Drugs of the Future 1998, 23(11): 1168-1175

CCC: 0377-8282/98

Fomivirsen Sodium

Antiviral

Prop INNM;USAN

ISIS-2922
Vitravene™

2'-Deoxy-*P*-thioguanylyl-(5'→3')-2'-deoxy-*P*-thiocytidylyl-(5'→3')-2'-deoxy-P-thioguanylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-2'-deoxy-*P*-thioguanylyl-(5'→3')-2'-deoxy-*P*-thiocytidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-2'-deoxy-*P*-thiocytidylyl-(5'→3')-*P*-thiothymidylyl(5'→3')-*P*-thiothymidylyl-(5'→3')-2'-deoxy-*P*-thiocytidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-2'-deoxy-*P*-thiocytidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-*P*-thiothymidylyl-(5'→3')-2'-deoxy-*P*-thioguanylyl-(5'→3')-2'-deoxy-*P*-thiocytidylyl-(5'→3')-2'-deoxyguanosine eicosasodium salt

d(P-thio)(G-C-G-T-T-T-T-G-C-T-C-T-T-C-T-T-C-T-T-G-C-G)deoxyribonucleic acid eicosasodium salt

$C_{204}H_{243}N_{63}Na_{20}O_{114}P_{20}S_{20}$ Mol wt: 7122.16

CAS: 160369-77-7
CAS: 144245-52-3 (as free acid)

EN: 196030

Synthesis

The synthesis of fomivirsen sodium has been performed by three similar ways:

1) On a Milligen 8800 automated synthesizer using a modified cycle in order to include the sulfurization using the Beaucage reagent. Standard, commercially available synthons were used in the synthesis. It has been shown that the use of only two equivalents of each amidite gives excellent average coupling efficiency (1).

2) Using an automated DNA synthesizer (Applied Biosystems model 380B) with the hydrogenphosphonate chemistry in a standard manner. After the final coupling step, the phosphorothioate linkages are generated by oxidizing the bound oligomer with sulfur in CS_2/triethylamine/pyridine. After the sulfur oxidation, standard deblocking procedures using NH_4OH are used to release the oligonucleotides from the support and to eliminate the blocking groups (2).

3) Using an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry and oxidation with 3*H*-1,2-benzodithiol-3-one 1,1-dioxide in order to obtain the sulfurization of the phosphite linkages. The deblocking and cleavage from the column is performed with concentrated NH_4OH (3, 4).

Introduction

Opportunistic infections are a concern due to the high incidence of diseases or states associated with immunosuppression such as those in AIDS patients, transplant recipients and the aged population. Cytomegalovirus (CMV) is the most common viral opportunistic infection in patients with AIDS, usually resulting in retinitis. The infectious cycle and molecular biology of CMV have been well characterized (5-7). These highly species-specific herpes viruses are very common and cause mild or subclinical infection in normal immunocompetent individuals usually not requiring treatment. Antibodies against human CMV (HCMV) have been observed in up to 90% of female prostitutes and homosexual males, and more than 50% of woman treated for other sexually transmitted diseases also have HCMV antibodies (8). In immunocompromised individuals incapable of suppressing the virus, HCMV is an opportunistic pathogen with severe ramifications including mortality. HCMV is the most common disease in HIV-infected patients with frequency of incidence related to CD4 cell counts; patients with CD4 counts >100 rarely develop active HCMV (9). The most frequent manifestation of HCMV in HIV-infected patients is cytomegalovirus retinitis (CMVR). It is estimated that unilateral or bilateral CMVR occurs in 15-40% of HIV-infected patients with a rise in incidence observed with increasing severity of immunosuppression (10). At present, no cure or long-term preventive therapy is available for CMVR. Although chronic daily infusion or oral therapy with approved antiviral agents such as ganciclovir, cidofovir and foscarnet is

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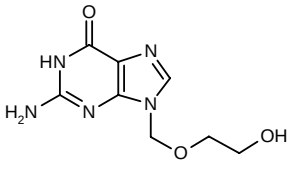
available, most patients diagnosed with CMVR experience progression of disease and eventual visual loss. In addition, a decrease in the quality of life is observed in patients due to the necessity of an indwelling catheter. Moreover, cross-resistance, reduced effectiveness due to decreased bioavailability and renal and other toxicities are associated with existing therapies (11). The chemical structures and status of development of anti-CMV agents are shown in Table I .

Based on antisense technology, scientists at Isis developed a phosphorothioate oligonucleotide, fomivirsen sodium (ISIS-2922), complementary to mRNA encoding the regulatory proteins of the immediate early region 2 of HCMV. Fomivirsen was chosen for further

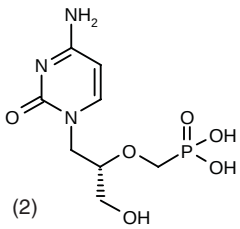
Table I: Anti-cytomegalovirus drugs launched and in clinical development.

Launched

1. Aciclovir (*Zovirax*)
   Glaxo Wellcome
   L-1981
2. Cidofovir (*Vistide*)
   Gilead; Pharmacia & Upjohn
   L-1996
3. Fomivirsen sodium (*Vitravene*)
   Isis Pharm.; Ciba Vision
   L-1998
4. Foscarnet sodium (*Foscavir*)
   Astra
   L-1989
5. Ganciclovir sodium (*Cytovene* capsules; *Vitrasert* implant)
   Roche; Chiron Vision
   L-1988



(1)



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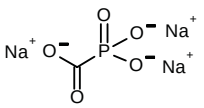

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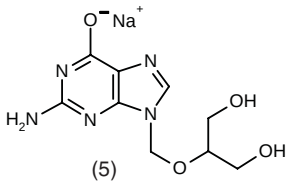

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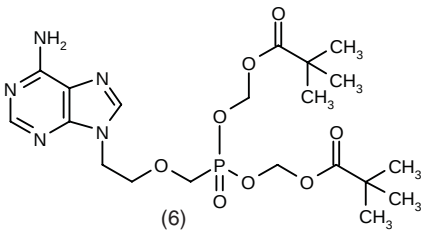
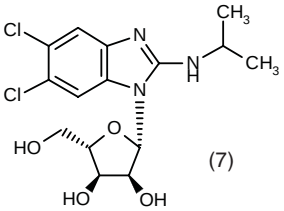


(6)

Clinical Trials

6. Adefovir dipivoxil
   Gilead; Bristol-Myers Squibb
   Phase III
7. Benzimidavir
   Glaxo Wellcome
   Phase II
8. BMS-200475, SQ-34676
   Bristol-Myers Squibb
   Phase I
9. Lobucavir
   Bristol-Myers Squibb
   Phase III
10. MDL-101731, KW-2331
    Hoechst Marion Roussel; Kyowa Hakko; Matrix
    Phase III
11. Penciclovir (*Vectavir*)
    SmithKline Beecham
    Clinical testing*
12. Valganciclovir (RS-79070)**
    Roche
    Phase II/III



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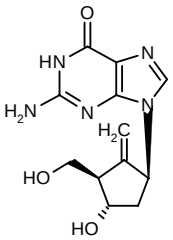

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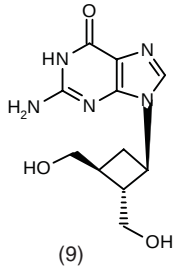

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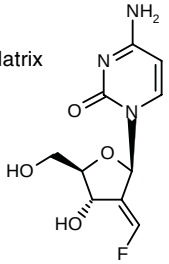

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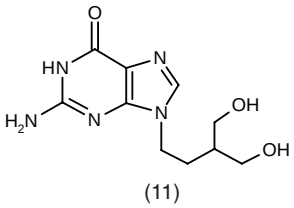
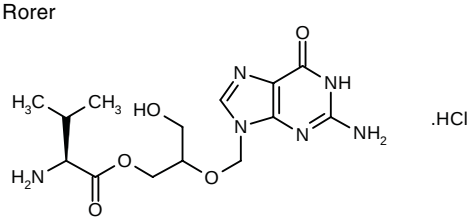


(11)

Preclinical Trials

13. RPR-111423
    Rhône-Poulenc Rorer



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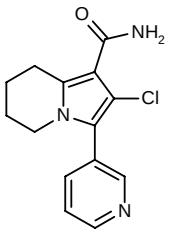

(13)

*Marketed for the treatment of herpes labialis. **Prodrug of ganciclovir. Source: Prous Science Ensemble database.

Table II: In vitro *antiviral activity of selected compounds against human cytomegalovirus.*

Compound	Antiviral activity IC_{50} (μM)	Cytotoxicity CC_{50} (μM)	Tissues Antiviral activity/Cytotoxicity	References
Aciclovir	50.7[b]	906	Human embryonic lung fibroblast/Human embryonic lung fibroblast	32-34,43
Adefovir	117	160	Human embryonic lung fibroblast/Human T-lymphoblastoid cells	34,44
Benzimidavir	0.03	-	Not reported	35
	0.11		Human fibroblast	36
Cidofovir	0.24[a]	298	Mouse mammary cancer cells/ Human embryonic lung fibroblast	37,45
Fomivirsen	0.2-0.7	300-500	Human dermal fibroblast/Human dermal fibroblast	9
	0.23[b]		Human dermal fibroblast	9,10
Foscarnet sodium	35[b]	-	Human dermal fibroblast/Human blood mononuclear cells	9,38,47
Ganciclovir	2.5[b]	159	Human fibroblast/Human blood mononuclear cells	9,10,47
	2.58	-	Human embryonic lung fibroblast	33
Lobucavir	1.9[b]	37	Human adenocarcinoma cells/Human lymphoblastic leukemia cells	39-41,48
MDL-101731	0.012	-	Human fibroblast	2
Penciclovir	125.6	132	Human embryonic lung fibroblast/Not reported	32,49
RPR-111423	0.022[c]	72.4	Human embryonic lung fibroblast/Human embryonic lung fibroblast	50
SQ-34676	15	120	Human fibroblast/Human lymphoblastoid cells	43

[a]Antiviral activity against murine cytomegalovirus.[b]Mean value calculated from different references that used the same experimental method. [c]Mean value calculated from activities against different virus strains (AD-169 and Davis strains). Source: Prous Science MFLine database.

development based on its ability to inhibit HCMV replication and infection with a higher potency than that of ganciclovir or foscarnet without interfering with anti-HIV agents such as AZT and dideoxycytidine (ddC). The *in vitro* antiviral activities of fomivirsen and other selected antiviral agents are shown in Table II.

Pharmacological Actions

Fomivirsen is a potent antiviral agent as assessed *in vitro* with a mechanism of action that is both sequence-dependent, resulting in loss of target RNA and protein, and sequence-independent due to inhibition of viral absorption (8). In an *in vitro* HCMV replication immunoassay using HCMV-infected primary human dermal fibroblasts, fomivirsen was found to be 30-fold more potent than ganciclovir with a 50% inhibition of viral antigen expression observed at concentrations of 0.2-0.5 μM. Intracellular infectious virus was inhibited by more than 90% even at the low dose of 0.3 μM fomivirsen (12). In another study, the antiviral activity of fomivirsen was shown to be accompanied by a dose-dependent reduction of major immediate early region protein synthesis and was not due to oligonucleotide-induced cytotoxicity since cell proliferation and viability were unaffected except in the presence of excessive concentrations of fomivirsen (13).

The interactions of fomivirsen with other antiviral agents approved for treatment of HCMV (*i.e.*, ganciclovir, foscarnet, ddC and AZT) were investigated *in vitro* using an immunoassay for HCMV replication and an acute infection assay for HIV replication. Results from the HCMV replication immunoassay, in which normal foreskin human dermal fibroblast cells were infected with HCMV (strain AD169) and treated with the antiviral agents, revealed that the antiviral EC_{50}s for treatment with fomivirsen (0.01-1.0 μM) in combination with ganciclovir (0.3-30 μM), foscarnet (3-300 μM) and ddC (3-300 μM) were 0.5 and 2 μM, 0.1 and 40 μM, and 0.3 and 300 μM, for fomivirsen and each agent, respectively. No antiviral response was observed with AZT and high concentrations of ddC inhibited the antiviral activity of fomivirsen. However, although neither AZT nor ddC interfered with the activity of fomivirsen, the antiviral effect was additive when the agent was given in combination with ganciclovir and foscarnet. In an acute infection assay for replication of HIV, a CEM-SS cell line was infected with HIV-1 strain IIIB and treated with 2-fold dilutions of combinations of fomivirsen (0.16-2.5 μM) and AZT (0.125-30 nM). Fomivirsen inhibited HIV replication in the presence and absence of AZT with an ED_{50} of 2 μM, while the ED_{50} for AZT was 26 nM. Significant additive interactions were observed, in addition to synergy with combination treatment (14, 15).

The interaction of fomivirsen (a 21-mer containing 5 purines) and ISIS-2105, another phosphorothioate oligonucleotide which is a 20-mer with 3 purines, with calcium was investigated, with results demonstrating that the stoichiometric ratio of calcium ion to the two agents was 12:1 and 3:1, respectively. The concentration at which the oligomer precipitated was sequence-dependent and the higher the purine content, the lower the concentration at which precipitation was observed (16).

The potent ability of fomivirsen to inhibit CMV replication was characterized using human retinal pigment epithelial cells (HRPE) and a human fibroblast cell line (MRC-5) infected with CMV (AD169 strain). When cells

were incubated for 20 days with fomivirsen (0.01, 0.1, 0.5, 1 or 2 μM), neither cell morphology nor cell growth were affected. In addition, fomivirsen pretreatment of cells 24 h prior to CMV infection resulted in inhibition of replication, with ED_{50}s of 0.5 and 0.1 μM for MRC-5 and HRPE cells, respectively. Moreover, when cells were exposed to fomivirsen 2 h following CMV infection, the ED_{50} was 0.5 μM in both cell types. Minimal virus-induced cytopathic effects were observed with a concentration of 2 μM in MRC-5 cells and no cytopathic effects were observed in HRPE cells (17). In a similar study, MRC-5 and HRPE cells were infected with a clinical CMV isolate in addition to CMV (AD169 strain) and treated with fomivirsen 2 h after infection. Replication of both CMV strains was inhibited in a dose-dependent manner, with 50% inhibition occurring at concentrations of 0.03 and 0.2 μM in HRPE and MRC-5 cells, respectively. Decreases in target gene expression were found to correlate with inhibition of replication. Fomivirsen continued to inhibit CMV replication even when treatment was delayed for 6 and 3 days after infection in HRPE and MRC-5 cells, respectively (18).

A study has described for the first time the isolation of a viral mutant with sequence-dependent resistance to an antisense oligonucleotide-based drug. An HCMV mutant was isolated with 10-fold resistance to fomivirsen and cross-resistance to a modified derivative of fomivirsen, ISIS-13312, containing the same base sequence; no resistance was observed to an oligonucleotide with an unrelated sequence (ISIS-3383). These results indicate that there is a possibility that resistant mutants may arise during fomivirsen administration, although determination as to whether the resistance observed *in vitro* is of clinical significance remains to be determined (19).

Pharmacokinetics and Metabolism

A pharmacokinetic study using vitreous humor and retina samples from rabbits injected with [^{14}C]-fomivirsen (66 μg/eye intravitreally) reported that at 4 h postdosing, the concentration of intact fomivirsen accumulated in the vitreous humor was 16% lower (3.3 μM) than the theoretical initial vitreal concentration; the $t_{1/2}$ beyond 4 h postdosing for elimination of intact fomivirsen from the vitreous humor was 62 h. The maximum concentration of intact fomivirsen accumulated in the retina over 5 days postdosing was 3.5 μM, with a $t_{1/2}$ for elimination of 79 h. At 10 days postdosing, the concentration of intact fomivirsen was 10 times higher in the retina (1.6 μM) than in the vitreous humor (0.17 μM). Since clearance of intact fomivirsen from the retina was faster than elimination of total radioactivity, it was suggested that metabolism may be involved in clearance of this agent (20, 21). When the in vitro metabolism of fomivirsen was investigated in normal dermal fibroblasts (NHDF) incubated with the agent, the phosphorothioate was found to be metabolized extremely rapidly. Stability against nucleases was inversely proportional to the concentration of phosphorothioate added to the media and to the intracellular concentration of accumulated fomivirsen (22).

The pharmacokinetics of fomivirsen in vitreous humor and retinal samples from rabbits (single 50-μg intravitreal injection) and monkeys (multiple intravitreal injections of 11, 57 or 155 μM weekly or biweekly) have been described. The half-life of fomivirsen clearance was found to be 60 and 22 h for rabbit and monkey vitreous humor, respectively, while in retina, fomivirsen accumulated over 3-4 days to maximum concentrations of 0.1-0.5 μM. The retinal elimination half-lives were 96 h for rabbits and 44 h and 74 h for 57- and 115-μg doses, respectively, in monkeys (23).

A method, including protocols for sample preparation and dilutions, was developed and reported in order to accurately measure concentrations of fomivirsen in its pharmaceutical intravitreal formulation and its metabolites. The method, known as quantitative capillary gel electrophoresis, has been validated for linearity, accuracy, selectivity, precision, stability and ruggedness for monitoring fomivirsen (24).

Toxicity

Fomivirsen was shown to have a very low intraocular therapeutic index in a study investigating the retinal toxicity of fomivirsen treatment. At concentrations of 10 and 5 μM administered intravitreally, the compound did not cause permanent toxic changes in rabbit retina or pig retina, respectively. While administration of 1 μM did not result in any retinal toxicity or inflammation, a transient inflammatory response was associated with a concentration of 3 μM (25). Monkeys administered once- or twice-weekly fomivirsen (3.3-33 μg/eye intravitreal injection) for 1 month were less sensitive to treatment than rabbits. Ocular inflammation including cyclitis and vasculitis was observed, although the incidence was sporadic and responses were not dose-dependent; inflammation was eliminated by topical treatment with steroids. In toxicity studies with rabbits, doses of up to 330 μg/eye were concluded to be safe for clinical evaluation in patients with CMV retinitis (8).

Clinical Studies

In a preliminary phase I clinical trial, 8 HIV-infected patients with intolerance to ganciclovir and foscarnet were administered (repeated dosing every week for 1 month followed by biweekly maintenance) 50-μl intravitreal injections of fomivirsen (75, 150 or 300 μg) to achieve final vitreal concentrations of 2, 4 and 8 μM, respectively. Positive responses to treatment were observed in 2/3 patients receiving 4 μM. Fomivirsen therapy was concluded to be well tolerated with some reversible, dose-independent ocular events observed with treatment (26) (Box 1).

Box 1: Safety and efficacy of fomivirsen (26).

Study Design	Open clinical trial
Study Population	Patients with AIDS and CMV retinitis showing intolerance or progression while on ganciclovir or foscarnet
Intervention Groups	Fomivirsen, 75-300 μg intravitreal 1x/week x 1 month → 1x/2 weeks
Results	Prolonged positive responses at fundus photography were noted in 2/3 patients on 150 μg and 3/3 patients on 300 μg
Conclusions	Fomivirsen is safe and appears to be effective in patients with CMV retinitis unresponsive to other drugs

Source: Prous Science CTLine database.

Box 2: Efficacy and safety of fomivirsen in patients with refractory CMV retinitis (27).

Study Design	Open clinical trial
Study Population	Patients with CMV retinitis refractory or intolerant to i.v. treatments
Intervention Groups	Fomivirsen, 75-450 μg intravitreal 3-4 x/week → 2x/week (n = 13 [17 eyes]) Fomivirsen, 75-450 μg intravitreal 2x/week (n = 9 [11 eyes])
Conclusions	Fomivirsen displays anti-CMV activity and was associated with an inflammatory response

Source: Prous Science CTLine database.

A phase I trial was also reported in which 22 patients (28 eyes) with CMVR were intravitreally administered 3-5 weekly doses of fomivirsen (75, 150, 300 or 450 μg/eye) followed by biweekly dosing. Out of 10 treated eyes displaying anterior chamber inflammation, 8 responded to topical steroid treatment; a higher incidence of inflammation was observed in patients receiving biweekly doses of fomivirsen. Vitritis was increased in 5 patients, with improvement observed in 4 following steroid treatment, and transient decreases in color vision intensity were noted in 4 patients. CMV activity was decreased in 2/4, 6/10, 7/11 and 1/1 eyes administered 150, 300 (weekly dosing), 300 (biweekly dosing) and 450 μg, respectively. Retinal toxicity was observed in patients receiving the high dose of fomivirsen (27) (Box 2).

In a randomized controlled phase II study, 107 HIV-infected patients with advanced CMVR who were unresponsive to established antiviral therapy were intravitreally administered 330 μg of fomivirsen as 3 weekly doses followed by biweekly maintenance; another group of patients with early and mid-stage disease received escalated doses of fomivirsen up to 150 μg. Although the study is ongoing, preliminary results show that anterior chamber inflammation is the most frequent side effect (28).

The safety and efficacy of fomivirsen treatment was demonstrated in several phase III studies. In one study with a 2:1 randomized scheme, 18 AIDS patients with previously untreated unilateral CMVR were administered intravitreal fomivirsen (150 μg) as 3 weekly doses, followed by biweekly maintenance dosing. Treatment was deferred in a second group of 10 patients until after disease progression. A significant difference was observed in the median time to observed progression for treated patients as compared to patients in the deferred treatment group (71 *vs.* 14 days). The most common side effects were increased intraocular pressure and mild to moderate reversible intraocular inflammation (29) (Box 3).

Phase III clinical studies in which active CMVR patients, unresponsive to previous established antiviral therapy with ganciclovir, foscarnet or cidofovir, were administered intravitreal fomivirsen (330 μg) at weekly intervals for 3 weeks, followed by biweekly maintenance, showed that the median progression-free survival period was 42 days. Clinically manageable adverse effects included transient increases in intraocular pressure and intraocular inflammation. Although a small percentage of patients experienced retinal detachment, risk was concluded to be low and acceptable (30-33) (Boxes 4 and 5).

A second generation of oligonucleotides for the treatment of CMVR are currently being developed using 2'-carbohydrate modifications with Isis' proprietary "Gapmer" technology. These agents may have a lower incidence of adverse effects and enhanced antiviral activity, with an increased residence time in the eye (34).

The U.S. FDA has recently approved fomivirsen sodium (Vitravene™ injectable) for the treatment of CMVR in patients with AIDS who are intolerant of or have a contraindication to other treatments for CMV retinitis, or who were insufficiently responsive to previous treatments for the condition. The drug, discovered by Isis Pharmaceuticals and codeveloped in collaboration with Ciba Vision (Novartis) (35), has been launched as a sterile aqueous solution for intravitreal injection in single-

Box 3: Efficacy and safety of fomivirsen in AIDS patients with CMV retinitis (29).

Study Design	Randomized clinical trial
Study Population	Patients with AIDS and untreated unilateral CMV retinitis (n = 28)
Intervention Groups	Fomivirsen (F), 150 μg intravitreal 1x/week x 3 weeks → 1x/2 months (n = 18) Deferred treatment (DT)
Adverse Events	Transient ocular hypertension, reversible intraocular inflammation
Results	Time to progression; F (71 days) > DT (14 days)
Conclusions	Fomivirsen is a safe and effective treatment for CMV retinitis

Source: Prous Science CTLine database.

Box 4: Efficacy and safety of fomivirsen in CMV retinitis (32).

Study Design	Open clinical trial
Study Population	Patients with history of CMV retinitis treated with ganciclovir or foscarnet
Intervention Groups	Fomivirsen, 330 mcg intravitreal 1x/week x 3 weeks → 1x/2 weeks x 77 days (median)
Withdrawals	5 patients died (3 with controlled CMV retinitis)
Adverse Events	Anterior chamber inflammation, vitreal haze
Results	Response rate, 100% Progression rate, 2/8 patients (25%)
Conclusions	Fomivirsen decreases CMV activity even in heavily pretreated patients

Source: Prous Science CTLine database.

Box 5: Risk of retinal detachment with fomivirsen in CMV retinitis (33).

Study Design	Open clinical trial
Study Population	Patients with AIDS and CMV retinitis uncontrolled with ganciclovir, foscarnet and cidofovir (n = 266 eyes)
Intervention Groups	Fomivirsen, 330 μg intravitreal 1x/week x 3 weeks → 1x/2 weeks
Results	Rate of retinal detachment, 4.9% Risk of retinal detachment, 1%
Conclusions	Fomivirsen intravitreal does not increase the risk of retinal detachment in patients with CMV retinitis

Source: Prous Science CTLine database.

use vials (0.25 ml) containing 6.6 mg/ml fomivirsen sodium (36). A Marketing Authorization Application, submitted in May, is currently under review by the European Agency for the Evaluation of Medicinal Products for the marketing of fomivirsen in Europe (35).

Manufacturer

Isis Pharmaceuticals, Inc. (US); Ciba Vision Corp. (US).

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