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THERAPEUTIC APPLICATIONS OF OLIGONUCLEOTIDES

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ABSTRACT

RNA is a unique therapeutics development target. Some of the advantages in using RNA as therapeutic target include applicability up to most RNAs in the cell, including noncoding RNAs, translation of genetic findings directly into drug discovery programs, and speeding and efficiency of the drug discovery process. While some promising advances have been made in the identification of small-molecule drugs that can modulate RNA function, oligonucleotides offer a much more direct—and, therefore, more expeditious—route. Antisense oligonucleotides are a new therapeutic platform for drug discovery with enormous potential to treat the multitude of diseases. Now, oligonucleotides stand to serve large populations of patients. Until now, their action used to be against diseases found in only a small number of patients. That being said, improvements can still be made using the oligonucleotide technology, with probably more discoveries set to be found in the coming years. Examples of the oligonucleotide therapeutics include antisense, oligonucleotides, small interfering RNA, microRNAs, aptamers, and decoys. The heterogeneity and feasibility of these diagnostically or therapeutically active drugs have increased incredibly over the past 25 years. There has been an emergence of several clinical and preclinical studies of oligonucleotides in patients with various neurodegenerative diseases, infectious diseases, respiratory diseases and in various types of cancer. This paper presents a summary of basic

information, mechanism of action and applications of antisense oligonucleotides, small interfering RNA, microRNAs, aptamers and decoys.

KEYWORDS: Antisense oligonucleotides, siRNAs, miRNAs, Aptamers, Decoys, Ribonucleic acid.

INTRODUCTION

For the past century, drug development efforts have targeted proteins with different compound types, from small molecules to monoclonal antibodies. Indeed, although some classes of proteins, such as membrane receptors, enzymes, ion channels, and transport proteins, can be approached therapeutically by conventional protein-targeting strategies, many other targets, such as transcription factors, scaffold proteins, and structural proteins, are much less druggable using traditional modalities. Alternatively, instead of modulating the function of a protein, modulation of its expression level may be achieved, and this can be done by acting on its mRNA.[1] Oligonucleotides are a class of single- or double-stranded small synthetic nucleic acid polymers (~≈20-mer) that can be used to modulate gene expression.[2] Typical oligonucleotide drugs include antisense oligonucleotides, siRNA, microRNAs, aptamers, and decoys. Among these, ASOs and siRNAs have been developed much further compared to others.[3]

Abbreviations: - RNA – Ribonucleic acid, ASO – Antisense Oligonucleotides, siRNA- Small interfering RNA, miRNA- Micro RNA, HCV- hepatitis C Virus, AGO – Argonaute protein, mRNA – Messenger RNA.

Different types of oligonucleotides

Antisense oligonucleotides

The wide variety of compounds and strategies developed in the last century for drug research allows the targeting of many different classes of proteins: from small molecules to monoclonal antibodies. Although some classes of proteins—in particular membrane receptors, enzymes, ion channels, and transport proteins—have an opportunity to be therapeutically tractable through conventional approaches to protein targeting, almost all the rest—transcription factors, scaffold proteins, and structural proteins—are far less readily druggable by traditional modalities. Instead, modulation of the expression level of a protein can be used instead of its function, which can be done by acting on its mRNA.[1] Oligonucleotides are a class of single- or double-stranded small synthetic nucleic acid

polymers (~≈20-mer) that can be used to modulate gene expression.[2] Examples of typical oligonucleotide drugs include antisense oligonucleotides, siRNA, microRNAs, aptamers, and decoys. Out of these ASOs and siRNAs have really got much more established than the rest.[3]

Mechanism of Actions

Detailed studies showed that the mechanism of synthetic ASOs can be of two types.

1. RNA cleavage
2. RNA blockage

1. RNA cleavage

ASOs target RNA, forming ASO-RNA heteroduplexes, which become substrates for RNase enzymes existing in the cytoplasm. The RNases hydrolyze the RNA in the heteroduplex.

2. RNA Blockage

a. Translation Arrest by Steric Hindrance

These ASO classes interact with target RNA sequence and result in translational arrest, caused by the inhibition of their binding to the 40S ribosomal subunit or, otherwise, block the assembly on the 40S or 60S ribosomal subunit. Since steric hindrance-based ASOs do not activate RNase H1-mediated cleavage, there is preservation of pre-mRNA structure. Steric hindrance depends directly on ASO binding affinity. An improvement in the binding affinity translates into better hybridization with target RNA, leading to an arrest in translation.[5]

b. Splice Modulation or Splice Switching-Based Mechanism

Another mechanism by which ASOs can exert their effects is through alternate splicing. The splice modulation may be of two kinds: exon skipping and exon inclusions. Frameshift mutations modify pre-messenger RNA splicing patterns, leading to the generation of abnormal proteins or the translation arrest of complete functional proteins. In exon skipping, the ASOs bind to the pre-mRNA transcripts, correct the disrupted reading frame, and produce a short but functional protein. Whereas in exon inclusion, ASOs bind to the pre-mRNA site, hence preventing the spliceosome and splicing factors from accessing the transcript sites.[5]

FDA approved ASO drugs

Table No. 1: US FDA approved Antisense oligonucleotide drugs.[5]

Drugs	Indications
Fomivirsen (VitraveneTM)	CMV infection
Mipomersen (KynamroTM)	Homozygous familial hypercholesterolaemia (HoFH)
Nusinersen (Spinraza®)	Spinal Mascular atrophy (SMA)
Patisiran (Onpattro®)	Hereditary transthyretin-mediated amyloidosis (hATTR)
Inotersen (Tegsedi®)	Hereditary transthyretin-mediated amyloidosis (hATTR)
Eteplirsen (Exondys 51®)	Muscle degenerative disorder
Golodirsen (Vyondys 53TM)	Muscle degenerative disorder
Givosiran (Givlaari®)	Acute hepatic porphyria
Milasen	Neuronal ceroid lipofuscinosis 7 (CLN7)

siRNAs

Over the last decade, nucleotide RNAs of ∼20−30 have emerged as potent regulators of eukaryotic genome expression and function. Two classes of small RNAs, siRNAs, and miRNAs, act in somatic and germline lineages of a wide spectrum of eukaryotic species to regulate endogenous genes and to defend the genome against invasive nucleic acids.[6] dsRNA is derived from transcription or by enzymes such as RNA-dependent RNA polymerases. This RNA is further processed to short interfering RNAs, siRNAs, that guide a nuclease complex, called RISC, that binds and sequence-specifically cleaves complementary target RNAs. This is also more commonly referred to as RNAi. Long dsRNA, however, is toxic for animal organisms with more sophisticated immune systems that are capable of sensing long dsRNA as "foreign," because such RNAs could, for example, result from viral infections. Subsequently, it was identified that small siRNAs evade immune sensing and can be utilized for gene knockdown in higher organisms e. mammals, and thus it was a breakthrough.

Mechanism of Action of siRNA

Small interfering RNA is a dsRNA molecule of length 21–23 nucleotides and acts post-transcriptionally. This siRNA duplex is the product of the cleavage of long dsRNA by Dicer, a member of the RNAse III family. The duplex is formed by a passenger strand, sense strand, a guide strand or antisense strand. The siRNA is incorporated into the RISC, which interacts with Argouate 2, whereupon the duplex unwinds and degrades the passenger strand. The guide strand then remains and guides the RISC complex to the mRNA; the m-RNA gets cleaved; thus, the protein synthesis gets inhibited.[6]

FDA approved siRNAs

Table No. 2: US- FDA approved siRNAs.[8]

siRNA Drugs	Indications
Patisiran	Polyneuropathy
Givosiran	Acute hepatic porphyria
Lumasiran	Primary hyperoxaluria
Inclisiran	Reducing LDL-C in subjects with Heterozygous familial hypercholesterolemia (HeFH)

miRNAs

miRNA is a small, evolutionarily conserved, single-stranded, noncoding RNA molecule that binds target mRNA to restrain protein production by either of the two distinct mechanisms. Two-step cleavage of primary miRNA, which harbors the effector complex RISC, generates the mature form of the miRNA. The miRNA functions as a guide to prevent the target mRNA from being translated into proteins.[10]

Mechanism of Action of miRNA

The minimal complex for miRNA-induced gene silencing includes the guide strand and AGO. The miRISC can identify its target mRNA because of complementary sequences with miRNA binding sites, on which the miRNA response elements reside. The degree of complementarity between MRE and miRISC makes a prior determination as to the occurrence of AGO2-dependent slicing of target mRNA or whether AGO2-independent miRISC-mediated translational repression takes place with target mRNA degradation. The interaction of the miRNA with a perfectly complementary MRE provokes the endonuclease activity of AGO2 and directs the mRNA for cleavage, but this interaction also unstable the AGO association with the 3 end of miRNA, which leads to the degradation of miRNA.[10]

Mi RNAs as a therapeutic agent

Table No. 3: Different miRNAs as a therapeutic agent.[11]

miRNAs	Indication
miR34	Cancer
MiR122	HCV
Miravirsen	HCV

Aptamers

Aptamers are small single-stranded RNA or DNA oligonucleotides that bind the target molecule with high affinity and specificity. By now, a huge number of generated aptamers have been shown to bind a wide range of targets, from simple inorganic molecules to large

protein complexes and even whole cells. In fact, aptamers are analogues of nucleotide antibodies, but aptamer generation is much easier and less expensive compared to the production of antibodies. In addition, aptamers have not shown any evidence of being immunogenic or toxic. All these properties of an aptamer make them ideal candidates for diagnostics and treatment.[12]

Mechanism of Action of Aptamers

Aptamer-based drug discovery generally utilizes one among three following strategies: an aptamer as an antagonist may block the interaction of disease-associated targets, such as protein–protein or receptor–ligand interaction; the aptamer may behave as an agonist and stimulate the activity of the target receptors; or a cell-type-specific aptamer acts as a carrier to deliver other therapeutic agents into the target cells or tissue.[13]

US FDA Approved aptamers

Table No. 4: US FDA approved aptamers.[14]

Aptamers	Indications
Macugen	Age related macular degeneration
Izervay	Geographic atrophy (GA)

Decoys

Decoys are inhibitory to DNA transcription because, by forming a double-stranded DNA structure, they inhibit the activity of dsDNA-binding transcription factors.[3]

Mechanism of action of decoys

Decoys are short double-stranded deoxyribonucleic acids of similar sequence to the DNA–binding site for transcription factors and are capable of selectively blocking transferase factors from activating target genes. In essence, the decoy ODN technology aims to repress expression of the target genes at the transcriptional level by competing for the transacting elements of the regulatory regions of the genes.[15]

Applications of Oligonucleotides

ASOs act directly at the genetic level in order to prevent the generation of an undesired or overproduced protein. Applications of ASOs are as mentioned below.

Spinal muscular atrophy (SMA): This is a very rare autosomal recessive neuromuscular disease caused by point mutations or deletions in the telomeric survival motor neuron 1 gene.

It is based on a range of disease characteristics underlying the phenotypic appearance whereby type I SMA is the most severe variant resulting in death soon after birth. This implies that in the absence of the SMN1 gene, there is a considerable reduction in the overall amount of expressed survival motor neuron protein. The splice-switching ASO Nusinersen, approved by the FDA, complexes with the intronic splicing suppressor site in intron 7 of SMN2 mRNA, allowing the inclusion of SMN2 exon 7 and expression of the full-length survival motor neuron protein.[17]

Alzheimer Disease (AD): It is a continuously progressive disease of cognition that ultimately renders the affected person fully dependent on someone else in most activities. AD exhibits characteristic neuropathology, signified by the presence of diffuse extracellular amyloid plaques containing β-amyloid, and intracellular neurofibrillary tangles, which are composed largely of the tau protein. ASOs represent a separate approach to decreasing the intracellular burden of tau, thereby decreasing the formation and spreading of intracellular neurofibrillary tangles.[18]

Huntington's Disease: According to the author, Huntington's Disease is an autosomal dominant neurodegenerative disorder that is quite common in middle-aged adults. However, juvenile variants are known to exist. The diseases present with motor decline, progressive brain atrophy, intellectual decline, and psychiatric symptoms. The molecular genetic etiology for the disease is an expansion of a CAG repeat that resides in the first exon of the Huntingtin gene. Tominersen is currently in clinical trials as an antisense oligonucleotide targeting the huntingtin gene mRNA, thus reducing gene expression and impeding protein production.[19]

Duchenne Muscular Dystrophy: This is a heritable disorder defined by large-scale muscle degradation and loss of function of the gene for the protein dystrophin. Of all the therapies in progress, exon-skipping ASO is the most promising because it will benefit in restoring and effectively improving dystrophin function in skeletal muscle.[20]

Osteoarthritis: Arthritis causes an initiation of the loss of proteoglycans due to the deterioration of cartilage and it is a process initiated by A disintegrin and metalloproteinase with thrombospondin motifs. This deterioration is reconstituted by LNA ASO. Combination of the ASO with hydrogels showed to indicate a sustained release for a period of 14 days as observed on confocal microscopy and flow cytometry.[21]

Hepatitis B: Hepatitis B is a liver-targeted infection in the indication of a locked nucleic acid single-stranded oligonucleotide, which is applied after treatment of chronic hepatitis B by incising it using RNase-H. The locked nucleic acid oligonucleotide is conjugated with three N-acetyl galactosamines by binding asialoglycoprotein, which particularly expresses in the liver for delivering pharmacological results in the liver. The antiviral effect of the conjugated locked nucleic acid is higher when compared with the nonconjugated effect.[22]

SARS-CoV-2 infection: Two types of ASOs have been reported, one being of the conventional modified phosphorothioate type, and the others are LNA GapmeR, which targets the 5′ unsaturated region, open reading frame 1a and 1b. The most putative GapmeR targets 5′-UTR in the virus cycle, and five ASO candidates target the ORF1a and ORF1b, and 5'-UTR. Another paper found that intranasal administration of locked nucleic acid, in reality, suppressed replication.[23]

Respiratory systems disorders: Oligonucleotides can be inhaled in the treatment of COPD and asthma. Because these drugs are targeted at the lungs, they have fewer side effects. Their duration of action is also extended as their absorption is usually increased at the target site.[24]

Cancer: Antisense oligonucleotides bind to mRNA and prevent gene translation, thus becoming the new medicine for the treatment of some cancers. The effective transportation and non-specific binding of the protein showed the main obstacle to their application in cancer therapy.[25]

CONCLUSION

Antisense oligonucleotides are a prospective novel therapeutic platform for discovering novel medicines that have the potential to cure most of the diseases. Advancements in ASO technology have been exceptional over the past decade, with ASO-based drugs that have been approved by both the FDA and EMA being registered for clinical application over the past few years. Many antisense oligonucleotides are approved by US- FDA for treatment of CMV infection, Homozygous familial hypercholesterolaemia (HoFH), Spinal Muskular atrophy (SMA), Hereditary transthyretin-mediated amyloidosis (hATTR), Muscle degenerative disorder and for Acute hepatic porphyria. Many siRNAs are approved by US FDA for treatment of Polyneuropathy, Acute hepatic porphyria and for Primary hyperoxaluria. Aptamers such as Macugen and Izervay are used for treatment of macular degeneration disorder and for geographic atrophy.

Conflict of Interest

The authors declared no conflict of interest in the manuscript**.**

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