

Rise and fall of fomivirsen, the first approved gene silencing medicine – A historical review

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Abstract: Fomivirsen was approved by the FDA in 1998 and by the EMA in 1999 as the very first antisense drug used to treat CMV retinitis in patients with AIDS. To date, it has been the only first generation antisense oligonucleotide used in therapy. Fomivirsen has been a pioneer in this field and has demonstrated the usefulness of the antisense technology for medicinal science. However, after three years of use, fomivirsen has been withdrawn from the market (in the US in 2001 and in the EU in 2002), and nowadays, gene silencing drugs with a more advanced chemical structure and more complex mechanism of action are used in medicine. On the occasion of the 20th anniversary of its European withdrawal, we briefly overview the history of fomivirsen.

Keywords: fomivirsen, antisense, oligonucleotide, phosphorothioate, Vitravene

Introduction

Acquired immunodeficiency syndrome (AIDS) is a severe disease, caused by human immunodeficiency virus (HIV). The disease is characterized by the suppression of the immune system (decreases level of CD4+ T cells), which can lead to infections by opportunistic pathogens such as cytomegalovirus (CMV). CMV is a β -herpesvirus which infects mostly immunosuppressed patients. CMV can cause the inflammation of the retina (CMV retinitis, or CMVR), leading even to vision loss [1]. The major intermediate-early gene of CMV codes the IE1 (isoforms 72 kDa, 19 kDa, 17.5 kDa and 9 kDa) and IE2 (isoforms 86 kDa, 55 kDa and 18 kDa) proteins. These proteins are derived from the same gene, by alternative splicing (two isoforms of late protein LP is also synthesised this way). Basically, IE1 and IE2 proteins have regulatory functions. They control the expression of viral genes, but also influence the activation of host genes, and play a role in the fight against the immune response. Therefore, these proteins are important in acute infection and virus reactivation. IE2 is essential for virus replication, while IE1 is dispensable [2]. In the treatment of CMVR, anti-herpesvirus drugs

can be used such as ganciclovir, valganciclovir, foscarnet or cidofovir [1]. In CMVR of AIDS patients, antiretroviral chemotherapy (ART) may be sufficient to prevent CMVR without specific anti-CMV medicine [3]. However some data suggest that in some cases specific anti-CMV therapy may be required to treat active CMVR, besides the ART [4].

An interesting method that can be used against viruses is gene silencing. Gene silencing means the blocking of gene expression by using short oligonucleotides, exploiting their ability to recognize and bind to complementary sequences. There are three main types of gene silencing, anti-gene strategy, RNA interference, and antisense strategy. In the anti-gene strategy, the target sequence is part of the gene, therefore the transcription is blocked sterically. The advantage of this method, that there are far fewer copies of DNAs (2 – few/cell) in the cell, than mRNAs (a few thousands copies/cell), which promises outstanding efficacy of this method. The disadvantages include that the oligonucleotide must be delivered into the nucleus, and anti-gene agents mostly work by triplex formation, which can be achieved on polypurine/polypyrimidine sequences. However these problems can be

overcome by using chemically modified antigenic agents [5]. RNA interference is performed using short, double-stranded RNA oligonucleotides. The double-stranded oligonucleotide can be integrated into a protein complex called RISC (RNA induced silencing complex). RISC uses the oligonucleotide as a „guide” to recognize and cleave the target mRNA. Because the guide complex is not degraded during the process, it can lead the RISC to another mRNA molecule multiple times, therefore a single molecule of the active ingredient can cause the degradation of multiple target molecules, therefore, this can be a very effective method [6]. Antisense strategy is the oldest and simplest way of gene silencing. Short, single-stranded oligonucleotides bind to the target mRNA and affect splicing, or sterically block translation. Also, under certain circumstances, the formed double stranded NA can activate RNase H, which leads to the degradation of the target mRNA [7-8]. Gene silencing can be used to treat genetic disorders or viral infections.

Natural oligonucleotides are not suitable for therapeutic use due to their short half life. Therefore, synthetic nucleic acid analogues (xeno nucleic acids, XNAs) were developed, to increase resistance to nucleases and improve other properties (e.g. hybridization strength or selectivity). There are many different types of XNAs with promising advantages, but three of them are cur-

rently used in gene silencing drugs (Figure 1), 2'-modified derivatives (with a methoxy (OMe) or methoxyethoxy (OME) group at 2'-position) [9-10], phosphorodiamidate morpholino oligomers (PMO) [9, 11] and phosphorothioates (PS) [12]. Among them, the phosphorothioates are the oldest derivatives applied. In the phosphorothioates, one of the non-bridging oxygen of the phosphate ester is replaced by a sulfur atom. This seemingly small modification significantly changes the properties of the oligomer. The advantages of the PS oligos include simple synthesis (conventional oligonucleotide synthesis methods can be used by changing the oxidation step for sulfurisation), better cellular uptake, high resistance to nucleases, and the ability of RNA/PS duplexes to activate RNase H. PS have two main disadvantages. One is, that the stability of PS/NA duplexes is slightly lower than that of unmodified NA/NA duplexes with the same sequence [12]. Another disadvantage is that, due to their polyanionic backbone, PS oligos interact non-specifically with a number of molecules, such as paraspeckle proteins, albumins, or Ku70 and Ku80 nuclear proteins [13]. PS oligomers also have immunostimulatory activity, partly because their polyanionic backbone is able to bind to cationic places on the cell surface, designed to bind the glycosaminoglycans of the ECM [14]. The CG sequence in an oligonucleotide is also recognized by TLR-9 receptor as a bacterial

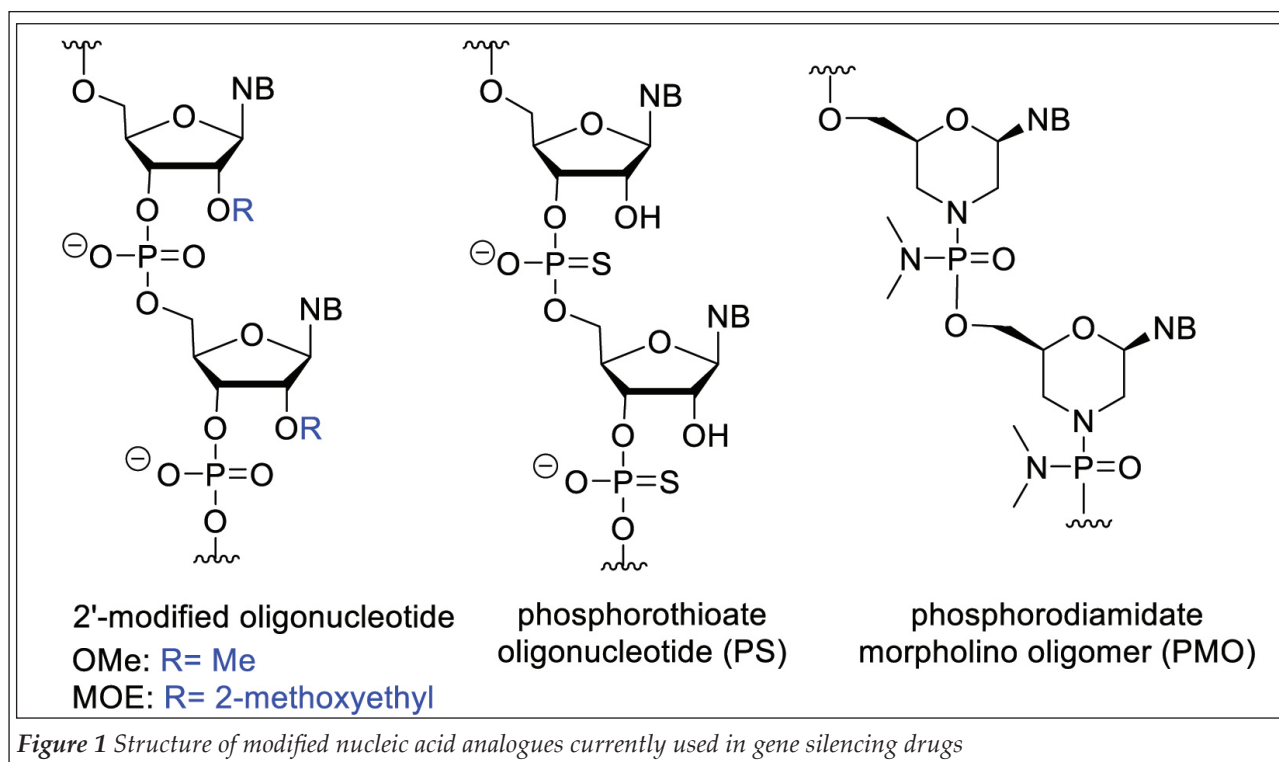


Figure 1 Structure of modified nucleic acid analogues currently used in gene silencing drugs

pattern, which also causes immunoactivation [12, 15]. Phosphorothioate oligomers also show antiviral effect against HIV [16] and herpes simplex virus 2 (HSV2) [17], probably based on inhibition of virus adsorption. This is due to the fact, that some viruses binds to negatively charged carbohydrates on the cell surfaces with their glycoproteins, and polyanionic PS oligomers bind to these viral envelope glycoproteins. PS can also compete with the RNA or DNA template for binding to viral polymerase/reverse transcriptase [17-18]. This mechanism is independent of sequence, but may be affected by the length of the oligomer and the base composition.

Chemical structure and mechanism of action

Fomivirsen (ISIS 2922) is a 21mer phosphorothioate oligodeoxynucleotide having the sequence 5'-GCG TTT GCT CTT CTT CTT GCG-3' (Figure 2, the CG motifs are highlighted in red). In the formulated medicine (Vitravene™), the sodium-salt of fomivirsen was used [19-20].

Mechanism of action of fomivirsen consists of three components as listed below [20]

Sequence dependent antisense effect: Fomivirsen is an antisense oligodeoxynucleotide (AON), targeting the MIE gene of CMV. Therefore it hybridizes to the mRNA transcribed from MIE and blocks the translation of IE2 proteins (the 86 kDa and 55 kDa isoforms), which causes a decrease in the level of these proteins. Fomivirsen also reduces the level of the targeted mRNA, which may be explained by the RNase-H-mediated cleavage, but a specific degradation product has not been observed, therefore, the role of the RNase H in the antisense effect is disputed.

Sequence dependent non-antisense effect: Incorporating mismatches into the sequence of fomivirsen, a decrease in antisense activity by destabilizing the formed duplexes was observed. However, in antiviral assays oligonucleotides with a few mismatches or non-complementary sequences also show moderate antiviral activity. The extent of this effect is sequence dependent, and the antiviral activity of these derivatives is significantly lower, than that of fomivirsen. This phenomenon suggests, that a sequence dependent, but non-antisense mechanism is involved in the antiviral effect of antisense oligonucleotides.

Sequence-independent non-antisense effect: At higher concentrations (in the μM range) PS oligomers can inhibit the adsorption of virus particles into

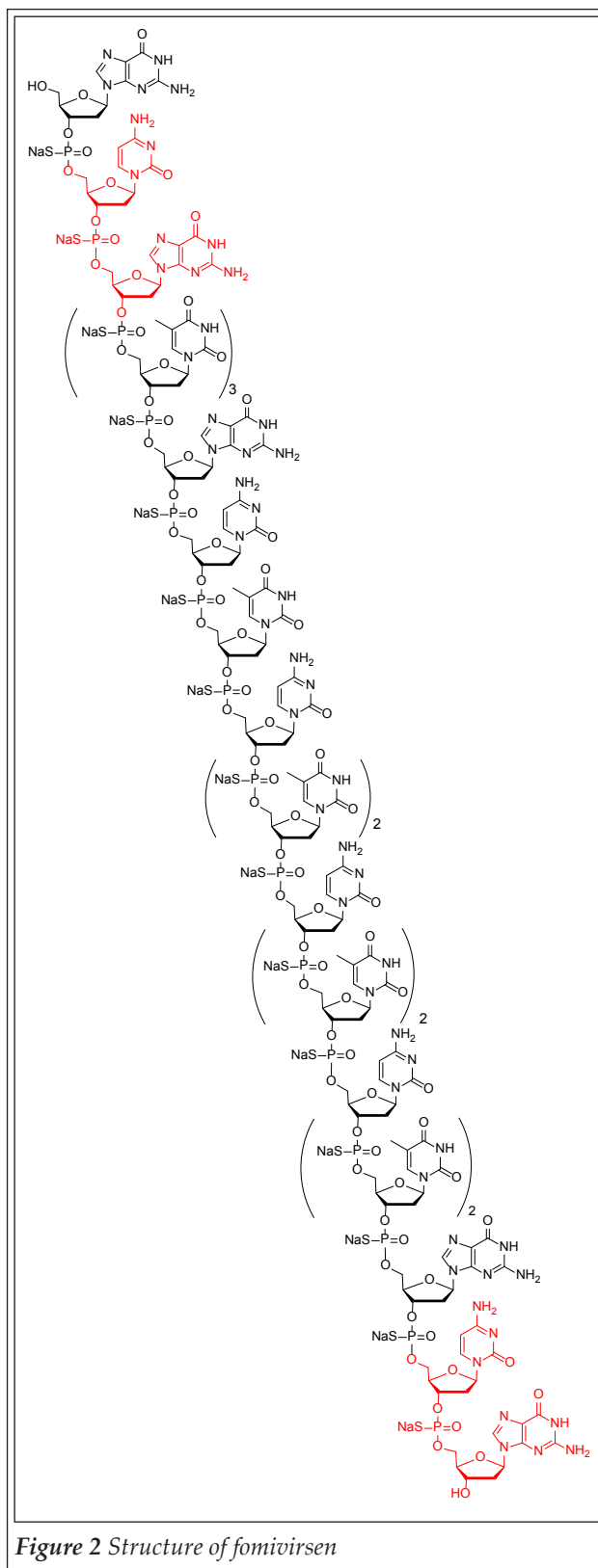


Figure 2 Structure of fomivirsen

the host cell in a sequence-independent manner. Because the antiviral EC_{50} value of fomivirsen against CMV is in the 100 nM range, this effect probably plays a modest role in activity.

Development and approval

Fomivirsen was developed by ISIS Pharmaceuticals (current name: IONIS Pharmaceuticals). Because of the higher order structure of the mRNA, not the entire molecule is approachable for antisense agents, therefore, the first step of development is to screen oligomers that target different sequences of mRNA. The first article on fomivirsen was published in 1993. 21 antisense PS oligonucleotides, (targeting different sequences in the mRNA of viral DNA polymerase, IE1 or IE2) in the length of 20-21 nucleotides were tested together with 4 nonspecific oligomers in a 96 well-plate immunoassay against CMV. Among them, ISIS-2922 was the most effective, therefore its activity was compared with that of ganciclovir. Ganciclovir had an EC_{50} of 3 μ M, while ISIS-2922 had an EC_{50} of 0.1 μ M, demonstrating the latter's superiority [21].

It was tested *in vitro* how drugs, applied to treat HIV and CMVR, affect the efficacy of fomivirsen. The anti-CMV effect of ganciclovir or foscarnet and fomivirsen was additive. Azidothymidine (AZT) and dideoxycytidine (ddC) have not shown antagonism to the antiviral activity of fomivirsen. In addition, because of its PS structure, fomivirsen has shown anti-HIV effect in high concentration, which can be useful in clinical practice [22].

In experiments in rabbits and pigs, fomivirsen did not show any toxicity or inflammatory effect below 1 μ M, and no permanent toxicity was ob-

served below 10 μ M (in rabbit) and 5 μ M (in pig), after intravitreal administration [23].

In clinical trials and in clinical practice, fomivirsen was administered by intravitreal injection. This way, the half-life is ~55 hours and shows no systemic distribution. This is advantageous, because it decreases the chances of adverse effects and drug interactions [24]. The only important drug interaction is, that administration of fomivirsen within 2-4 weeks after cidofovir therapy may cause excessive inflammation [25].

Fomivirsen was approved by FDA in August 1998 and by EMA in July 1999. Unfortunately, limited data are available in the literature on clinical trials of fomivirsen before 1998. We found 3 reviews [26-28] from this time mentioning this topic, but mainly referring to unedited sources (e.g. conference abstracts). In phase I/II studies, late stage AIDS patients with CMVR were treated with different doses of fomivirsen, which significantly reduced the progression of CMVR, however local inflammation reactions were observed, which were then treated with steroids [26]. Fomivirsen was effective when ganciclovir or foscarnet did not help. The phase III trials were started in 1995. Because the high starting dose caused adverse effects, it was paused and re-started with lower dose. Fomivirsen was proved to be useful in early stage patients [27-28]. Progression of CMVR was measured by masked reading of fundus photographs. Different doses and dosing frequencies were tested. There was a significant difference in

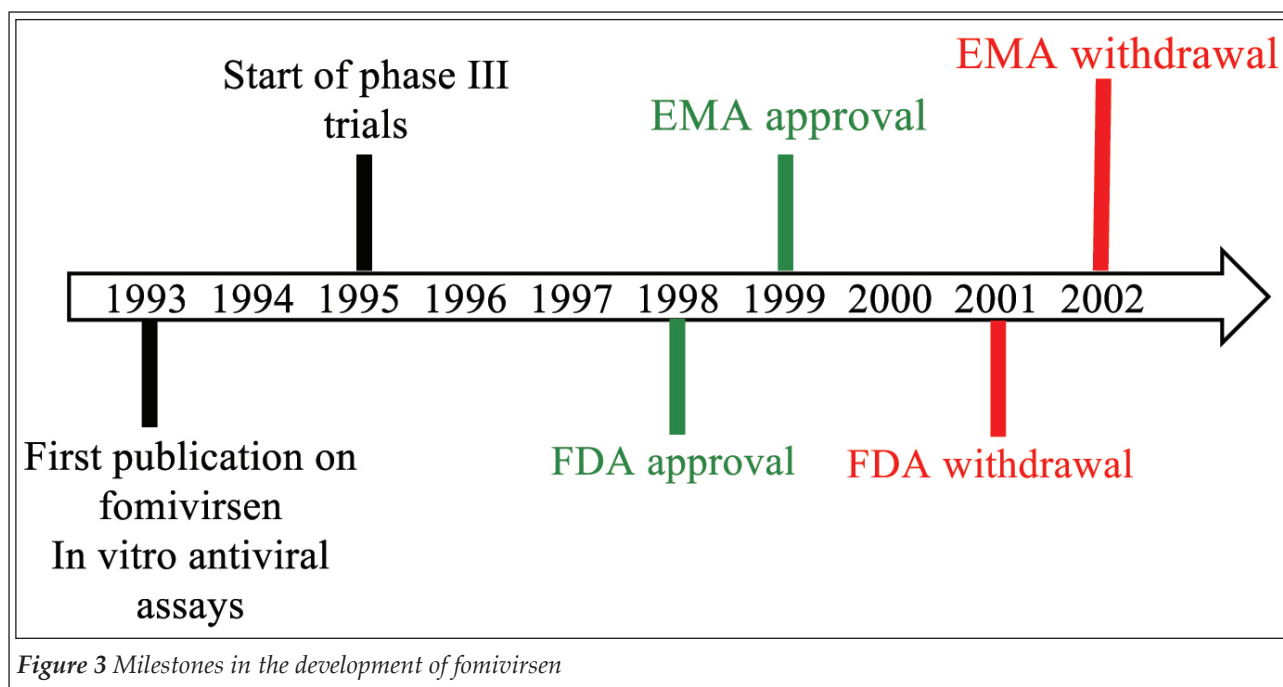


Figure 3 Milestones in the development of fomivirsen

Table 1 Currently approved gene silencing medicines

INN name	Code	Brand name	Chemical structure	Mechanism of action	Disease
Mipomersen	ISIS-301012	Kynamro	PS, 2'-O-MOE gapmer, 5-Me-C	antisense	familial hypercholesterolemia
Eteplirsen	AVI-4658	Exondys 51	PMO	antisense, splicing modulation	Duchenne muscular dystrophy
Nusinersen	ISIS-396443	Spinraza	2'-O-MOE, PS, 5-Me-C	antisense, splicing modulation	Spinal muscular atrophy
Patisiran	ALN-18328	Onpattro	2'-OMe	RNA interference	Hereditary transthyretin mediated amyloidosis
Inotersen	ISIS-420915	Tegsedi	PS, 2'-O-MOE, 5-Me-C	antisense	Hereditary transthyretin mediated amyloidosis
Milasen	TY777	-	2'-O-MOE	antisense, splicing modulation	Batten's disease
Volanesorsen	ISIS-304801	Waylivra	PS, 2'-O-MOE, 5-Me-C	antisense	Familial chylomicronemia
Givosiran	ALN-AS1	Givlaari	PS, 2'-F, 2'-OMe, GalNAc-conjugate	RNA interference	Acute hepatic porphyria
Golodirsen	SRP-4053	Vyondys 53	PMO	antisense, splicing modulation	Duchenne muscular dystrophy
Viltolarsen	NS-065/ NCNP-01	Viltepso	PMO	antisense, splicing modulation	Duchenne muscular dystrophy
Casimersen	SRP 4045	Amondys 45	PMO	antisense, splicing modulation	Duchenne muscular dystrophy

the delay of progression between the groups treated immediately and delayedly [29-31]. In 2002, the Vitravene study group published 3 articles to give better access to clinicians on safety and efficacy of fomivirsen [32-34]. In the first article, a multicenter RCT was described in which 165 µg of fomivirsen was administered to patients with newly diagnosed peripheral CMVR. The time until first progression of disease was determined, and it was significantly longer in the immediately treated group (71 days) than in the deferral of treatment group (13 days) [32]. In the second, two trials were described, using two different dosing methods (the more intense method: induction therapy for 3 weeks with weekly injections, maintenance therapy with injections on every second week; the less intense method: induction with 2 injections on day 1 and day 15 and maintenance therapy with monthly injections) with 330 µg fomivirsen in both cases. Both regimens showed similar results [33]. Finally, safety data were reported, based on 3 RCTs. The main adverse effects were local reactions (anterior chamber inflammation and intraocular pressure increasing) and were controllable [34]. About adverse effects, a case report was published on the development of reversible bull's eye maculopathy after fomivirsen treatment [35]. Despite the critics of the trials (because of the small number of patients and deficiencies of analysis) [36-37] fomivirsen was approved as a second-line treatment of CMVR of HIV patients [38].

Withdrawal

Highly active antiretroviral therapy (HAART) is a term for treatment protocols, combining anti-HIV drugs, which target different molecular targets of the virus (e.g. reverse transcriptase inhibitors and protease inhibitors) [39]. As mentioned above, adequate anti-HIV therapy is sufficient to cure or prevent CMVR in AIDS patients. Therefore, the prevalence of HAART has dramatically reduced the incidence of AIDS-related CMVR [40]. As a result, the necessity of fomivirsen decreased and it was withdrawn in 2002 [9,41].

Conclusion and outlook

Development of fomivirsen started in the early 1990's, it was approved in late 1990's as the first antisense drug, and was withdrawn in the early 2000's. Despite its withdrawal - which was caused by commercial reasons and not serious adverse effects - fomivirsen had a major impact on medicinal sciences because it proved the concept of gene silencing in the medicine [42]. Therefore, this indirectly opened the door to a new generation of gene silencing medicines. There are currently 11 approved gene silencing medicines, these are shown in Table 1 [9, 43-45]. Among these, 5 carry PS modification and 9 are based on the antisense mechanism, which means that the pathway, shown by fomivirsen was not bad at all.

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