

REVIEW

Targeting RNA: A Transformative Therapeutic Strategy

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The therapeutic pathways that modulate transcription mechanisms currently include gene knockdown and splicing modulation. However, additional mechanisms may come into play as more understanding of molecular biology and disease etiology emerge. Building on advances in chemistry and delivery technology, oligonucleotide therapeutics is emerging as an established, validated class of drugs that can modulate a multitude of genetic targets. These targets include over 10,000 proteins in the human genome that have hitherto been considered undruggable by small molecules and protein therapeutics. The approval of five oligonucleotides within the last 2 years elicited unprecedented excitement in the field. However, there are remaining challenges to overcome and significant room for future innovation to fully realize the potential of oligonucleotide therapeutics. In this review, we focus on the translational strategies encompassing preclinical evaluation and clinical development in the context of approved oligonucleotide therapeutics. Translational approaches with respect to pharmacology, pharmacokinetics, cardiac safety evaluation, and dose selection that are specific to this class of drugs are reviewed with examples. The mechanism of action, chemical evolution, and intracellular delivery of oligonucleotide therapies are only briefly reviewed to provide a general background for this class of drugs.

The concept of a synthetic oligonucleotide to control the expression of selected genes was first demonstrated 4 decades ago by Stephenson and Zamecnik.¹ Since then, it has been recognized that oligonucleotide therapeutics can be highly specific and can target disease-relevant proteins or genes that are inaccessible by small molecules and proteins.² However, the anticipated clinical success was not achieved until recently after innovation and technology breakthroughs overcame some of the major hurdles of these therapeutics.³ These hurdles include poor pharmacokinetics (PKs), inefficient tissue and cellular delivery to reach intracellular targets, insufficient biological activity, immune stimulation, and off-target toxicity. Since 2016, five oligonucleotides (defibrotide, eteplirsen, nusinersen, inotersen, and patisiran) have been approved to treat a range of diseases. This success provides momentum for continued development of oligonucleotide therapeutics into a next major class of drugs following small molecules and protein therapeutics.

In this review, we focus on the translational strategies encompassing preclinical evaluation and clinical development in the context of approved oligonucleotide therapeutics. The mechanism of action, chemical evolution, and intracellular delivery of oligonucleotide therapies are only briefly reviewed to provide a background for this class of therapies. Reviews specific in these areas have been published elsewhere and the readers are encouraged to review them.^{3–16}

MECHANISM OF ACTION

Landmark events, such as the discovery of the helical structure of DNA¹⁷ and the completion of the human genome project,¹⁸ led to the development of oligonucleotide medicines in the postgenomic era (**Figure 1**). It has been postulated and generally recognized that only one-third of

the roughly 20,000 proteins in the human genome are druggable by small molecules and protein-based drugs (e.g., monoclonal antibodies.² This leaves a large gap in treating human disease, and this gap, in part, could be filled by therapeutic oligonucleotides. In principle, oligonucleotides can be rationally designed against virtually any genetic target.⁴ Their unique mechanism of action differentiates this class of therapeutics from small molecules and protein therapeutics^{2,3,7–10,14,19} (**Table 1**). Oligonucleotides bind to their cognate RNA target by Watson-Crick hybridization with high selectivity and affinity. By exploiting known degradation and maturation pathways, these therapeutics can either utilize the endogenous nucleases to degrade the target RNA or modulate RNA splicing and translation by sterically blocking the ribosomal machinery^{2,3,7–10,14,19} (**Figure 2**).

Degradation mechanism

The concept of using a synthetic antisense oligonucleotide to control the gene expression, known as the antisense intervention, was demonstrated in 1978.¹ Shortly thereafter, the RNase H mechanism for site-specific cleavage of RNA was discovered in 1979²⁰ (**Figure 1**). This remains the most utilized antisense mechanism despite the rapid growth in the discovery of new RNA biology.³ RNase H enzymes are a family of endogenous catalytic nucleases that are present in both the cytoplasm and the nucleus. Among them, the RNase H1 mechanism is harnessed by designing an antisense oligonucleotide with a central “gap” of 8–10 DNA bases. Following binding to the target messenger RNA (mRNA), the RNA–DNA heteroduplex can be recognized by RNase H1, resulting in the selective cleavage of the RNA strand while leaving the synthetic DNA strand intact to bind additional target mRNA.¹⁴ The knockdown of the target mRNA ultimately downregulates production of the

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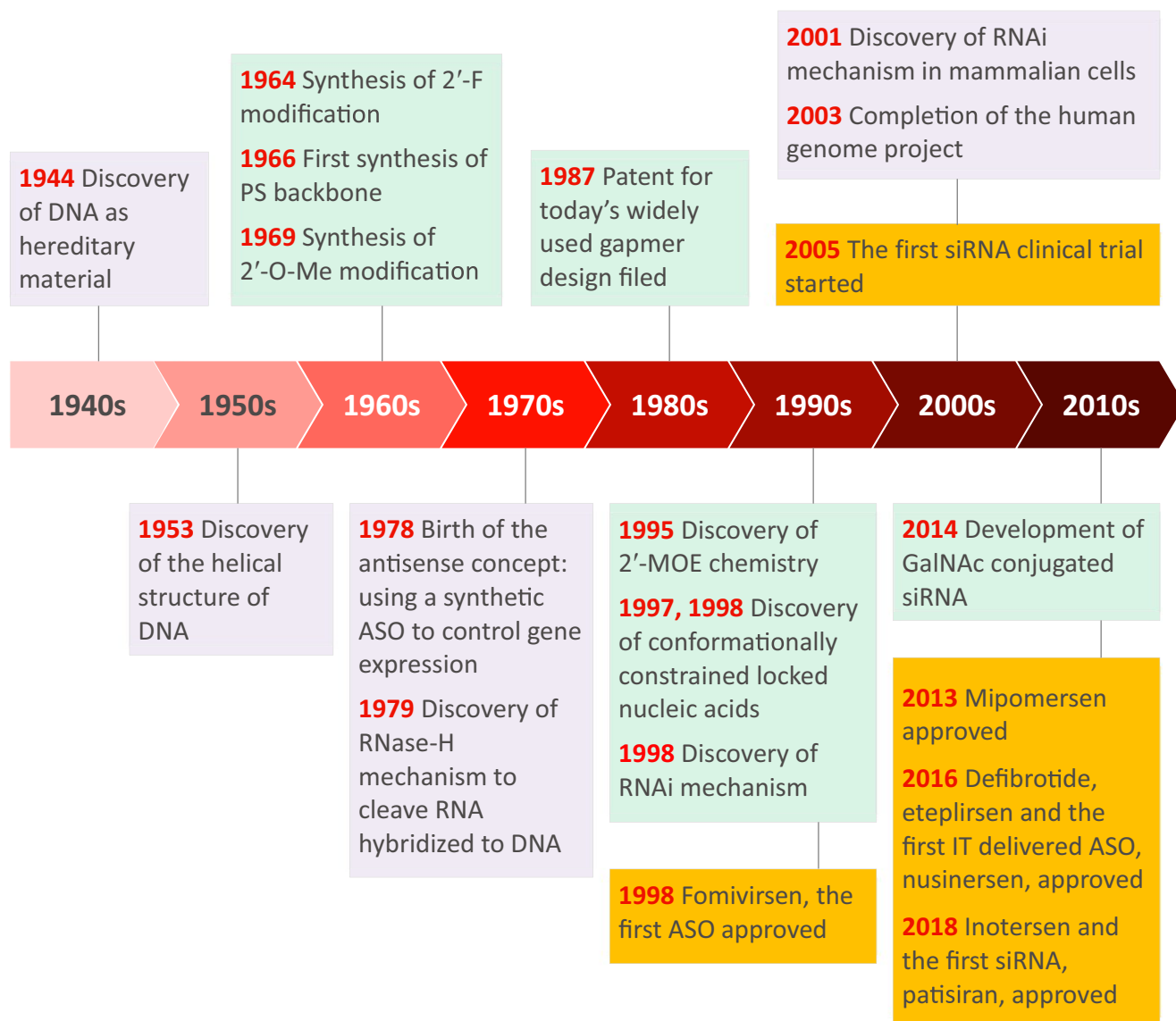


Figure 1 Selected key milestones in the development of oligonucleotide therapeutics. **Purple box:** milestones in biology; **green box:** milestones in chemistry; **orange box:** clinical milestones. 2'-F, 2'-fluoro; PS, phosphorothioate; 2'-MOE, 2'-O-methoxyethyl; 2'-O-Me, 2'-O-methyl; ASO, antisense oligonucleotide; GalNAc, *N*-acetylgalactosamine; IT, intrathecal; RNAi, interference RNA; siRNA, short interference RNA.

disease-associated protein^{3,9} (**Figure 2**). This pathway has been validated by the approval of three antisense oligonucleotides that utilize the RNase H1 mechanism (fomivirsen, mipomersen, and inotersen) to treat a range of diseases.

Another mRNA degradation mechanism widely utilized for designing oligonucleotide therapeutics is the endogenous RNA interference mechanism.^{21,22} RNA interference activity is driven by the argonaute-2 (Ago2) enzyme, which forms the catalytic core of the RNA-induced silencing complex.²³ Different from RNase H1 that recognizes the RNA-DNA heteroduplex, Ago2 recognizes the RNA-RNA duplex and cleaves the target RNA through an RNase H-like enzyme mechanism.¹⁴ Another important aspect by which RNA interference differs from the RNase H1 mechanism is its activation by double-stranded RNA molecules. To harness

this ubiquitous pathway, double-stranded short interference RNAs (siRNAs) have been designed to include two complementary strands: a sense “passenger” strand and an antisense or “guide” strand. The siRNAs are recognized by the RNA-induced silencing complex complex, where unwinding and release of the sense strand is accomplished by Ago2.²⁴ The antisense strand is left bound to Ago2 protein to form a ribonucleoprotein complex. The Ago2 complex facilitates hybridization of the antisense strand to the target RNA, resulting in site-specific cleavage of the target RNA while retaining the antisense strand for a prolonged time period permitting it to bind additional target RNA^{3,8,9} (**Figure 2**). The impact of RNA interference is well recognized by the scientific community. In 2006, Fire and Mello received the Nobel Prize for their 1998 discovery of this mechanism.²² The approval of

Table 1 Comparison of key properties of small molecules, oligonucleotide therapeutics, and mAbs

	Small molecule	Oligonucleotide therapeutics		mAb
		ASO	siRNA	
MW ^{15,86}	~ 200–500 Da	6,000–10,000 Da	12,000–13,300 Da	~ 150,000 Da
Manufacture ^{3,87}	Chemical synthesis	<Chemical synthesis>		Bioprocessing based on mammalian cell
Physicochemical properties ^{3,86}	Well-defined; driven by chemistry	Well-defined; similar for each chemical class	Well-defined; used for delivery strategy	Complex; heterogeneous product
Site of action ^{2,9}	Extracellular and intracellular	<Intracellular>		Extracellular
Selectivity and potency ^{2,86}	Generally less selective but potent	<High selectivity and potency>		High selectivity and potency
Intracellular delivery ^{2,9}	Generally good	Sufficient by endocytosis	Limited; must be encapsulated or conjugated	Uncommon
Route of administration ^{3,9,86}	Primarily oral	i.v., s.c., IT, and IVT; not orally bioavailable	i.v., s.c., and IVT; not orally bioavailable	Primarily i.v., s.c., and i.m.; not orally bioavailable
Dosing frequency ^{3,86,88}	Often daily	Less frequent; weekly to once every 4 months	Less frequent; weekly to once every 3–6 months	Less frequent; often weekly to monthly
BA ^{40,49,53,88}	Generally good	Good for s.c., 50–100%; no oral BA	Not reported	Good for s.c. and i.m., 50–100%; no oral BA
T _{max} , s.c. or i.m. ^{15,88}	NA; primarily given orally	0.25–5 hour after s.c.	Not reported	1–8 days after s.c. or i.m.
V _d ^{15,54,86}	Generally high, with distribution to organs and tissues	High; extensive distribution to kidneys and liver	Extensive distribution to kidneys and liver	Lower, often limited to plasma or extracellular fluids
Metabolism ^{15,54,86}	Primarily by CYP and phase II enzymes	<By nucleases to shorter ONs>		Catabolized to peptides or amino acids
Excretion ^{15,54,86}	Primarily excreted in bile and urine	<Primarily excreted in urine>		Very limited
CL ^{15,54,86}	Often linear CL	Rapid plasma CL due to distribution to tissues; slow clearance from tissues	More rapid clearance than ASO	Slow CL
t _{1/2} ^{15,54,86}	Short, often several hours to a day	Long, 2 weeks to 6 months	Shorter than ASO, up to a few days; prolonged t _{1/2} by formulations	Long, days to weeks
Immunogenicity ^{3,16,86}	No	<Yes; low risk of an impact on PK and PD>		Yes, and high risk of an impact on PK and PD
PD duration ^{3,86}	Generally short	<Long>		Long
DDI ^{15,46,54,62,86}	High risk	<Very low risk through interaction with CYPs, transporters and PPB>		Uncommon
Off-target toxicity ^{16,86,89}	High risk	<Low risk>		Uncommon

The symbol of “< >” denotes properties for both ASO and siRNA.

ASO, antisense oligonucleotide; BA, bioavailability; CL, clearance; CYP, cytochrome P450; DDI, drug–drug interaction; IT, intrathecal; IVT, intravitreal; mAbs, monoclonal antibodies; MW, molecular weight; NA, not applicable; ONs, oligonucleotides; PD, pharmacodynamic; PK, pharmacokinetic; PPB, plasma protein binding; siRNA, short interference RNA; t_{1/2}, terminal elimination half-life; T_{max}, time to reach the maximum plasma concentration; V_d, volume of distribution.

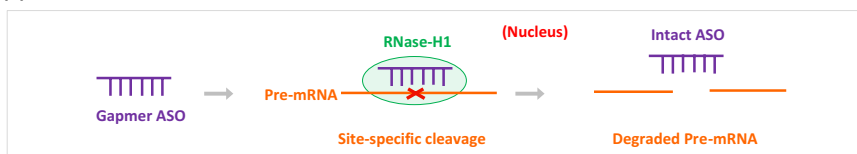
the first siRNA drug (patisiran) and current testing of over 20 siRNAs in the clinic validates the strategy of harnessing RNA interference as a mechanism to treat diseases caused by dysfunctional protein.

Numerous other nucleases and natural RNA-degrading pathways, such as nonsense-mediated decay, may be utilized to design new classes of oligonucleotide therapeutics that selectively knock down RNA.¹⁴ In addition, clustered regularly interspaced short palindromic repeats-Cas technology utilizes the antisense pairing of a single guide RNA to a specific DNA target site to directly edit genomic DNA.²⁵ As a result, the functional output of the DNA sequences within

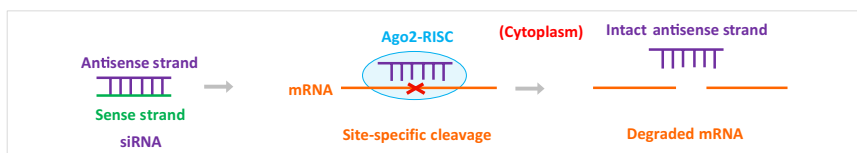
the endogenous genome can be modified as a means to cure genetic disease.²

Nondegradation mechanism

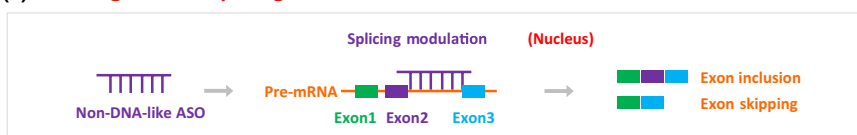
Oligonucleotide therapeutics can also act as a steric blocker to alter the mRNA maturation process. This includes modulation of splicing to reduce dysfunctional protein translation, restore function to a protein, and to obstruct interactions of the target RNA with key proteins, among others¹⁹ (Figure 2). For this mechanism, oligonucleotides are designed to avoid formation of oligonucleotide–RNA hybrids that are substrates for RNase H1 or Ago2 cleavage.

(a) RNase-H1 degradation mechanism

Approvals: fomivirsen, mipomersen and inotersen.

(b) Ago2 degradation mechanism

Approval: patisiran.

(c) Non-degradation splicing modulation mechanism

Approvals: eteplirsen and nusinersen.

Figure 2 Schematic illustration of three common mechanisms adopted by the approved ASOs and siRNA. (a) An ASO with a central “gap” of DNA bases (gapmer ASO) binds to target mRNA by Watson-Crick hybridization; RNase-H1 recognizes an RNA–DNA heteroduplex, cleaving the target RNA strand selectively while leaving ASO strand intact to bind to additional target RNA. (b) An siRNA is recognized by the RISC complex, where the sense strand is degraded and removed, and the antisense strand is left bound to Ago2 protein to form a ribonucleoprotein complex. The Ago2 complex facilitates hybridization of the antisense strand to the target RNA, cleaving the target RNA selectively while leaving the antisense strand intact to bind to additional target RNA. (c) An ASO modified to remove any potential to form RNA–DNA hybrids (non-DNA-like ASO) acts as a steric blocker to alter RNA maturation process, including modulation of splicing. Ago2, argonaute-2; ASO, antisense oligonucleotide; mRNA, messenger RNA; siRNA, short interference RNA; RISC, RNA-induced silencing complex.

For example, eteplirsen, approved by the US Food and Drug Administration (FDA) to treat Duchenne muscular dystrophy (DMD), contains morpholino modifications.⁹ Another example is nusinersen, an 18-mer antisense oligonucleotide that contains fully modified ribose (i.e., no “gap” in central region). Nusinersen has been approved worldwide to treat spinal muscular atrophy (SMA), the most common genetic cause of infant death. The genetic cause of the disease, homozygous loss, or mutation of the survival motor neuron 1 (*SMN1*) gene was identified in 1995.²⁶ Nusinersen is designed to alter splicing of the pre-mRNA of a paralogous gene, *SMN2*, to promote inclusion of exon 7, thus forming full-length *SMN1*-like mRNA. This ultimately compensates for the loss of full-length *SMN* protein production in patients with SMA leading to profound modification of the disease.³ Nusinersen was approved by the FDA in 2016, only 21 years after the discovery of the genetic cause.

CHEMICAL EVOLUTION

Unmodified oligonucleotides do not possess desired drug-like properties. They have poor PK properties (e.g., poor stability and poor distribution), poor intracellular uptake, and suboptimal binding affinity toward target mRNA.⁹ To achieve clinical utility, oligonucleotides require chemical

modifications. As this is a chemically synthesized drug platform, advances in medicinal chemistry have been crucial in the emerging success of oligonucleotide therapeutics. Today’s most widely used single alteration, phosphorothioate (PS) modification (replacement of a nonbridging phosphodiester oxygen by sulfur), was first studied in the 1960s.²⁷ This backbone modification reduces hydrophilicity and increases resistance to nuclease degradation and binding to plasma proteins.³ This modification consequently enhances stability and decreases glomerular filtration and excretion into urine.^{4,9} Through improved PK properties, PS modification maintains the systemic drug exposure that ultimately leads to enhanced cellular uptake and trafficking. However, PS modification alone does not fully protect the oligonucleotide from degradation. Moreover, it reduces affinity toward its target and generates potential inflammatory responses at high concentrations.^{3,4,28} To further improve binding affinity and nuclease resistance and limit pro-inflammatory effects, a range of second-generation oligonucleotides with sugar modifications were developed in the 1990s.^{3,4,7,9} Replacing the 2′-hydroxyl by 2′-O-methyl (2′-O-Me), 2′-O-methoxyethyl (2′-MOE), and 2′-fluoro (2′-F) and introducing conformationally constrained modifications, such as locked nucleic acid (LNA) and its methylated analog, known as constrained ethyl (cEt) are the common sugar

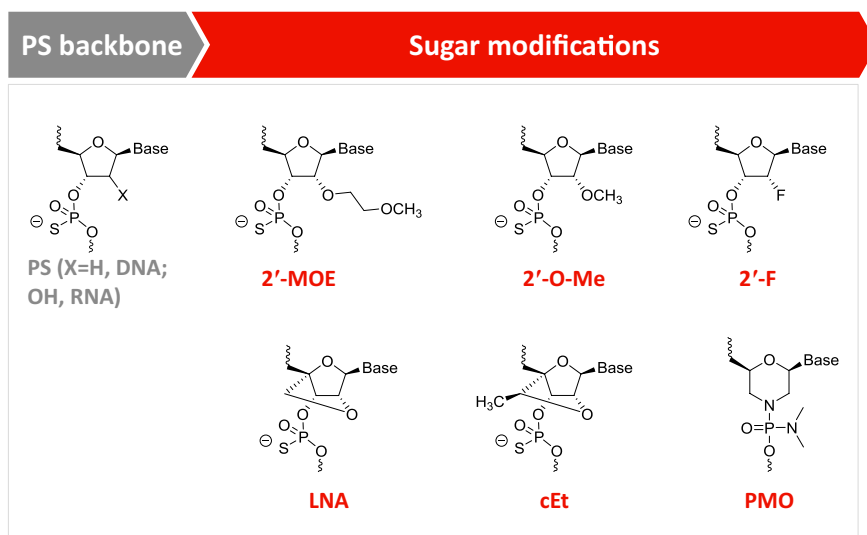


Figure 3 Common chemical modifications for the ASOs and siRNAs approved and in the clinic. The modifications utilized in the approved ASOs and siRNA (fomivirsen, mipomersen, eteplirsen, nusinersen, inotersen, and patisiran) are PS, 2'-MOE, 2'-O-Me, 2'-F, and PMO. Fomivirsen: PS DNA, no sugar modification; mipomersen and inotersen: PS and 2'-MOE modified gapmer ASOs; nusinersen: PS and 2'-MOE fully modified ASO; patisiran: PS, 2'-F and 2'-O-Me modified siRNA; eteplirsen: PMO, 2'-F, 2'-fluoro; 2'-O-Me, 2'-O-methyl; 2'-MOE, 2'-O-methoxyethyl; ASO, antisense oligonucleotide; cEt, constrained ethyl; LNA, locked nucleic acid; PMO, phosphorodiamidate morpholino oligomer; PS, phosphorothioate; siRNA, short interference RNA.

modifications.^{4,29–31} The synthesis of 2'-F and 2'-O-Me modifications first occurred in the 1960s, 3 decades before their realization for therapeutic use.^{32–34} The 2'-O-Me modification improves binding affinity and nuclease resistance and reduces immunostimulatory properties of the PS backbone.^{4,28} Building on 2'-O-Me chemistry, the binding affinity and nuclease resistance were further improved by 2'-MOE modification.^{4,29} Through reducing conformational flexibility of nucleotides, LNA and cEt dramatically improved affinity and 5-fold to 10-fold in potency relative to a 2'-MOE analog.^{3,4,28,30,31} However, LNA and cEt modifications have been associated with liver toxicity. The liver toxicity is sequence dependent and caused by off-target cleavage of mismatched transcripts.⁴ To reduce the toxicity, computational tools can be utilized to select a sequence that has minimal complementarity to off-target transcripts.⁴ Although chemistry continues to evolve and new analogs continue to be explored, 2'-MOE remains the leading analog used for antisense oligonucleotides. Three 2'-MOE modified antisense oligonucleotide have been approved for commercial use (mipomersen, nusinersen, and inotersen) (**Figure 3**) and over 20 are in clinical development. However, this is not the case for siRNAs. The structural requirement to utilize RNA interference pathways limits the usage of 2'-MOE modification on siRNAs. Instead, siRNAs use extensively 2'-F and 2'-O-Me modifications. The approved siRNA drug, patisiran, has a combination of 2'-F and 2'-O-Me analogs³ (**Figure 3**).

The critical PS backbone modification introduces a chiral center at the phosphorus loci, and consequently mixtures of up to 2^n stereoisomers exist in any PS-modified oligonucleotides, where n represents the number of PS linkages.³⁵ Although stereochemistry is generally controlled for small molecule drugs to optimize potency and efficacy,

it has not been widely adopted in the clinic for oligonucleotide therapeutics. It was not considered feasible to separate or synthesize stereopure oligonucleotides for a clinical setting.³⁵ All oligonucleotide therapeutics approved to date are stereoisomeric mixtures. However, recent developments in chemistry overcome the feasibility hurdle, and a scalable synthetic process has been reported to yield stereopure oligonucleotides.³⁵ A different phosphorus(V)-based reagent platform has also demonstrated diastereoselective phosphorus–sulfur incorporation and can, in principle, synthesize stereopure oligonucleotides via an efficient and inexpensive protocol.³⁶ The stereochemistry of a PS oligonucleotide has been demonstrated to have a substantial impact on stability, specificity, and efficacy of the oligonucleotide.^{4,35,37–39} Building on this concept, a new generation of antisense oligonucleotides is being designed with controlled stereochemistry. Recently, two stereo-defined antisense oligonucleotide drugs have been advanced to the clinic to treat Huntington's disease (PRECISION-HD1 and PRECISION-HD2). As single isomers, it is also anticipated that greater discriminatory control between toxic and non-toxic RNA can be achieved, thus improving clinical safety margins. Wider use of stereoselective PS oligonucleotides to treat diseases that require discrimination between functional and dysfunctional proteins will continue when these next-generation therapeutics progress from promise to success.⁴

INTRACELLULAR DELIVERY

Unlike protein-based drugs that generally only bind to extracellular soluble targets or targets on the cell surface, oligonucleotide therapeutics must reach the intracellular targets in the cytoplasm and nucleus to exert pharmacological activities (**Table 1**). The cellular uptake pathways

have been well studied for single-stranded antisense oligonucleotides and to a lesser extent for double-stranded siRNAs.^{7–11,40} Without the inclusion of a delivery system, antisense oligonucleotides are taken up by most cells through endocytosis, a mechanism that requires binding of the antisense oligonucleotide to surface proteins, such as clathrin and caveolin.^{10,11,40} Multiple pathways are involved in antisense oligonucleotide internalization, with several pathways considered as “productive,” meaning that these pathways can direct antisense oligonucleotides to the cytoplasm or ultimately the nucleus to bind to their specific targets. The productive pathways have been characterized^{10,11} and rely on nonmacropinocytotic, endosomal intracellular distribution.⁴¹ The nonproductive pathways (e.g., macropinocytosis pathway) result in the accumulation of antisense oligonucleotides in lysosomes where antisense oligonucleotides are degraded, sequestered, and cleared from the cells.¹² Tackling the issues of nonproductive intracellular distribution has been an active area of research.^{3,10–12}

Compared with an antisense oligonucleotide (~ 7 kDa), cellular uptake of a double-stranded siRNA is limited by its larger molecular size (~ 13 kDa; **Table 1**) and the hydrophilic nature due to its duplex structure. To enhance cellular uptake, siRNAs must either be complexed within nanoparticles^{5,42} or be conjugated to a targeting ligand, such as *N*-acetylgalactosamine (GalNAc).⁴³ The GalNAc ligand is recognized by highly expressed asialoglycoprotein receptor in hepatocytes, which consequently enhances their potency toward the liver targets.^{2,3,6} The first approved siRNA, patisiran, is delivered primarily to the liver by lipid nanoparticles (LNPs). However, to overcome the pro-inflammatory effect of the nanoparticle formulation, direct conjugation strategies have become increasingly important for siRNA delivery. To date, over 10 siRNAs complexed within nanoparticles and even more GalNAc conjugated siRNAs are being tested in clinical trials.³ Identifying ligands and a delivery strategy outside the liver remains a daunting but active area of research.^{2,4}

TRANSLATIONAL STRATEGIES ENCOMPASSING PRECLINICAL EVALUATION AND CLINICAL DEVELOPMENT

As a chemically synthesized drug platform, oligonucleotide therapeutics are regulated as small molecules, although they differ in many aspects (**Table 1**). The preclinical evaluation of this class of drugs has generally followed regulatory guidelines for small molecules. However, they share attributes with protein therapeutics, such as high selectivity, long half-lives, infrequent dosing, and low risk of drug–drug interaction (**Table 1**).

Pharmacology

Because oligonucleotide therapeutics modulates mRNA to either reduce or increase protein production, the mRNA and protein levels can be measured in animal models to demonstrate pharmacological effect. Oligonucleotides bind to the target RNA with high specificity, and a single base mismatch could decrease the affinity by ~ 500-fold.⁴⁴ For this reason, human transgenic mouse models

are often used for pharmacological testing.⁴⁵ However, the translational success from animal models to humans differs between diseases. The challenge remains for many diseases. For example, Alzheimer’s disease and related dementias do not have animal models that robustly recapitulate human disease and the long-term protein-lowering effect is not fully understood.²⁸ In addition, although testing a species-specific oligonucleotide may be viable to evaluate direct pharmacological activities, the animal sequence, physiology, and disease may differ sufficiently from the human setting that interpretation of results may become problematic.⁴⁶

Animal models have successfully demonstrated pharmacological activities for the approved oligonucleotide drugs. Mipomersen, for example, was designed to target human apolipoprotein B (apoB) mRNA to lower apoB-100 and subsequently lower low-density lipoprotein cholesterol (LDL-C). LDL-C is a major risk factor for coronary heart disease and has been implicated in the inflammation associated with atherosclerosis.⁴⁶ Mipomersen is 100% complementary to a 20-base-pair portion of the coding region of human apoB. However, the mouse and monkey apoB sequences are only 81% and 95% homologous, respectively, to the human sequence. Because of the lack of homology and base-pair mismatches between human and animal sequences, mipomersen was evaluated in mice containing the human apoB genomic transgene. Species-specific surrogate oligonucleotides with 100% complementarity to the animal sequences were also used to examine the inhibitory effect of apoB in mouse, hamster, rabbit, and monkey models. Studies were performed in animals with normal cholesterol levels, as well as several rodent disease models of hyperlipidemia and atherosclerosis. In these studies, mipomersen and its species-specific analogs reduced hepatic apoB mRNA and protein, and serum apoB, LDL-C, and total cholesterol level in a dose-dependent, concentration-dependent, and time-dependent manner. In addition to effects on lipids, reduction of apoB resulted in the reduction of atherosclerosis in three mouse models, and this reduction correlates with decreases in apoB-100 expression (KYNAMRO; Pharmacology Review FDA 2013). The reductions in serum apoB mRNA and protein, serum LDL-C, and total cholesterol were concurrently observed in humans with similar half-maximal effective concentration (EC₅₀) for serum apoB reduction obtained across species.^{40,46}

Nusinersen is another example. As mentioned in **Mechanism of Action**, nusinersen alters the splicing of the SMN2 pre-mRNA to promote inclusion of exon 7 to form a full-length SMN1-like mRNA. Humans are the only species known to have the SMN2 gene; therefore, the preclinical pharmacological effects of nusinersen can only be studied in genetically modified animal models or human cells. Several SMA mouse models have been developed and have a broad range of phenotypic severity.⁴⁷ The general approach to replicating human disease has been to remove the endogenous functional mouse gene and add various copy numbers of the human SMN2 gene. Those models with more copies of the SMN2 gene typically have milder phenotypes than those with fewer copies.⁴⁷ The pharmacological properties of nusinersen were assessed in multiple models with varying degrees of phenotypic

severity. For PK and pharmacodynamic (PD) relationships, a mild model expressing four copies of the human *SMN2* gene was used. The use of the mild model to assess PK/PD relationships minimized potential complications associated with the rapid deterioration and morbidity found in the severe models. Mouse models with more severe phenotypes were used to assess efficacy of nusinersen. In the mild model, 50–90% exon 7 inclusion was observed in the central nervous system (CNS) when nusinersen was present at tissue concentrations of 2–10 µg/g (SPINRAZA, Pharmacology Review FDA 2016, SPINRAZA Clinical Pharmacology and Biopharmaceutics Review FDA 2016). To the extent PK assessments could be conducted in severe mouse models, they, too, showed efficacy in the 2–10 µg/g tissue concentration range.⁴⁸ These concentrations were utilized to select the clinical doses and were achieved in patients with SMA (SPINRAZA Pharmacology Review FDA 2016, SPINRAZA Clinical Pharmacology and Biopharmaceutics Review FDA 2016).

Pharmacokinetics

The PK properties of oligonucleotide therapeutics depend on the type of chemical modifications, the carriers, or conjugates, and are mostly sequence independent.^{3,7,15,46,49–52} For example, antisense oligonucleotides with 2'-MOE modifications have similar physicochemical characteristics and, thus, similar PK properties in rats, monkeys, dogs, and humans.^{15,46,49–52} The human systemic exposure following s.c. or i.v. injection can be predicted directly from monkey exposure based on body weight adjusted dose (e.g., in mg per kg dose). Approximately fivefold allometric scaling factor should be added to the body weight adjusted dose when the prediction is made from mouse exposure.^{49,52} This class of oligonucleotides are readily absorbed following s.c. administration with peak plasma concentrations (C_{max}) achieved within 3–4 hours and bioavailability ranging from 53% to nearly complete^{40,49,53} (Table 1). Following either i.v. or s.c. administration, plasma concentrations decline rapidly from C_{max} in a multiphasic manner with a rapid distribution phase from plasma to tissues within a few minutes to a few hours. This is followed by one or more slower disposition phases and a terminal elimination half-life of up to 4 weeks to 2 months.^{40,49,53} The long terminal elimination half-life from plasma following initial tissue distribution likely represents redistribution from tissues and reflects the slow transfer from cell to extracellular fluid to plasma. In this phase, plasma concentrations seem directly proportional to tissue concentrations (e.g., ~ 1:5,000 between plasma and liver for 2'-MOE modified antisense oligonucleotides). This ratio is consistently observed across species, and, thus, it enables plasma to serve as a potential biomarker of tissue kinetics.^{40,54} This is critical for understanding the PD effect in humans where the target tissues are often inaccessible. For example, the liver EC_{50} of mipomersen cannot be measured in humans but can be estimated from the plasma EC_{50} by applying a liver/plasma partition ratio of 5,800. The estimated liver EC_{50} in humans is 81 µg/g, which is similar to those determined from the mouse models (101–109 µg/g).⁴⁶

Oligonucleotide therapeutics are metabolized by ubiquitous nucleases; parent compound and metabolites are

then primarily excreted in urine.^{15,54} Antisense oligonucleotides that contain a PS backbone are extensively bound to plasma proteins ($\geq 85\%$) and mainly to albumin across all species including man.⁵⁴ This high degree of binding to human plasma proteins is expected to reduce urinary excretion and renal clearance of the drugs due to glomerular filtration. The binding affinity to albumin is relatively low, allowing uptake into tissues. Other classes of oligonucleotides that are less charged or less bound to plasma proteins (e.g., siRNAs or morpholino nucleotide oligomers) are cleared rapidly from blood because of either nuclease metabolism in blood or excretion in urine. For example, $< 2.1\%$ of patisiran, an siRNA, is bound to plasma proteins, and the terminal elimination half-life of patisiran is only 3 days (ONPATTRO (patisiran) Highlights of Prescribing Information 2018). This results in much lower tissue uptake in comparison to PS oligonucleotides. Thus, as mentioned in **Intracellular Delivery**, patisiran must be formulated in LNPs to enhance cellular uptake into hepatocytes. To enhance stability against nucleases, GalNAc-conjugated siRNAs must be chemically modified and typically have 4–12 PS substitutions as well as 2'-F and 2'-O-Me sugar modifications.³ GalNAc siRNAs can be given by s.c. injection, and the absorption is rapid. The elimination half-life from hepatocytes is several weeks, supporting infrequent weekly to quarterly dosing.³ Another example is eteplirsen, a phosphorodiamidate morpholino oligomer (PMO) with low plasma protein binding (6.1–16.5%; EXONDYS 51 Clinical Pharmacology and Biopharmaceutics Review FDA 2016). Although eteplirsen is metabolically stable because of the nuclease-resistant morpholino chemistry, low protein binding results in high renal clearance of 60–70% of the dose within 24 hours of i.v. administration. The elimination is much faster than a typical PS 2'-MOE analog resulting in a shorter elimination half-life of only 3–4 hours. The high clearance and short half-life might result in suboptimal target distribution and cellular uptake, which may partially account for the marginal efficacy. Although eteplirsen was granted conditional approval by the FDA, it is stated in the drug label that a clinical benefit of eteplirsen has not been established. FDA reviewers recommended evaluation of higher doses or a more frequent dosing regimen to help to increase the production of dystrophin in skeletal muscle, a pharmacological biomarker of eteplirsen activity (EXONDYS 51 (Eteplirsen) Highlights of Prescribing Information 2016; EXONDYS 51 Clinical Pharmacology and Biopharmaceutics Review FDA 2016).

Following systemic s.c. or i.v. injection, oligonucleotide therapeutics distribute extensively in the kidneys and liver with over 80% of the dose accounted for in these two organs.^{40,54,55} However, these molecules are large, highly charged, and do not cross the blood brain barrier. There is no distribution in the CNS following systemic delivery.^{40,54} To reach targets in the brain, oligonucleotide therapeutics must be delivered directly into cerebrospinal fluid (CSF). Once delivered to the CSF using intraventricular or intrathecal (IT) injection, antisense oligonucleotides distribute throughout the brain and spinal cord in mice, rats, monkeys, and humans.^{28,40,56}

As mentioned earlier, nusinersen was approved to treat SMA by modifying the splicing of *SMN2* pre-mRNA, an

intended target in the CNS. To bypass the blood brain barrier, it is delivered directly to the CSF by IT injection, thus achieving complete bioavailability in the CSF (SPINRAZA, Pharmacology Review FDA 2016). The PK characteristics of nusinersen have been well characterized in both monkeys and humans (SPINRAZA, Pharmacology Review FDA 2016; SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016), representing the PK of this class of oligonucleotides when delivered IT. Following IT injection to monkeys, peak CSF concentrations occurred at the first evaluated timepoint (15 minutes to 1 hour). The CSF concentrations declined from the peak concentration in a multiphasic manner: a relatively rapid declining phase (up to 1–2 days or 2–7 days depending on the sampling scheme of individual studies) followed by a very slow terminal elimination phase (over > 70 days). The rapid decline is likely due to distribution to CNS tissues as well as CSF turnover to the systemic circulation. In a single-dose study, the mean residence time over the first 48 hours was 5.4 hours. The CSF clearance over the first 48 hours was 2.0 mL/hour, a value similar to the CSF turnover rate (1.8–2.4 mL/hour)⁵⁷ suggesting a relatively large proportion of CSF drug clearance is due to CSF turnover. Consistent with this phenomenon, peak plasma levels of nusinersen occurred at 2–5 hours. Similar to the PK profile in the CSF, a multiphasic profile was seen in the plasma, with a relatively rapid decline phase and a much slower terminal elimination phase. The rapid decline phase likely represents distribution into peripheral tissues. The slow terminal elimination phase in both the plasma and CSF represents equilibrium with tissue, combined with clearance from the peripheral and CNS compartments, respectively. Longer half-lives were observed in CNS tissues (117–195 days) than the liver and kidneys (23–35 days). This is likely a result of slower degradation in these tissues (SPINRAZA, Pharmacology Review FDA 2016).

In humans, the apparent volume of distribution in the CSF was estimated for nusinersen using a population PK analysis.⁵⁸ The estimate was greater than the CSF volume, suggesting significant distribution into CNS tissues. Consistent with this observation, extensive distribution into monkey CNS tissues was observed following IT dosing to monkeys. Tissues proximal to the injection site (e.g., lumbar and thoracic regions) had higher levels of nusinersen than more distal sites. After equilibrium was reached with CNS tissues, the partition ratio of CNS tissues/CSF was high and ranged from 1,600–4,500. As expected, the terminal elimination half-lives were similar between CSF and CNS tissues, being 111 days for CSF and 117–195 days for CNS tissues. This scaling method enabled CSF to serve as a potential biomarker of CNS tissue kinetics in humans. For example, by applying a CNS tissue/CSF partition ratio obtained in monkeys, the clinical CNS tissue levels could be estimated from the CSF levels in humans. The human CNS tissue exposure following the clinical dose was estimated as 6–16 µg/g, which is consistent with the CNS tissue exposure determined from the autopsy samples of deceased patients (2–26 µg/g). The estimated CSF half-life in humans was 135–177 days, which is similar to the CSF and CNS tissue half-lives in

monkeys. These observations provided further evidence of the translatability of PK from monkeys to humans (SPINRAZA, Pharmacology Review FDA 2016; SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016). In a dedicated PK study in monkeys that were dosed IT and i.v., a high plasma exposure ratio (IT/i.v.) was observed and indicated nearly complete transfer of nusinersen from CNS to plasma following an IT dose. No specific studies investigated the extent of systemic distribution, although high levels were observed in the kidneys and to a lesser extent in the liver of monkeys following IT dosing (SPINRAZA, Pharmacology Review FDA 2016).

In a recently developed semimechanistic population PK model of nusinersen, data from 92 nonhuman primates following IT doses of 0.3–7 mg were used to characterize the PKs in CSF, plasma, spinal cord, brain, and pons. Simulations using an allometric model agreed with clinical observations from 52 pediatric patients in phase I studies. This finding supports efforts to utilize more sophisticated quantitative models, such as mechanism-based population PK as a means to guide dose selection and trial design.⁵⁹

IT delivery through lumbar puncture or use of s.c. IT catheters to circumvent spinal pathology (e.g., scoliosis and spinal fusion) are the common delivery methods of antisense oligonucleotides in the clinic.^{28,60} However, less invasive methods, such as i.v. delivery, have potential. For example, conjugated heteroduplex oligonucleotide is composed of an antisense oligonucleotide and its complementary RNA. These complexes have demonstrated CNS penetration and are being explored for future clinical trials.⁶¹

A significant advantage of oligonucleotide therapy is low risk of drug interactions. Oligonucleotide therapeutics are not substrates, inhibitors, or inducers of common transporters and cytochrome P450 enzymes.^{15,46,54,62} Clinical drug interaction studies that are often conducted for small molecules are not typically necessary for this class of drugs. By design, antisense oligonucleotides are highly bound to plasma proteins to reduce glomerular filtration. However, the binding sites differ from those of hydrophobic small molecule drugs.⁶³ At typical therapeutic oligonucleotide doses, plasma protein binding sites are not saturated because of the large capacity of binding proteins in plasma.⁴⁰ For these reasons, the drug interaction risk due to competition with plasma protein binding is also low (**Table 1**).

Cardiac safety

Waivers of a thorough QT (TQT) study were granted for recently approved oligonucleotide therapies, including both antisense oligonucleotides (e.g., nusinersen and inotersen) and an siRNA (i.e., patisiran; SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016; TEGSEDI Clinical Pharmacology and Biopharmaceutics Review FDA 2018; ONPATTRO, Multi-Discipline Review FDA 2018). The low likelihood of direct ion channel interactions and no evidence of altered corrected QT (QTc) interval from nonclinical or clinical data supported the waivers.^{64–67} Oligonucleotide drugs are large and highly charged, which are attributes that limit the potential for direct inhibition of the human ether-a-go-go-related gene (hERG) potassium channel current through direct actions within the channel

pore.⁶⁴ Effects on hERG were evaluated *in vitro* for seven antisense oligonucleotides of the same chemical class (i.e., PS 2'-MOE modification). As expected, there were no biologically meaningful changes in hERG function.⁶⁵ Evaluation of oligonucleotide cardiac effects in monkeys with implanted telemetry units demonstrated no drug-related changes in blood pressures, heart rate, or electrocardiography and associated parameters (i.e., QRS duration), consistent with an overall absence of cardiac arrhythmic potential for this chemical class.⁶⁵

A TQT study was conducted for an earlier approval, mipomersen, which is also the first approved 2'-MOE-modified oligonucleotide. In the initial phase I dose escalation study in healthy subjects, there was no effect on cardiac repolarization at doses up to 400 mg (i.e., twofold the therapeutic dose) for 4 weeks and no positive correlation between corrected QT Fridericia's formula (QTcF) and plasma concentration.⁶⁶ This observation on QT interval was confirmed in the subsequent TQT study. The predicted upper bounds of the 90% confidence interval for the baseline-corrected and placebo-corrected QTcF ($\Delta\Delta\text{QTcF}$) at C_{max} of therapeutic and suprathreshold dose were approximately -1.7 and 2.9 ms, respectively.⁶⁶ As more phase I studies on other antisense oligonucleotides were completed, a retrospective exposure/response analysis was conducted using data from 10 single and multiple ascending dose studies of 2'-MOE-modified antisense oligonucleotides.⁶⁷ As with the original observations, there was no evidence for QTc prolongation associated with increasing plasma concentrations for all 10 compounds evaluated. Here, the oligonucleotide concentrations were up to 20 times the C_{max} of the therapeutic dose. Using a linear mixed effect approach to analyze the relationship between plasma concentration and $\Delta\Delta\text{QTcF}$, the slope of the regression line was close to zero, indicating virtually no relationship. The upper bound of the 90% confidence interval at C_{max} for suprathreshold doses ranged from -2.2 to 7.3 ms. None exceeded the 10 ms threshold.⁶⁷

In addition, a TQT study was conducted for defibrotide, a natural product containing a mixture of single-stranded (90%) and double-stranded (10%) phosphodiester oligonucleotides. No clinically relevant changes in the QTc interval were observed at a dose of 2.4 times the maximum recommended dose (DEFITELIO (defibrotide sodium), Highlights of Prescribing Information 2016).

Dose selection

Dose selection plays a critical role in the development of oligonucleotide therapeutics. The first-in-human dose is generally selected by computational integration of preclinical pharmacology, PK, and toxicology characterization.^{59,68} As mentioned in **Pharmacology**, the unique mechanism of action of this class of drugs enables straightforward measurement of the effect on mRNA and protein levels in animal models and potential relevance to human disease modulation.⁶⁸ Although target tissues are often inaccessible in the clinical setting, the plasma or CSF PK can often serve as a biomarker for target tissue PK (see **Pharmacokinetics**). Depending on the target, the mRNA and/or protein levels can also be measured in the plasma or CSF to serve as a biomarker

for target tissue PD effect.^{40,46} Based on these pharmacologic biomarker changes, a relationship can be further established with disease-related biomarkers and clinical outcomes^{40,46} (SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016). If a plasma or CSF biomarker is not predictive, a direct PK relationship with clinical outcomes can also be established for dose optimization (SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016).

As mentioned in **Pharmacology**, the translatable PD effect from animals to humans was demonstrated for mipomersen. This was enabled by the translatable PK properties across species (see **Pharmacokinetics**) and direct gene knockdown mechanism of action of the drug. Similar PK/PD relationships have been observed across species in mice (both wild type and transgenic), monkeys, and humans (healthy volunteers and patients) for mipomersen or its species-specific analogs.^{40,46}

Another example is inotersen, a recently approved antisense oligonucleotide to treat polyneuropathy of hereditary transthyretin amyloidosis (hATTR) in adults. A dose-response pharmacology study was conducted in a human transthyretin (hTTR) transgenic mouse model and demonstrated dose-dependent and time-dependent reduction of liver hTTR mRNA and plasma hTTR protein. Relationships were found between liver drug concentration and reduction in both liver hTTR mRNA and plasma hTTR protein levels. Maximum reductions of 90% and 94% in liver hTTR mRNA and plasma hTTR protein, respectively, were achieved at the high dose. These reductions corresponded to a liver concentration of 631 $\mu\text{g/g}$ inotersen. Time-dependent target reduction was also demonstrated in monkeys. Because of the high cost associated with monkey studies, only one dose level of 25 mg/kg was tested. This dose resulted in ~80% reduction in liver hTTR mRNA and plasma hTTR protein levels. Plasma retinol binding protein 4, another TTR-related PD biomarker, was also reduced by 60% (TEGSEDI, Pharmacology Review FDA 2018). Based on the PK and PD characterized in mice and monkeys with support from preclinical toxicology studies, five dose levels (50, 100, 200, 300, and 400 mg) were tested in the first-in-human study. Similar transthyretin (TTR) reduction (75–76%) was observed for the 300-mg and 400-mg dose levels after 3 weeks of treatment, and about 80% steady-state TTR reduction was predicted with both 300 and 400 mg/week regimens. Because 400 mg/week dose did not result in better PD effect and a higher dose was associated with safety risk (e.g., thrombocytopenia), a 300 mg/week dosing regimen was selected for the pivotal study. In the pivotal trial, the inotersen-treated group showed ~70% reduction in TTR levels from baseline, as compared with about 8% reduction in the placebo arm. As a result, the clinical outcomes measured as changes from baseline in both coprimary end points (modified Neuropathy Impairment Score+7 composite score and the Norfolk Quality of Life – Diabetic Neuropathy total score) significantly favored inotersen. Although a 300-mg weekly dose was approved for commercial use, the reported clinical PK/PD and observed safety profile of inotersen supported evaluation of the efficacy at a lower dose or less frequent regimen to improve the benefit/

risk profile of inotersen (TEGSEDI, Clinical Pharmacology and Biopharmaceutics Review FDA 2018).

Patisiran, an siRNA directed to the same TTR target to treat the same disease, adopted a slightly different pre-clinical approach to assess pharmacological activities. Biological activity was only evaluated in monkeys because patisiran is not active in rodents. Applying the correlations found in TTR reductions in the plasma and the liver, only serum TTR reduction was measured to determine the pharmacological effect of patisiran-LNP in monkeys. A range of i.v. doses (0.15–0.5 mg/kg) and two dosing regimens (every 4 weeks and every 3 weeks) were evaluated in monkeys to determine the dose response. Reductions in serum TTR protein were observed in all treatment groups, with greater suppression observed at higher doses when given at the same frequency. The same dose but with greater frequency (every 3 weeks rather than every 4 weeks) resulted in similar maximum but more prolonged reduction of TTR (ONPATTRO, Multi-Discipline Review FDA 2018). As expected, a similar dose response was then observed in humans.⁶⁹ In a phase II, multiple-dose study, patients with TTR-mediated familial amyloid polyneuropathy received two i.v. infusions of patisiran: 0.01–0.3 mg/kg every 4 weeks or 0.3 mg/kg every 3 weeks. Administration of patisiran led to rapid, dose-dependent, and durable TTR knockdown, with the maximum effect of 96% reduction observed at the highest dose (0.3 mg/kg) with greater frequency (every 3 weeks).⁶⁹ As patisiran was generally well tolerated, 0.3 mg/kg every 3 weeks was selected for the pivotal phase III study. In this study, patisiran reduced serum TTR by 81%, and the effect was rapid and sustained. A significant clinical benefit with respect to polyneuropathy was also demonstrated for patisiran.⁷⁰ Based on the results from the pivotal study, 0.3 mg/kg every 3 weeks was approved for commercial use (ONPATTRO, Multi-Discipline Review FDA 2018).

One example of dose selection for IT delivered oligonucleotides is nusinersen.²⁸ Previously described, CNS tissue concentrations of 2–10 µg/g were associated with 50–90% SMN2 exon 7 inclusion in SMA transgenic mice (SPINRAZA, Pharmacology Review FDA 2016; SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016). When considered with results from preclinical PK and toxicology studies, the clinical doses were selected to achieve these CNS tissue concentrations in patients. Appropriate dose selection was confirmed by the CNS tissue exposure and related PD effect determined from the autopsy samples of deceased patients with SMA.⁵⁶ The CNS tissue exposure of 2–26 µg/g (also see **Pharmacokinetics**) and 50–69% of SMN2 exon 7 inclusion in thoracic spinal cord and other brain regions were observed following 6–12 mg doses. Imaging analysis directly supported these doses by illustrating a 64% increase in SMA protein staining of the thoracic spinal cord anterior horn when compared with untreated patients.⁵⁶ As nusinersen was well tolerated, a dose of 12 mg was selected for the pivotal studies and ultimately demonstrated robust clinical efficacy of improved motor functions and prolonged survival in patients with SMA.^{71,72} Although SMN protein levels in the CSF were measured in phase I and phase II studies as a potential PD biomarker of nusinersen

activity, the concentrations were quite variable. A clear and consistent relationship between SMN protein in the CSF and nusinersen dose was not established. This may be, in part, due to the lack of data on SMN protein levels from control subjects (SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016). Other questions, such as the source of SMN protein in the CSF and optimal time to measure the change, remain unanswered. Because SMN protein in the CSF was not found predictive of clinical outcome, the exposure–response analysis was conducted with the clinical end points in infantile-onset patients with SMA (i.e., Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders score, compound muscle action potential, and motor milestones). The overall findings reflect dose–response using these disease severity end points. The CSF exposures were also significantly related to the probability of being a motor milestone responder at 6 months and a year (SPINRAZA, Clinical Pharmacology and Biopharmaceutics Review FDA 2016). As nusinersen was well tolerated, these relationships supported a fixed dose for all patients with SMA (i.e., 12 mg, four loading doses followed by maintenance dosing every 4 months thereafter). This is the highest dose and most frequent dosing regimen evaluated in clinical trials (SPINRAZA, Cross Discipline Team Leader Review FDA 2016; SPINRAZA (nusinersen), Highlights of Prescribing Information 2016).

Safety

Although oligonucleotide therapeutics are becoming a promising drug platform, several safety concerns have been raised in nonclinical and clinical studies.^{15,16} For example, severe thrombocytopenia has been reported for inotersen and volanesorsen; both are antisense oligonucleotides with 2'-MOE PS modifications. As the disease indication for inotersen (i.e., hATTR) is rare, progressive, and life-threatening, inotersen was approved to treat the disease with appropriate monitoring. However, the risk-benefit assessment did not support the approval of volanesorsen. Despite the clinical benefit demonstrated in phase III trials, the FDA rejected approval of this product, which was developed to treat familial chylomicronemia syndrome.

Factors that might contribute to the specific toxicities observed with oligonucleotide therapeutics can include dose, treatment duration, target potency, specific chemical classes, sequence, or sequence motif, etc. The potential mechanisms responsible for serious adverse events, the preclinical discovery and screening strategies to reduce the liability of selected oligonucleotides, and the extrapolation of some of these effects from nonclinical studies to humans have been specifically reported elsewhere.^{15,16,73} The readers are encouraged to read those reports.

APPROVED OLIGONUCLEOTIDE THERAPEUTICS

As of November 2018, eight oligonucleotide therapeutics have been approved (fomivirsin, pegaptanib, mipomersen, defibrotide, eteplirsin, nusinersen, inotersen, and patisiran; **Table 2**). However, it was not until July 11, 2018, that the FDA released a series of six draft guidance documents intended to advance development of gene

Table 2 Approved oligonucleotide therapeutics

Drug	Year of approval/ indication	Target/tissue/dosing	Chemistry/mechanism	Key observations and notes
Fomivirsen ^{75,76}	1998/CMV retinitis	CMV IE-2/eye/300 µg every 4 weeks, IVT	21-mer PS ONs/RNase H1	Clinical efficacy was demonstrated to treat CMV retinitis; however, marketing of the drug was stopped because of dramatic decrease in CMV cases
Pegaptanib ^{76,77}	2004/Neovascular AMD	VEGF165/eye/0.3 mg every 6 weeks, IVT	27-mer aptamer/VEGF antagonist by binding to VEGF165	Clinical efficacy was demonstrated to treat neovascular AMD, and no systemic toxicity was observed; however, market share declined after 2011 because of competition from ranibizumab and bevacizumab
Mipomersen ^{76,78–81}	2013/HoFH	ApoB-100/liver/200 mg once weekly, s.c.	20-mer PS 2'-MOE/RNase H1	Clinical efficacy was demonstrated to treat HoFH; however, EMA did not approve the drug, citing safety concerns. The drug did not achieve marketing success because of competition from other therapeutics
Defibrotide ^{76,82}	2016/Hepatic VOD	Proteins, nonspecific/liver/6.25 mg/kg every 6 hours, i.v. infusion	Natural product, ON mixture/nonspecific interaction with proteins	Although the mechanism of action is very complex and has not been fully elucidated, defibrotide demonstrated improved survival rate and complete response rate in a phase III trial when compared with historical controls
Eteplirsen ^{76,83}	2016/DMD	Dystrophin (Exon 51)/muscle/30 mg/kg once weekly, i.v. infusion	30-mer PMO/splicing modification (exon skipping)	Controversy exists on the amount of evidence required to demonstrate efficacy and PD effect. The FDA approved the drug under conditional approval. In 2018, the CHMP of the EMA refused the approval of eteplirsen in Europe
Nusinersen ^{9,71,72,85}	2016/SMA	SMN2/CNS/12 mg once every 4 months, IT	18-mer PS 2'-MOE/splicing modification (exon inclusion)	Profound clinical benefit of prolonged survival and improved motor function evident during interim analysis of two phase III studies. The FDA approved the drug based on the interim results
Inotersen ^{3,9}	2018/hATTR	TTR/liver/300 mg once weekly, s.c.	20-mer PS 2'-MOE/RNase H1	Robust efficacy was demonstrated in a phase III study; however, two significant adverse events were observed during the study: thrombocytopenia and renal dysfunction. One patient with observed thrombocytopenia died because of intracranial hemorrhage
Patisiran ^{3,9,70}	2018/hATTR	TTR/liver/0.3 mg/kg for BW < 100 kg or 30 mg for BW ≥ 100 kg, once every 3 weeks, i.v. infusion	PS, 2'-O-Me and 2'-F siRNA/Ago2	The first approved siRNA. Robust efficacy was demonstrated in a phase III study, and there has been little or no evidence of safety concerns related to thrombocytopenia, renal dysfunction, or liver enzyme elevations. However, premedication with a corticosteroid, acetaminophen, and antihistamines is required to mitigate pro-inflammatory effect of the LNP formulation

2'-F, 2'-fluoro; 2'-MOE, 2'-O-methoxyethyl; 2'-O-Me, 2'-O-methyl; Ago2, argonaute-2; AMD, age-related macular degeneration; apoB, apolipoprotein B; BW, body weight; CHMP, Committee for Medicinal Products for Human Use; CMV, cytomegalovirus; CNS, central nervous system; DMD, Duchenne muscular dystrophy; EMA, European Medicines Agency; FDA, US Food and Drug Administration; hATTR, hereditary transthyretin amyloidosis; HoFH, homozygous familial hypercholesterolemia; IE-2, immediate-early-2; IT, intrathecal; IVT, intravitreal; LNPs, lipid nanoparticles; ON, oligonucleotide; PD, pharmacodynamic; PMO, phosphorodiamidate morpholino oligomer; PS, phosphorothioate; siRNA, short interference RNAs; SMA, spinal muscular atrophy; SMN2, survival of motor neuron 2; TTR, transthyretin; VEGF, vascular endothelial growth factor; VOD, veno-occlusive disease.

therapy products. They include nucleic acids in their definition of gene products. Among the issued documents, there are three disease-specific gene therapy guidances (Human Gene Therapy for Hemophilia; Human Gene Therapy for Rare Diseases; and Human Gene Therapy for Retinal Disorders) and three guidances on manufacturing gene therapies (Chemistry, Manufacturing, and Control Information for Human Gene Therapy Investigational New Drug Applications; Testing of Retroviral-Based Human Gene

Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up; and Long-Term Follow-Up After Administration of Human Gene Therapy Products). Although not specific for oligonucleotide therapeutics, these guidance documents encourage innovation in this area and set the stage for continued advancement of this new class of drugs. As stated by the FDA Commissioner Scott Gottlieb, "In the future, we expect this field to continue to expand, with the potential approval of

new treatments for many debilitating diseases. These therapies hold great promise. Our new steps are aimed at fostering developments in this innovative field.”⁷⁴

Earlier approvals

The first approved oligonucleotide therapy was fomivirsen by the FDA in 1998, a 21-mer PS-modified antisense oligonucleotide administered locally into the eye by intravitreal injection to treat cytomegalovirus retinitis.⁷⁵ Although a milestone in the history of oligonucleotide therapeutics, fomivirsen was short-lived because the incidence of cytomegalovirus retinitis was drastically reduced following the development of highly active antiretroviral therapies. The marketing of fomivirsen was eventually halted.^{9,76} The second FDA-approved oligonucleotide therapy, pegaptanib (Macugen; 2004), is also locally administered by intravitreal injection to treat age-related macular degeneration of the retina.⁷⁷ This is the leading cause of blindness in people over the age of 50. Although still in the market, the sale of pegaptanib has largely decreased since 2010 because of the emergence of more effective (i.e., ranibizumab) or less expensive (i.e., bevacizumab) drugs.⁷⁶

In addition to local administration, mipomersen (Kynamro) was the first approved systemically delivered oligonucleotide therapy by s.c. injection to treat homozygous familial hypercholesterolemia.^{78,79} Mipomersen was approved by the FDA in 2013. However, the European Medicines Agency (EMA) refused marketing authorization in 2012 citing safety concerns, such as injection site reactions, liver toxicity, and cardiovascular risk. Like pegaptanib, mipomersen did not achieve marketing success, and its future is unclear because of competition from small molecule and proprotein convertase subtilisin/kexin type 9-inhibiting monoclonal antibodies.^{76,80,81}

More recent approvals

After a slow period for earlier approvals, innovations in science and technology have overcome some major hurdles and hastened the development of oligonucleotide therapeutics. Since 2016, five oligonucleotides, defibrotide, eteplirsen, nusinersen, inotersen, and patisiran, have been approved by the FDA and/or EMA.

In April 2016, the FDA approved defibrotide to treat severe hepatic veno-occlusive disease consequent to high-dose chemotherapy and autologous bone marrow transplantation. Unlike chemically synthesized oligonucleotide therapeutics that dominate this class of drugs, defibrotide is a natural product with a mixture of single-stranded (90%) and double-stranded (10%) phosphodiester oligonucleotides (on average, 50-mer, with a range of 9–80-mer). Defibrotide demonstrated improved survival rate and complete response rate in a phase III trial when compared with historical controls.^{76,82}

In the same year, eteplirsen was given conditional approval by the FDA to treat DMD; the decision was based on an increase in dystrophin in skeletal muscle. Eteplirsen is a 30-mer PMO that was designed to bind to exon 51 of dystrophin pre-mRNA, resulting in exclusion of this exon during mRNA processing. Because of PMO chemistry, it is not a substrate for nucleases, an important property for splice-modifying activity.^{76,83} To receive eteplirsen

treatment, patients need to have a confirmed mutation of the *DMD* gene that is amenable to exon 51 skipping. However, there was wide public and regulatory controversy related to the amount of evidence on efficacy and relevance of the PD biomarker. Although approved, it is communicated in the drug label that a clinical benefit of eteplirsen has not been established and continued approval for this indication may be contingent upon verification of a clinical benefit in confirmatory trials (EXONDYS 51 (Eteplirsen), Highlights of Prescribing Information 2016). Because of the lack of evidence on clinical benefit, in May 2018, the Committee for Medicinal Products for Human Use of the EMA adopted a negative opinion and refused approval of eteplirsen to treat patients with DMD in Europe.⁸⁴

In contrast, profound clinical benefit, as evidenced by prolonged survival and improved motor function, was demonstrated for nusinersen in two randomized, double-blinded, sham-controlled phase III studies, ENDEAR and CHERISH.^{71,72} Because remarkable clinical benefit was evident during interim analysis, these placebo-controlled studies were stopped early and all participants were transitioned into an open-label study to receive active treatment (Biogen Press Releases on August 1, 2016, and November 7, 2016). The FDA acted rapidly on the interim results and approved nusinersen on December 23, 2016, to treat SMA in pediatric and adult patients (SPINRAZA (nusinersen), Highlights of Prescribing Information 2016). Subsequently, nusinersen was approved by the EMA in May 2017, in Canada and Japan in July 2017, and in many other countries since then. To date, nusinersen has been the most recognized success story for an oligonucleotide therapy. It has generated renewed and significant enthusiasm for treating other devastating or life-threatening diseases currently with limited treatment options.^{9,85}

The side-by-side approval of an antisense oligonucleotide and an siRNA in 2018 (i.e., inotersen and patisiran) to treat the same disease by inhibiting the same target makes it possible to directly compare the two major classes of oligonucleotide therapeutics.⁹ With the approval received in July 2018 from the European Commission (EC) for the treatment of stage 1 or stage 2 hATTR, inotersen became the world's first RNA-targeted therapeutic approved to treat hATTR (Akcea Therapeutics and Ionis Pharmaceuticals Press Release on July 11, 2018). Robust efficacy was demonstrated in a randomized, double-blinded, placebo-controlled phase III study of inotersen. However, two significant adverse events that are related to this class of drugs were observed during the study that required changes to the monitoring schedule: thrombocytopenia and renal dysfunction. One patient with observed thrombocytopenia died because of intracranial hemorrhage (Ionis Pharmaceuticals Press Release on May 15, 2017). After changes were made to platelet monitoring, no additional serious sequelae secondary to thrombocytopenia have been observed.³ Inotersen was subsequently approved by the FDA and Health Canada in October 2018 (Akcea Therapeutics and Ionis Pharmaceuticals Press Releases on October 5, 2018, and October 4, 2018, respectively).

One month after the approval of inotersen by the EC, in August 2018, and only days apart, the FDA and the EC granted the first-ever approval of an siRNA,

patisiran (ONPATTRO), to treat the same disease (Alnylam Pharmaceutical Press Releases on August 10, 2018, and August 30, 2018, respectively). Similar to inotersen, the approval was based on robust efficacy demonstrated in a randomized, double-blinded, placebo-controlled phase III study of patisiran in patients with hATTR. Thus far, there has been no significant evidence of safety concern related to thrombocytopenia, renal dysfunction, or liver enzyme elevations. This is a distinct advantage of patisiran over inotersen.^{9,70} However, as patisiran is delivered in an LNP formulation, pretreatment with antihistamines, nonsteroidal antihistamines, and a glucocorticoid are required to mitigate the pro-inflammatory effect of this formulation. This pretreatment imparts a disadvantage for patisiran. In addition, s.c. delivered inotersen provides convenience for patients as compared with patisiran administered through i.v. infusion.^{3,9} Nevertheless, the milestone approval of the first-of-its-kind treatment by patisiran marks the arrival of a new treatment approach for patients with rare and devastating diseases (Table 2).

CONCLUDING REMARKS

The therapeutic strategy to harness new RNA mechanisms has expanded beyond gene knockdown and splicing modulation. Building on the advances in chemistry, oligonucleotide therapeutics are emerging as an established, validated class of drugs that could modulate nearly any genetic target. These targets include over 10,000 proteins in the human genome that have hitherto been considered undruggable by small molecules and protein therapeutics. The approval of five oligonucleotide therapies within the last 2 years elicited unprecedented excitement in the field. However, there are remaining challenges to overcome and significant room for future innovation to fully realize the potential of oligonucleotide therapeutics.

Safety of these products demands attention. To mitigate toxicity, innovations on chemistry and delivery tools remain essential in enhancing the drug-like profiles of oligonucleotide therapies. As GalNAc conjugation has enabled targeted delivery of siRNAs to hepatocytes, the effort to identify ligands outside the liver needs to be accelerated to broaden the range of therapeutic benefit. It is also highly desirable to develop robust delivery technologies to enhance endosomal escape and to increase the productive intracellular distribution to the cytosol and the nucleus. In addition, knowledge gained from developing oligonucleotide therapies may accelerate the translation of clustered regularly interspaced short palindromic repeats-Cas gene-editing technology to successful clinical outcomes.

In conclusion, continued advancements in science and technology will further expand the scope of oligonucleotide therapeutics. The anticipated expansion will bring effective treatments for diseases that currently remain therapeutically elusive.

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- Stephenson, M.L. & Zamecnik, P.C. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proc. Natl. Acad. Sci. USA* **75**, 285–288 (1978).
- Lieberman, J. Tapping the RNA world for therapeutics. *Nat. Struct. Mol. Biol.* **25**, 357–364 (2018).
- Crooke, S.T., Witztum, J.L., Bennett, C.F. & Baker, B.F. RNA-targeted therapeutics. *Cell Metab.* **27**, 714–739 (2018).
- Khvorov, A. & Watts, J.K. The chemical evolution of oligonucleotide therapies of clinical utility. *Nat. Biotechnol.* **35**, 238–248 (2017).
- Juliano, R.L. The delivery of therapeutic oligonucleotides. *Nucleic Acids Res.* **44**, 6518–6548 (2016).
- Springer, A.D. & Dowdy, S.F. GalNAc-siRNA conjugates: leading the way for delivery of RNAi therapeutics. *Nucleic Acid Ther.* **28**, 109–118 (2018).
- Bennett, C.F., Baker, B.F., Pham, N., Swayze, E. & Geary, R.S. Pharmacology of antisense drugs. *Annu. Rev. Pharmacol. Toxicol.* **57**, 81–105 (2017).
- Ozcan, G., Ozpolat, B., Coleman, R.L., Sood, A.K. & Lopez-Berstein, G. Preclinical and clinical development of siRNA-based therapeutics. *Adv. Drug Deliv. Rev.* **87**, 108–119 (2015).
- Shen, X. & Corey, D.R. Chemistry, mechanism and clinical status of antisense oligonucleotides and duplex RNAs. *Nucleic Acids Res.* **46**, 1584–1600 (2018).
- Crooke, S.T. Molecular mechanisms of antisense oligonucleotides. *Nucleic Acid Ther.* **27**, 70–77 (2017).
- Crooke, S.T., Wang, S., Vickers, T.A., Shen, W. & Liang, X.H. Cellular uptake and trafficking of antisense oligonucleotides. *Nat. Biotechnol.* **35**, 230–237 (2017).
- Sharma, V.K. & Watts, J.K. Oligonucleotide therapeutics: chemistry, delivery and clinical progress. *Future Med. Chem.* **7**, 2221–2242 (2015).
- Kaczmarek, J.C., Kowalski, P.S. & Anderson, D.G. Advances in the delivery of RNA therapeutics: from concept to clinical reality. *Genome Med.* **9**, 60 (2017).
- Bennett, C.F. & Swayze, E.E. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. *Annu. Rev. Pharmacol. Toxicol.* **50**, 259–293 (2010).
- Mustonen, E.K., Palomäki, T. & Pasanen, M. Oligonucleotide-based pharmaceuticals: Non-clinical and clinical safety signals and non-clinical testing strategies. *Regul. Toxicol. Pharmacol.* **90**, 328–341 (2017).
- Chi, X., Gatti, P. & Papoian, T. Safety of antisense oligonucleotide and siRNA-based therapeutics. *Drug Discov. Today* **22**, 823–833 (2017).
- Watson, J.D. & Crick, F.H. Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature* **171**, 737–738 (1953).
- Goodwin, S., McPherson, J.D. & McCombie, W.R. Coming of age: ten years of next-generation sequencing technologies. *Nat. Rev. Genet.* **17**, 333–351 (2016).
- Rinaldi, C. & Wood, M.J.A. Antisense oligonucleotides: the next frontier for treatment of neurological disorders. *Nat. Rev. Neurol.* **14**, 9–21 (2018).
- Donis-Keller, H. Site specific enzymatic cleavage of RNA. *Nucleic Acids Res.* **7**, 179–192 (1979).
- Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. & Tuschl, T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **24**, 494–498 (2001).
- Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. & Mello, C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **19**, 806–811 (1998).
- Meister, G., Landthaler, M., Patkaniowska, A., Dorsett, Y., Teng, G. & Tuschl, T. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell* **15**, 185–197 (2004).
- Matranga, C., Tomari, Y., Shin, C., Bartel, D.P. & Zamore, P.D. Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell* **123**, 1–14 (2005).
- Cong, L. *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819–823 (2013).
- Lefebvre, S. *et al.* Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155–165 (1995).
- Eckstein, F. Nucleoside phosphorothioates. *J. Am. Chem. Soc.* **88**, 4292–4294 (1966).
- Schoch, K.M. & Miller, T.M. Antisense oligonucleotides: translation from mouse models to human neurodegenerative diseases. *Neuron* **94**, 1056–1070 (2017).
- Martin, P.A. New access to 2'-O-alkylated ribonucleosides and properties of 2'-O-alkylated oligoribonucleotides. *Helv. Chim. Acta* **78**, 486–504 (1995).
- Obika, S. *et al.* Synthesis of 2'-O,4'-C-methyleneuridine and -cytidine. Novel bicyclic nucleosides having a fixed C3'-endo sugar pucker. *Tetrahedron Lett.* **38**, 8735–8738 (1997).
- Koshkin, A.A. *et al.* Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition. *Tetrahedron* **54**, 3607–3630 (1998).
- Codington, J.F., Doerr, I.L. & Fox, J.J. Nucleosides. XVIII synthesis of 2'-fluorothymidine, 2'-fluorodeoxyuridine, and other 2'-halogeno-2'-deoxy nucleosides. *J. Org. Chem.* **29**, 558–564 (1964).
- Reist, E.J., Benitez, A. & Goodman, L. The synthesis of some 5' thiopentofurano-silylpyrimidines. *J. Org. Chem.* **29**, 554–558 (1964).

34. Bobst, A.M., Rottman, F. & Cerutti, P.A. Effect of the methylation of the 2'-hydroxyl groups in polyadenylic acid on its structure in weakly acidic and neutral solutions and on its capability to form ordered complexes with polyuridylic acid. *J. Mol. Biol.* **46**, 221–234 (1969).
35. Iwamoto, N. *et al.* Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. *Nat. Biotechnol.* **35**, 845–851 (2017).
36. Kyle, W. *et al.* Unlocking P(V): reagents for chiral phosphorothioate synthesis. *Science* **361**, 1234–1238 (2018).
37. Boczkowska, M., Guga, P. & Stec, W.J. Stereodefined phosphorothioate analogues of DNA: relative thermodynamic stability of the model PS-DNA/DNA and PS-DNA/RNA complexes. *Biochemistry* **41**, 12483–12487 (2002).
38. Koziolkiewicz, M., Krakowiak, A., Kwinkowski, M., Boczkowska, M. & Stec, W.J. Stereodifferentiation—the effect of P chirality of oligo(nucleoside phosphorothioates) on the activity of bacterial RNase H. *Nucleic Acids Res.* **23**, 5000–5005 (1995).
39. Stec, W.J. *et al.* Stereodependent inhibition of plasminogen activator inhibitor type 1 by phosphorothioate oligonucleotides: proof of sequence specificity in cell culture and in vivo rat experiments. *Antisense Nucleic Acid Drug Dev.* **7**, 567–573 (1997).
40. Geary, R.S., Norris, D., Yu, R. & Bennett, C.F. Pharmacokinetics, biodistribution and cell uptake of antisense oligonucleotides. *Adv. Drug Deliv. Rev.* **87**, 46–51 (2015).
41. Wang, S., Sun, H., Tanowitz, M., Liang, X.H. & Crooke, S.T. Intraendosomal trafficking mediated by lysobisphosphatidic acid contributes to intracellular release of phosphorothioate-modified antisense oligonucleotides. *Nucleic Acids Res.* **45**, 5309–5322 (2017).
42. Zatselin, T.S., Kotelevtsev, Y.V. & Koteliensky, V. Lipid nanoparticles for targeted siRNA delivery – going from bench to bedside. *Int. J. Nanomedicine* **11**, 3077–3086 (2016).
43. Nair, J.K. *et al.* Multivalent N-acylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. *J. Am. Chem. Soc.* **136**, 16958–16961 (2014).
44. Crooke, S.T., Vickers, T., Lima, W. & Wu, H. Mechanism of antisense drug action, an introduction. In *Antisense Drug Technology Principles, Strategies, and Applications* 2nd edn. (ed. Crooke, S.T.) 5–46 (CRC Press, Boca Raton, FL, 2008).
45. Church, D.M. *et al.* Lineage-specific biology revealed by a finished genome assembly of the mouse. *PLoS Biol.* **7**, e1000112 (2009).
46. Yu, R.Z. *et al.* Cross-species comparison of in vivo PK/PD relationships for second-generation antisense oligonucleotides targeting apolipoprotein B-100. *Biochem. Pharmacol.* **77**, 910–919 (2009).
47. Bebee, T.W., Dominguez, C.E. & Chandler, D.S. Mouse models of SMA: tools for disease characterization and therapeutic development. *Hum. Genet.* **131**, 1277–1293 (2012).
48. Passini, M.A. *et al.* Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Sci. Transl. Med.* **3**, 72ra18 (2011).
49. Yu, R.Z., Kim, T.W., Hong, A., Watanabe, T.A., Gaus, H.J. & Geary, R.S. Cross-species pharmacokinetic comparison from mouse to man of a second-generation antisense oligonucleotide, ISIS 301012, targeting human apolipoprotein B-100. *Drug Metab. Dispos.* **35**, 460–468 (2007).
50. Geary, R.S. *et al.* Effect of dose and plasma concentration on liver uptake and pharmacologic activity of a 2'-methoxyethyl modified chimeric antisense oligonucleotide targeting PTEN. *Biochem. Pharmacol.* **78**, 284–291 (2009).
51. Yu, R.Z., Grundy, J.S. & Geary, R.S. Clinical pharmacokinetics of second generation antisense oligonucleotides. *Expert Opin. Drug Metab. Toxicol.* **9**, 169–182 (2013).
52. Yu, R.Z. *et al.* Predictive dose-based estimation of systemic exposure multiples in mouse and monkey relative to human for antisense oligonucleotides with 2'-o-(2-methoxyethyl) modifications. *Mol. Ther. Nucleic Acids* **4**, e218 (2015).
53. Crooke, S.T. & Geary, R.S. Clinical pharmacological properties of mipomersen (Kynamro), a second generation of antisense inhibitor of apolipoprotein B. *Br. J. Clin. Pharmacol.* **76**, 269–276 (2012).
54. Geary, R.S. Antisense oligonucleotide pharmacokinetics and metabolism. *Expert Opin. Drug Metab. Toxicol.* **5**, 381–391 (2009).
55. White, P.J., Anastopoulos, F., Pouton, C.W. & Boyd, B.J. Overcoming biological barriers to in vivo efficacy of antisense oligonucleotides. *Expert Rev. Mol. Med.* **11**, e10 (2009).
56. Finkel, R.S. *et al.* Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. *Lancet* **388**, 3017–3026 (2016).
57. Artru, A.A. Spinal cerebrospinal fluid chemistry and physiology. In *Spinal Drug Delivery* (ed. Yaksh, T.I.) 177–237 (Elsevier, New York, NY, 1999).
58. Luu, K.T., Norris, D.A., Gunawan, R., Henry, S., Geary, R. & Wang, Y. Population pharmacokinetics of nusinersen in the cerebral spinal fluid and plasma of pediatric patients with spinal muscular atrophy following intrathecal administrations. *J. Clin. Pharmacol.* **57**, 1031–1041 (2017).
59. Biliouris, K. *et al.* A semi-mechanistic population pharmacokinetic model of nusinersen: an antisense oligonucleotide for the treatment of spinal muscular atrophy. *CPT Pharmacometrics Syst. Pharmacol.* **7**, 581–592 (2018).
60. Strauss, K.A. *et al.* Preliminary safety and tolerability of a novel subcutaneous intrathecal catheter system for repeated outpatient dosing of nusinersen to children and adults with spinal muscular atrophy. *J. Pediatr. Orthop.* **38**, e610–e617 (2018).
61. Kuwahara, H. *et al.* Modulation of blood-brain barrier function by a heteroduplex oligonucleotide in vivo. *Sci. Rep.* **8**, 4377 (2018).
62. Yu, R.Z. *et al.* Lack of interactions between an antisense oligonucleotide with 2'-O-(2-methoxyethyl) modifications and major drug transporters. *Nucleic Acid Ther.* **26**, 111–117 (2016).
63. Watanabe, T.A., Geary, R.S. & Levin, A.A. Plasma protein binding of an antisense oligonucleotide targeting human ICAM-1 (ISIS 2302). *Oligonucleotides* **16**, 169–180 (2006).
64. Stanfield, P.J., Sutcliffe, M.J. & Mitcheson, J.S. Molecular mechanisms for drug interactions with hERG that cause long QT syndrome. *Expert Opin. Drug Metab. Toxicol.* **2**, 81–94 (2006).
65. Kim, T.W., Kim, K.S., Seo, J.W., Park, S.Y. & Henry, S.P. Antisense oligonucleotides on neurobehavior, respiratory, and cardiovascular function, and hERG channel current studies. *J. Pharmacol. Toxicol. Methods* **69**, 49–60 (2014).
66. Yu, R.Z. *et al.* No effect on QT intervals of mipomersen, a 2'-O-methoxyethyl modified antisense oligonucleotide targeting ApoB-100 mRNA, in a phase I dose escalation placebo-controlled study, and confirmed by a thorough QT (TQT) study, in healthy subjects. *Eur. J. Clin. Pharmacol.* **72**, 267–275 (2016).
67. Yu, R.Z., Gunawan, R., Geary, R.S., Hughes, S.G., Henry, S.P. & Wang, Y. Lack of QT prolongation for 2'-O-methoxyethyl-modified antisense oligonucleotides based on retrospective exposure/response analysis of ten phase 1 dose-escalation placebo-controlled studies in healthy subjects. *Nucleic Acid Ther.* **27**, 285–294 (2017).
68. Geary, R.S., Yu, R.Z., Siwkowski, A. & Levin, A. Pharmacokinetic/pharmacodynamic properties of phosphorothioate 2'-O-(2-methoxyethyl)-modified antisense oligonucleotides in animals and man. In *Antisense Drug Technology Principles, Strategies, and Applications* 2nd edn. (ed. Crooke, S.T.) 305–326 (CRC Press, Boca Raton, FL, 2008).
69. Suhr, O.B. *et al.* Efficacy and safety of patisiran for familial amyloidotic polyneuropathy: a phase II multi-dose study. *Orphanet. J. Rare Dis.* **10**, 109 (2015).
70. Adams, D. *et al.* Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N. Engl. J. Med.* **379**, 11–21 (2018).
71. Finkel, R.S. *et al.* ENDEAR Study Group, Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N. Engl. J. Med.* **377**, 1723–1732 (2017).
72. Mercuri, E.; CHERISH Study Group *et al.* Nusinersen versus sham control in later-onset spinal muscular atrophy. *N. Engl. J. Med.* **378**, 625–635 (2018).
73. Sewing, S. *et al.* Assessing single-stranded oligonucleotide drug-induced effects in vitro reveals key risk factors for thrombocytopenia. *PLoS One* **12**, e0187574 (2017).
74. Statement from FDA Commissioner Scott Gottlieb, M.D. on agency's efforts to advance development of gene therapies. FDA Statement <<https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm613026.htm>> (July 11, 2018).
75. Jabs, D.A. & Griffiths, P.D. Fomivirsen for the treatment of cytomegalovirus retinitis. *Am. J. Ophthalmol.* **133**, 552–556 (2002).
76. Stein, C.A. & Castanotto, D. FDA-approved oligonucleotide therapies in 2017. *Mol. Ther.* **25**, 1069–1075 (2017).
77. Gragoudas, E.S., Adamis, A.P., Cunningham, E.T. Jr. & Feinsod, M.; VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. *N. Engl. J. Med.* **351**, 2805–2816 (2004).
78. Stein, E.A. *et al.* Apolipoprotein B synthesis inhibition with mipomersen in heterozygous familial hypercholesterolemia: results of a randomized, double-blind, placebo-controlled trial to assess efficacy and safety as add-on therapy in patients with coronary artery disease. *Circulation* **126**, 2283–2292 (2012).
79. Thomas, G.S., Cromwell, W.C., Ali, S., Chin, W., Flaim, J.D. & Davidson, M. Mipomersen, an apolipoprotein B synthesis inhibitor, reduces atherogenic lipoproteins in patients with severe hypercholesterolemia at high cardiovascular risk: a randomized, double-blind, placebo-controlled trial. *J. Am. Coll. Cardiol.* **62**, 2178–2184 (2013).
80. Paton, D.M. PCSK9 inhibitors: monoclonal antibodies for the treatment of hypercholesterolemia. *Drugs Today (Barc.)* **52**, 183–192 (2016).
81. Chaudhary, R., Garg, J., Shah, N. & Sumner, A. PCSK9 inhibitors: a new era of lipid lowering therapy. *World J. Cardiol.* **9**, 76–91 (2017).
82. Richardson, P.G. *et al.* Phase 3 trial of defibrotide for the treatment of severe veno-occlusive disease and multi-organ failure. *Blood* **127**, 1656–1665 (2016).
83. Mendell, J.R.; Eteplirsen Study Group and Telethon Foundation DMD Italian Network *et al.* Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. *Ann. Neurol.* **79**, 257–271 (2016).
84. EMA refusal of the marketing authorisation for Exondys (eteplirsen) – outcome of re-examination <<https://www.ema.europa.eu/en/medicines/human/EPAR/exondys>> (September 20, 2018).

85. Aartsma-Rus, A. FDA approval of nusinersen for spinal muscular atrophy makes 2016 the year of splice modulating oligonucleotides. *Nucleic Acid Ther.* **27**, 67–69 (2017).
86. Wan, H. An overall comparison of small molecules and large biologics in ADME testing. *ADMET DMPK* **4**, 1–22 (2016).
87. Kelley, B. Industrialization of mAb production technology: the bioprocessing industry at a crossroads. *MAbs* **1**, 443–452 (2009).
88. Lobo, E.D., Hansen, R.J. & Balthasar, J.P. Antibody pharmacokinetics and pharmacodynamics. *J. Pharm. Sci.* **93**, 2645–2668 (2004).
89. Hansel, T.T., Kropshofer, H., Singer, T., Mitchell, J.A. & George, A.J. The safety and side effects of monoclonal antibodies. *Nat. Rev. Drug Discov.* **9**, 325–338 (2010).

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