TOWARDS AN EFFECTIVE CHEMOTHERAPY OF VIRUS INFECTIONS: THERAPEUTIC POTENTIAL OF CIDOFOVIR [(*S*)-1-[3-HYDROXY-2-(PHOSPHONOMETHOXY)PROPYL]CYTOSINE, HPMPC] FOR THE TREATMENT OF DNA VIRUS INFECTIONS*

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The acyclic nucleoside phosphonate HPMPC [(*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine, cidofovir, Vistide®] has a unique profile among the antiviral agents in that it is active against a much broader spectrum of DNA viruses than any other antiviral agent, and, furthermore, shows a long-lasting antiviral activity, thus enabling infrequent dosing (for intravenous administration, as infrequent as once a week or every other week). HPMPC owes its antiviral activity to a selective inhibitory effect on viral DNA synthesis: as has been demonstrated for human cytomegalovirus (CMV), HPMPC leads to DNA chain termination following the incorporation of two consecutive HPMPC residues. The activity spectrum of HPMPC encompasses herpes-, adeno-, polyoma-, papilloma-, and poxviruses. It has been approved for the treatment of CMV retinitis in AIDS patients and has proved effective in the treatment of herpes simplex virus (HSV) infections (particularly those that are resistant to acyclovir), human papilloma virus (HPV) infections (*e.g.* anogenital warts and recurrent laryngeal papillomatosis) and poxvirus infections (*e.g.* molluscum contagiosum). It is now further explored for its therapeutic potential in the treatment of HSV, CMV and HPV infections, and various other DNA virus infections, including adenovirus infections (*e.g.* keratoconjunctivitis), polyomavirus infections such as PML (progressive multifocal leukoencephalopathy), poxvirus infections (*e.g.* molluscum contagiosum), Epstein–Barr virus (EBV)-associated infections, and human herpesvirus type 8 (HHV-8)-associated infections (*e.g.* Kaposi's sarcoma).
Key words: HPMPC; Cidofovir, Vistide®; DNA viruses; Herpesvirus; Polyomavirus; Adenovirus; Poxvirus; Papillomavirus.

Emanating from a collaborative undertaking that Dr A. Holy and I initiated in 1976, DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine was described a few years later[1] as the first acyclic nucleoside analogue with broad-spectrum antiviral activity. This followed shortly after the description of acyclovir as the first acyclic nucleoside analogue with

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narrow-spectrum antiviral activity, specifically, against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) (refs[2,3]). While the specific activity of acyclovir against HSV-1 and HSV-2 is dependent on the phosphorylation of acyclovir to its monophosphate by the virus-induced thymidine kinase (TK) (which can also be regarded as a deoxycytidine kinase[4,5]), DHPA owes its broad-spectrum antiviral activity to its action targeted at (*S*)-adenosylhomocysteine (SAH) hydrolase, a key enzyme involved in (*S*)-adenosylmethionine (SAM)-dependent transmethylation reactions required for the maturation of viral mRNAs (ref.[6]).

Although active against a broad spectrum of viruses, including, in particular, pox-, rhabdo-, paramyxo-, and arenaviruses, DHPA is not very potent against herpesviruses. In attempts to increase its potency against this important group of viral pathogens, we extended our studies to (*S*)-{[1-(adenin-9-yl)-3-hydroxyprop-2-yl]oxy}methylphosphonic acid or (*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine[7] (HPMPA)

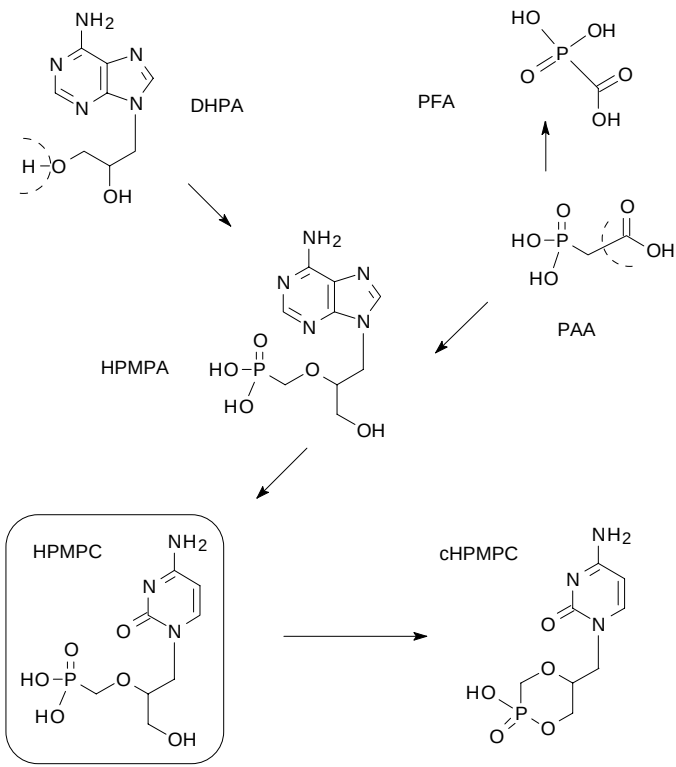

FIG. 1
HPMPA and HPMPC (cidofovir, Vistide®)

(Fig. 1). HPMPA can be regarded upon as a DHPA derivative bearing a phosphonomethyl ether moiety[7]. The phosphonate analogues PAA (phosphonoacetic acid) and PFA (phosphonoformic acid), now licensed for the treatment of certain herpesvirus infections) had been known for several decades as specific, although not very potent, anti-herpesvirus agents[8].

HPMPA was designed as a representative of enzymatically non-degradable isopolar and isosteric nucleotide analogues. Originally, this principle was expected to afford stable analogues of active antimetabolites (araAMP, araCMP, BrdUMP, *etc.*) (refs[9–17]). Later it was applied to acyclic nucleoside analogues, *e.g.* DHPA.

In contrast to acyclovir, HPMPA no longer needs the first phosphorylation step, that quite often represents the bottleneck in the intracellular phosphorylation pathway of nucleoside analogues and that for acyclovir is accomplished by the viral TK. Furthermore, the phosphonate group represents an isosteric, isopolar and non-degradable version of the phosphate group. If this group was to be attached to DHPA, it may be readily split off by cellular esterases.

HPMPA turned out to be effective against a broad range of DNA viruses, including the TK-deficient (TK^-) herpesviruses[18]. From HPMPA, HPMPC [(*S*)-1-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine] was derived by substituting the cytosine for the adenine ring, and this molecule (Fig. 2) was found to exhibit a similar potency and activity spectrum as HPMPA (ref.[19]).

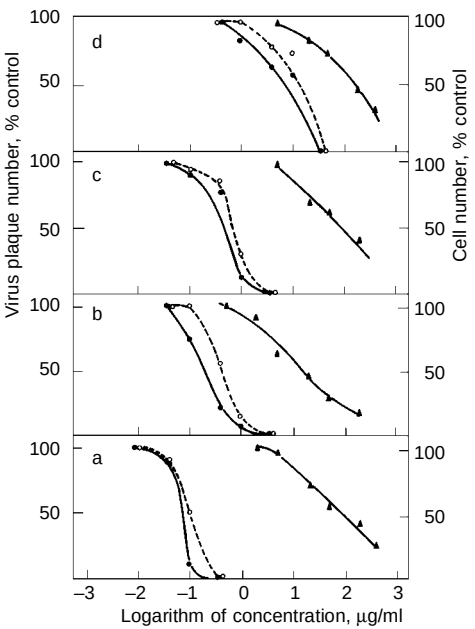

FIG. 2
Concentration-dependent inhibition of CMV plaque formation and host cell proliferation by (*S*)-HPMPC (a), (*S*)-HPMPA (b), ganciclovir (DHPG, c) and foscarnet (PFA, d) (ref.[30]). ● Davis strain, ❍ AD-169 strain, ▲ cell number

Antiviral Activity Spectrum

The antiviral activity spectrum of HPMPC is unique in that it extends to virtually all DNA viruses, including polyoma-, papilloma-, adeno-, herpes-, irido-, and poxviridae (Table I, ref.[20]). (For the inhibitory effects of HPMPC on polyomaviruses, see ref.[21].) Among the herpesviruses, all human herpesviral pathogens [including human herpes virus (HHV-8)] as well as veterinarily important herpesviral pathogens that were examined proved sensitive to HPMPC (Table II). (For the inhibitory effects of HPMPC on HHV-8, see refs[22,23].)

Of significant interest is the activity of HPMPC against those HSV and CMV strains that, because of a deficiency in either the thymidine kinase or protein kinase activity, resist the antiviral action of acyclovir and ganciclovir, respectively[24–26]. HPMPC has only slight activity against HBV (hepatitis B virus) (IC_{50}: 25 μg/ml, ref.[27]).

It should be pointed out that HPMPC is not active against HIV (human immunodeficiency virus) [although a recent study has indicated that in some cell lines (*i.e.* HeLa-CD4) HPMPC may effectively inhibit HIV replication[28]] and, in this respect, it differs from the other acyclic nucleoside phosphonates PMEA [9-(2-phosphonomethoxyethyl)adenine] and PMPA [(*R*)-9-(2-phosphonomethoxypropyl)adenine]. PMEA has marked activity against herpes-, hepadna-, and retroviruses, whereas the antiviral activity spectrum of PMPA is confined to retro- and hepadnaviruses. Both PMEA and

TABLE I
Antiviral activity spectrum of HPMPC[a] (cidofovir)

Polyomaviridae	
	Murine polyoma virus
	Human polyoma virus
Papillomaviridae	
	Rabbit papilloma virus
	Human papilloma virus (several types)
Adenoviridae	
	Human adenovirus (several types)
Iridoviridae	
	African swine fever virus (ASFV)
Hepadnaviridae	
	Hepatitis B virus (HBV)
Poxviridae	
	Vaccinia virus (VV)
	Molluscum contagiosum virus (MCV)

[a] Refs[20–23].

PMPA are under clinical development in their oral prodrug form [bis(POM)PMEA and bis(POC)PMPA, representing the bis(pivaloyloxymethyl) and bis(isopropyloxycarbonyloxymethyl) esters of PMEA and PMPA, respectively] for the treatment of HIV and HBV infections[29]. Bis(POM)PMEA is also referred to as adefovir dipivoxil (Preveon™).

Antiviral Selectivity and Mode of Action

The potential of HPMPC as an antiviral drug became apparent when its potency and selectivity against human cytomegalovirus (CMV) was compared to that of PFA (phosphonoformic acid, foscarnet) and DHPG [9-(1,3-dihydroxypropoxymethyl)guanine, ganciclovir], two compounds which in the mean time have been licensed for the treatment of CMV infections in humans[30]. In these experiments (Fig. 2), the anti-CMV activity was determined against two different strains (Davis, AD-169) and the cytotoxicity was monitored by the inhibitory effects of the compounds on host cell proliferation. From the dose-response curves for both antiviral activity and cytotoxicity (Fig. 2), selectivity indexes (safety margins) could be calculated as the ratios of the 50% cyto-

TABLE II
Antiviral activity spectrum of HPMPC (cidofovir) against herpesviruses[a]

Herpesviridae
Herpes simplex virus type 1 (HSV-1)
Herpes simplex virus type 2 (HSV-2)
Varicella-zoster virus (VZV)
Epstein–Barr virus (EBV)
Human cytomegalovirus (HCMV)
Human herpesvirus type 6 (HHV-6)
Human herpesvirus type 7 (HHV-7)
Human herpesvirus type 8 (HHV-8)
Thymidine kinase-deficient HSV (TK^- HSV)
Thymidine kinase-deficient VZV (TK^- VZV)
Protein kinase-deficient HCMV (PK^- HCMV)
Simian varicella virus (SVV)
Equine herpesvirus type 1 (EHV-1)
Bovine herpesvirus type 1 (BHV-1)
Bovine herpesvirus type 2 (BHV-2)
Murine cytomegalovirus (MCMV)
Rat cytomegalovirus (RCMV)
Guinea pig cytomegalovirus (GPCMV)
Murine herpesvirus type 68 (MHV-68)

[a] Refs[20,22,23].

toxic concentrations (CC_{50}, required to inhibit host cell proliferation by 50%) to the 50% antivirally effective concentrations (EC_{50}, required to inhibit virus plaque formation by 50%). The selectivity index for HPMPC was approximately 1 000, as compared to 100 for HPMPA, 100 for DHPG and only 10 for PFA (Fig. 2). This thus means that HPMPC inhibits virus replication at a concentration that is 1 000 times lower than the concentration required to inhibit host cell growth.

The apparent selectivity of HPMPC as an anti-CMV agent resides in the drug capacity to interfere specifically with viral DNA synthesis in the CMV-infected cells[31]. In uninfected human embryonic lung (HEL) cells, HPMPC inhibits DNA synthesis by 50% at a concentration of 100 μg/ml (Fig. 3). In CMV-infected HEL cells, HPMPC reduces viral DNA synthesis by 50% at a concentration of 0.1 μg/ml (Fig. 3), a concentration that is 1 000 times lower than the concentration required to inhibit normal cellular DNA synthesis. From these experiments it would appear that the anti-CMV activity of HPMPC is targeted at viral DNA synthesis.

How would HPMPC be able to achieve its suppressive effect on viral DNA synthesis? HPMPC as such is taken up by the cells (presumably by an endocytosis-like process) and intracellularly converted to three metabolites: HPMPC monophosphate (HPMPCp), HPMPC diphosphate (HPMPCpp) and HPMPCp-choline (Fig. 4, refs[32,33]). HPMPCp-choline may serve as an intracellular depot (or reservoir) form from which

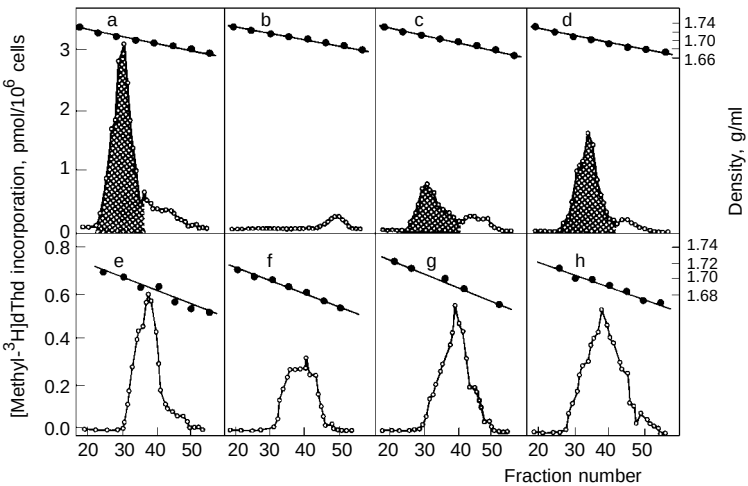

FIG. 3

Inhibitory effects of different concentrations of HPMPC [0.1 (d), 0.4 (c), 4, (b) 10 (h), 40 (g) and 100 (f) μg/ml] on viral DNA synthesis [in human embryonic lung fibroblasts infected with CMV (a–d)] and cellular DNA synthesis [in mock-infected cells (e–h)] (ref.[31]); a, e control. ● Density, ❍ [methyl-^{3}H]d Thd incorporation

the antivirally active metabolite HPMPCpp would be continuously generated, thus explaining the prolonged antiviral activity of HPMPC.

The main pathway in the intracellular anabolism of HPMPC would encompass two phosphorylation steps, catalyzed by the pyrimidine nucleoside monophosphate (PNM) kinase and a number of other phosphorylating enzymes [*i.e.* pyruvate kinase, creatine kinase and nucleoside diphosphate kinase), respectively[34]. The resulting HPMPCpp may then interact in one of the following ways at the level of viral DNA synthesis: (i) as a competitive inhibitor with respect to the normal substrate (dCTP); (ii) as an alternative substrate, being incorporated at either the 3′-end thus terminating the DNA chain, or *via* an internucleotide linkage in the interior of the DNA chain (Fig. 5). In fact, HPMPCpp has been shown to serve as an alternative substrate for the human CMV DNA polymerase[35].

Although, theoretically, HPMPCpp may interact with the viral DNA polymerase in one of the three ways illustrated in Fig. 5, its inhibitory effect on the human CMV DNA synthesis would mainly result from the incorporation of two consecutive HPMPC molecules (Fig. 6). Incorporation of one HPMPC molecule would cause a decrease in the rate of DNA elongation, without affecting the fidelity of the CMV DNA polymerase for the addition of the natural nucleotides; incorporation of a second HPMPC molecule immediately following the first would prevent further chain elongation, thus ensuring

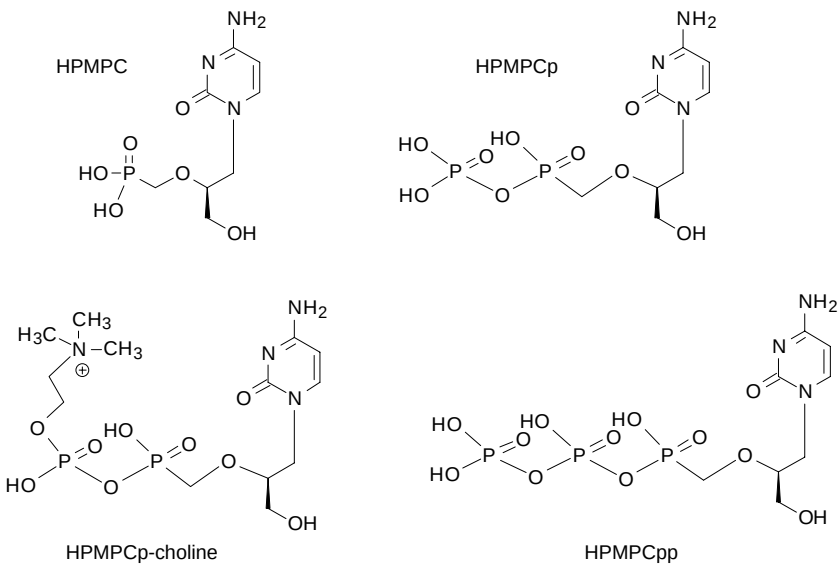

FIG. 4
HPMPC and the intracellular metabolites (HPMPCp, HPMPCpp and HPMPCp-choline) formed from HPMPC

chain termination[36] (Fig. 7). Human CMV DNA polymerase would be unable to excise incorporated HPMPC from DNA. The lack of repair of the viral DNA once it has incorporated HPMPC, together with the continuous regeneration of HPMPCpp from its HPMPCp-choline reservoir, may explain the long-lasting anti-CMV activity of HPMPC.

Animal Models

The efficacy of HPMPC has been demonstrated in a large number of animal models for herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infections, including thymidine kinase-deficient (TK^-) HSV-1 and HSV-2 infections, simian varicella virus (SVV) infections, murine cytomegalovirus (MCMV) infections, murine herpesvirus type 68 (MHV-68) infections, rat cytomegalovirus (RCMV) infections, equine herpes-

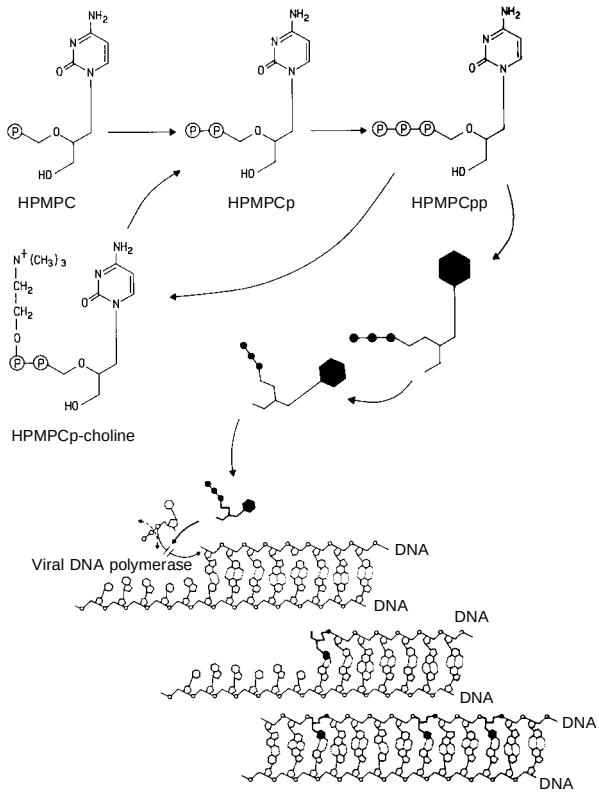

FIG. 5
Theoretically possible modes of action of HPMPC at the DNA level (competitive inhibition with respect to the natural substrate, dCTP, or incorporation at the 3′-end of the DNA chain (leading to chain termination), or incorporation internally into the DNA chain (allowing further chain elongation)

virus type 1 (EHV-1) infections, bovine herpesvirus type 1 (BHV-1) infections, vaccinia virus (VV) infections, adenovirus infections, polyomavirus infections and rabbit papillomavirus infections[20,21,37–40] (Table III).

The experimental animal infections in which HPMPC was found to be efficacious are reminiscent of all major DNA virus infections in humans, *viz.* mucocutaneous HSV-1 infections (primary herpetic gingivostomatitis, recurrent herpes labialis), mucocutaneous HSV-2 infections (primary and recurrent genital herpes), herpetic encephalitis, herpetic keratitis, adenovirus keratoconjunctivitis, varicella-zoster, human CMV disease manifestations (in the immunosuppressed host), Epstein–Barr virus (EBV)-associated diseases, poxvirus infections (*i.e.* molluscum contagiosum, monkey pox), polyomavirus infections [*i.e.* progressive multifocal leukoencephalopathy (PML)] and papilloma virus infections [human papilloma virus (HPV)-associated lesions]. If, therefore, HPMPC would be used in humans under the same conditions where it has been found effective against the animal model infections, it may be expected to be effective against their human counterparts as well.

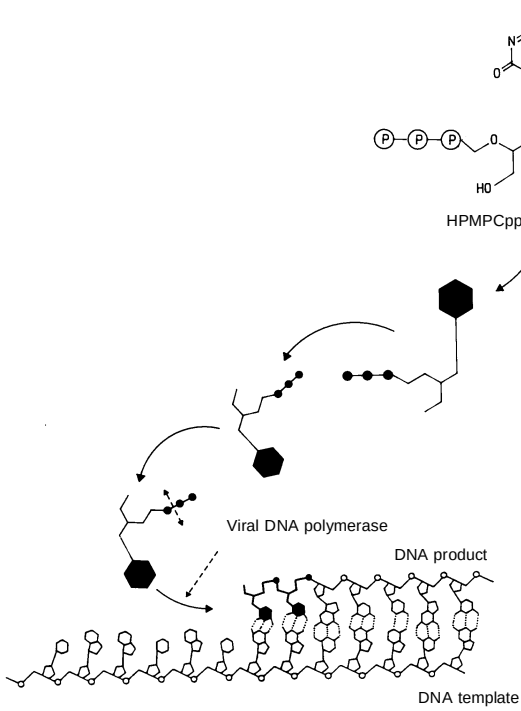

FIG. 6
In the specific case of human cytomegalovirus (CMV), HPMPCpp blocks CMV DNA chain elongation following two consecutive incorporations of HPMPC in the DNA product[36]

TABLE III
Animal models in which efficacy of HPMPC has been shown

1. Intracutaneous HSV-1 or HSV-2 infection in hairless mice
2. Intracutaneous TK^- HSV-1 infection in athymic-nude mice
3. Intracutaneous HSV-1 infection in guinea pigs
4. Intraperitoneal HSV-1 infection in NMRI mice
5. Intraperitoneal HSV-2 infection in Swiss mice
6. Intracerebral HSV-2 infection in NMRI mice
7. Intravaginal TK^+ HSV-2 or TK^- HSV-2 infection in mice
8. Herpetic (TK^+ HSV-1 or TK^- HSV-1) keratitis in rabbits
9. Herpetic (HSV-1) retinitis in rabbits
10. Intratracheal SVV infection in African green monkeys
11. Intraperitoneal MCMV infection in NMRI mice
12. Intracerebral MCMV infection in NMRI mice
13. Intrapritoneal MCMV infection in SCID mice
14. Intraperitoneal MCMV infection in LP-BM5 virus-infected C57BL/6 mice
15. Intraperitoneal RCMV infection in X-irradiated rats
16. Intraperitoneal RCMV infection in X-irradiated, allogeneic bone marrow-transplanted rats
17. Intranasal EHV-1 infection in Balb/c mice
18. Intranasal EHV-1 infection horses (foals)
19. Intanasal BHV-1 infection in calves
20. Intavenous VV infection in SCID mice
21. Murine herpesvirus type 68 infection in SCID mice
22. Murine polyomavirus infection in mice
23. Adenovirus keratoconjunctivitis in rabbits
24. Rabbir (Shope) papillomavirus infection in rabbits

Refs[20,21,37–40].

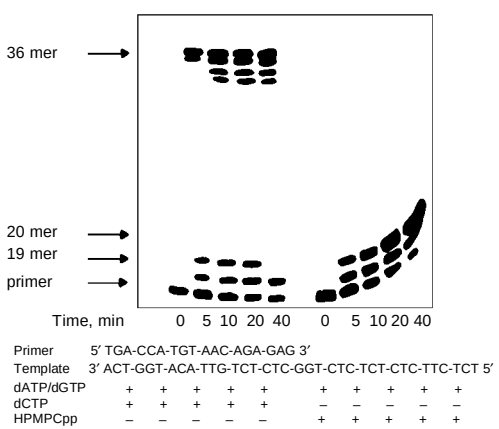

FIG. 7
Mechanism of inhibition of CMV DNA polymerase by HPMPCpp: incorporation of two consecutive molecules of HPMPC stops the DNA chain elongation by CMV DNA polymerase[36]

The effectiveness of HPMPC in one particular animal model, *i.e.* murine CMV infection in severe combined immune deficient (SCID) mice, is illustrated in Table IV. This experimental animal CMV infection can be considered as representative for human CMV disease in the immunocompromised host. In this model infection, HPMPC was directly compared to ganciclovir, since the latter has been the drug of choice for several years for the treatment of CMV complications (*i.e.* retinitis) in immunosuppressed (*i.e.* AIDS) patients. From the comparative study of HPMPC and ganciclovir in the murine CMV/SCID mouse model, it appears that HPMPC if administered at a dose of 2 mg/kg once weekly is almost as effective, and if administered at a dose of 10 mg/kg once weekly significantly more effective, than ganciclovir administered daily (5 times a week) at a dose of 20 or 50 mg/kg (Table IV). These data clearly indicate that HPMPC is far superior to ganciclovir in the treatment of (murine) CMV in the immunocompromised (murine) host[41].

Clinical Data

In a phase I/II clinical trial with intravenous HPMPC in patients with human immunodeficiency virus infection and asymptomatic CMV infection, prolonged and dose-dependent anti-CMV activity (demonstrated by clearing of CMV viruria), was observed[42] with cidofovir regimens of 3, 5 and 10 mg/kg. These observations have been confirmed in another phase I/II trial[43]. The dose-limiting toxicity was nephrotoxicity, which was dose-dependent and could be counteracted by the concomitant administration of oral probenecid (4 g: 2 g at 3 h before HPMPC injection, and 1 g at 2 h and again at 8 h

TABLE IV
Inhibitory effects of subcutaneous injection of HPMPC (as compared to ganciclovir) on mortality of SCID mice infected intraperitoneally with MCMV[a]

Compound	Number of injections per week	Dose mg/kg	Mean day of death
Ganciclovir	5	0	6.5 ± 1.2
	5	10	19 ± 0.57
	5	20	24.1 ± 5.8
	5	50	27.0 ± 3.0
HPMPC	1	0	6.3 ± 1.2
	1	2	23.4 ± 0.8
	1	10	55.0 ± 3.3
	1	20	82.0 ± 10.6
	1	50	>140

[a] According to data published in ref.[41].

after HPMPC injection), intravenous hydration (1 l saline) and extended dosing intervals (every 1 to 3 weeks) and early discontinuation/interruption for proteinuria. The highly tolerated weekly intravenous dose of HPMPC (under probenecid cover) was estimated[43] at approximately 5 mg/kg. Pharmacokinetics studies in HIV-infected patients indicated that approximately 90% of the intravenously administered HPMPC was recovered unchanged in urine within 24 h, and that active tubular secretion played a significant role in the renal clearance of HPMPC (ref.[44]). Concomitant oral probenecid decreased the renal clearance of HPMPC presumably by blocking its active tubular secretion. This observation provided further support for the clinical use of concomitant probenecid as a nephroprotectant during HPMPC therapy[44].

In a prospective, multicentered, randomized, controlled trial, forty-eight patients with AIDS and previously untreated peripheral *CMV retinitis* (Fig. 8) were randomized to immediate ($n = 25$) or deferred ($n = 23$) intravenous HPMPC 5 mg/kg once a week for 2 weeks (induction therapy), then once every other week (maintenance therapy), until retinitis progression or treatment-limiting toxicity. Intravenous saline and oral probenecid were administered concomitantly with each infusion to minimize nephrotoxic potential. Progression of retinitis was assessed through retinal photographs read by an ophthalmologist masked to randomization assignment[45]. The median time to progression of retinitis was 22 days in the deferred group *versus* 120 days in the immediate group ($p < 0.0001$) (Fig. 9a). Following progression of retinitis in the deferred group, 16 of the 23 patients crossed over to HPMPC treatment. The median time to progression for crossover patients was not reached (>140 days), as compared with 21 days for the deferred group ($p < 0.0002$) (Fig. 9b). Of note, only 1 of the 41 patients developed a new retinal lesion while receiving HPMPC. Neutropenia (15%) and proteinuria (12%), both asymptomatic, were the most common adverse events felt to be related to HPMPC treatment. Thus, HPMPC proved efficacious in delaying progression of previously untreated CMV retinitis in patients with AIDS (ref.[45]). In a further trial, HPMPC has also proved efficacious in delaying progression of relapsing CMV retinitis,

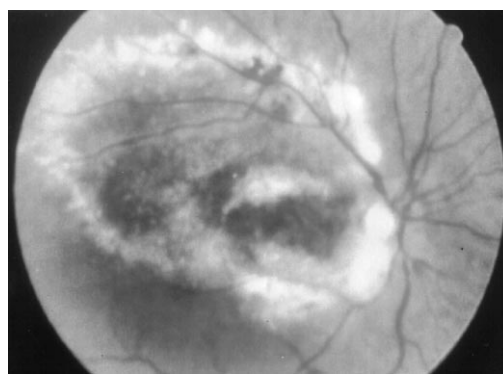

FIG. 8
Primary endpoint of phase II/III clinical trial of immediate *versus* deferred HPMPC in patients with AIDS and previously untreated peripheral CMV retinitis[45]

originally treated with ganciclovir [median time to progression: 49 or 115 days, following treatment with HPMPC at 3 or 5 mg/kg, respectively (data not shown).

Another trial, the HPMPC Peripheral Cytomegalovirus Retinitis Trial[46], was designed to evaluate the efficacy and safety of intravenous HPMPC in the treatment of CMV retinitis in patients with AIDS. In this randomized controlled trial only patients with previously untreated, small, peripheral CMV retinitis lesions (that is, patients at low risk of loss of visual acuity) were enrolled. Patients were randomly assigned to one of three groups: the deferred group, in which treatment was deferred until retinitis progressed; the low-dose group which received HPMPC at 3 mg/kg once every 2 weeks and the high-dose group which received HPMPC at 5 mg/kg once every 2 weeks (following the induction therapy in both the low-dose and high-dose group with HPMPC at 5 mg/kg once weekly for 2 weeks). To minimize nephrotoxicity, HPMPC was adminis-

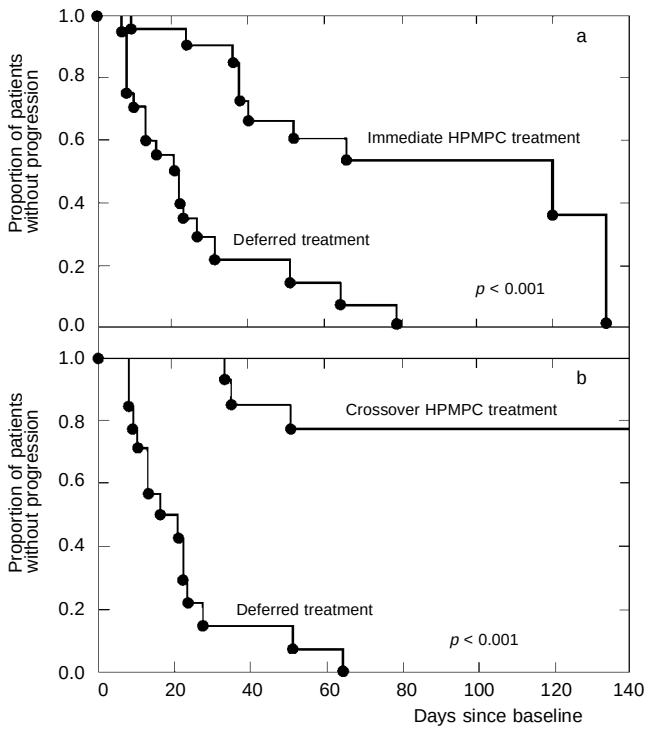

FIG. 9

Time of retinitis progression in phase II/III clinical trial in patients with AIDS and previously untreated peripheral CMV retinitis (Kaplan–Meier estimates)[45]. a HPMPC immediate vs deferred treatment, $p < 0.0001$; b HPMPC crossover *vs* deferred treatment, $p = 0.0002$

tered with hydration and probenecid. As shown in Figs 10 and 11, both the low-dose and, to a more pronounced extent, the high-dose HPMPC (cidofovir, CDV) slowed down the progression of CMV retinitis. Concomitant probenecid and hydration therapy minimized the risk of nephrotoxicity[46].

As compared to the two other drugs, ganciclovir and foscarnet (Fig. 12), that have been formally approved for the treatment of CMV retinitis in AIDS patients, HPMPC offers a much more convenient treatment regimen: *i.e.* for induction therapy (first three weeks) only two doses of HPMPC are required, as compared to 42 doses (2 doses daily) for ganciclovir and 63 doses (3 doses daily) for foscarnet; all the compounds to be given by the intravenous route. The fact that HPMPC has to be given only once a

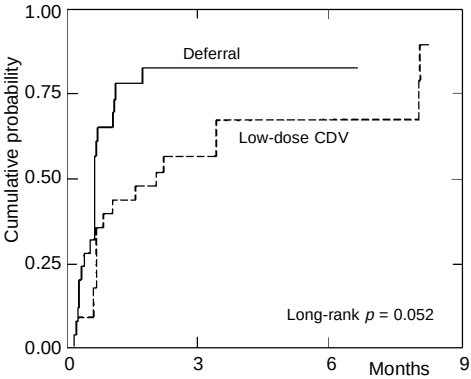

FIG. 10
Cumulative probability of progression of cytomegalovirus retinitis. Cidofovir (CDV) at a maintenance dose of 3 mg/kg once every 2 weeks (low-dose CDV), as compared with observations alone[46]

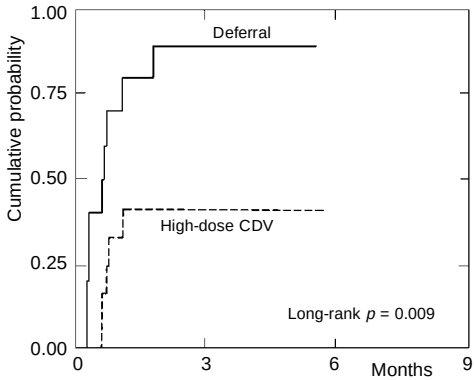

FIG. 11
Cumulative probability of progression of cytomegalovirus retinitis. Cidofovir (CDV) at a maintenance dose of 5 mg/kg once every 2 weeks (high-dose CDV), as compared with observations alone[46]

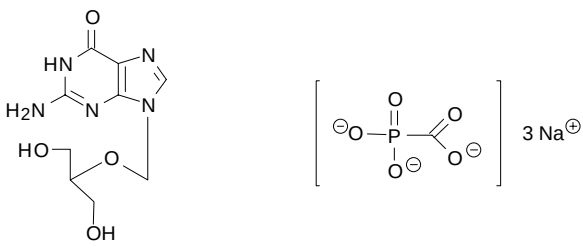

FIG. 12
Chemical structure of ganciclovir [GCV, 9-(1,3-dihydroxy-2-propoxymethyl)guanine, DHPG, Cytovene®] and foscarnet (trisodium phosphonoformate, PFA, Foscavir®)

week for induction, and only once every 2 weeks for maintenance obviates the need for long-term use of indwelling catheters. It should be noted that HPMPC must be given by the parenteral (*i.e.* intravenous) route, since its oral bioavailability is poor[47] (less than 5%).

A number of adverse events may possibly or probably be related to HPMPC (Table V). Of these adverse events, proteinuria (5 of 41 patients), neutropenia (6 of 41 patients), elevated creatinine levels (2 of 41 patients) were considered as serious[45]. Strict adherence to monitoring of renal function before HPMPC is implemented and concomitant administration of probenecid and saline hydration[48] (Fig. 13) should minimize the risk of drug-related nephrotoxicity.

Prolonged arrest of the progression of CMV retinitis was also obtained following intravitreal injection of HPMPC in patients with AIDS (refs[49,50]). Following a single intravitreal injection of 20 μg of HPMPC, progression of the disease was stopped for a median time of 55 days. If this injection was repeated, it took a median time of 63 days for the disease to progress[50]. As additional effects of the drug, a decrease in intraocular pressure, and a mild to moderate iritis in some eyes (that responded well to topical medication with corticosteroids) were observed. The ocular hypotensive effect of the drug merits further investigation.

TABLE V
Adverse events considered to be possibly or probably related to cidofovir[a]

Adverse event	Patients who received immediate treatment (n = 25)	Patient who crossed over to treatment (n = 16)	Total (n = 41)
	N		*n* (%)
Proteinuria	8	8	16 (39)
Asthenia	11	5	16 (39)
Neutropenia	7	4	11 (27)
Fever	6	4	10 (24)
Alopecia	4	6	10 (24)
Nausea with emesis	4	4	8 (20)
Nausea without emesis	6	2	8 (20)
Headache	6	1	7 (17)
Chills	5	1	6 (15)
Increased creatinine level	2	3	5 (12)
Pain	4	1	5 (12)

[a] All adverse events occurred in at least 10% of patients receiving cidofovir. According to data from the study of Lalezari *et al.*[45].

HPMPC has also proved useful in the treatment of HSV and VZV infections that had become resistant to acyclovir because of a deficiency in their thymidine kinase (TK^- HSV and TK^- VZV strains). Thus HPMPC was mentioned as a new topical drug, to be used as such, or alternating with systemic acyclovir, in the treatment of *severe mucocutaneous herpesvirus infections* in severely immunocompromised patients[51]. In two patients, an AIDS patient with a perineal HSV-2 infection resistant to acyclovir and a BMT (bone marrow transplant) recipient with an orofacial HSV-1 infection resistant to both acyclovir and foscarnet, topical treatment with 1% HPMPC (in Beeler base or Orabase) once a day for three consecutive days resulted in a complete regression of the lesions[52]. When the herpesvirus lesions recurred (because of the severely immunocompromised state of these patients), they again responded to the HPMPC treatment. Following the HPMPC treatment, the recurring virus appeared to have regained sensitivity to acyclovir, but then again developed resistance to acyclovir, so that HPMPC had to be reinstalled. At no point of the recurrent herpesvirus episodes had the virus developed resistance to HPMPC. These observations clearly indicated that HPMPC may be useful

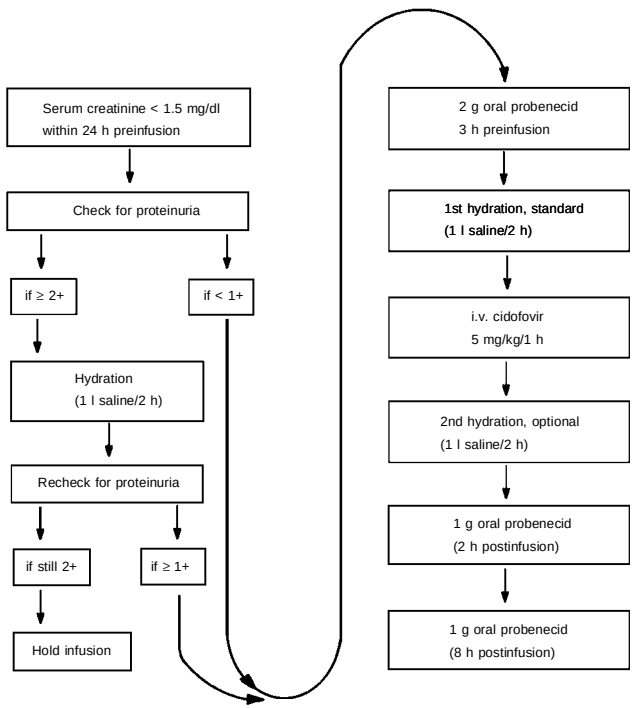

FIG. 13
Algorithm for cidofovir infusion[48]

in the treatment of acyclovir-resistant HSV infections and that topical HPMPC may be used alternately with systemic acyclovir in the treatment of alternately acyclovir-resistant and acyclovir-sensitive HSV infections[52]. The fact that TK^- VZV, while resistant to acyclovir, remains sensitive to HPMPC (ref.[53]), also points to the potential usefulness of HPMPC for the treatment of acyclovir-resistant VZV infections.

Lalezari *et al.*[54] have described the case of a patient with AIDS that had a six-month history of acyclovir-resistant perineal HSV-2 infection. Following four weekly doses of intravenous HPMPC at 5 mg/kg/week with concomitant prehydration (1 liter saline) and oral probenecid (4 g), an almost complete healing of the lesions was noted (Fig. 14). The perineal lesions recurred after the HPMPC treatment was interrupted (because of hypersensitivity to the concomitant probenecid treatment), but, again, responded to HPMPC when this was re-installed[54].

One of the patients that had entered the study to quantify the anti-CMV activity (monitored by CMV viruria) of HPMPC (ref.[42]), had bilateral *oral hairy leukoplakia*, presumably due to Epstein–Barr virus infection (Fig. 15). The lesions resolved within one week after a single intravenous administration of 5 mg/kg of HPMPC. This is the first (anecdotal) demonstration of the *in vivo* anti-EBV effect of HPMPC.

Patients with AIDS and clinical evidence of mucocutaneous HSV infection unresponsive to acyclovir were randomized to receive either placebo gel or cidofovir topical gel at 0.3 or 1% applied once a day for 5 days[55]. Primary endpoints included complete healing (*i.e.* complete re-epithelialization) and good healing (*i.e.* >50% decrease of total lesion area) rate and conversion to culture negativity; additional endpoints included complete healing rate, pain score changes (using a 0–10 scale) and safety. As shown in Table VI, cidofovir topical gel provided statistically significant effects on the rate of healing of the HSV lesions and the time to cessation of viral shedding and pain

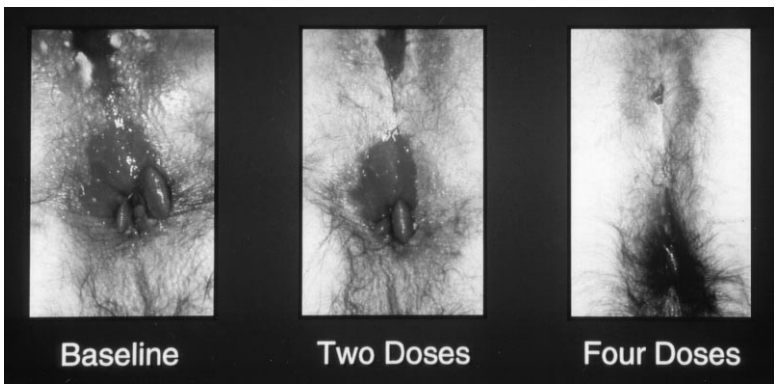

FIG. 14
Response of perineal acyclovir-resistant HSV-2 infection to intravenous HPMPC following two doses and four doses of HPMPC at 5 mg/kg/week (refs[42,54])

diminution. The cidofovir gel was extremely well tolerated with no evidence of significant systemic or local toxicity. From this study[55] it was concluded that cidofovir topical gel is safe and effective for the treatment of HSV lesions unresponsive to acyclovir.

In a randomized, double-blind, placebo-controlled trial in immunocompetent patients with *recurrent genital herpes*, presenting themselves within 12 h of lesions outbreak, cidofovir topical gel was administered at three dose levels (1, 3 or 5%) as a single

TABLE VI
Randomized, double-blind, placebo-controlled study of cidofovir topical gel in AIDS patients in the treatment of mucocutaneous HSV lesions that were refractory to acyclovir[a]

	Placebo	Cidofovir			All cidofovir *vs* placebo
		0.3%	1%	all	
Number of patients	10	11	9	20	–
Complete + good healing	0	6	4	10	$p = 0.008$
Complete healing	0	3	3	6	$p = 0.047$
Conversion to HSV culture negativity/number evaluable	0	7/9	6/6	13/15	$p = 0.00004$
Days to culture negativity (median)	–	7	2	2	$p = 0.0001$
Change in mean pain (area under the pain intensity––time curve)	–0.34	–2.29	–1.34	–1.86	$p = 0.039$

[a] According to Lalezari *et al.*[55].

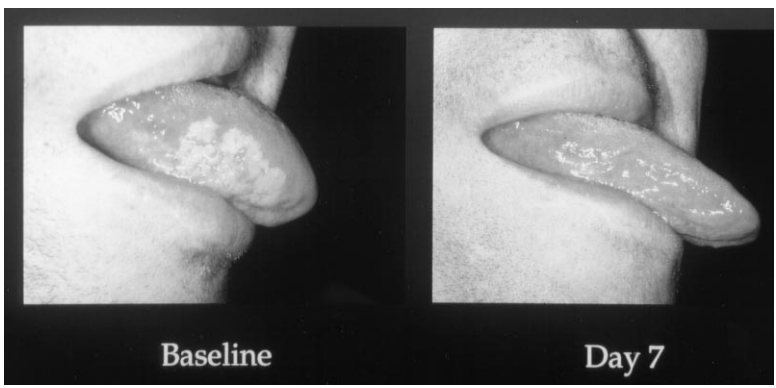

FIG. 15
Resolution of oral hairy leukoplakia (presumably caused by EBV) following a single intravenous HPMPC dose of 5 mg/kg (ref.[42])

application on the first day of the study[56]. Observations were made twice a day through complete healing, and endpoints included the time to HSV culture negativity, the time to complete healing, the duration of lesion-associated symptoms, and safety. As shown in Table VII, this single-dose cidofovir topical gel therapy significantly decreased time to HSV culture negativity. It was also associated with the evidence of more rapid healing of the genital herpes lesions. Cidofovir topical gel was well tolerated at all dose levels, although delayed healing at a high (5%) dose was observed in some patients[56].

Prompted by the knowledge that HPMPC had a suppressive effect on the growth of papilloma in rabbits[40] (Fig. 16), and that it could be safely administered to humans at doses not exceeding 3–5 mg/kg (intravenously), we endeavored to treat a 69-years-old woman with an enormous *squamous papillomatous tumor of the hypopharynx* (Fig. 17a). The tumor showed evidence of malignant degeneration, it was positive for HPV types 16 and 18 [as based on the polymerase chain reaction (PCR)], had relapsed after surgery and had also failed to respond to laser photocoagulation and α-interferon treatment[57]. The patient was treated with local injections of HPMPC (diluted in 20 ml saline), injected at 1.25 mg/kg body weight with a sclerosing needle, through the biopsy channel of a video-endoscope, directly into the tumor. The injections were given from March 23, 1993 to July 13, 1993 on seven different occasions, the first four injections at an interval of 1 week, the next three injections at 3- to 5-weeks intervals[48]. A signi-

TABLE VII
Randomized, double-blind, placebo-controlled study of single-dose cidofovir topical gel in the treatment of recurrent genital herpes in immunocompetent patients[a]

	Placebo	Cidovir, %			All cidovir
		1	3	5	
Time conversion to HSV culture negativity					
Number of patients	27	15	15	20	50
Median days	3.04	2.15 (p = 0.018)	1.31 (p = 0.0001)	1.10 (p = 0.0003)	1.30 (p = 0.0001)
Time to complete healing					
Number of patients	32	20	21	23	64
Median days	5.00	4.25 (p = 0.12)	4.07 (p = 0.16)	4.58 (p = 0.57)	4.22 (p = 0.49)
Application site reactions (*i.e.* erythema, ulcer, discoloration, pain, *etc.*)					
Number of patients with reaction/total	1/32	1/20	4/21	5/23	–

[a] According to Sacks *et al.*[56].

ficant decrease in the volume of the tumor was seen already after the second HPMPC injection (Fig. 17b). Upon the subsequent injections, the lesions became smaller (Figs 17c–17e) until they completely disappeared (Fig. 17f). Now, more than 4 years later, the patient is still disease-free[57].

Respiratory papillomatosis is a rare and often severe disease, usually localized in the larynx. It may cause respiratory distress and even life-threatening obstruction of the airways. Treatment is generally based on the evaporation of the lesions with a CO_2-laser, but microsurgery, cytotoxic and/or cytostatic drugs, interferons and vaccines are also used. Cidofovir was shown to suppress the growth of tumors induced by rabbit papillomavirus as well as human papillomavirus (HPV). The efficacy of cidofovir was assessed in 17 patients with severe respiratory papillomatosis[58]. Cidofovir at a concentration of 2.5 mg/ml was injected directly in the different laryngeal papillomatous lesions during microlaryngoscopy, the patient being under general anaesthesia. Biopsies were taken before the treatment was started both for anatomopathology and viral typing. HPMPC kinetics in serum was monitored in three patients, the drug levels being determined by HPLC. Complete disappearance of the papillomatosis was observed in 14 patients. Of them, four developed relapses that were again successfully treated with cidofovir. Of the three remaining patients, one progressed while under treatment with cidofovir, after an initial spectacular response. One patient had a partial remission and remained stable for more than one year after the last injection. He had a very aggressive and extensive disease originally. Finally, one patient was lost for the follow-up after 4 injections. Intratumoral injections of cidofovir for the treatment of severe laryngeal papillomatosis proves to be a potent new therapeutic approach for this disease. Treatment was well tolerated and no significant side effects were noted[58].

HPMPC has also been successfully used in the topical treatment (1% ointment or gel) of *anogenital HPV infections, i.e.* in three AIDS patients with severe, relapsing, penile, perigenital/intraanal or cervical/vulvar condylomata, respectively[59]. In all three cases, HPV type 16 was identified. Following topical HPMPC treatment the lesions disappeared, and the patients have remained free of disease during the 6- to 12-months follow-up period.

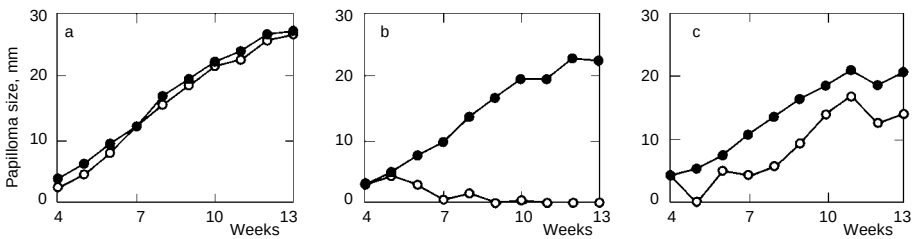

FIG. 16
Cidofovir (1%) topical gel (b), in comparison with Condilox (0.5% podophyllotoxin) (c), treatment of rabbit papillomavirus infection. a Vehicle. Treatment (twice daily for 5 days a week) started at 4 weeks post infection and continued for 8 weeks[40]

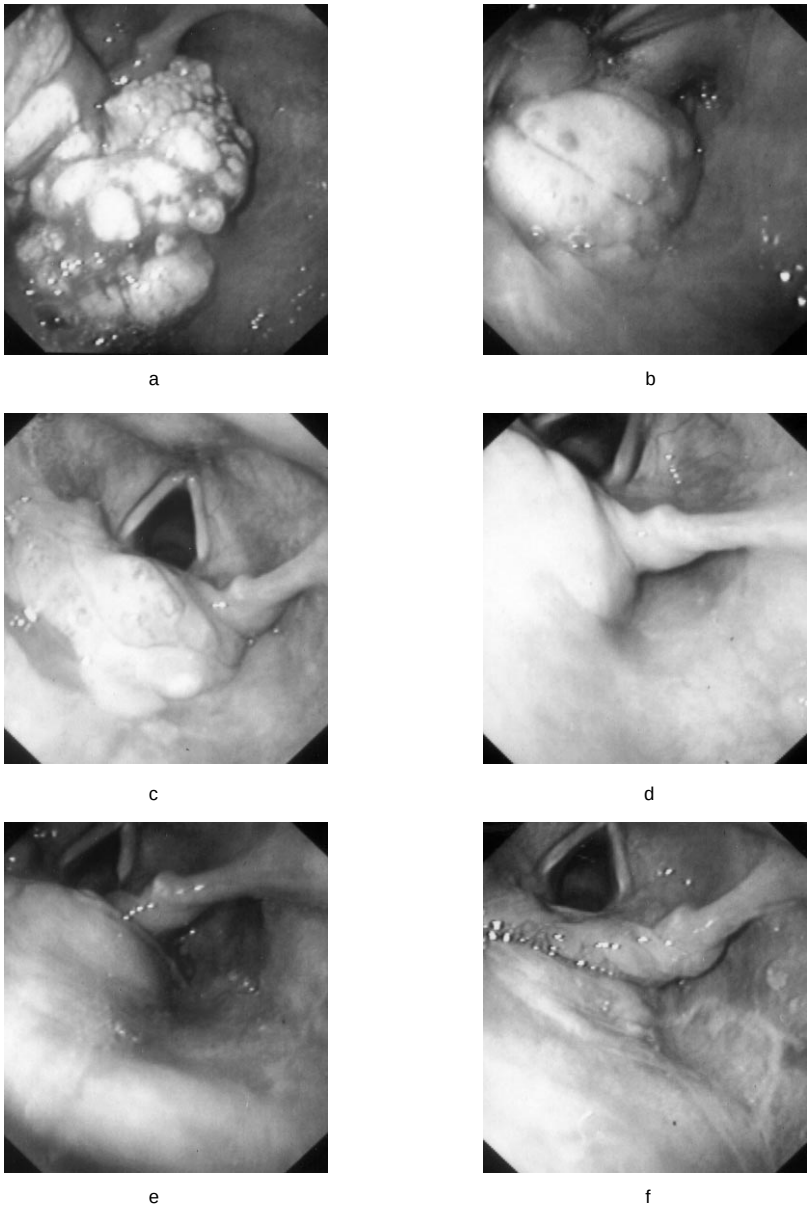

FIG. 17

Total regression of squamous papilloma of the hypopharynx following 7 local intratumoral injections of HPMPC (at 1.25 mg/kg body weight) (from March 23, 1993 to July 13, 1993) (ref.[57]). Pictures taken on: a March 23, 1993 (before the first injection); b April 8, 1993; c April 15, 1993; d May 6, 1993; e June 8, 1993 and f September 2, 1993

Recent case reports point to the therapeutic potential of HPMPC in the treatment of CMV encephalitis, CMV oesophagitis, molluscum contagiosum and progressive multifocal leukoencephalopathy. In one case[60], cidofovir effected a complete resolution of CMV retinitis, *CMV encephalitis and CMV oesophagitis* after only 2 months of intravenous cidofovir therapy and after initial attempts to stop the disease with ganciclovir and foscarnet had been unsuccessful.

In another three patients with HIV infection[61], cidofovir (given intravenously at 2 or 5 mg/kg, once a week for 2 weeks, and thereafter once every 2 weeks, or when applied topically as a 3% cream) led to a complete resolution of advanced *molluscum contagiosum* lesions. The lesions that had not responded to any other treatment resolved completely within 1 month of cidofovir treatment (as illustrated[61] for one of the patients treated with intravenous cidofovir in Figs 18 and 19). This impressive, albeit anecdotal, response of molluscum contagiosum justifies further controlled trials with cidofovir in

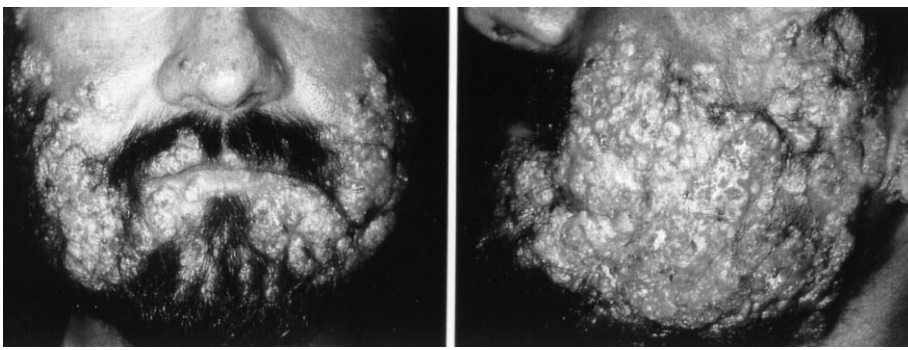

FIG. 18
Molluscum contagiosum in a HIV-positive individual (front and side view)[61]

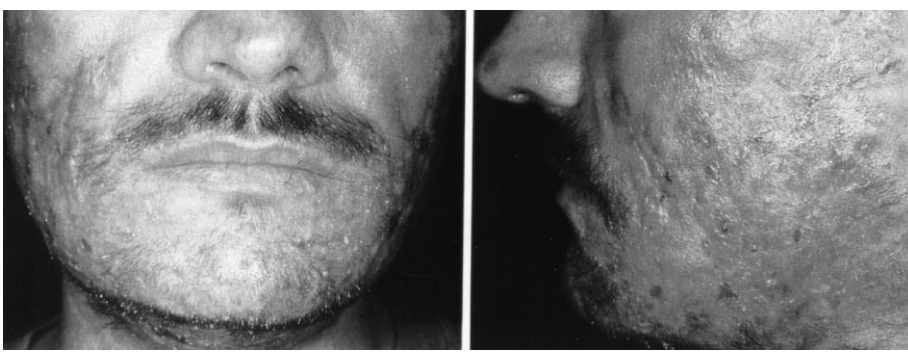

FIG. 19
Same patient as in Fig. 18, 2 months after treatment with intravenous cidofovir (2 mg/kg once a week for 2 weeks, followed by 2 mg/kg every 2 weeks) was started (front and side view)[61]

the treatment of molluscum contagiosum, especially (but not exclusively) in HIV-infected persons.

Finally, two patients with PML (progressive multifocal leukoencephalopathy) and rapidly progressive neurologic deterioration showed resolution of neurologic abnormalities within 2 months after intravenous treatment with cidofovir (5 mg/kg weekly for 2 weeks, then biweekly) and remained stable for at least 7–9 months[62]. These observations warrant further evaluation of cidofovir in the therapy of PML.

Resistance

Following exposure to increasing concentrations of HPMPC *in vitro*, human CMV can develop moderately reduced susceptibility to HPMPC (ref.[63]), due to a mutation (K513N) in the CMV DNA polymerase gene[64]. *In vitro* selected ganciclovir-resistant mutants carry mutations in both the DNA polymerase gene (K513N, M812L) and protein kinase UL97 gene (M460V). *In vivo*, the UL97 mutation would arise first, conferring low-level ganciclovir resistance. Upon further ganciclovir therapy, the DNA polymerase mutation may develop, and both the UL97 and DNA polymerase mutations combined may then develop high-level ganciclovir resistance.

To date, there has been no demonstration of development of resistance to HPMPC resulting from the HPMPC treatment *in vivo* (ref.[65]). All human CMV isolates obtained from patients receiving first-line HPMPC showed complete susceptibility to HPMPC (ref.[64]). Some of the isolates obtained from patients that had received second-line HPMPC (following ganciclovir) therapy did show reduced susceptibility to cidofovir and high-level resistance to ganciclovir. It is not clear, however, whether HPMPC or ganciclovir are responsible for this resistance pattern. Importantly though, there was no difference in time to retinitis progression on cidofovir therapy when comparing patients with CMV isolates that were sensitive or showed reduced susceptibility to HPMPC. This contrasts with the findings for ganciclovir, where patients with ganciclovir-resistant isolates progressed more quickly than patients with sensitive isolates. While for ganciclovir development of (high-level) resistance has been associated with clinical failure, it has so far not proved possible to demonstrate such association for cidofovir.

Conclusion

HPMPC (cidofovir, Vistide®) has been approved for the treatment of CMV retinitis in AIDS patients. It has also proved efficacious in the treatment of mucocutaneous HSV infections (particularly when resistant to acyclovir) and HPV infections (particularly recurrent laryngeal and hypopharyngeal/esophageal papillomatosis, anogenital warts and CIN grade III, although all these efficacy findings remain to be confirmed by placebo-controlled trials). There are various other DNA virus infections for which HPMPC seems to be indicated, such as adenovirus-, VZV-, EBV-, CMV- and HHV-8-

associated infections, Kaposi's sarcoma, molluscum contagiosum and progressive multifocal leukoencephalopathy (PML) (Table VIII). HPMPC is now under investigation, as either systemic (intravenous) or topical formulation (gel, eyedrops, solution for direct intralesional injection) for the treatment of these different DNA virus infections.

TABLE VIII
Major clinical indications for which the efficacy/usefulness of HPMPC has been investigated, demonstrated and approved

	Investigated	Demonstrated	Approved
Systemic (intravenous)			
CMV retinitis (in AIDS patients)	X	X	X
HSV and VZV infections (particularly when resistant to acyclovir) in immunocompromised patients	X	X	
EBV-associated diseases	X (to be explored)	X (suggested)	
CMV-associated diseases (other than retinitis)	X (to be explored)		
HHV-8-associated diseases (*i.e.* Kaposi's sarcoma)	X		
Polyomavirus infections (progressive multifocal leukoencephalopathy, PML)	X	X (suggested)	
Molluscum contagiosum (in AIDS patient)	X	X	
Topical			
HPMPC gel: Mucocutaneous HSV infections (particularly when resistant to acyclovir)	X	X	
HPMP gel: Recurrent genital herpes	X	X	
HPMP gel: Molluscum contagiosum	X	X	
HPMPC gel: Anogenital warts	X	X	
HPMPC gel: CIN (Cervical intraepithelial neoplasia grade III)	X	X	
HPMPC intratumoral: Recurrent laryngeal papillomatosis	X	X	
HPMPC intratumoral: Hypopharyngeal/esophageal pappilomatosis	X	X	
HPMPC intravitreal: CMV retinitis (in AIDS patients)	X	X	
HPMPC eye drops: Herpetic keratitis/adenovirus keratoconjunctivitis	X	X (suggested)	

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