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RNA therapeutics: RNAi and antisense mechanisms and clinical applications

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Abstract

RNA therapeutics refers to the use of oligonucleotides to target primarily ribonucleic acids (RNA) for therapeutic efforts or in research studies to elucidate functions of genes. Oligonucleotides are distinct from other pharmacological modalities, such as small molecules and antibodies that target mainly proteins, due to their mechanisms of action and chemical properties. Nucleic acids come in two forms: deoxyribonucleic acids (DNA) and ribonucleic acids (RNA). Although DNA is more stable, RNA offers more structural variety ranging from messenger RNA (mRNA) that codes for protein to non-coding RNAs, microRNA (miRNA), transfer RNA (tRNA), short interfering RNAs (siRNAs), ribosomal RNA (rRNA), and long-noncoding RNAs (lncRNAs). As our understanding of the wide variety of RNAs deepens, researchers have sought to target RNA since >80% of the genome is estimated to be transcribed. These transcripts include non-coding RNAs such as miRNAs and siRNAs that function in gene regulation by playing key roles in the transfer of genetic information from DNA to protein, the final product of the central dogma in biology[1]. Currently there are two main approaches used to target RNA: double stranded RNA-mediated interference (RNAi) and antisense oligonucleotides (ASO). Both approaches are currently in clinical trials for targeting of RNAs involved in various diseases, such as cancer and neurodegeneration. In fact, ASOs targeting spinal muscular atrophy and amyotrophic lateral sclerosis have shown positive results in clinical trials[2]. Advantages of ASOs include higher affinity due to the development of chemical modifications that increase affinity, selectivity while decreasing toxicity due to off-target effects. This review will highlight the major therapeutic approaches of RNA medicine currently being applied with a focus on RNAi and ASOs.

Keywords

RNA therapeutics; Locked Nucleic Acids (LNAs); siRNAs; Antisense oligonucleotides (ASOs)

Introduction

Transcription is the process by which RNA polymerase II transcribes a gene from DNA into messenger RNA (mRNA) in the nucleus. mRNA must then be made into protein - a key workhorse of the cell that performs many of the functions essential for cell viability. In order for mRNA to be made into protein, mRNA must first be transported from the nucleus to the

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cytoplasm via the nuclear pore complex - a huge complex composed of about 30 different proteins - that helps direct the mRNA towards the cytoplasm, the site of protein synthesis. Once the mRNA has reached the cytoplasm, ribosomes, which tend to associate with the endoplasmic reticulum and are composed of proteins and small RNAs (ribosomal RNAs/ rRNAs), translate the mRNA into a polypeptide chain that folds into a protein structure. It is this process of protein synthesis that RNA based therapeutics usually try to inhibit.

RNA medicine is the therapeutic targeting of RNA. Two major methods are employed in RNA medicine: double stranded RNA-mediated interference (RNAi) and antisense oligonucleotides (ASO). Broadly speaking, RNAi operates sequence specifically and post-transcriptionally by activating ribonucleases which, along with other enzymes and complexes, coordinately degrade the RNA after the original RNA target has been cut into smaller pieces[3]. Antisense oligonucleotides bind to their target nucleic acid via Watson-Crick base pairing, and inhibit or alter gene expression via steric hindrance, splicing alterations, initiation of target degradation, or other events.

Conventionally, most drugs are small molecules designed to bind to proteins and often times have toxic off-target effects[4]. In contrast, RNA therapeutics offer the promise of uniquely targeting the precise nucleic acids involved in a particular disease with greater specificity, immproved potency, and decreased toxicity. This could be particularly powerful for genetic diseases where it is most advantageous to aim for the RNA as opposed to the protein. Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA) are two examples of this which are more extensively reviewed elsewhere[5]. In spite of the tremendous potential of RNA-based therapies, there are also challenges to bear in mind. For example, RNAs are inherently unstable, and therefore difficult to deliver in high enough amounts to the target tissue due to clearance by the renal system and degradation by nucleases in the blood stream[6,7]. In addition, toxicity due to off-target effects and activation of the immune system are also pressing concerns[7,8]. This review will highlight some of the methods being developed to overcome these obstacles.

Within the scope of RNA medicine, there are several types of therapeutic approaches categorized by their mechanism of action. This includes antisense oligonucleotides (ASOs) that inhibit mRNA translation, oligonucleotides that function via RNA interference (RNAi) pathway, RNA molecules that behave like enzymes (ribozymes), RNA oligonucleotides that bind to proteins and other cellular molecules, and ASOs that bind to mRNA and form a structure that is recognized by RNase H resulting in cleavage of the mRNA target[6]. The predominant focus of this review will be RNAi and ASOs that inhibit mRNA translation, including oligonucleotides that alter splicing.

RNAi

RNAi is a naturally occurring process used by cells to regulate gene expression; it can prevent genes from being translated into protein. In addition, RNAi is used in the innate immune response of cells as a defense mechanism against foreign nucleic acids from viruses and bacteria[9]. RNAi was first identified in plants[10]. Even though the effects of suppressing gene expression by the introduction of a transgene had been previously observed[11,12,13], it was the landmark paper of Fire and Mello in 1998 that documented a clear mechanism of

action whereby double strand RNA (dsRNA) most effectively silenced mRNA in *Caenorhabditis elegans* (*C.elegans*)[13,14]. Normally triggered by endogenous dsRNA[15], RNAi can also be activated by injection of exogenous dsRNA[16,17]. As mammalian cells cannot usually cleave dsRNA into small interfering RNA (siRNA), injection of long dsRNA triggers an interferon response. It was later discovered however that 21 nucleotide-long RNAs can use the RNAi machinery in mammalian cells to silence gene expression[18], allowing researchers to transfect short dsRNA in order to silence genes in mammalian cells[19]. There are three types of small RNAs that can silence mRNA in the cytoplasm with RNAi[20,21]: piwi-interacting RNAs (piRNAs), microRNA (miRNAs), and siRNAs. piRNAs (reviewed elsewhere[19,20]) are small noncoding RNAs 21-24 nucleotides in length that primarily regulate transposon activity in germline development by binding to Piwi proteins, a subset of the Argonaute class of proteins.

A. siRNAs—siRNAs come from the family of non-coding RNAs (ncRNAs) which include long ncRNAs (lncRNAs) broadly defined as 200 nucleotide to 100 kilobases ncRNAs[10], and small ncRNAs. Many small ncRNAs have been ascribed with precise functions in transcriptional regulation, signaling, post-transcriptional gene silencing, and cell communication. The most well-known small ncRNAs are siRNAs and miRNAs.

Long dsRNAs, which can be encoded in the genome or transfected into the cell in shorter sequences, are precursors for siRNAs. In the nucleus, these long dsRNAs are cleaved by Dicer, a dsRNA-specific ribonuclease, into 21-25 nucleotide-long double-strand siRNAs with 2 nucleotides in their 3’ overhang and 5’ phosphate groups[22,23,17]. siRNAs then bind to the AGO2-RISC complex: a combination of the RNA-induced silencing complex (RISC) and the endonuclease Argonaute 2 (AGO2)[19]. Once siRNAs are bound to RISC, they are unwound into their single-strand components: the complementary or antisense strand binds to the target mRNA sequence and the sense strand is degraded. After siRNAs bind to the target sequence with perfect complementarity, AGO2 cleaves the mRNA about 10 to 11 nucleotides downstream from the 5’ end of where the antisense strand binds[8]. Cleavage of the mRNA triggers its degradation by exonucleases[24].

Challenges faced by siRNA-based therapeutic approaches include off-target effects, efficacy, delivery, and immune system activation. Off- target effects arise through two hypothesized means[25,26]. First, siRNAs could bind to non-targeted mRNAs with imperfect complementarity leading to silencing of these mRNAs. Second, siRNAs could enter endogenous miRNA systems that tolerate imperfect binding to target mRNAs outside the miRNA seed region, thereby silencing those targets. Addressing efficacy is challenging because in spite of the extensive process of selecting and designing siRNAs, they can still lack function in mammalian cells. In fact, few have been proven functional in mammalian cells[27]. Additionally, siRNAs targeting the same region can work with varying efficacies. This may be due to a variety of reasons, including accessibility of the sequence to be targeted, stability of the siRNA-target sequence hybrid, chemical modifications of the siRNA, and thermodynamics of siRNA integration into the RISC complex[27]. Delivery of siRNAs to the target tissue is a challenge because siRNAs are easily filtered out by the renal system[28]. In addition, nucleases in the bloodstream can quickly degrade siRNAs, resulting in a short half-life. Chemical modifications such as phosphorothioate (PS) modification and

cholesterol/ligand conjugation, as well as encapsulation of siRNAs in nanoparticles have demonstrated protection from nucleases, higher affinity, and longer half- life[29,30,31]. siRNAs have been shown to induce interferon response by activating the innate immune system and can induce proinflammatory cytokines[9,32]. Chemical modifications have been identified that prevent the siRNA from inducing this immune response[9]. However, caution must be taken when designing these chemically modified siRNAs, as certain modifications can inhibit the siRNA from properly integrating into the RISC complex, resulting in loss of mRNA targeting. In spite of these challenges, there have been 26 different siRNAs tested in over 50 clinical trials involving diseases such as age-related macular degeneration (AMD), diabetic macular edema (DME), glaucoma, hypercholesterolemia and human solid tumor (melanoma)[9]. There are also some companies with promising siRNA based drugs, such as Arrowhead which is using siRNA based drug as a therapeutic for chronic Hepatitis B infection, as well as other ailments such as cardiovascular diseases, clear cell and renal cell carcinoma. Phase II clinical results for the siRNA based drug for Hepatitis B have been very positive. Additionally, Alnylam Pharmaceuticals is another company taking advantage of RNAi to treat genetic diseases, cardio-metabolic diseases, and hepatic infectious diseases. In fact, two such drugs targeting Hereditary ATTR Amyloidosis have made it into Phase III clinical trials, suggesting their proximity to coming to market. Wittrup and Lieberman 2015 provide a detailed review of the efforts to bring siRNAs to the clinic[33].

B. miRNAs—Similar to siRNAs, miRNAs silence gene expression post-transcriptionally[8], and are thought to regulate roughly 30% of human genes[21,34]. miRNA precursors are naturally encoded in the genome, but miRNAs themselves can be artificially synthesized for therapeutic strategies. In the cell, RNA polymerase II transcribes DNA into primary miRNA (pri-miRNA). The pri-miRNA forms a hairpin structure that is cleaved by Drosha, a ribonuclease with dsRNA specificity, into the precursor miRNA (pre- miRNA). The pre-miRNA is generally more than 100 nucleotides long and contains a hairpin loop and a double-strand region where the miRNA resides. Pre-miRNAs are transported by exportin 5 from the nucleus to the cytoplasm via the nuclear pore complex[19]. Pre-miRNAs are then further processed by Drosha to release the hairpin[19,6]. The RNAse III enzyme Dicer then cleaves the loop of the hairpin in the pre-mRNAs generating the double-strand segment known as the miRNA[23,21]. miRNA is then incorporated into the RISC complex by binding to AGO2[19,21]. The guide strand is kept, and the other strand (“the passenger”) is degraded. Within the miRNA guide strand lies a seed sequence near the 5’-end; this seed sequence is composed of seven bases that are crucial for the complementary binding to the target mRNA[34]. Sequences outside this seed region are less significant for binding. The guide strand of the miRNA leads the AGO2-RISC complex to the target mRNA[35]. Generally, the complementary sequence lies in the 3’ untranslated region (UTR) of the mRNA, but it can also be found in the 5’ UTR or coding region. The mRNA is silenced by inhibition of translation and/or degradation by exonucleases[36].

Like siRNAs, miRNAs can also be designed to target a gene of interest. However, the same challenges of delivery, specificity, toxicity, and immune response are present. While chemical modifications can optimize miRNAs to overcome these hurdles, they must be done in a way that still permits the miRNA to integrate into the RISC complex. Currently,

targeting of miRNAs with ASOs appears to be a more common and effective approach as many genes are regulated by miRNAs. In fact, ASOs that target miRNAs have been demonstrated to be effective and safe in mice, non-human primates, and humans[37,38,39]

Antisense Oligonucleotides (ASOs)

Many types of oligonucleotides have been referenced to as ASOs. Within the scope of this review, ASOs are short oligonucleotides with RNA/DNA-based structures that can sequence-specifically bind to RNA via Watson-Crick hybridization. ASOs must be able to cross the cell membrane to bind to the target RNA either in the nucleus (pre-mRNA, mRNA, noncoding RNA, etc.) or cytoplasm (miRNA, mRNA, etc.)[40]. ASOs are very stable without refrigeration, highly soluble in water, and used in saline solution -attributes that bode well for their therapeutic potential.

Antisense technology was first introduced in 1977 by Paterson et al. who used recombinant nucleic acid molecules to inhibit translation[41]. Since then, many advances such as modifications to the backbone, sugar moieties, and base have been made to optimize the technology for therapeutic purposes. Currently, there are three oligonucleotide drugs that have been approved by the Federal Drug Administration (FDA) for use in the clinic: Formivirsen (Vitravene), Mipomersen and Macugen. Formivirsen targets the immediate-early 2 (IE2) gene for cytomegalovirus retinitis treatment in patients with AIDS (Acquired Immunodeficiency Syndrome)[7,4]. Mipomersen is a 20 nucleotide-long Antisense oligonucleotide that targets apolipoprotein B (ApoB100) mRNA in homozygous familial hypercholesterolemia (HoFH)[4,42,7,43]. ApoB100 is a core protein in low-density lipoprotein (LDL) cholesterol[43]. Individuals with HoFH have increased levels of LDL, a known risk factor for atherosclerosis and cardiovascular disease, but are unable to discard excess LDL due to genetic mutations. Treatment with Mipomersen decreases ApoB100 levels without significant deleterious effects on high-density lipoprotein cholesterol (HDL-C), the ‘good’ cholesterol[43]. Macugen is a 28-mer oligonucleotide that binds to extracellular vascular endothelial growth factor (VEGF) to inhibit the progression of neovascular (wet) age-related macular degeneration (AMD), a primary cause of blindness[4,44,45,46]. Macugen binding to VEGF inhibits VEGF binding to its receptors, thereby preventing the activation of angiogenesis and increased vascular permeability and inflammation[46,45].

ASO mechanisms of action

ASOs can work through many mechanisms depending, in part, on the region in the RNA sequence that is targeted and ASO design/chemical properties. The mRNA sequences targeted by ASOs are chosen based on their binding accessibility. Terminal sequences, sequences within internal loops, hairpins, joint sequences and bulges of 10 or more bases have been determined to be ideal[47]. The two most widely-used ASOs are double-stranded ASOs that use the RISC complex to degrade RNA and single-stranded ASOs that silence gene expression by a variety of mechanisms, including: 1) inhibiting 5’cap formation, 2) steric blocking of protein translation, 3) inhibiting or altering RNA splicing, and 4) activation of RNase H, which degrades the target mRNA[47,48,49].

1. Inhibition of 5' cap formation—Formation of the 5'cap can be prevented by targeting the ASO to sequences in the 5' UTR [47,48]. Oligonucleotide binding near the cap site of pre-mRNA prevents the binding of proteins needed for cap formation, as demonstrated when oligonucleotides designed to bind the 5'cap prevented binding of the translation initiation factor eIF-4α[50]. EIF-4α binds to the 7-methyl guanosine base at the 5'end of all mRNA sequences and associates with eIF4-G which is bound by ribosome. Ribosome bound eIF4-G association with eIF4- α triggers recruitment of the translation machinery. Therefore, inhibition of eIF-4α binding prohibits 5'cap dependent translation.

2. Steric blocking of translation—Steric blocking of mRNA translation is usually achieved by designing ASOs that bind at or near the initiation codon of the mRNA sequence and hinder the translation machinery, such as the ribosomal subunit, from binding [47,48]. These ASOs block access to pre-mRNA and mRNA and can prevent RNA folding but do not activate degradation of the target mRNA [5]. These ASOs tend to possess more extensive chemical modifications than those that must be incorporated into RNAi machinery because they are not structurally constrained by the need to integrate[5]. Additionally, such oligonucleotides can work in identical fashion to splice-switching oligonucleotides, discussed below.

3. Alteration of Splicing—After DNA is transcribed into the precursor form of mRNA (pre-mRNA), the pre-mRNA must be spliced to exclude non-coding introns and exclude or include specific exons, generating the mature mRNA. This process is moderated by proteins and small nuclear RNAs in the spliceosome[51]. Conserved sequences in the splice junctions and enhancer and silencer sequences in the introns and exons also play key roles in this process.

Alterations in the appropriate splicing pattern can lead to disease. Duchenne muscular dystrophy (DMD) is a clear example of this. Inappropriate deletions alter the translational reading frame of the protein dystrophin, which is required for the integrity of the sarcolemma membrane[5]. Males with DMD lose mobility within 10-12 years of birth and die in their mid-20's due to respiratory and/or cardiac failure[5]. ASOs that block the splicing machinery from inappropriately deleting certain sequences in the DMD/dystrophin pre-mRNA have the unique ability to repair the RNA by promoting splicing that leads to the correct RNA sequence, that is inclusion of the right exons. The correct form of the protein can then be produced from the normal mRNA sequence, thereby potentially ameliorating the disease.

These splice-switching oligonucleotides have been demonstrated to be effective in animal models for the genetic diseases β-thalassaemia[52,53], spinal muscular atrophy (SMA)[54,55,56], and DMD[57]. In fact, oligonucleotides for DMD in phase 2 clinical trials have shown promising results with abrogation of dystrophy observed in some patients[58,59]. SMA is a disease where mutations or deletions of the SMN1 gene lead to loss of SMN protein, leading to disruption of motor neuron function and controlled movement. ASOs designed to favor inclusion of the right exon, thereby restoring the SMN protein in SMA patients, have shown some muscle function improvement in patients[60].

4. Activation of RNase H—The most commonly used mechanism of action of ASOs is recruitment of the enzyme RNase H which degrades the RNA in a RNA-DNA hybrid[48]. Two forms of RNase H are expressed in human cells: RNase H1 and RNase H2[61]. ASOs that work through RNase H recruit RNase H1 to RNA-DNA hybrids resulting in degradation of the targeted RNA molecule. The DNA strand (or ASO in the case of ASO targeting) is released intact. Antisense oligonucleotide recruitment of RNase H1 for mRNA cleavage can occur both in the nucleus and cytoplasm[61]. The rate-limiting step for this process is the concentration of RNase H1 relative to the activity of the ASOs[61].

ASO modifications

Some of the obstacles that have slowed the pace of bringing more RNA-based therapies to the clinic include: 1) instability/high vulnerability of the oligonucleotides to degradation by nucleases, 2) off-target/toxicity effects, 3) poor delivery to the right tissues or low cellular uptake, and 4) low affinity for the target mRNA[7,4,5]. ASOs have been extensively chemically modified to bypass these constraints and increase stability, affinity, specificity and delivery while decreasing the potential for off-target effects[5,4]. Without these modifications, the nucleic acid molecules are degraded by exo- and endonucleases in the blood stream[7] and are unable to cross the plasma membrane, since unmodified ASOs carry a net negative charge[47].

The chemical modifications of ASOs are generally classified as first generation, second generation, and third generation. First generation modifications include changes made to the phosphodiester backbone, heterocyclic nucleobase and sugar moiety to increase affinity and specificity of the ASO[4]. The predominant type of first generation modification is phosphorothioate (PS) bond introduction, which helps protect the oligonucleotide from degradation by nucleases[4]. Additionally, by modifying the charge of the ASO, PS increases the amount of ASO available to reach the target tissue by increasing binding to receptor sites and plasma proteins[40]. Appropriate binding by plasma proteins decreases rapid filtration from the blood by the renal system, facilitating optimal distribution[40]. Vitravene, the first ASO-based drug approved by the FDA for use in the clinic is a first generation PS-ASO. In fact, most of the ASO-based drugs that have made it to Phase I clinical trials are based on PS modifications (**Table 1**).

Although first generation modifications improve nuclease resistance and bioavailability, PS-ASOs generally bind poorly to the target RNA, have low specificity, and low cellular uptake. These limitations are addressed by second generation modifications that modify the sugar moiety of the nucleobase (pyrimidine, purine) thereby increasing binding affinity to the target RNA[4]. Other modifications to the heterocyclic portion of the nucleobase that improve the base-stacking capabilities also increase affinity and specificity. Modifications such as conjugation to cell-penetrating peptides also increase delivery to the cells[62].

2’-O-Methyl (2’-OMe), 2’-O-methoxyethyl (2’- OMOE), and Locked Nucleic Acids (LNAs) are the most prominent types of second generation modifications[4]. The leading company for 2’-OME chemistry is Ionis pharmaceuticals whose chemical developments have enabled drugs to resemble RNAs thereby increasing affinity and protecting from nuclease degradation. Other chemistries have been developed by Ionis which improve delivery, potency, and distribution to tissues. Ionis pharmaceuticals currently has antisense based

drugs in phase II clinical trials for TTR amyloid cardiomyopathy, promising results from a phase II study for Type 2 Diabetes, an early stage oral delivery antisense drug for gastrointestinal autoimmune disease, and other diseases.

The LNA chemistry has the 2' oxygen and 4'carbon of the RNA ribose sugar locked in a ring structure. This modification increases specificity, affinity, and half-life allowing effective delivery to tissues of interest with lower toxicity. Additionally, the chemistry does not interfere with the ability of RNA molecules that contain LNA to incorporate into the RNAi machinery.

LNA is one of the most promising chemistry for overcoming the challenges of tissue delivery and efficacy faced by antisense therapeutics. Currently, there are a number of companies attempting to bring LNA oligonucleotides to the clinic. These include Santaris Pharma, miRagen which is using LNAs to target miRNAs in cardiovascular disease, Shire which is attempting to target rare genetic disorders, Pfizer that is targeting multiple diseases including cancer, GlaxoSmithKline which is targeting viral diseases, and Enzon which is targeting cancer. In fact, Santaris Pharma, recently purchased by Roche, exclusively makes LNA based drug design and currently has an LNA drug that has shown promise in Phase IIa clinical trials for Hepatitis C. RaNA is a company using LNAs to target long non-coding RNAs involved in various diseases, such as SMA and Friedreich's Ataxia.

Third generation ASOs such as peptide nucleic acid (PNA) and phosphorodiamidate morpholino oligomer (PMO) carry modifications to the furanose ring of the nucleotide[4]. PNAs bind with greater affinity and specificity to target DNA or RNA than unmodified oligonucleotides, and reduce protein expression by sterically inhibiting the translation machinery[63,64,65]. Like PNAs, PMOs do not activate RNase H cleavage. Rather PMOs have a six-member morpholino ring in place of the ribose sugar and what's called a phosphorodiamidate linkage substitute for the phosphodiester bond[66]. PMOs inhibit protein production by steric hindrance and also increase nuclease resistance.

ASO naming

ASOs are named based on the chemical modifications and how the modifications are integrated into their structure. One increasingly popular structure is to have a DNA segment in the middle to activate RNase H for cleavage of target mRNA. Modifications are then added at the end of the sequence to increase affinity and stability while protecting the internal DNA sequence from degradation. Due to the limits of this review, only a few will be highlighted here. For example, single strand oligonucleotides that form stable three dimensional structures able to bind with high affinity and specificity to many targets are called aptamers[4]. Aptamers bind directly to the protein as opposed to RNA. Macugen, another FDA-approved ASO drug, is a second-generation aptamer. Gapmers are ASOs with a DNA stretch in the middle typically flanked by LNAs or other proprietary chemistries that protect from nuclease degradation and increase affinity[67]. The central DNA piece helps trigger RNase H degradation of the target RNA. Mipomersen, the second FDA-approved ASO drug previously mentioned, is a gapmer. Mixmers are ASOs with chemistries such as LNAs distributed throughout the sequence[67], and are ideal for targeting miRNAs[67].

ASO delivery

ASO delivery has been previously and comprehensively reviewed[40] and will only be very briefly highlighted here. Parenteral injection, such as intravenous infusion or subcutaneous injection, is the main method of delivery of PS modified single-stranded ASOs. These ASOs are usually transferred from the blood to the tissues in minutes to hours, regardless of the recipient's gender or speciation[40]. Endocytosis facilitates delivery into the cells where the ASO can reside for 2-4 weeks before degradation[40].

Although ASO activity has been observed in many tissues such as lung, stomach, bladder, and heart, ASOs predominantly accumulate in liver, kidney then bone marrow, adipocytes, and lymph nodes[40]. ASOs cannot cross the blood-brain barrier due to their size and charge. The one exception to this is intrathecal injection of single- stranded ASOs with precise chemical modifications into the cerebrospinal fluid (CSF), which allows ASOs into the central nervous system (CNS)[40]. Oligonucleotide uptake into tissues such as the spinal cord and brain within a couple hours has been observed with this approach. Delivery appears to be enhanced most strongly by chemical modifications to the phosphate bridge in the backbone[40].

Conclusion

This review provides a brief overview of RNA therapeutics – a field that is incredibly vast and quickly growing. Antisense oligonucleotides are being used in therapeutic attempts for a wide variety of diseases, especially cancers. In cancer, ASOs face the challenges of non-specific targeting that can lead to greater clotting times and undesirable immune activation[7]. ASO use in cancer is also hindered by inefficient and ineffective delivery that lend to some cancer cells escaping treatment[7]. While some chemical modifications such as nanoparticle carriers have been developed to overcome these limitations, there is still some ways to go for ASOs to become effective cancer treatments.

Overall, RNA therapeutics has tremendous potential as a means to target genes within the context of an individual's genetic background and lifestyle – a field known as precision medicine. Table 1 provides a recent list of Antisense and RNA based drugs that are being applied in clinical trials according to clinicaltrials.gov. Since the first report of antisense inhibition of genes, new chemistries have been developed to optimize the properties of the oligonucleotides with improvements such as enhanced delivery, specificity, affinity, and nuclease resistance with decreased toxicity. A complete history of all the work done for each of these is beyond the limits of one review. This summary however is intended to give a flavor of the general challenges and advances in the field.

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Table 1

RNA based drugs currently in Clinical Trials

Drug	Mechanism	Target	Condition	Phase	Sponsor	Clinical Trial ID
Isis 420915/GSK 299872	Antisense	TTR	Wild-Type Transthyretin Amyloid Cardiomyopathy	II	Ionis Pharmaceuticals (Isis); GlaxoSmithKline	NCT02627820
ISTH0036 (TGF-β2 Antisense oligonucleotide)	Antisense	TGF-β2	Primary Open Angle Glaucoma	I	Isarna Therapeutics (GmbH)	NCT02406833
c-myb ASODN	Antisense		Hematologic Malignancies	I completed	University of Pennsylvania	NCT00780052
AVI-4658 (PMO)	PMO	Dystrophinexon 51	Duchenne muscular dystrophy (DMD)	I/II	Imperial College London	NCT00159250
IGF-1R/ASODN	Antisense	Insulin-like Growth Factor Receptor 1	Gliomas	I/recruiting	Thomas Jefferson University	NCT02507583
Intravenous EZN-2968	LNA Antisense	hypoxia-inducible factor 1α	Solid Tumors/Lymphoma	I	Enzon Pharmaceuticals, Inc.	NCT00466583
LErafAON-ETU	Antisense	c-raf	Neoplasms	I	INSYS Therapeutics Inc	NCT00100672
EZN-2968		HIF	Neoplasms Liver Metastases	I	National Cancer Institute (NCI)	NCT01120288
BP1001	Antisense oligodeoxynucleotide		Recurrent Adult Acute Myeloid Leukemia Acute Lymphoblastic Leukemia Myelodysplastic Syndrome Ph1 Positive CML	I	Bio-Path Holdings, Inc.	NCT01159028
ISIS 104838	Antisense	tumor necrosis factor (TNF-alpha)	Rheumatoid Arthritis	II	Ionis Pharmaceuticals, Inc	NCT00048321
miR 29a	miRNA		Stiffness of Shoulder	I/recruiting	Chang Gung Memorial Hospital	NCT02534558
Apatorsen (OGX-427)	Antisense	Hsp27	Squamous Cell Lung Cancer	II	Queen Mary University of London	NCT02423590
Diabetes-suppressive dendritic cell vaccine		CD40, CD80 and CD86	Type 1 Diabetes	I	University of Pittsburg	NCT00445913
SB010	DNA (deoxyribonucleic acid) zymes (Antisense oligonucleotide)	GATA-3 mRNA	Asthma	I	Sterna Biologicals GmbH & Co. KG	NCT01470911
QR-010	Single-stranded RNA Antisense oligonucleotide	Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)	Cystic Fibrosis	I	ProQR Therapeutics	NCT02564354
Oblimerson sodium, G3139	Antisense	Bcl-2	Chronic Lymphocytic Leukemia CLL	I/II	Genta Incorporated	NCT00021749

Drug	Mechanism	Target	Condition	Phase	Sponsor	Clinical Trial ID
G4460	Antisense	c-myb	Leukemia	II	Abramson Cancer Center of the University of Pennsylvania	NCT00002592
OGX-427	Antisense	Hsp27	Neoplasms	I	OncoGenex Technologies	NCT00487786
G3139	Antisense	Bcl-2	Lung Cancer	I/II	University of Chicago	NCT00005032
G3139 (Genasense)	Antisense	Bcl-2	Recurrent Renal Cell Cancer, Stage IV Renal Cell Cancer	II	National Cancer Institute (NCI)	NCT00059813
OGX-011	Antisense		Bladder Cancer Breast Cancer Kidney Cancer Lung Cancer Ovarian Cancer Prostate Cancer Unspecified Adult Solid Tumor	I	NCIC Clinical Trials Group	NCT00471432
IONIS-STAT3Rx	Antisense	STAT3	Advanced Cancers DLBCL Lymphoma	I/II	Ionis Pharmaceuticals, Inc.	NCT01563302
oblimersen sodium (G3139)	Antisense	Bcl-2	Melanoma (Skin)	III	Genta Incorporated	NCT00016263
OGX-011			Breast Cancer	II	NCIC Clinical Trials Group	NCT00258375
G3139	Antisense		Lymphoma	I	British Columbia Cancer Agency	NCT00070083
Genasense	Antisense	Bcl-2	Multiple Myeloma and Plasma Cell Neoplasm	III	Genta Incorporated	NCT00017602
ISIS 113715	Antisense	Protein Tyrosine Phosphatase 1B	Type 2 Diabetes Mellitus	I	Ionis Pharmaceuticals, Inc.	NCT00365781
oblimersen sodium	Antisense	Bcl-2	Prostate Cancer	II	European Organisation for Research and Treatment of Cancer - EORTC	NCT00085228
OGX-011	Antisense	clusterin	Prostate Cancer	I	NCIC Clinical Trials Group	NCT00054106
ISIS CRP Rx	Antisense	CRP	Paroxysmal Atrial Fibrillation	II	Ionis Pharmaceuticals, Inc.	NCT01710852
G3139, NSC #683428 (oblimersen sodium)	Phosphorothioate Antisense	Bcl-2	Colorectal Cancer	I/II	The University of Texas Health Science Center at San Antonio	NCT00004870
ISIS 5132/ISIS 3521	Antisense	Human C-raf Kinase/ Human Pkc-Alpha	Breast Cancer	II	Eastern Cooperative Oncology Group	NCT00003236
OGX-427	Antisense	HSP-27	Castration Resistant Prostate Cancer	II	British Columbia Cancer Agency	NCT01120470
G3139, Oblimersen sodium,	Antisense	Bcl-2	Tumors	I	Genta Incorporated	NCT00543231
ASM8	Antisense		Asthma	I/II	Pharmaxis	NCT00264966

Drug	Mechanism	Target	Condition	Phase	Sponsor	Clinical Trial ID
GTI-2040	Antisense	R2 component of ribonucleotide reductase (RNR) mRNA	Carcinoma, Renal Cell Metastases, Neoplasm	I/II	Aptose Biosciences Inc.	NCT00056173
ISIS 2302 (Alicaforsen)	Antisense	intercellular adhesion molecule (ICAM-1)	Crohn's Disease	III	Ionis Pharmaceuticals, Inc.	NCT00048113
OGX-427	Antisense	HSP-27	Bladder Cancer Urothelial Carcinoma	II	Noah Hahn, M.D	NCT01780545
AZD9150	Antisense		Diffuse Large B-Cell Lymphoma	I	MedImmune LLC	NCT02549651
cenersen	Antisense	p53	Myelodysplastic Syndromes	I	Eleos, Inc.	NCT02243124
AZD5312	Antisense	Androgen Receptor (AR)	Advanced Solid Tumours With Androgen Receptor Pathway as a Potential Factor	I	AstraZeneca	NCT02144051
AEG35156	Antisense	XIAP	Leukemia, Myelomonocytic, Acute	I/II	Aegera Therapeutics	NCT00363974
G3139	Antisense	Bcl-2	Unspecified Adult Solid Tumor	I	National Cancer Institute (NCI)	NCT00054548
DS-5141b	Antisense		Duchenne Muscular Dystrophy	I/II	Daiichi Sankyo Co., Ltd.	NCT02667483