Oligonucleotide therapy: regulatory & development challenge

The various advantages and therapeutic potential of oligonucleotides have led to great interest in these molecules. Currently, as many as seventeen oligonucleotide drugs received regulatory approval from the FDA and the EMA. In addition, multiple clinical trials with oligonucleotides are ongoing. In recent years, both industry and regulatory agencies have gained extensive expertise with this type of products. However, there are challenges, both at the product quality/control, nonclinical and clinical as well as regulatory level, that must be considered for the successful development of oligonucleotide therapies.

Oligonucleotide therapies are typically synthetically modified, single-stranded or double-stranded RNAs or RNA/DNA hybrids. Based on their mechanism of action, we can distinguish antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), anti-microRNAs, and antagomirs. Oligonucleotides therapies are designed to hybridize to complementary targets to modulate specific gene expression across the genome [1].

A main *advantage* of oligonucleotides is that they can modulate a variety of disease targets, including more than 10,000 proteins in the human genome that have been thought to be undruggable by small molecules. Also, the inherent ease of synthesizing new libraries of complementary oligonucleotides targeting the intended protein highly facilitates the optimal design of these products.

Despite their great therapeutic potential, there are several *challenges* in the development of oligonucleotides, including appropriate drug delivery and untoward effects. After parenteral administration, oligonucleotide drugs must travel through the bloodstream, pass through the biological membranes, and be taken up by the target cells. Various strategies are employed to promote the delivery of the oligonucleotides to target tissues and enhance their stability in this respect. These strategies consist of: a) introducing specific structural modifications in the nucleotide backbone or sugar moieties; b) conjugation to different moieties (polymers, peptides, lipids, antibodies, etc); c) formulation into delivery vectors (i.e. lipid-based nanoparticles) [2]. Safety aspects that should be considered include off-target toxicity, immune-stimulatory responses, thrombocytopenia, inhibition of coagulation and renal accumulation resulting in renal damage [1, 3].

Currently, up to seventeen oligonucleotide drugs were approved by the FDA and the EMA **(Table 1)**. These oligonucleotides are antisense oligonucleotides (ASO) or small interference RNAs (siRNAs) administered via intravenous (IV, to target the liver or muscle), subcutaneous (SC, to target the liver), intrathecal or intravitreal routes. Oligonucleotide drugs are generally indicated for orphan conditions, like Duchenne muscular dystrophy, spinal muscular atrophy or hereditary transthyretin amyloidosis. The most recently approved oligonucleotide product

was tofersen (Qalsody, FDA, April 2023[1]), for the treatment of amyotrophic lateral sclerosis. Tofersen is currently under review by the EMA[2].

Table 1. Oligonucleotide drugs approved by the EMA and FDA as of June 2023.

Drug	Brand name	Approval	Indication	RoA	Target	Type
Fomivirsen†	Vitravene	US, 1998; EU, 1999	Cytomegalovirus retinitis	Intra-vitreal	Eye	ASO
Pegaptanib	Macugen	US, 2004; EU, 2006	Neovascular, age-related macular degeneration	Intra-vitreal	Eye	Aptamer
Mipomersen ‡	Kynamro	US,2013	Homozygous familial hypercholesterolemia	SC	Liver	ASO
Defibrotide	Defitelio	US,2016	Hepatic veno-occlusive disease	IV	Liver	ON mixture
Eteplirsen	Exondys 51	US, 2016	Duchenne muscular dystrophy	IV	Muscle	ASO
Nusinersen	Spinraza	US, 2016; EU, 2017	Spinal muscular atrophy	Intra-thecal	CNS	ASO
Inotersen	Tegsedi	EU, 2018; US, 2018	Human hereditary transthyretin amyloidosis	SC	Liver	ASO
Patisiran	Onpattro	US, 2018; EU, 2018	Human hereditary transthyretin amyloidosis	IV	Liver	siRNA
Volanesorsen	Waylivra	EU, 2019	Familial chylomicronemia syndrome	SC	Liver	ASO
Givosiran	Givlaari	US, 2019; EU, 2020	Acute hepatic porphyria	SC	Liver	siRNA
Golodirsen	Vyondys 53	US, 2019	Duchenne muscular dystrophy	IV	Muscle	ASO
Viltolarsen	Viltepso	US, 2020	Duchenne muscular dystrophy	IV	Muscle	ASO
Lumasiran	Oxlumo	US, 2020; EU, 2020	Primary hyperoxaluria type 1	SC	Liver	siRNA
Inclisiran	Leqvio	EU, 2020; US, 2021	Atherosclerotic cardiovascular disease and heterozygous familial hypercholesterolemia	SC	Liver	siRNA
Casimersen	Amondys 45	US, 2021	Duchenne muscular dystrophy	IV	Muscle	ASO
Vutrisiran	Amvuttra	US, 2022; EU, 2022	Hereditary transthyretin-mediated amyloidosis	SC	Liver	siRNA
Tofersen	Qalsody	US, 2023	Amyotrophic lateral sclerosis	Intra-thecal	CNS	ASO

Abbreviations: ASO, antisense oligonucleotide; CNS, central nervous system; IV: intravenous; SC: subcutaneous; siRNA: small interference RNA. Based on Igarashi et al, 2021 and Thakur, 2022. Updated as of June 9th 2023. †The sale is currently discontinued. ‡It is still available on a very restricted basis due to side effects.

Beyond these approvals, multiple clinical trials with oligonucleotides are ongoing, including Phase III trials in neurological, cardiovascular, metabolic or ophthalmic indications, among

[1] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2023/215887Orig1s000TOC.cfm
[2]

others [1]. Altogether, during the recent years, both industry and regulators have gained a thorough expertise on this type of products. There are, however, challenges that should be considered for the successful development of oligonucleotide therapies.

Regulatory challenges

There are only few specific regulatory guidelines addressing the development of oligonucleotides.

- From the *quality/control* point of view, synthetic oligonucleotides are at the interface of small molecules and biologicals, therefore specific considerations apply to this class of therapeutics. Indeed, oligonucleotides are fully or partially excluded from the scope of ICH Q3A/B, ICH Q6A/B and ICH M7. Of note, a recent concept paper published by the EMA anticipates a new guideline addressing specific aspects of the manufacturing process, characterization, specifications and analytical control for synthetic oligonucleotides [4].
- Even though they are not biological products, for *nonclinical* development, oligonucleotides should follow recommendations in the ICH S6 guideline *Preclinical safety evaluation of biotechnology-derived pharmaceuticals* [5]. Besides this regulatory guideline, the Oligonucleotide Safety Working Group (OSWG) has published several articles discussing toxicology assessments for oligonucleotides (genotoxicity, repeat dose toxicity, reproduction toxicity, etc) [6-8].
- As for any other products, the clinical development plan of oligonucleotide therapies should follow the regulatory guidelines for the intended therapeutic indication, when available. In addition, the FDA has recently published a draft guidance for the industry on the *Clinical Pharmacology Considerations for the Development of Oligonucleotide Therapeutics*. Among others, this guideline provides recommendations on immunogenicity risk assessments or how to characterize the impact of hepatic or renal impairment [9].

Moreover, the FDA recently published three guidelines on the CMC, nonclinical and clinical development of individualized antisense oligonucleotides for treatment of a severely debilitating or life-threatening diseases caused by a unique genetic variant identified in very small patient numbers (usually one or two) [10-12]. While these guidelines can be considered a reference, additional assessments to evaluate efficacy and safety may be expected for products to be used in wider populations. Specifically for nusinersen, the FDA has published a draft guidance for the development of generic versions, which includes recommendations for demonstrating sameness and supporting a waiver for *in vivo* bioequivalence study [13].

Quality/control development

For the CMC development of synthetic oligonucleotides, major issues relate to the control of the starting materials of the drug substance and of the drug product [14, 15]. Some major challenges are as follows:

- *Starting materials* are an important part of the overall control strategy. Phosphoramidites, the building blocks used in chemical synthesis of oligonucleotides, already have a complex chemistry and syntheses. Criticality assessment of their impurity profile is essential, and impurity profiles for phosphoramidites from different suppliers should be compared.
- A good understanding of the *impurity profile* in the *drug substance* should be demonstrated. As most impurities exist as mixtures of closely related molecules (i.e.: diastereomers, n-1, n-2, n+1, n+2), many impurities coelute with the active ingredient, and there is a lack of analytical methods to adequately resolve impurities. Particularly, genotoxic impurities need to be comprehensively discussed in the dossier, and the presence of nitrosamines should be controlled and kept as low as possible.
- For the *control of the drug product*, usually ±3 times standard deviation is considered adequate for setting of specifications. Early development batches should not be the basis of specification setting; in fact, post-approval adjustment of specifications may be an option. Usually for antisense and siRNA molecules no bioassay is expected; however, a justification for omission of bioassay should be provided.

Nonclinical development

Toxicology packages should be designed to assess, as far as possible, the expected toxicities associated to the therapeutic class (i.e.: off-target toxicities, immune-stimulation or inhibition of coagulation and renal damage). Importantly, some of these assessments can be done (at least preliminarily) using *in silico* and *in vitro* approaches [3].

As for other biological products, a main challenge for the nonclinical development of oligonucleotides is the identification of relevant species for toxicology assessments, as oligonucleotide hybridization to the target sequence is required to assess on-target toxicities. Moreover, the route of administration used in the nonclinical studies should mimic, as far as possible the intended clinical route, which is an additional challenge for complex routes of administration, like intravitreal or intrathecal.

Clinical development

The therapeutic effect of the oligonucleotides requires a specific level of *target engagement* in the intended cells. Identifying the therapeutic dose in this respect is specially challenging when estimation of clinical doses relies on nonclinical data; especially if differences in the pharmacokinetic profile and biodistribution of the oligonucleotide between humans and animal models exist.

An unwanted immune response to an oligonucleotide can be generated to the carrier, backbone, oligonucleotide sequence, or any novel epitopes created from the whole drug. The clinical immunogenicity assessment for an oligonucleotide therapeutic should follow a risk-based approach. For clinical immunogenicity assessments, immunogenicity sample collection should coincide with pharmacokinetic and pharmacodynamic sampling time points to evaluate whether anti-drug antibodies (ADAs) impact the pharmacokinetics, pharmacodynamics, and any immune-mediated adverse events [9].

An additional challenge often faced during the development of oligonucleotides is demonstrating efficacy based on a *relevant clinical outcome*. Oligonucleotides are generally developed for the treatment of rare genetic conditions affecting multiple systems and patients may have very diverse symptoms. Often, these conditions have a slow and complex evolution, and their natural history is poorly understood. In this scenario, demonstration of efficacy often relies on *surrogate biomarkers* for which clinical relevance has not always been demonstrated. This could be a major issue, as clinical data may fail to demonstrate the positive benefit/risk expected by regulators.

Conclusion

There is only a limited number of regulatory guidelines on oligonucleotides therapies. However, key experience has been gained evaluating therapies already approved and currently under development. Nevertheless, there are still major challenges for the successful development of these therapies, both at quality/control as well as nonclinical and clinical level. It is therefore strongly recommended to discuss the development plan with the regulatory authorities and to agree on major aspects including, among others, specifications (mainly impurities), selection of relevant species, design of the toxicology plan, patient selection, and clinical outcomes of efficacy.

Keen to know how to design an optimal development plan for your oligonucleotide? Planning to discuss your oligonucleotide program with the regulatory authorities? We can support you!
Building on decades of experience in regulatory interactions and requirements, Zwiers Regulatory Consultancy, a ProductLife Group Company, provides up-to-date support and expert advice on drug development and regulatory strategy.

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