New inhibitors of human cytomegalovirus (HCMV) on the horizon

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There are at present five compounds that have been officially licensed for the treatment of human cytomegalovirus (HCMV) infections: ganciclovir [GCV, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, Cymevene, Cytovene] (1; Figure 1), which is administered intravenously at 10 mg/kg per day (2 x 5 mg/kg, every 12 h) for induction therapy and intravenously at 5 mg/kg once daily or orally at 3000 mg/day (3 x 4 x 250 mg capsules) for maintenance therapy (the latter also for prevention); ganciclovir can also be implanted intravitreally (Vitrasyset); foscarnet [trisodium phosphonoformate, phosphonoformic acid (PFA), Foscavir] (2), which is administered intravenously at 180 mg/kg per day (3 x 60 mg/kg, every 8 h) for induction therapy and at 120 mg/kg per day (3 x 40 mg/kg, every 8 h) for maintenance therapy; cidofovir [CDV, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, Vistide] (3), which is administered intravenously at 5 mg/kg per week during the first 2 weeks (induction therapy), and then 5 mg/kg every other week (maintenance therapy) with sufficient hydration and under cover of probenecid to prevent nephrotoxicity; fomivirsen (ISIS 2922, an antisense oligodeoxynucleotide composed of 21 phosphorothioate-linked nucleosides, Vitravene) (4), which is administered intraocularly (i.e. intravitreally); and valganciclovir (the valine ester of ganciclovir) (Valcyte) (5), which is administered orally (two 450 mg tablets twice daily, which gives equivalent systemic ganciclovir levels as intravenous ganciclovir 5 mg/kg twice daily). Oral valganciclovir is expected to replace intravenous ganciclovir, in both the therapy and prevention of HCMV infections. The principal indication for all these agents is HCMV retinitis in AIDS patients.1

All HCMV inhibitors that are currently approved for the treatment of HCMV infections, with the exception of fomivirsen, are targeted at the viral DNA polymerase. Foscarnet interacts directly with the pyrophosphate binding site of the DNA polymerase, whereas ganciclovir and cidofovir must first be phosphorylated, ganciclovir in three steps and cidofovir in two steps, to their triphosphate and diphosphate derivatives, which then interact as competitive inhibitors/alternate substrates (with respect to dGTP and dCTP, respectively) with the substrate binding site of the DNA polymerase. As alternate substrates, ganciclovir triphosphate and cidofovir diphosphate, will be incorporated (as ganciclovir monophosphate and cidofovir, respectively) into the growing DNA chain and block chain elongation. The first phosphorylation step in the intracellular metabolism of ganciclovir is catalysed by the HCMV-encoded protein kinase, a UL97 gene product, which thus accounts for a preferential phosphorylation of ganciclovir in the HCMV-infected cells. UL97 is also a primary site for mutations that may engender resistance towards ganciclovir. Obviously, such mutations do not lead to resistance to other anti-HCMV drugs such as foscarnet and cidofovir, as they do not require the intervention of UL97. Fomivirsen does not interfere with viral DNA synthesis: as it is complementary in base sequence, it hybridizes with, and thus blocks the expression (i.e. translation) of, the HCMV immediate early mRNA.2

The anti-HCMV drugs currently available suffer from a number of drawbacks that limit their clinical usefulness. Fomivirsen has to be injected intraocularly, foscarnet must be given intravenously three times daily, and cidofovir must also be administered intravenously albeit once weekly or every other week. Development of nephrotoxicity is the principal risk factor encountered with patients receiving cidofovir or foscarnet, whereas bone marrow suppression resulting in granulocytopenia and thrombocytopenia is the most common dose-limiting toxic effect seen with ganciclovir. Drug-resistant HCMV clinical isolates through mutations within the DNA polymerase gene (UL54) have been reported for both ganciclovir and foscarnet, and mutations affecting the protein kinase gene UL97 activity (see above) have proven to be predominantly present among clinical isolates recovered from patients after long-term ganciclovir therapy. Taken

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Figure 1. Inhibitors of HCMV. (1) Ganciclovir; (2) foscarnet; (3) cidofovir; (4) fomivirsen; (5) valganciclovir; (6) CMV423: 2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide; (7) pyrrolo[2,3-d]pyrimidines; (8) 7,8-dihydroisoquinoline-6-carboxyl [2-(1-indol-3-yl)ethyl]amide; (9) 4-hydroxyquinoline-3-carboxylic acid; PNU-181465; (10) 3-hydroxy-2,2-dimethyl-N-[4-[[5-dimethylamino]-1-naphthyl]sulphonyl]amino)-phenyl]propanamide: BAY 38-4766; (11) 1-(β-D-ribofuranosyl)-2-bromo-5,6-dichlorobenzimidazole: BDCRB; (12) 1-(β-L-ribofuranosyl)-2-isopropylamino-5,6-dichlorobenzimidazole: 1263W94, maribavir; (13) Gis 6976; (14) aminothiazolylphenyl derivative: BILS 179 BS; (15) N-[5-(aminosulphonyl)-4-methyl-1,3-thiazol-2-yl]-N-methyl-2-[4-(2-pyridinyl)phenyl]acetamide: BAY 57-1293; (16) dL-cyclohexenyl guanine; (17) HDP-cidofovir.
together, all these considerations justify the search for new anti-HCMV agents that are less toxic, more effective and orally bioavailable.

Snoeck et al. have recently described 2-chloro-3-pyridin-3-yl-5,6,7,8-tetrahydroindolizine-1-carboxamide (CMV423) (6) as a new lead compound for the treatment of HCMV infections: CMV423 showed very potent in vitro activity against a wide range of HCMV reference strains and clinical isolates, including those that had acquired resistance to ganciclovir, foscarnet or cidofovir. CMV423 also gave highly synergic activity when combined with ganciclovir, foscarnet or cidofovir. It was ascertained that CMV423 acts on a step of the viral replicative cycle that precedes the DNA polymerase step and, most likely, coincides with immediate early antigen expression.3

The antiviral activity profile of CMV423 is reminiscent of that of the pyrrolo[2,3-d]pyrimidines 828, 951 and 1028 (7), reported by Jacobson et al. to inhibit HCMV replication. Like CMV423, the pyrrolopyrimidines were found to be active against ganciclovir-resistant virus, showed a strict dependence on the multiplicity of virus infection and were assumed to interfere with an early step in the HCMV replicative cycle, before viral DNA synthesis and presumably coinciding with immediate early antigen expression (or functioning).4

Another series of compounds exhibiting potent activity against HCMV is that of the 1,6-naphthyridine and 7,8-dihydroisoquinoline carboxamides (8).5 Again, activity against ganciclovir-resistant HCMV strains was noted: synergic activity with ganciclovir was observed, and to the extent that the target of anti-HCMV action of the naphthyridines and dihydroisoquinolines was resolved, it could well correspond, as surmised for 6 and 7, to an early (post-adsorption) event of the HCMV replication cycle.5

Whatever the mechanism of action of 6, 7 and 8, it must be different from that of the 4-hydroxyquinoline-3-carboxamides, such as PNU-181465 (9), which were recently reported as broad-spectrum anti-herpesvirus agents, active against HCMV, as well as other herpesviruses, such as herpes simplex virus (HSV-1 and HSV-2) and varicella-zoster virus (VZV).6 The 4-hydroxyquinoline carboxamides also demonstrated potent inhibition of HCMV, HSV-1 and VZV DNA polymerases, and a strong correlation was found between their inhibitory effect on the viral DNA polymerases and their inhibitory effect on viral replication, pointing to the viral DNA polymerase as their target of action.6 Thus, 4-hydroxyquinoline carboxamides may represent a novel class of non-nucleoside inhibitors of herpesvirus DNA polymerase, and, in this sense, they bear some resemblance to the NNRTIs (non-nucleoside reverse transcriptase inhibitors) that have been described in the case of human immunodeficiency virus (HIV).

Further down the HCMV replicative pathway, the HCMV ‘terminase’, composed of the UL89 and UL56 gene products, has been recognized as a target for chemotherapeutic inter-

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vention. The UL89 and UL56 gene products are responsible for the cleavage of viral high-molecular-weight DNA concatemers and packaging of monomeric genomes into procapsids. Intervention at this stage may be expected to block DNA cleavage and packaging, leading to an accumulation of empty procapsids and unprocessed concatemeric DNA,7 as has been demonstrated with the (naphthylsulphonylamine)phenylpropanamide BAY 38-4766 (10). The fact that BAY 38-4766 targets a DNA maturation step that does not occur in normal eukaryotic cells makes this a highly specific strategy for combating HCMV infections.

BAY 38-4766 is not the first compound postulated to interfere with HCMV DNA maturation and processing. In 1998, Krosky et al. had already mapped resistance to TCRB (2,5,6-trichloro-1-β-D-ribofuranosyl benzimidazole) (another specific anti-HCMV agent) to mutations in the UL89 and UL56 gene products. That the class of β-D-ribofuranosyl benzimidazoles is really targeted at viral DNA maturation and processing was ascertained in a recent study by Biron et al. with another such derivative, namely BDCRB or 1-β-D-ribofuranosyl)-2-bromo-5,6-dichlorobenzimidazole (11). Also, this compound exhibited specific and potent HCMV activity, but, reportedly, proved unsatisfactory for further development because of its rapid metabolism to the inactive and toxic glycine.

Instead, the L-counterpart of BDCRB, namely 1263W94 (maribavir) (12), in which, in addition to the D→L configuration switch, the bromine was replaced by an isopropylamine moiety, was chosen for further development. Maribavir proved clearly more potent against HCMV than either BDCRB or ganciclovir, and it was also less toxic than ganciclovir to bone marrow cells in vitro.9 Also, maribavir proved active against HCMV strains resistant to ganciclovir. Surprisingly, maribavir appears to be targeted at the UL97 protein kinase, i.e. the same enzyme that is required for the phosphorylation of ganciclovir to its monophosphate (see above).

The UL97 protein kinase is not only able to phosphorylate ganciclovir, it is also capable of autophosphorylation, and UL97 autophosphorylation is a prerequisite for ganciclovir phosphorylation: both processes can be inhibited by indolocarbazoles,10 such as Gö 6976 (13). Like 1263W94, indolocarbazoles proved to be highly effective inhibitors of both ganciclovir-sensitive and ganciclovir-resistant HCMV strains, while not being effective against HSV.10

Although they are both targeted at the UL97 protein kinase, it is not clear whether maribavir and indolocarbazole Gö 6976 act by the same mechanism. The function of the UL97 gene product has been recently clarified;11 it is responsible for nuclear egress, i.e. the release of HCMV nucleocapsids from the nucleus. If so, maribavir and indolocarbazoles may be assumed to interfere with an even later stage of the HCMV replication cycle than BAY 38-4766 or BDCRB, that is the
exit of the nucleocapsids from the nucleus after DNA maturation and packaging have been completed. This, however, seems somewhat at odds with the purported inhibitory effect of 1263W94 on viral DNA synthesis in a single HCMV replication cycle assay.9

Maribavir has been the subject of preclinical pharmacokinetic and toxicological studies in mouse, rat and monkeys.12 These studies demonstrated a favourable safety profile, good to excellent oral bioavailability and lower toxicity than currently available anti-HCMV agents. Phase I/II dose escalation studies in HIV-infected men with asymptomatic HCMV 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 was rapidly absorbed following oral dosing and demonstrated in vivo anti-HCMV activity in semen, with mean reductions in semen HCMV titres of 2.9–3.7 log_{10} pfu/mL; maribavir was generally well tolerated, taste disturbance being the most frequently reported adverse event over the 28 day dosing period.13

Remarkable in vivo efficacy, in animal models of HSV-1 and HCMV-2 infection, has recently been reported for two classes of compounds (that bear a number of structural similarities), represented by BILS 179 BS (14) and BAY 57-1293 (15). These compounds also showed a highly similar activity profile, oral bioavailability and mechanism of action: they appear to be specifically targeted at the HSV DNA helicase–RNA polymerase (primase), an enzyme composed of the HSV UL5, UL8 and UL52 gene products, that precedes the DNA polymerase activity.14,15 The antiviral activity of BAY 57-1293 was quoted as superior to all compounds currently used to treat HSV infections based on its antiviral efficacy in various models of HSV infection.16 These data obviously validate the use of helicase–primase inhibitors for the treatment of HSV infections, but leave us with the question of whether this strategy would also work with herpesvirus infections other than HSV, in particular HCMV. This question has so far not been addressed.

The currently accredited anti-HCMV drugs, here represented by ganciclovir, are generally perceived as ‘nucleoside analogues’ with toxic side effects, poor oral bioavailability and risk for emergence of drug resistance. This pretext should, however, not deter the development of new nucleoside analogues as broad-spectrum anti-herpesvirus agents. In addition to ganciclovir, various other guanosine analogues have been found to exhibit interesting anti-HCMV properties,17 such as synguanol, anhydrohexitol guanine and, in particular, D- and L-cyclohexenyl guanine (16). A distinct advantage of these compounds is that, in principle, their activity also extends to the HCMV-related human herpesvirus type 6 (HHV-6).18

For cidofovir, the poor oral bioavailability has been recognized as a disadvantage limiting the wide usefulness of the compound. This problem can now be overcome by using alkoxyalkyl esters of cidofovir such as HDP-cidofovir (hexadecyloxypropyl-HPMPC) (17). This oral lipid produg of cidofovir not only has greater oral bioavailability, it also shows enhanced inhibition of virus replication in vitro compared with that of the parental cidofovir.19 This might make it feasible to use HDP-cidofovir for the oral treatment of all virus infections that are sensitive to the drug, including poxvirus infections,20 as well as herpesvirus (i.e. HCMV, HSV, VZV, HHV-6, etc.) infections.

In conclusion, the currently available anti-HCMV drugs (i.e. ganciclovir, foscarnet, cidofovir, fomivirsen, valganciclovir) suffer from a number of shortcomings (i.e. toxic side effects, poor oral bioavailability and/or risk for emergence of drug-resistant virus strains). This has prompted the search for new anti-HCMV agents that are more specific in their anti-HCMV action and, hopefully, will not show the shortcomings of the ‘older’ compounds. This search has yielded a wealth of novel compounds, most of which are, or behave as, non-nucleoside, i.e. CMV423, pyrrolopyrimidines, dihydroisoquinolines, 4-hydroxyquinoline carboxamides, (naphthylsulphonylamino)phenyl propanamide, β-D- and β-L-ribofuranosyl benzimidazoles and indolocarbazoles. All of these compounds act on a molecular target that is different from that of the ‘older’ compounds, targeted at the viral DNA polymerase. Of all these compounds, only one, i.e. the β-L-ribofuranosyl benzimidazole maribavir (1263W94), has so far been the subject of clinical trials. As these studies provided promising results, they should, hopefully, stimulate the development of other candidate anti-HCMV drugs as well.

References


