciclovir to bone marrow cells *in vitro* [171]. No adverse pharmacological effects were demonstrated in safety pharmacology studies with maribavir [181]. Toxic effects were limited to increases in liver weights, neutrophils, and monocytes at higher doses in female rats. Maribavir was not genotoxic in the Ames or micronucleus assays [181].

In phase I clinical trials, maribavir was rapidly absorbed following oral administration, with peak concentrations in plasma occurring 1-3 h after dosing. In humans, approximately 25-45% of oral maribavir was found to be absorbed, based on the urinary recovery of maribavir [180]. Metabolism of the drug occurs via the cytochrome P450 (CYP3A4) to an N-alkylated metabolite [181]. Maribavir decreases or

inhibits CYP2C19 and CYP2D6 activities but does not affect the CYP1A2, CYP2C9, CYP3A, NAT-2, or XO activities [182]. Patients who receive maribavir concomitantly with medications predominantly metabolized by CYP2C19 or CYP2D6 (eg omeprazol or dextromethorphan) may need additional monitoring. Maribavir has no effect on the metabolism of HIV protease inhibitors despite the fact that many of the compounds in this drug class are also metabolized by the same CYP system. Conversely, only few antiretroviral drugs exert effects on the potency of maribavir. Zidovudine and abacavir, reverse transcriptase inhibitors, and indinavir, a protease inhibitor, potentiated slightly the effect of maribavir on CMV [179].

Maribavir is rapidly eliminated, with a mean half-life in plasma of 3-5 h; the half-life is independent of the dose level. Less than 2% of the maribavir dose administered is eliminated unchanged in urine [180]. Clearance in rats and monkeys is primarily by biliary secretion, with evidence of enterohepatic recirculation. Maribavir is primarily distributed in the gastrointestinal tract of rats but does not cross the blood-brain barrier. In monkeys, maribavir levels in the brain, cerebrospinal fluid, and vitreous humor ranges from 4-20%, 1-2%, and <1%, of corresponding concentrations in plasma, respectively.

Four clinical trials of maribavir have taken place to date and the drug appears to be well tolerated with appreciable concentrations able to reach the eye (important for the treatment of CMV retinitis). Phase I/II dose escalation studies in HIV-infected men with asymptomatic CMV shedding have indicated that at the six dosage regimens used (100, 200 or 400 mg three times a day, or 600, 900 or 1200 mg twice a day), maribavir is rapidly absorbed following oral dosing. Maribavir was generally well tolerated. Taste disturbance was the most frequently reported adverse event over the 28 day dosing period [183]. Currently, phase II clinical trials under way investigate the drug's potential for prevention of CMV after bone-marrow transplantation (clinicaltrials.gov NCT00078533 and NCT00223925). A phase I clinical trial in the treatment of CMV retinitis in HIV-infected patients has been completed recently (clinicaltrials.gov NCT00002373).

Maribavir has many characteristics that make it an attractive candidate for development; namely, high potency *in vitro*, selectivity, good oral bioavailability, and lower toxicity than current therapies. Furthermore, its hepatic metabolism makes it an attractive alternative to currently licensed drugs in patients with renal impairment. Initial clinical trials have provided encouraging results, including good tolerability and linear pharmacokinetics over a wide dose range [170].

#### GW275175X

GW275175X is a D-ribosepyranosyl derivative of BDCRB (2-bromo-5,6-dichloro-1-*D*-ribosepyranosyl-1*H*-benzimidazole, ViroPharma and GlaxoSmithKline). It acts as a DNA maturation inhibitor like the parent compound, BDCRB, rather than via the mechanisms of action of Maribavir or any other anti-CMV drug approved for marketing. GW275175X displays comparable antiviral activity as BDCRB without the *in vivo* instability mentioned above. In a murine model,

GW275175X was inhibited CMV replication by approximately 30- to 3,000-fold in comparison to the placebo group [178]. GW275175X is *in vitro* more active against CMV than ganciclovir, approximately fourfold less active than its close analog BDCRB and from three- to sevenfold less active than maribavir [184].

All CMV strains tested, including those resistant to ganciclovir and foscarnet, were sensitive to maribavir and GW275175X, with mean EC50% of about 1-5  $\mu$ M compared to that of ganciclovir at 6  $\mu$ M. The toxicity of GW275175X in tissue culture cells appeared to be similar to that observed with ganciclovir [185]. GW275175X is a promising candidate for clinical development as an anti-CMV agent.

#### 4-Hydroxyquinoline Carboxamides

The 4-hydroxyquinolines represent a novel class of nonnucleoside DNA polymerase inhibitors. This class of compounds was discovered through a combination of high-throughput screening for small-molecule inhibitors of herpesvirus DNA polymerases and structure activity relationship studies to improve the characteristics of the initial lead templates of naphthalene carboxamides. Structure-activity relationship studies demonstrated that a quinoline ring could be substituted for naphthalene, resulting in the discovery of a 4-hydroxyquinoline-3-carboxamide class of antiviral agents (Pharmacia)[186].

Antiviral cell culture assays have confirmed that these compounds are active against CMV, HSV-1, HSV-2, VZV, and many animal herpesviruses. A strong correlation between the viral DNA polymerase and antiviral activity for this class of compounds supports inhibition of the viral DNA polymerase as the mechanism of antiviral activity [186]. Northern blot analysis of immediate-early and late viral transcripts also pointed to a block in the viral life cycle consistent with inhibition of viral DNA replication. *in vitro*, CMV polymerase assays indicate that the 4-hydroxyquinolines are competitive inhibitors of nucleoside binding. Thus, 4-hydroxyquinoline carboxamides may represent a novel class of nonnucleoside inhibitors of herpesvirus DNA polymerase, and, in this sense, they bear some resemblance to the NNRTIs (nonnucleoside reverse transcriptase inhibitors) that are used in the therapy of human immunodeficiency virus (HIV).

#### CMV 423

Snoeck *et al.* [187] described 2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide (CMV423) as a new compound for the treatment of CMV infections. CMV423 showed potent *in vitro* activity against a wide range of CMV reference strains and clinical isolates, including those that had acquired resistance to ganciclovir, foscarnet, or cidofovir. CMV423 appears to act on a step of the viral replicative cycle that precedes the DNA polymerase step and likely coincides with immediate early antigen expression [187]. CMV423 exhibits in combination with ganciclovir, foscarnet, or cidofovir highly synergic activity. CMV423 is oxidized by CYP1A2- and, to a minor extent, by CYP3A4-isoenzyme of the cytochrome P450 [188]. CMV423 is expected to affect CYP1A2 and -1A1 activities *in vivo*, but not the metabolism of other drugs. In patients

with renal impairment, CMV 423 may become an interesting treatment option.

### Derivatives of Naphthyridines and Isoquinolones

Another series of compounds exhibiting potent activity against CMV includes derivatives of naphthyridines and isoquinolones, particularly 1,6-naphthyridine and the isoquinoline-6-carboxamides [189-191]. A subset of these derivatives has been studied in more detail for their antiviral properties and was found to be highly effective *in vitro* as indicated by an IC<sub>50</sub> up to 39- to 223-fold lower than that of ganciclovir [191, 192]. Activity against ganciclovir-resistant CMV strains and synergic activity with ganciclovir was noted. The most likely target of these two compounds appears to be an early (post-adsorption) event of the CMV replication cycle [191].

However, during preliminary pharmacological evaluation, the 1,6-naphthyridine derivatives and isoquinoline-6-carboxamides were found to be unstable in mouse or monkey S9 liver preparations indicating that they are likely to be subject to first pass metabolism [193]. *in vivo* Studies in mice confirmed further this hypothesis; no parent compound was detected 20 minutes after 1,6-naphthyridine was given orally.

The 7,8-dihydroisoquinoline class of compounds were found to be a metabolically stable and orally bioavailable alternative to the 1,6-naphthyridines and isoquinoline-6-carboxamides [193]. Several compounds of this class were found to be stable towards monkey and mouse S9 homogenate and *in vivo* experiments also indicated that these compounds were stable and well absorbed when administered per os in mice. The oral bioavailability in mice was determined to be 60.4%. The issue of stability *in vivo* was not addressed yet in the recent development of further derivatives of the 1,6-naphthyridine class [192].

### Tricyclic Inhibitors

A novel, non-nucleoside inhibitor of CMV replication, PD0084430, was identified by high-throughput screening of approximately 1200 compounds [194]. The anti-CMV activity of the compound was confirmed in both yield and plaque reduction assays. Times of addition and Western blot assays have shown that this compound inhibits CMV at or before viral DNA replication [194]. Its site of action appears to be distinct from ganciclovir since CMV strains with mutations in either the UL97 or UL54 genes displayed no cross-resistance to this compound.

PD0084430 is rapidly glucuronidated on the OH-group, which is known to be essential for anti-CMV activity [195], and so further structure activity relationships need to be explored before these compounds enter human trials. In mice dosed with PD0084430, the compound was rapidly metabolized and, therefore, likely targeted for elimination [194].

### Acyclonucleosides

Acyclonucleosides are derivatives of benzothiadiazine dioxides and non-nucleoside inhibitors of CMV. They are characterized by a heterocyclic base but lack the 5'-OH mimetic group that is characteristic for ganciclovir. In order for the compounds to have antiviral activity, a double

substitution in the heterocycle is required, and to maintain anti-CMV activity, lipophilicity in the acyclic side chain is important. Benzothiadiazine dioxide-modified acyclonucleosides show good activity against CMV *in vitro* [196]. IC<sub>50</sub> values were similar to that of ganciclovir. Structure-activity relationships with these compounds against prototype and clinical strains of CMV showed that the acyclonucleosides had an IC<sub>50</sub> against CMV of 3-10  $\mu$ M. Oral bioavailability was predicted to be significantly higher than that for ganciclovir [197]. Acyclonucleosides, however, were not compared with valganciclovir.

Martinez *et al.* [198] have reported the synthesis of *N,N*- and *N,O*-dibenzyl derivatives of these compounds with a variety of para-substitutions (alkyl, phenyl, electron donating, electronwithdrawing) in the phenyl ring and benzyl derivatives of thiadiazines, thienothiadiazines, benzothienothiadiazines and quinazolines [199]. While the molecular targets of acyclonucleosides have yet to be fully characterised, time of addition experiments indicate that they are likely to act in the first stages of the viral replicative cycle [198] and are still effective when viral resistance to ganciclovir emerges [196].

### Naphthalene Derivatives

BAY 38-4766 (3-hydroxy-2,2-dimethyl-N-[4([5-(dimethylamino)-1-naphthyl] sulfonylamino) -phenyl]propanamide, tomglovir®, Bayer AG) and its prodrug, BAY 40-1007, are members of a new class of non-nucleosidic anti-CMV agents. They are based on a 4-substituted naphthalene nucleus. Resistance to this drug was mapped to mutations in UL89 and UL104, proteins known to be involved in viral DNA cleavage and packaging (see also benzimidazoles) [200]. CMV isolates resistant to the benzimidazoles TCRB and BDCRB and ganciclovir were sensitive to tomglovir [201], which suggest a different drug target than for benzimidazoles.

Tomeglovir exhibited comparable antiviral efficacy in a rodent model *in vivo* in comparison to ganciclovir (IC<sub>50</sub> 0.34  $\mu$ M vs 0.74  $\mu$ M) [200]; however, both drugs were administered orally with the well-known poor bioavailability of ganciclovir. Combination of tomglovir with ganciclovir showed antagonism, which indicated a limited usefulness of these two drugs in combination therapy [201].

Pharmacokinetics of this compound in dogs showed that absorption from the gastrointestinal tract was approximately 65% with a rapid half-life (approximately 1 hour). Tomeglovir has a pronounced protein binding (the free fraction of compound in the cell culture medium was estimated to be 29% and in SCID mouse plasma 0.8%). High total plasma concentrations are consequently required to meet the IC<sub>50</sub> *in vitro*. Metabolism occurs via the CYP3A4 system - the effects of tomglovir on the metabolism of other drugs metabolised by these enzyme, for example HIV protease inhibitors, remain to be evaluated. Nevertheless, tomglovir may become an interesting treatment option in patients with renal impairment.

### Indolocarbazoles

The UL97 protein kinase phosphorylates ganciclovir, but is also capable of autophosphorylation, which is again a

prerequisite for ganciclovir phosphorylation; both processes can be inhibited by indolocarbazoles, [202] such as Gö 6976. Indolocarbazoles proved to be highly effective inhibitors of both ganciclovir-sensitive and ganciclovir-resistant CMV strains, while not being effective against HSV [202]. Although both, maribavir and indolocarbazole Gö 6976, target the UL97 protein kinase, it is not clear whether they act by the same mechanism. A strong antiviral effect of indolocarbazole compounds (e.g., NGIC-I) on the *in vitro* replication of CMV was reported [174, 202, 203]. However, the excellent antiviral potencies of distinct indolocarbazoles *in vitro* seemed to be accompanied by unfavorable pharmacological properties *in vivo*, such as poor pharmacokinetics and bioavailability [174]; thus, further preclinical developments await continuation.

### Quinazolines

Quinazolines are potent inhibitors of phosphorylation mediated by pUL97, such as the phosphorylation of various substrates (H2B and MBP) as well as autophosphorylation. Remarkably, the drug gefitinib (Iressa®; AstraZeneca Pharmaceuticals), a protein kinase inhibitor approved for use in antitumor therapy, also belongs to this class. Several compounds from this group were found to be effective in inhibiting laboratory CMV strains as well as clinical isolates of CMV resistant to ganciclovir and cidofovir [204]. Inhibition of CMV replication occurs during the early-late phase but not at the level of viral entry. This group of compounds is awaiting further pharmacological, toxicological, and possibly *in vivo* evaluation.

### US28 agonists

CMV US28 (and the related open reading frame [ORF] US27) is a chemokine receptor homolog believed to promote viral replication and maintain persistence by interference with leukocyte trafficking [205]. *In vitro*, US28 has been shown to bind and internalize ligands, as well as activate intracellular signaling in response to certain chemokines, and to initiate the migration of smooth muscle cells to chemokine gradients. US28 shows high homology with  $\beta$ -mammalian chemokine receptors, binds several CC-chemokines with high affinity [206-208], and is able to sequester CC-chemokines from the extracellular environment via endocytosis [209, 210]. This feature appears to be a putative strategy of the virus to escape immune surveillance by reducing the immune response to sites of CMV infection [211]. The US28 gene is abundantly expressed on the cell surface during the early stages of clinical infection and is therefore easily accessible to antiviral drugs [212]. Nevertheless, the exact role of US28 in the pathogenesis of CMV infection still remains to be elucidated.

Recently, [5-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2-diphenylpentanenitrile], a nonpeptidergic inverse agonist for the CMV-encoded chemokine receptor US28, was described [213]. The characterisation of this compound may provide the basis for the clinical application of recently licensed agonists of US28 [214]. The consequences of inhibition of the CMV encoded chemokine receptor US28, however, are hardly to fathom, as the role of US28 and its interaction with human chemokines are still in doubt.

## CURRENT & FUTURE DEVELOPMENTS

Prevention and therapy of CMV disease remains a challenge despite the availability of highly effective drugs. Emergence of drug resistant CMV strains, toxicities, poor oral bioavailability, and drug-drug interactions detract from the clinical usefulness of currently licensed drugs. Several promising antiviral drugs are on the horizon that differ significantly from approved drugs by mode of action, bioavailability, and metabolism. In the near future, some of these drugs may be useful alternatives or supplements in the prevention and therapy of CMV disease.

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