RNA-based Therapies for Monogenic Disorders

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Abstract

Monogenic disorders are conditions caused by variants in single genes. The current treatment strategies for monogenic disorders include DNA, RNA, or protein-based therapies. RNA-based therapeutics can be divided into four main categories namely antisense oligonucleotides, RNA interference, aptamers, and messenger RNA (mRNA) based therapies. Here, we discuss the RNA therapies that have emerged as promising strategies for the treatment of several monogenic disorders in recent years.

Keywords: RNA-based therapies, antisense oligonucleotides, small interfering RNAs (siRNA), aptamers

Introduction

Monogenic disorders are hereditary disorders that are caused by variations in single genes. These disorders can follow autosomal dominant, autosomal recessive, or X-linked modes of inheritance. These conditions are rare, but cumulatively affect more than 6% of the world's population which accounts for hundreds of millions of people (Clarke et al., 2023). Thus, the management of these conditions presents a vast global challenge.

Currently, the key challenges in the treatment of monogenic disorders are the high cost of treatment. Moreover, these drugs are not targeted against all genotypes. Also, only 10% of the genes are estimated to produce druggable target proteins (Clarke et al., 2023).

Current therapeutic strategies for monogenic disorders

The treatment strategies, at present, are aimed to treat monogenic disorders by targeting the DNA (gene therapy, gene editing), RNA (antisense oligonucleotides, RNA interference, readthrough compounds), or the protein encoded by the mutated gene (Zhu et al., 2022). In this review, we will be focussing on RNA-based therapies for monogenic disorders.

An overview of the major developments in the RNA targeting field

RNA-based therapies have emerged as promising strategies for the treatment of a number of monogenic disorders in recent years. The vital role of RNA in the flow of genetic information was first described by Francis Crick in his study on the 'Central Dogma of Molecular Biology' and was later confirmed by the discovery of mRNA in 1961.

The first application of RNA base-pairing for therapeutic purposes was described by Stephenson and Zamecnik in 1978 when they designed an antisense oligonucleotide targeting the 35S RNA sequence of the Rous sarcoma virus (RSV) thereby inhibiting its replication. In the year 1998, the first antisense oligonucleotide-based drug (Fomivirsen) was approved by the United States Food and Drug Administration (US FDA) for the treatment of cytomegalovirus retinitis. The first aptamer drug (Pegaptanib) was approved by US FDA in 2004 for the treatment of neovascular age-related macular degeneration (AMD). Patisiran was the first siRNA-based drug that was approved by the US FDA (2018) for the treatment of hereditary transthyretin-related (hATTR) amyloidosis (Kim, 2022).
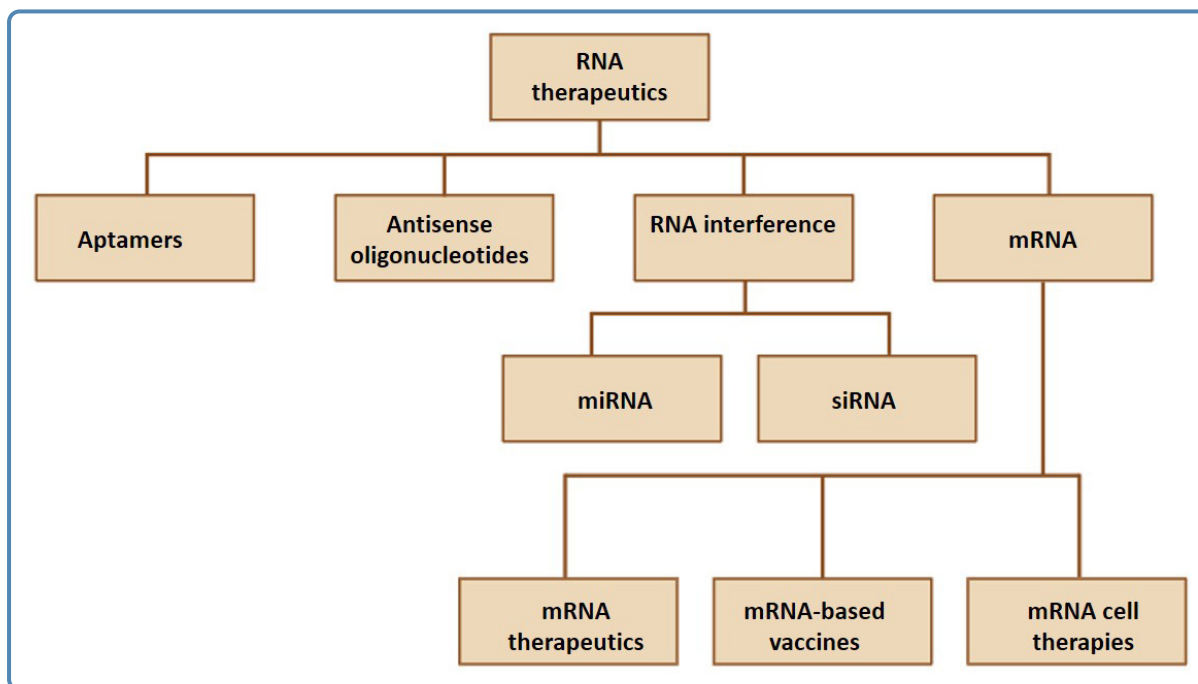
Figure 1 Classification of RNA-based therapies (Adapted from Zhou et al., 2019)

A few mRNA vaccines (Comirnaty by Pfizer, and BioNTech and Spikevax developed by Moderna) also got emergency approval in the year 2020 (final approval 2021) for use in the recent COVID-19 pandemic caused by SARS-CoV-2 (Kim, 2022).

Types of RNA therapies

Based on the structural characteristics and mode of action, RNA-based therapies can be broadly classified into four main categories namely, antisense oligonucleotides (ASO), RNA interference (siRNA, miRNA), aptamers, and messenger RNA-based therapeutics (**Figure 1**). The various FDA-approved RNA-based therapies for different genetic and non-genetic disorders are listed in **Table 1**.

Antisense oligonucleotides (ASO)

Antisense oligonucleotides (ASO) are single-stranded nucleotide sequences that modulate the expression of target RNAs via sequence-specific binding. Therapeutic ASOs range from 12 to 24 bp in length. Although the structure of these antisense oligonucleotides is determined primarily by their specific sequence, their chemistry can be modulated to produce novel effects with an increase in their specificity and stability (Zhu et al., 2023).

Antisense oligonucleotides that have been approved by the US FDA can be divided into two broad categories depending on their mechanism of action. The first group induces the cleavage of RNA by binding to the target sequence. Once these ASOs form a DNA-RNA duplex with their target RNA, it is recognized by the RNase H enzyme which leads to the degradation of the target RNA (**Figure 2**). Because these therapeutics utilize RNase H, which is active in both the nucleus and cytoplasm, these agents can be used to target noncoding elements as well (Dhuri et al.,2020). This provides an advantage over siRNA-based drugs which act primarily in the cytoplasm (Kim, 2022).
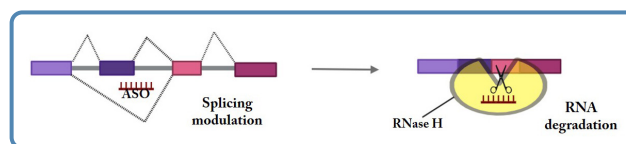
Figure 2 Antisense oligonucleotide-based RNA drugs that induce RNase H-mediated degradation of target mRNA (Adapted from Dhuri et al., 2020)
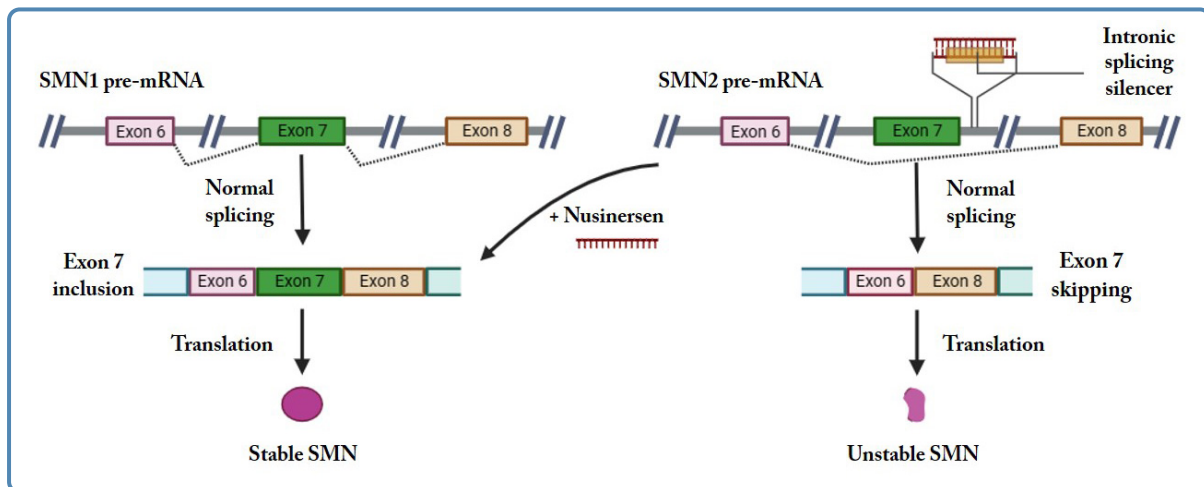
Several antisense oligonucleotide drugs using this type of cleavage have been approved

Table 1 FDA-approved RNA-based therapies (Adapted from Zhang et al., 2023; Kim, 2022)

RNA-based drug	Brand name	Year of FDA approval	Mechanism of action	Target disease
Antisense oligonucleotides				
Fomivirsen	Vitravene	1998	Inhibition of translation of viral mRNA encoding IE2 protein	CMV retinitis
Mipomersen	Kynamro	2013	Induction of the degradation of ApoB-100 mRNA	Homozygous familial hypercholesterolemia (HoFH)
Nusinersen	Spinraza	2016	Induction of exon 7 inclusion in SMN2 mRNA	Spinal muscular atrophy
Eteplirsen	Exondys 51	2016	Induction of exon 51 skipping in DMD mRNA	Duchenne muscular dystrophy
Inotersen	Tegsedi	2018	Induction of the degradation of TTR mRNA	Hereditary transthyretin-mediated (hATTR) amyloidosis
Golodirsen	Vyondys 53	2019	Induction of exon 53 skipping in DMD mRNA	Duchenne muscular dystrophy
Small interfering RNAs				
Patisiran	Onpattro	2018	RNA interference-mediated cleavage of TTR mRNA	Hereditary transthyretin-mediated (hATTR) amyloidosis
Givosiran	Givlaari	2019	RNA interference-mediated cleavage of ALAS1 mRNA	Acute hepatic porphyria
Lumasiran	Oxlumo	2020	RNA interference-mediated cleavage of HAO1 mRNA	Primary hyperoxaluria type 1
Inclisiran	Leqvio	2021	RNA interference-mediated cleavage of PCSK9 mRNA	Heterozygous familial hypercholesterolemia (HeFH)
RNA aptamers				
Pegaptanib	Macugen	2004	Antagonistic binding to VEGF protein	Neovascular age-related macular degeneration

by the US FDA including mipomersen and inotersen. Mipomersen is the second antisense oligonucleotide drug approved by the FDA (2013) as an adjunct to lipid-lowering therapy for the treatment of homozygous familial hypercholesterolemia (HoFH) (**Figure 3**). Apolipoprotein B-100 (ApoB-100) is the main component of low-density lipoprotein and its precursor very low-density lipoprotein (VLDL). Mipomersen binds to ApoB-100 mRNA and cleaves

its sequence, thereby reducing lipid levels in these individuals (Clarke et al.,2023).

The second group of antisense oligonucleotide drugs primarily regulates the splicing of pre-mRNAs by utilizing a steric hindrance-based mechanism. These ASOs bind to specific sequences within the pre-mRNA transcripts, and subsequently, modulate the other splicing factors to produce alternative splicing (**Figure 3**). A few FDA-approved ASOs which work on this mechanism include nusinersen, eteplirsen, golodirsen, and casimersen (Kim, 2022).

Individuals with spinal muscular atrophy have variations in *SMN1,* which encodes the survival motor neuron (SMN) protein. These variations prevent the expression of a functional SMN protein from the SMN1 gene locus but some amount of SMN protein is still produced from the *SMN2* locus. However, this protein product is smaller and less stable because of exon 7 skipping in the SMN2 pre-mRNA. As a result, there is loss of motor neurons eventually leading to muscle wasting. The intron splicing enhancer located between exons 7 and 8 in SMN2 pre-mRNA leads to this exon 7 skipping during the splicing of SMN2 pre-mRNA. Nusinersen is an antisense oligonucleotide that binds to this element and blocks its recognition by the splicing factors. As a result, the SMN2 pre-mRNA is spliced like SMN1 pre-mRNA leading to the production of a more stable SMN protein. This drug has shown improved motor function in infants with spinal muscular atrophy in a phase III clinical trial (Clarke

et al.,2023) and was approved by the US FDA for clinical use in 2016.

Exon skipping approach has been utilized by a series of antisense oligonucleotide-based drugs such as eteplirsen, golodirsen, and casimersen that have been developed for the treatment of Duchenne muscular dystrophy (DMD) (Zhang et al.,2023). In individuals with DMD, the mRNA coding for dystrophin protein usually harbours a variation that can alter the reading frame, thereby producing a truncated (non-functional) protein in these individuals. Eteplirsen binds to the exonic splicing enhancer present in exon 51 of DMD pre-mRNA. Exon splicing enhancer element is required for the inclusion of this exon in the mature mRNA. Thus, binding of eteplirsen to this element leads to exon 51 skipping and correcting the reading frame in the mature mRNA. As a result, there is production of a short but functional dystrophin protein in these individuals. Exon 51 skipping may be beneficial for individuals with DMD with exon deletions ending at exon 50 or starting at exon 52 (Łoboda et al.,2020). Golodirsen and casimersen also utilize a similar mechanism in a different subset of individuals. Golodirsen induces exon 53 skipping while casimersen leads to exon 45 skipping to produce a functional version of the dystrophin protein (Kim, 2022).

Several clinical trials using antisense oligonucleotides such as IONIS-HTTRx for Hungtington disease and Tofersen, which targets the SOD1 protein frequently implicated in familial amyotrophic lateral sclerosis are underway (Clarke

et al.,2023).

Small interfering RNAs (siRNA) Small interfering RNAs (siRNAs) and micro RNAs (miRNAs) are small duplex RNA molecules that target messenger RNA (mRNA) leading to post-transcriptional gene silencing. siRNAs and miRNAs utilize the RNA interference (RNAi) pathway to modulate the expression of their target mRNA. RNAi is the endogenous and intrinsic defence mechanism of the body against invading viruses and transposable elements (Chen et al.,2018). In the RNAi pathway, endogenous small RNAs (siRNA or miRNA) form a complex with Argonaute 2 protein (Ago2) to produce an RNA-induced silencing complex (RISC) (**Figure 4**). This complex binds to the target mRNA via sequence-specific binding leading to mRNA cleavage (Zhang et al.,2023).
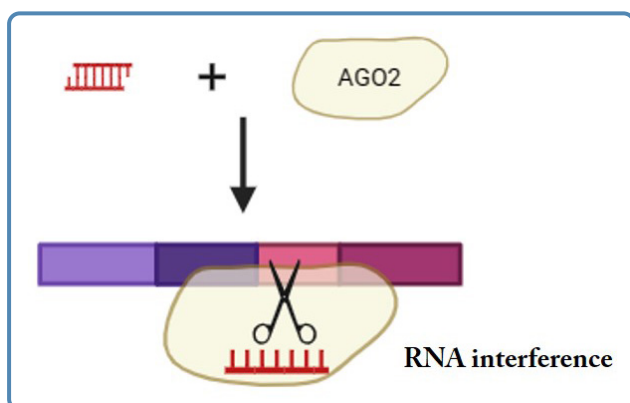

Figure 4 Mechanism of action of small interfering RNA-based drugs (Adapted from Kim, 2022)

siRNAs are double-stranded RNA molecules about 18 to 25 nucleotides in length. Four siRNA-based drugs have been approved by the US FDA namely patisiran, givosiran, inclisiran, and lumasiran for the treatment of hereditary transthyretin-mediated (hATTR) amyloidosis, acute hepatic porphyria, heterozygous familial hypercholesterolemia (HeFH) and primary hyperoxaluria type 1, respectively (Kim, 2022). However, no miRNA-based drug has been by approved so far by the US FDA for the treatment of monogenic disorders.

Patisiran is the first FDA-approved siRNA-based drug (2018) for treating hereditary transthyretin-mediated (hATTR) amyloidosis. This condition is caused by variation in the transthyretin (TTR) gene leading to the production of a misfolded transthyretin protein and it eventually leads to amyloid deposition in various tissues of the body. Patisiran utilizes a lipid nanoparticle-based delivery system and is injected into the body via intravenous infusion. These particles enter the hepatocytes via ApoE (apolipoprotein E) receptors. In the hepatocytes, patisiran combines with RISC and this complex binds to the 3' untranslated regions (UTR) of both the wild-type as well as mutant TTR mRNA leading to the suppression of TTR protein translation and an overall reduction in the amyloid deposition in the tissues (Zhang et al.,2023).
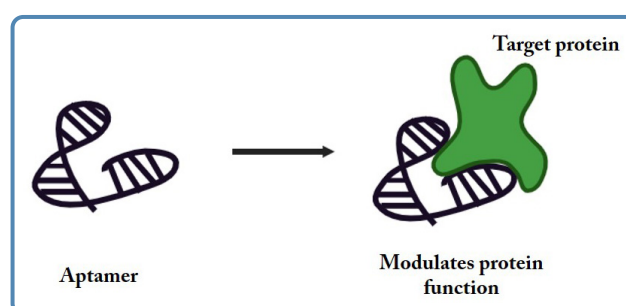
Figure 5 Mechanism of action of aptamer-based drugs (Adapted from Kim, 2022)

Aptamers Aptamers are small, single-stranded oligonucleotides (DNA or RNA) that bind to their targets (proteins, peptides, or nucleic acids) with high specificity and affinity and modulate their functions (**Figure 5**). Pegaptanib is the only aptamer drug that has been approved by the US FDA. It is a 28-nucleotide construct with two polyethylene glycol moieties (PEG) attached at its end. It binds to the vascular endothelial growth factor (VEGF) thereby inhibiting the interaction of VEGF with its receptor leading to the suppression of downstream VEGF signalling and cell proliferation. Pegaptanib was developed for the treatment of neovascular age-related macular degeneration, but it is rarely used nowadays because of the availability of several antibody-based drugs with similar efficacy. Nevertheless, it is a promising therapeutic strategy, and several RNA-based aptamers are under development for the treatment of various monogenic disorders (Zhu et al.,2022; Kim, 2022).

Conclusion

After several decades of development, RNA-based therapeutics are now becoming a clinical reality.

The field of RNA-based therapy is undergoing a major expansion and the underlying potential of these therapies for personalized medicine will certainly ensure the continued development of RNA-based therapeutics for years to come.

References

1. Chen X, et al. RNA Interference–Based Therapy and Its Delivery Systems. Cancer Metastasis Rev. 2018; 37(1): 107–124.
2. Clarke LA, Amaral MD. What Can RNA-Based Therapy Do for Monogenic Diseases? Pharmaceutics. 2023; 15: 260.
3. Dhuri K, et al. Antisense Oligonucleotides: An Emerging Area in Drug Discovery and Development. J Clin Med. 2020; 9(6):2004.
4. Kim Y-K. RNA therapy: rich history, various applications and unlimited future prospects. Exp Mol Med. 2022; 54: 455–465.
5. Zhang C, Zhang B. RNA therapeutics: updates and future potential. Sci China Life Sci. 2023; 66: 12–30.
6. Zhou L-Y, et al. Current RNA based therapeutics in clinical trials. Curr Gene Ther. 2019; 19(3): 172-196.
7. Zhu Y, et al. RNA-based therapeutics: an overview and prospectus. Cell Death Dis. 2022; 13: 644.

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