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Antisense Oligonucleotides: Adding Sense to Therapeutic Medicine

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Abstract

Rapid progress in the field of molecular biology has led to the development of numerous genetic therapies. Among these, antisense oligonucleotide (ASO) therapeutics have recently gained momentum due to their application in the spectrum of disorders ranging from neurodegenerative disorders to malignancies. This brief review discusses the principle of ASO based therapies, mechanism of action and their current role in the field of therapeutic medicine.

Introduction

Antisense Oligonucleotide (ASO) therapeutics is a well-recognized class of drugs exploiting the Watson and Crick's base pairing rules to target disease-related RNAs. Although the concept of using synthetic oligonucleotides to modulate RNA function dates back to 1978, the anticipated clinical success was achieved only after recent advances in genomics, chemistry and pharmacology.

Oligonucleotides are unmodified or chemically modified single stranded DNA molecules which are 8-50 bp in length. They hybridize to target RNA and alter its original function through an array of mechanisms. With the knowledge of gene sequences, ASOs directed at specific target sequences are being utilized to understand gene functions. Simplicity of the concept has led to its use in knock-down experiments, target validation, drug therapy, and other applications. The same principle is applicable to the use of ASOs in therapeutics. Another advantage of ASOs is the reversibility of effects as opposed to gene therapy and genomic editing. The current success in the treatment of neuromuscular disorders especially spinal muscular atrophy has proved the potential of ASO based therapies.

The major hurdles in the designing of ASOs include rapid degradation by intracellular exonucleases and endonucleases, inefficient uptake in certain tissues, nonspecific effects and adverse immune responses. However, these issues are being actively addressed to enhance efficacy and specificity of ASOs.

Mechanisms of action of ASOs

ASOs modulate the transfer of genetic information to protein in multiple ways-

a) *RNase-H mediated site specific degradation of mRNA:* This remains the most utilized antisense mechanism despite major advances in the field of RNA biology (Crooke et al., 2018). RNase-H causes degradation of DNA/RNA heteroduplex when DNA based ASO binds to its RNA targets. Activation of RNase-H is extremely sequence specific. Mismatch of ≥ 3 base pairs results in complete loss of RNase-H activation. Hence extreme caution is required in designing ASO for this action. Nevertheless, this remains the most efficient mechanism which causes 80-95% downregulation of mRNA and protein expression and acts effectively even when targeted at any region of mRNA (Dias et al., 2002)

b) *Steric block of ribosome binding:* This includes interruption of RNA translation by preventing the movement of ribosomes onto mRNA thereby inhibiting assembly of 40s, 60s ribosomal subunits.

c) *Modulation of splicing:* Some ASOs function by binding to regulatory sequences, masking splicing enhancers or repressor sequences causing exon skipping and forcing inclusion of otherwise alternatively spliced exons. ASOs can also modulate polyadenylation selection in

those transcripts with > 1 poly A site at 3′ untranslated region (Vickers et al.,2001) This in turn creates alternative transcript and increases mRNA stability and alters protein expression.

d) *Targeting miRNA and Natural Antisense Transcript (NAT):* Recently discovered ASOs are designed in such a way that they can directly bind to miRNA and NAT and prohibit them from binding to their own mRNA specific targets. This in turn causes upregulation of genes targeted by miRNA and NAT (Davis et al., 2009)

- Generations of ASOs:

1st generation ASOs: These compounds have phosphorothioate backbone only, limiting the function to RNase-H degradation. In addition, these compounds have nonspecific interaction with cell surface and intracellular proteins (Kurreck, 2003).

2nd generation ASOs: These compounds have 2′-sugar modifications like 2′-*O*-methyl and 2′-*O*-methoxyethyl (MOE) additions. This makes them resistant to degradation by cell nucleases and increases their affinity and target specificity.

3rd generation ASOs: These include Locked Nucleic Acid (LNA) and Morpholino modifications. Morpholinos display greater potency in altering splicing and inhibiting translation in vivo but do not activate RNase-H. LNA compounds exhibit enhanced potency and are known for their robust binding improvement and nuclease resistance when compared to other 2′ modified compounds (Swayze et al., 2007).

- Delivery of Oligonucleotides to Cells:

Adsorptive endocytosis and fluid phase pinocytosis appear to be the major mechanisms for oligonucleotide internalization. The proportion of internalization depends on the concentration of oligonucleotide. At low concentrations, the likely mechanism of internalization occurs via interaction with membrane bound receptors. At high concentrations, the receptors get saturated and pinocytic process assumes greater importance.

- Applications in the field of therapeutic medicine:

The first ASO approved for clinical use was Fomivirsen for cytomegalovirus retinitis. Since then numerous oligonucleotides targeting a wide spectrum of disorders have been studied in various clinical trials. A summary of the various ASOs approved by United States Food and Drug Administration (USFDA) is given in Table I. The recent approvals of Eteplersin for Duchenne muscular dystrophy and Nusinersen for SMA are briefly reviewed.

- Nusinersen for spinal muscular atrophy (SMA):

SMA is an autosomal recessive neuromuscular disorder caused by a mutation in the *SMN1* gene. Absence of functional SMN protein leads to degeneration of motor neurons in the spinal cord, resulting in progressive muscle weakness. SMN2 gene on chromosome 5q13 is identical to SMN1 except for a C-to-T transition within exon 7. This base substitution by disrupting a splicing enhancer or creating a splicing silencer, results in the exclusion of exon 7. *SMN2*, therefore produces only 10% properly spliced mRNA. The remaining 90% lack exon 7 and the resultant protein becomes unstable and is quickly degraded. Antisense oligonucleotide (Nusinersen) complementary to ISS-N1 (intronic splicing silencer) blocks its ability to exclude exon 7, resulting in full-length mRNA containing exon 7 (Figure 1).

In the interim analysis of clinical trial, 21 of 51 infants in the nusinersen group had a motor-milestone response as against 0 of 27 in control group ($p<0.001$), and this result prompted early termination of the trial (Finkel et al., 2018). The efficacy of nusinersen has also been observed in late onset SMA (Montes et al., 2019).

- Eteplirsen for Duchenne Muscular Dystrophy (DMD):

DMD is a fatal neuromuscular disorder caused by progressive muscle degeneration due to defective dystrophin protein. Eteplirsen functions by hybridizing to a site within exon 51, thereby blocking the splicing machinery from binding and forcing it to “skip” the exon. Exon 52 is spliced to exon 48, which restores the reading frame, generating a shortened but functional dystrophin (Figure 2). This is expected to benefit 14% of the entire DMD population.

USFDA approved the drug for DMD in 2016. However, it created a lot of controversies due to the lack of conclusive evidence regarding the efficacy of the drug. However, European Medical Agency (EMA) did not approve the drug stating that the study was done on only 12 patients with no control group and historical data was used for comparison. Following this, confirmatory phase 3 study using a larger sample size with a control group was performed.

Table 1 Antisense oligonucleotides approved by USFDA and their clinical indications (Modified from Yin., 2019).

Drug	Year of approval	Indication	Target	Tissue	Dosing	Results and conclusions
Fomivirsen	1998	CMV retinitis	CMV IE-2 (immediate early-2)	Eye	300 μg every 4 weeks, intravitreal.	Clinical efficacy was witnessed but the drug marketing got hampered by dramatic decrease in CMV cases.
Pegaptanib	2004	Neovascular Age related macular degeneration (AMD)	VEGF165	Eye	0.3 mg every 6 weeks, intravitreal	Clinical efficacy was present and no systemic toxicity was observed. Faced tough competition with ranibizumab and bevacizumab manufacturing companies.
Mipomersen	2013	Homozygous familial hyper-cholesterolemia	ApoB-100	Liver	200 mg once weekly, subcuta-neous	Clinical efficacy was demonstrated but safety concerns were present.
Defibrotide	2016	Hepatic veno-occlusive disease	Proteins, nonspecific	Liver	6.25 mg/kg every 6 hours, i.v. infusion	Defibrotide demonstrated improved survival rate and complete response rate in phase III trial when compared with historical controls.
Eteplirsen	2016	Duchenne muscular dystrophy	Dystrophin (Exon 51)	Muscle	30 mg/kg once weekly, i.v. infusion	Controversy exists on the level of evidence demonstrating drug efficacy. The FDA approved the drug under conditional approval. In 2018, the EMA refused the approval of eteplirsen.
Nusinersen	2016	Spinal muscular atrophy	SMN2	CNS	12 mg once every 4 months, Intrathe-cal	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	2018	Hereditary transthyretin amyloidosis	TTR	Liver	300 mg once weekly, s.c.	Robust efficacy was demonstrated in a phase III study; however, two significant adverse events were observed during the study: thrombocytopenia causing death due to intracranial hemorrhage and renal dysfunction.
Patisiran	2018	Hereditary transthyretin amyloidosis	TTR	Liver	0.3 mg/kg or 30 mg based on BW, once every 3 weeks, i.v. infusion	The first approved siRNA. Robust efficacy was demonstrated in a phase III study with no safety concerns.

i.v. – intravenous; s.c. – subcutaneous

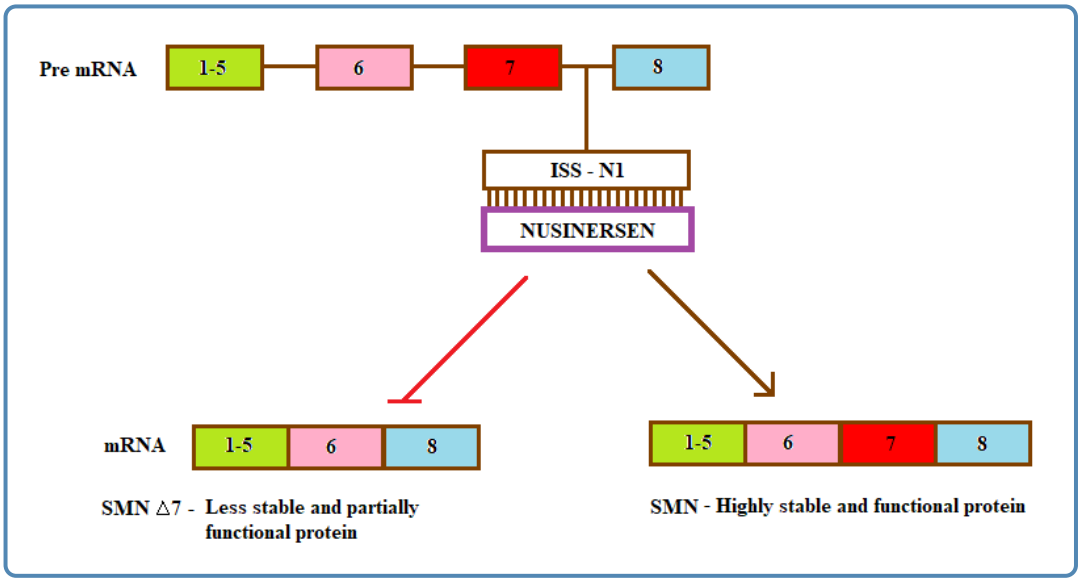

Figure 1 Mechanism of nusinersen in causing exon inclusion in *SMN2* gene.

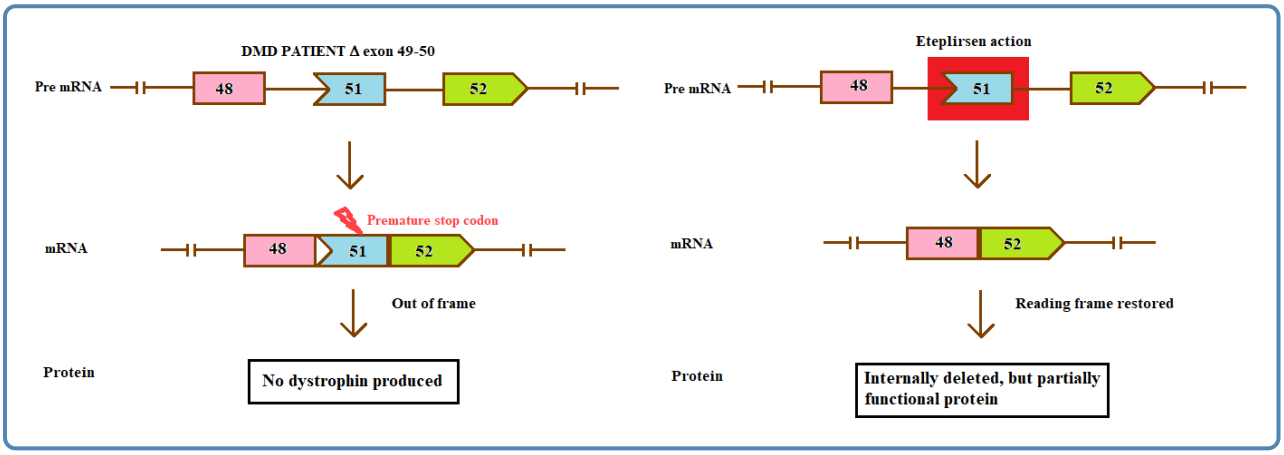

Figure 2 Mechanism of eteplirsen in causing exon 51 skipping in *DMD* gene.

- Challenges for ASO agents:

The two major hurdles that hamper the widespread application of oligonucleotide therapeutics include drug safety and delivery.

Some oligonucleotides bind to Toll-like receptors and induce immune responses. Single-stranded phosphorothioate oligonucleotides are known for their renal accumulation causing glomerulonephritis in some individuals and a rare but notable reduction in platelet count (Crooke et al., 2017). Drug delivery also remains a significant challenge in ASO therapeutics because of its limitation in penetrating cell membrane due to their high molecular weight (5-15 kDa). Systemic delivery to most organs and tissues, with the exception of the liver, has proved to be exigent. All these observed effects can be minimized by the advent of newer versions of ASOs.

Emerging as a valid approach to selectively modulate gene expression, therapeutics with oligonucleotides has a great potential of being used as an ardent tool in drug designing. It has great potential in cancer therapeutics as well (Harada et al., 2019). The enhanced biological activity and efficient target delivery will pave the way for the apparently endless ASO therapeutic approaches in the near future.

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