FDA-Approved Oligonucleotide Therapies in 2017

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Oligonucleotides (oligos) have been under clinical development for approximately the past 30 years, beginning with antisense oligonucleotides (ASOs) and apatmers and followed about 15 years ago by siRNAs. During that lengthy period of time, numerous clinical trials have been performed and thousands of trial participants accrued onto studies. Of all the molecules evaluated as of January 2017, the regulatory authorities assessed that six provided clear clinical benefit in rigorously controlled trials. The story of these six is given in this review.

The Earlier Food and Drug Administration Approvals
Vitravene, Also Known as Fomivirsen

The first antisense oligonucleotide approved for marketing by the Food and Drug Administration (FDA) was fomivirsen, a drug developed in a collaboration of Isis Pharmaeeuticals with Novartis Ophthalmics. This 21-mer phosphorothioate oligodeoxynucleotide (which contains a CpG motif near its 5′ terminus) was intended for treatment of patients with cytomegalovirus (CMV) retinitis. The target was the mRNA that encoded the CMV immediate-early (IE)-2 protein, which is required for viral replication.

In a study from the Vitravene Study Group,[1] fomivirsen was highly successful at ameliorating the signs and symptoms of CMV retinitis. Treatment consisted of weekly injections of 165 μg of fomivirsen injected directly into the vitreous humor, followed by maintenance injections every other week until evidence of retinitis progression. The median time to disease progression for the fomivirsen-treated group (n = 18) was 71 days versus 13 days for the untreated group (p = 0.0001). Overall during the course of the study, progression occurred in 44% of the treated versus 70% of the untreated patients.

Vitravene was approved by the FDA in 1998 and by EMEA (European Agency for the Evaluation of Medicinal Products) in 1999. At the time, there was a high unmet need for an anti-cytomegalovirus retinitis drug. Subsequently, due to the development of high-activity anti-retroviral therapy (HAART), the number of CMV cases dramatically decreased. Novartis stopped marketing the drug in 2002 in Europe and in 2006 in the United States.

Macugen, Also Known as Pegaptanib

Macugen (formerly pegaptanib) is an aptamer targeted to vascular endothelial growth factor (VEGF165). The molecule was first produced by the systematic evolution of ligands by exponential enrichment (SELEX)[2,3] strategy pioneered by Larry Gold (an excellent review by Ng et al.[4] provides a brief, concise description of the SELEX approach).

Pegaptanib is 27 nucleobases in length. The molecule contains a phosphorothioate 3′-3′ deoxythymidine cap to promote nuclease stability; all of the purine ribose sugars are 2′-O-methylated and the pyrimidine ribose sugars all 2′-fluorinated.[5] A 40 kDa polyethylene glycol substituent was linked to the 5′ molecular terminus. Pegaptanib binds to the heparin binding site of VEGF165[6] with an affinity in the picomolar range and inhibits its binding to both VEGFR1 and VEGFR2, though to a lesser extent for the latter.

Macugen was approved by the FDA to treat age-related macular degeneration (AMD) of the retina. This disease is the leading cause of blindness in people over the age of 50. At least in part, it's caused by the VEGF165-stimulated growth of blood vessels (neovascularization) of the choroid (the vascular tissue beneath the retina) of the eye. Disease activity is especially severe in the macula of the retina and can result in central blindness.

The safety and efficacy of pegaptanib was evaluated in two identical trials. These trials were prospective, randomized, double-masked, multi-centered, and dose finding and have been referred to as the VISION trials.[7,8] There were 1,186 patients that received at least one treatment of pegatinib. The drug was injected into the ocular vitreous humor (after the application of the appropriate anesthesia) at doses of 0.3, 1.0, or 3.0 mg every 6 weeks for 48 weeks (nine treatments total). An equivalent number of patients received a sham treatment. Efficacy was determined by the ability of patients to visually read the letters on eye charts, a metric that is currently the standard in clinical ophthalmology trials.

There were 70%, 71%, and 65% of patients receiving the 0.3, 1.0, and 3.0 mg doses of pegaptanib, respectively, compared to 55% of patients receiving the sham injections (p > 0.001, > 0.001, and > 0.03, respectively) that lost less than 15 letters of visual acuity, or about three lines on the study eye chart, in the 54 week duration of the trial. Because higher doses of pegaptanib did not lead to greater activity, the FDA approved the 0.3 mg dose in late 2004.

All the doses of pegaptanib were safe. Side effects were mild to moderate in intensity and related to the injection process rather than the drug. These included five cases each of traumatic injury and lens

detachment and 12 cases of endophthalmitis (most of these patients nevertheless remained in the trial). All these adverse events were seen in the pegaptanib-injected patients. As anticipated, there was no systemic toxicity. The results after 2 years of pegaptanib treatment were similar to those observed after 1 year, with a 45% mean relative benefit change in vision noted in drug versus sham injected patients (p < 0.01).

However, since the FDA approval date in 2004, Macugen has led a peripatetic existence. Initially developed by NeXstar, Macugen was eventually acquired by Eyetech Pharmaceuticals, which in collaboration with Pfizer performed the randomized trials above. In 2005, Eyetech Pharmaceuticals was acquired by OSI Pharmaceuticals. However, in 2008, former Eyetech employees purchased the drug from OSI and established Eyetech, Inc., which in turn was acquired by Valeant Pharmaceuticals in 2010. The drug is currently marketed by Bausch and Lomb, which was acquired in 2013 by Valeant.[9]

Sales of Macugen were satisfactory from 2004 through 2011. However, by 2010, sales had declined to only $12 million/year in the face of stiff competition from Lucentis (ranibizumab; Novartis). This drug appears to be more effective than Macugen. Competition also came from the off-label use of the anti-VEGF mAb bevacizumab (Avastin; Genentech), which is less expensive. Nevertheless, as of 2017, Macugen still retains a relatively small, though somewhat precarious, market share for the treatment of AMD.

More Recent FDA Approvals
Kynamro, Also Known as Mipomersen
Mipomersen is approved in the United States (but not in the European Union) for homozygous familial hypercholesterolemia (HoFH). This is a disease associated with very high plasma concentration of low-density lipoprotein (LDL). Molecularly, the disease is distinguished by loss of function mutations in both LDL-receptor genes. This results in the reduced liver uptake of plasma LDL cholesterol (LDL-C).

Because of the lack of clearance of LDL-C, patients with this ailment develop cutaneous and tendinous xanthomatas (benign cholesterol-laden tumors), in addition to cardiovascular disease at a very young age. Patients not treated usually do not live beyond the age of about 30.[10]

LDL-C levels can be dramatically reduced by statins,[11] but even high dose intensity statins are often insufficient to reach therapeutic goals; i.e., elimination of the risk for coronary heart disease. The core protein of the LDL particle is apolipoprotein B (apoB). In an attempt to diminish circulating LDL-C, Crooke et al.,[12] synthesized mipomersen (Isis 147764), an antisense 20-mer phosphorothioate 2′-methoxyethoxy (MOE) gapmer targeted to the coding region of the apoB mRNA (see below). The MOE groups were incorporated at positions 1–5 and 15–20 of the oligonucleotide chain, where they block exonuclease activity and increase the Tm of the apoB mRNA/ASO duplex by 1.1°C per MOE residue.[13] This stabilizes the duplex and helps to enable cleavage of the mRNA by RNase H1.

apoB is the critical protein of atherogenic lipoprotein particles[11] and is central to the clearance of these particles through its binding to the LDL receptor on hepatocytes.[14] This protein also plays a role in the production of very low-density lipoprotein (VLDL) particles by the liver and is associated with the VLDL particle. VLDL particles, in turn, are metabolic precursors of LDL particles.[15]

After completion of studies in healthy volunteers and phase I and II clinical studies, four different phase III clinical trials were performed.[10,16–18] Mipomersen was evaluated in distinct sets of patients: trial #1 required genetic confirmation of HoFH or untreated LDL-C >500 mg/dL with either xanthoma before 10 years of age or heterozygous FH (HeFH) in both parents;[10] trial #2 required LDL-C >200 mg/dL;[16] trial #3 required HeFH plus LDL-C >100 mg/dL + serum triglycerides <200 mg/dL and a diagnosis of coronary artery disease;[17] and trial #4 required LDL-C >100 mg/dL + serum triglycerides <200 mg/dL.[18] In every trial, patients were 2:1 randomized to 26 weekly, subcutaneous injections of either 200 mg mipomersen or placebo. All patients were maintained on maximally tolerated lipid lowering therapy. Most patients were middle aged, overweight, and about 50% had metabolic syndrome.

Trial #1 (HoFH) assigned 34 patients to mipomersen and 17 to placebo. The mean percentage change from baseline in serum LDL-C levels was about 25% in the mipomersen group versus about 3% in the placebo group, p = 0.0003, as measured 28 weeks after the initiation of treatment. Similar changes in total cholesterol, non-HDL cholesterol, and, to a lesser extent triglycerides, were also seen. In trial #2, 39 patients were treated with mipomersen and 19 with placebo. In the mipomersen-treated patients (n = 27), LDL-C was reduced by 36%, as compared with an increase of about 13% in n = 12 placebo patients (p < 0.001). Changes in serum apoB, non-HDL-C, and Lp(a) (see below) were similar, though serum triglyceride levels were reduced to a much lesser extent. A >15% decrease in LDL-C was seen in almost 80% versus 17% of the mipomersen versus placebo-treated patients and about 25% of the mipomersen patients achieved a >50% reduction in LDL-C.

In trial #3 (HeFH), 83 of the randomized patients received mipomersen and 41 received placebo. LDL-C was decreased by a mean of 28% in the 73 mipomersen patients who completed the study, compared to an increase of 5% in those treated with placebo. Total cholesterol, apoB, non-HDL-C, and Lp(a) levels were also reduced (all p < 0.001), though HDL-C levels were unaltered. As in the other trials above, triglyceride levels were reduced to a lesser extent.

Finally, in trial #4, in patients with severe hypercholesterolemia at high cardiovascular risk, 60 mipomersen patients and 44 placebo (out of 158 randomized) patients completed treatment. Mipomersen reduced LDL-C by 36% versus 4.5% (p < 0.001) and a targeted LDL-C level of <100 mg/dL was achieved in 76% versus 38% of placebo treated patients. Reductions in apoB and non-HDL-C were similar to that observed for LDL-C, but reductions in triglycerides and total cholesterol were somewhat smaller.

Despite the wealth of efficacy data, initial regulatory opinion of mipomersen was not favorable. On December 13, 2012, the European Medicines Agency (EMA) refused marketing authorization for the drug.[15] The EMA acknowledged that the drug reduced average LDL-C in patients with HoFH and in severe heterozygous hypercholesterolemia by 25%–36%. However, the agency also observed that a "high proportion" of patients discontinued the drug by 2 years, mostly due to adverse events such as injection site reactions and liver toxicity. The EMA was also concerned about the increasing possibility of developing hepatic steatosis (fatty liver), and that the probability of developing serious cardiovascular events after mipomersen treatment was in fact greater than placebo. The EMA therefore concluded that the mipomersen induced reduction of cholesterol levels did not outweigh its cardiovascular risk.

However, on January 13, 2013 the U.S. FDA granted approval for the marketing of Kynamro. Nevertheless, the drug failed to generate much in the way of sales. This was probably due to competition from a small molecule, which seems to have garnered most of the tiny group of patients with HoFH. By the beginning of 2016, the collaboration of Isis (now renamed Ionis) and Genzyme to market the drug had dissolved. By May, 2016, Kynamro's rights were sold by Ionis to Kastle Therapeutics. At the time of this writing (January, 2017), the clinical fate of mipomersen is not clear.

Exondys 51, Also Known as Eteplirsen

Eteplirsen (Sarepta) is a 30-mer phosphomorpholidate oligonucleotide.[19] Unlike charged phosphorothioate oligonucleotides, uncharged phosphomorpholidates, which were first introduced more than 35 years ago, are not substrates for nucleases and cannot elicit RNase H activity. These properties are important for oligonucleotide-induced splice-switching activity.

Oligonucleotide-directed splice switching is used to manipulate alternative pre-mRNA splicing. Splice-switching oligos (SSOs) can be employed to correct an irregular splicing event or to induce the expression of a new splicing variant, which may have a therapeutic function. The SSOs are generally targeted to bind one of the key splicing sequences (usually the donor or the acceptor splice site) that define the exons' boundaries within the gene pre-mRNA. When the exon boundary is masked, the splicing machinery searches downstream for another suitable site, thus skipping the problematic exon. Eteplirsen is designed to skip exon 51 of the dystrophin protein.[19]

Eteplirsen has been evaluated in young male patients with the devastating disease known as Duchenne muscular dystrophy (DMD). This X-linked disease is found in approximately 1/3,500–1/5,000 live male births[20] and is characterized by mutations (such as intragenic deletions, duplications, and point mutations) in the dystrophin gene. These mutations disrupt the reading frame of the dystrophin mRNA and cause the introduction of premature stop codons,[20] leading to mRNA degradation and the loss of protein synthesis[21,22] in striated muscle. The clinical consequences of these mutations are catastrophic for the patient.

The dystrophin protein is located beneath the sarcolemma (the muscle cell membrane). Via the binding of its N-terminal to F-actin and its C-terminal to β-dystroglycan, it connects the cellular cytoskeleton to the muscle cell membrane, the sarcolemma. Histologically, the loss of dystrophin results in inflammation, muscle degeneration, and its replacement with fibrotic tissue and fat.[23]

Children and young adults who suffer from DMD progressively lose neuromuscular function as they age. This often culminates in their inability to walk by their mid-teens. Sadly, muscle deterioration is progressive: The inevitable result is cardiomyopathy, respiratory failure, and death by the time DMD patients are in their twenties.[24] It is not possible to express in words the anguish of the parents of DMD patients nor the suffering those who are afflicted by this terrible ailment must stoically endure.

The molecular lesion, in about 13% of the patients with DMD, is an exon deletion that more commonly occurs in exons 47–63.[25] Eteplirsen, targeted to the splice-donor region of exon 51, induces skipping of exon 51.[26] This produces a truncated, though in-frame, partially functional dystrophin protein, similar to what is found in Becker's muscular dystrophy. This variant leads to a disease in which the clinical symptoms can be much less severe than those of patients with Duchenne's. The age of onset of Becker's can be variable and many sufferers retain both their ability to ambulate and their active life style for many decades.

As of 2013, numerous in vitro and in vivo studies had demonstrated restoration of dystrophin protein via an exon-skipping strategy.[21–25] In 2016, a phase III study[24] was published in which patients with DMD were treated with eteplirsen. The primary functional assessment examined in this trial was the 6-minute walk test (6MWT). This metric is currently the sole primary validated efficacy endpoint accepted by regulatory authorities.[22] Treatments were double blinded; patients were randomly assigned to three cohorts (n = 4 each): (1) placebo; (2) eteplirsen 30 mg/kg; or (3) 50 mg/kg every week for 24 weeks. Patients then received open-label drug, while placebo patients also received open-label drug (30 or 50 mg/kg) for over 3 years. Because all placebo patients eventually received active drug treatment, pooled historical controls were used as the basis of comparison. The extraordinary and provocative results of this trial were that the distance lost on the 6MWT test at 36 months was 151 m less than in the historical controls (p < 0.01; from about 350 to 250 m in the eteplirsen-treated patients to about 350 to 100 m in the historical controls). After 3 years, only 16.7% of the eteplirsen-treated patients lost ambulation, compared with 46.2% of the historical control patients. Several treating physicians have privately voiced their opinion that eteplirsen substantially altered the natural history of the disease in responding patients. In addition, there were no reports of treatment-related serious adverse events. None of the observed adverse events led to treatment interruptions or dose adjustments.

However, despite the chorus of numerous eteplersen advocates, including treating physicians, parents, and over 100 members of

Congress, an FDA advisory panel, by a 6–7 vote, wasn't convinced: Sarepta had disregarded repeated FDA warnings not to perform a trial based on such a small number (n = 11) of historical controls. The entire study, the FDA stated, was too small: A larger, placebo-controlled trial would now be necessary to determine if eteplirsen had truly helped anyone.[27] Further, Sarepta had apparently never demonstrated much of an increase in dystrophin production in affected muscle tissue (0.28% of normal on average, far less than the 10% of normal deemed by some necessary for a clinical impact), even in patients who had been clinically helped by their drug. Fortunately, by June, 2016, Sarepta and the FDA had settled their argument: The company will submit biopsy results to the FDA from treated patients as part of an additional, larger placebo controlled trial that is now accruing patients. How much dystrophin production will be sufficient to convince the FDA that Sarepta's claims about eteplirsen are reasonable? It has been suggested[28] that only 0.9% of the amount of dystrophin protein found in normal muscle cells will be necessary. But is this idea reasonable based on our knowledge of how biological systems are engineered?

The FDA's position was not popular among the drug advocates. A recent opinion in the Wall Street Journal[29] berated the agency for "checking off the procedural boxes" rather than confronting the realities of a deadly disease. On the other side, many FDA scientists defended their original analysis, insisting there wasn't sufficient scientific information to justify the marketing of a drug at the stratospheric price of $300,000/patient/year. Anthem, a leading insurance carrier, concurred, eventually refusing to cover drug cost for their insured patient. Other carriers, however, have been willing to approve coverage.

On September 19, 2016, the FDA overruled its own scientists. Eteplirsen, renamed Exondys 51, was approved by the FDA, though some at the agency lamented the decision as "this isn't even science".[30] It seems many treating physicians do not agree with this sentiment. Nevertheless, Sarepta must now produce data on more than 12 patients or run the risk of losing their hard-won FDA approval. We should know if Exondys 51 will produce in about the year 2020. For the sake of the children, we all hope it will.

Defitelio, Also Known as Defibrotide
On April 1, 2016, the FDA approved defibrotide (now known as Defitelio, Jazz Pharmaceuticals) for marketing in the United States.[31] The drug is indicated for severe hepatic veno-occlusive disease (sVOD) occurring after high dose chemotherapy and autologous bone marrow transplantation. sVOD is a toxicity of therapy with a high mortality.

Defibrotide (DF) is an oligonucleotide drug with a very complicated non-specific mechanism of action, which is almost certainly based on the charge-charge interactions of its phosphodiester (PO) constituents with proteins. Far from existing as a discrete molecule, DF is a polydisperse mixture of single-stranded (90%) and double-stranded (10%) PO oligonucleotides (approximate length 9-80-mer; average 50-mer; average molecular mass 16.5 ± 2.5 kDa).[32,33] However, PO oligonucleotides are rapidly degraded in plasma. Thus, the active oligomers in DF may well be double stranded, probably forming intra-strand stem loop structures or interstrand concatamers. These higher order structures may be relatively nuclease resistant, which probably stabilizes the individual DNA strands for long enough for them to reach their target, the liver.

DF cannot be produced by DNA synthesizers. The drug is a natural product obtained by the controlled depolymerization of porcine intestinal mucosal DNA. How this came to be discovered is a complex story that for reasons of space will not be discussed here. The concentration of any specific sequence in the DF mixture is approximately in the femtomolar range. Therefore, it is not possible that the activity of DF is via an antisense-type mechanism. Further, DF's individual DNA strands cannot be resolved by known physical separation methods, including capillary gel electrophoresis.

Veno-occlusive disease (VOD) of the liver, also known as sinusoidal obstruction syndrome (SOS), is characterized by damage and occlusion of small hepatic venules.[34–36] The pathophysiology of VOD/SOS appears to be related to the activation of endothelial cells by locally released cytokines in the setting of pro-inflammatory and pro-thrombotic states during hematopoietic stem cell transplantation (HSCT). Endothelial cell damage occurs, followed by activation of the fibrinolytic pathway. The hepatic sinusoids then become fibrosed; perivascular hepatocyte necrosis is the end result.[34–36] It is not uncommon that endothelial cell toxicity results from the myeloablative conditioning regimen.

The estimated incidence rate of VOD/SOS in patients undergoing HSCT is approximately 10%–15%. Typically, it occurs within 20–30 days of the transplant. sVOD is associated with progressive multi-organ failure and a mortality rate of over 80%. Patients can develop jaundice, tender hepatomegaly, fluid retention, ascites, and weight gain of more than 5% of baseline. sVOD is associated with progressive multi-organ failure and a mortality rate of over 80%.

The pathophysiology of VOD/SOS is not completely understood. As mentioned above, it appears to initially be related to endothelial cell activation by locally released cytokines in the setting of pro-inflammatory and pro-thrombotic states during HSCT. This leads to endothelial cell damage. DeLeve et al.,[33,37,38] have provided evidence that the endothelial cells round up, detach, and eventually occlude the microvascular lumina. Occlusion of the vessel lumina is followed by hepatic stellate cell activation and by the subsequent deposition of collagen in the hepatic venules,[38] culminating in perivascular hepatocyte necrosis. Sinusoidal obstruction leads to a reduction in hepatic venous outflow and development of post-sinusoidal hypertension and further liver damage.[36,39,40]

The effects of DF in sVOD post-HSCT were evaluated by Richardson and colleagues.[41] In a phase III multi-center clinical trial, an intravenous dose of 25 mg/kg/day of the drug was administered to 102 patients with multi-organ failure. However, there was no contemporaneous

comparator arm. Similar to what was done in the phase III Eteplirsen trial, patients treated with DF were compared to 32 case-matched historical-controlled patients, culled from the medical records over 6,880 cases of VOD. It appears the FDA accepted a historically controlled trial because none of the local principle investigators had faith in the efficacy of standard therapy for sVOD, which is often low molecular weight heparin, N-acetylcysteine or ursodeoxycholic acid.

The primary endpoint of the trial was patient survival rate at day +100 post-HSCT. There were 38.2% of patients that were alive in the DF group compared to 25% in the control group. The secondary endpoint was the complete response rate (i.e., complete resolution of all signs and symptoms attributable to sVOD), with a 25.5% rate observed in the DF-treated cohort and a 12.5% rate in the control group. The reported adverse events with the use of DF included hemorrhagic events and hypotension.[36,39–42]

What is the mechanism of action of DF? DNA oligomers can mimic several of the features of heparin because both are polyanions. The proteins that bind PO oligomers are also heparin-binding proteins. The presence of the nucleobases in the DF strands is also critical for high affinity binding to protein: They provide a degree of rigidity to the individual strands, limiting their extent of rotational freedom.

A recent hypothesis that comments on the mechanism of action of DF centers on its interaction with FGF2. This heparin binding protein promotes microvessel formation,[33,43–45] both directly and by[46] inducing expression of VEGF 16, which is also highly pro-angiogenic.

DF binds FGF2 and releases it from its low-affinity binding sites on extracellular matrix. This has been shown to promote endothelial cell proliferation.[47–50] On the other hand, DF does not release FGF2 from its high affinity, low picomolar-affinity cell surface receptors nor does it block the binding of FGF2 to these receptors.

In fact, the opposite situation occurs. Heparin forms a bridge between FGF2 and its cell surface receptors, which increases receptor-ligand affinity and stabilizes the interaction between them. DF can substitute for heparin, as both potentiated the proliferative effects of FGF2 on endothelial cells.[33,51] DF could also promote the growth of human vascular endothelial cells (HUVECs) both on plastic and underneath collagen I gels. In 3D-collagen I gels, DF stimulated both the proliferation and a dramatic increase (6–7- fold) in the tubular morphogenesis of human microvascular endothelial cells-1 (HMEC cells).

However, as stated above, the mechanism of DF is complex, controversial, and not entirely understood. A study by Palomo et al.,[52] showed that the DF uptake in endothelial cells was concentration, time, and temperature dependent. However, these observations could not be extended to other cell types.[52] These authors also demonstrated that the interaction of DF with the cell membrane was sufficient to produce anti-inflammatory and anti-oxidant effects. Further, the uptake of DF was not dependent on the presence of adenosine receptors.

This contradicts previous observations[53,54] and highlights the complexity of the mechanism of action of DF.

As mentioned above, DF binds to and protects FGF2, which stimulates endothelial cell mitogenesis.[33] Endothelial tubular morphogenesis was also promoted. Therefore, in some systems, DF seems to promote angiogenesis.[33] However, it is also plausible that DF's pro-angiogenesis activity is in part a result of an antagonistic action on the apoptotic pathway. Consistent with this idea is a study[55] that demonstrated anti-apoptotic effects of DF on fludarabine-treated HMECs, and its ability to downregulate the cytotoxic T lymphocyte response against endothelial cells.[55]

The fact that DF can also display anti-angiogenic potential[56] emphasizes that the activity of this drug is probably cell/system and concentration dependent.[52] The observation that the anti-angiogenic activity detected in HUVEC and HMEC cells[56] seems to develop into pro-angiogenic (and/or anti-apoptotic) activity at an approximately 4-fold higher concentration in the identical cell types[33] is also a notable one.

But the mechanism of action of DF is even far more complex than noted above. The excellent review by Pescador and colleagues[32] in which many of the other activities of DF are discussed is highly recommended. Briefly, DF is potently antithrombotic[32,33] and fibrinolytic.[32,57] DF increases plasma tissue plasminogen activator activity and decreases the activity of its inhibitor (PAI-1). It can also release tissue-factor pathway inhibitor (TFPI) from endothelial cells[58] and inhibit platelet aggregation by increasing the plasma concentration of prostaglandin E2.[59] These effects, and others described by Pescador et al.,[32] may be anti-coagulating at the site where DF concentrations are highest and where DF is needed most, in the hepatic sinusoidal endothelium.

Much of the confusion about the mechanism of DF in sVOD has been engendered because of our lack of a fundamental understanding of the disease process. Is sVOD a coagulopathy or is it caused by obstruction by endothelial cells, as suggested by the work of DeLeve et al.[37,38] or is it a combination of both and more besides? While these questions are of keen academic importance, to the patient whose life has been saved by this drug, it probably makes very little difference.

Spinraza, Also Known as Nusinersen

Of all the oligonucleotide therapeutics approved to date for marketing, this drug, approved by the FDA on December 23, 2016, seems to be the most exciting. Nusinersen is a 18-mer phosphorothioate 2′-O-methoxyethyl antisense oligonucleotide with all cytidines methyl-modified at the 5-position. The oligo induces the inclusion of exon 7 in the SMN1 and SMN2 mRNA by targeting and blocking an intron 7 internal splice site.[60] Nusinersen is now indicated in infants with types 1, 2, and 3 spinal muscular atrophy (SMA). About 400 infants are born with this disease in the United States every year.

SMA is caused by a mutation in the SNM1 gene on chromosome 5, leading to a deficiency of the survival of motor neuron (SNM) protein. When the disease presents in very young infants, it is referred to as type 1 SMA. These infants have generalized muscle weakness and difficulty breathing. They may also have difficulty swallowing and fail to reach the developmental milestone of sitting upright.[61] Later onset of the disease in infants and toddlers is referred to as SMA2.

At the time of this writing (early January, 2017), the results of the pivotal ENDEAR trial in infants with SMA have not yet been made public. The ENDEAR trial was a randomized, double-blinded, sham-controlled study in children with infantile SMA. Most of these infants were likely to develop type 1 SMA. There were 82 infants that were accrued onto trial. Those randomized to Spinraza received one intrathecal injection (i.e., into the cerebrospinal fluid) every 2 months. So far, the information made public indicates that at a planned interim analysis, a greater percentage (40% Spinraza treated versus 0% sham treated, $p < 0.0001$) of Spinraza-treated infants achieved a motor milestone response as measured by the Hammersmith Infant Neurological Examination (HINE). Further, a smaller number of patients treated with Spinraza (29%) died during the course of the study compared to those who were untreated (43%). The most common side effects were respiratory infections and constipation, and the FDA has warned about the possibility of thrombocytopenia and renal toxicity.

This potentially life-saving drug comes at an extremely high cost from its developer, Biogen, who licensed it from Ionis (formerly Isis, as already mentioned). Biogen has priced Spinraza at $750,000 for the first year's treatment ($125,000 per injection) and $350,000 per year subsequently. What if any pushback will emerge from insurance carriers or governmental authorities is currently unknown.

CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

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