Chapter

Antisense Therapy: An Overview

Shashwat Sharad

Abstract

Nucleic acids are the backbone of antisense therapy. Antisense oligonucleotide-based therapeutics involves downregulation of gene expression. RNA-based drugs that include antisense oligonucleotides bear great therapeutic potential toward treatment of various diseases by altering RNA and/or reducing, restoring, and modifying protein expression through multiple molecular mechanisms. Pharmacology of targeted antisense therapy has provided the platform to translate its utility to the clinic. Over the years, chemical modifications of antisense oligonucleotides have not only enhanced the specificity and efficacy but also reduced the side effects. These have changed the whole clinical trial design and provide newer strategies for therapies. Improvement in antisense oligonucleotide therapy technology has allowed and brought research from bench to clinic. Additionally, the use of small interfering RNAs, micro RNAs, ribozymes, and other antisense compounds toward the treatment of deadly diseases like cancers have demonstrated both preclinical and clinical responses. Furthermore, antisense therapy has great potential to target specific genes of interest in the context of precision medicine. Optimization of enhanced delivery, specificity, affinity, and nuclease resistance with reduced toxicity is underway in different disease context. This chapter gives a complete overview of antisense therapy and highlights its potential. Here, we focused on the advances of the antisense technology, pharmacology, therapeutics, and drug discovery.

Keywords: antisense oligonucleotides, antisense therapy, antisense drug, antisense therapeutic, gene therapy

1. Introduction: antisense technology

The advancement in the next-generation sequencing enables us to identify the genetic heritages of several diseases, such as cancer, Parkinson's, rheumatoid arthritis, and Alzheimer's, which brings to attention the development of personalized medicine [1]. This knowledge has been well adapted and accepted for diagnosis, but the field still lags toward pharmaceutical interventions to address the genetic defects underlying diseases. At present, small molecules and proteins are the two major classes of US Food and Drug Administration (FDA)-approved drugs [2]. Small-molecule drugs inhibit target proteins through competitive binding, whereas protein-based drugs (such as antibodies) can bind with high specificity to several targets. The size and stability of proteins are the major limitations of their utility for majority of disease targets [2], and both protein and small-molecule drugs cannot target every disease-relevant protein or gene. Thus, there is a current need to develop the drugs for personalized genomics. The mRNA- and DNA-based drugs are therapeutically more promising and have the great potential to cure the genetic defect [1]. The RNA-based drugs have emerged as a promising candidate to treat

diseases at the genetic (gene and RNA) levels. The delivery of therapeutic RNA has been limited due to several numbers of factors such as nucleic acid design, delivery methods, and materials for transport of RNA drugs to the site of interest [1]. The current advancement in RNA and RNA-protein therapy has shown the great potential for the development of RNA delivery, and the clinical applications of RNA-based drugs have been proven by modulating gene/protein expression and gene editing [1].

An approach to fight disease by utilizing short DNA-like molecules is known as antisense oligonucleotides. This is the most effective and commonly used technology to regulate the gene expression and drugs for targeted gene therapy. These antisense oligonucleotides bind to messenger RNA (mRNA) and impair the protein production and inhibit the gene expression. The antisense molecules are synthetic replica of specific mRNA sequence to block the function of the specific target gene of interest in the human genome. Recently, antisense therapy has emerged as a promising tool to treat various diseases, and for treatment, several antisense drugs have been approved by the FDA. For antisense gene therapy, chemically engineered oligonucleotides complementary to specific mRNA are inserted into the cells which stop the translation of the specific protein. Similarly, the antisense drug contains the vital molecule—"the noncoding mRNA"—which blocks the translation of a specific protein. The antisense oligonucleotides could be very useful to treat the viral diseases, genetic/hereditary diseases, as well as cancers. The naturally occurring oligonucleotides bear poor stability and very low specificity and have a lot of side effects in vivo. The therapeutic use of oligonucleotides can be achieved by enhancing the stability and specificity of the molecules and reducing the side effects by chemical modification. The most common therapeutic oligonucleotides are small interfering RNA, ribozyme, DNAzyme, anti-gene, CpG, decoy, and aptamer. The chemical modification of antisense oligonucleotides can improve their ability to enter the cells to bind the specific target gene sequences which further disrupt the targeted gene function. Several antisense RNA and antisense oligonucleotide delivery systems such as virus vectors (retrovirus, adenovirus, and adeno-associated virus) and liposomes have been developed to carry the antisense RNA or oligonucleotides through the cell membrane into the cytoplasm and nucleus. The oligonucleotides mainly target the ribonucleic acid (RNA), whereas small molecules and antibodies primarily target proteins due to their chemical properties and distinct molecular mechanism of action. The mRNA codes for protein to noncoding RNAs (such as microRNA, transfer RNA, small interfering RNAs, ribosomal RNA, and long noncoding RNAs). The main function of noncoding RNAs is the transfer of genetic information from DNA to protein [3]. The major therapeutic approach to target RNA-based therapy is antisense oligonucleotides because of their high affinity, selectivity, ease of chemical modifications, and less toxicity. This chapter will provide a comprehensive overview of antisense therapy and their major therapeutic approaches.

The remarkable progress in the field of gene therapy and antisense therapy is apparent from numerous gene therapy- and antisense therapy-based clinical trials that are currently underway worldwide.

Gendicine (Ad-p53), the first gene therapy-based product, was approved in China for the treatment of head and neck squamous cell carcinoma in conjunction with radiotherapy. One AON drug, Vitravene, had been also approved for the local treatment of cytomegalovirus-induced retinitis, and several others are in clinical trials, including those siRNAs, miRNAs, and ribozymes that are targeting the mRNA of different oncogenes and other cancer-promoting genes.

Although the application of gene therapy and antisense therapy to mediate tumor regression is well demonstrated in experimental and clinical settings, impediment

remains when translating this into large clinical application. The main obstacles that remained in cancer gene therapy and antisense therapy are the lack of delivery systems that successfully deliver an efficacious dose of a therapeutic gene (s) or antisense drug(s) to the targeted tumor site. Targeted gene or antisense drug delivery to distant tumors for therapeutic approaches is a demanding task that urges the development of delivery vectors capable of overcoming many barriers. Many scientists have used viral and non-viral vectors to deliver the therapeutic gene or antisense compound in to the targeted tumor cells or tissues. Although the results of early gene therapy- and antisense therapy-based clinical trials using either viral or non-viral vectors have been encouraging, still it is difficult to find a single method that meets all the conditions for an ideal gene transfer and vector expression.

Limitations of the present vector technologies have slowed the progress of gene therapy and antisense therapy for cancer to the clinic. Thus, the development of appropriate delivery systems for targeting therapeutic genes and antisense agents into targeted tumor cells and tissues is one of the potential approaches that have to be further explored in the future in order to augment gene therapy and antisense therapy against a wide range of cancers. It is hoped that the next generation of carriers could be a promising technology for systemic cancer gene therapy and antisense therapy.

2. Pharmacology of antisense drugs

The antisense oligonucleotides have the potential to manipulate the gene expression which prompted the field toward the therapeutic application and value of oligonucleotides as potential drugs and their targets [4]. The direct route to target RNA in a selective way is a well-established platform for drug discovery. The well-defined mechanisms, uncomplicated and easy to design, bring antisense oligonucleotides as a promising candidate for therapeutic development. The therapeutic potential of antisense drugs for the treatment of several diseases is already translated from bench to bedside, and many antisense drugs have entered into clinical trials for the treatment. The first patent on antisense therapy was granted to Molecular Biosystems company in 1991 for developing the antisense compounds. The first FDA-approved antisense product drug was afovirsen developed by Ionis Pharmaceuticals in 1992 which was a phosphorothioate oligonucleotide that targeted mRNA sequence of the E2 gene, which is associated with human papillomavirus transcription and replication. Later oblimersen, a phosphorothioate oligonucleotide, was designed to target the Bcl-2 protein for the treatment of melanoma and certain leukemias. Unfortunately, both the drugs failed in the clinical trial programs due to lack of efficacy and failure to demonstrate overall survival benefits and dose-limiting toxicity. Currently, several gene therapy- and antisense therapy-based clinical trials are ongoing. The major challenge of antisense drugs is effective and safe delivery to the target. The advancement toward antisense-based drug delivery is in progress. Several chemical modifications, novel chemistries, better formulation, and design of oligonucleotide not only have improved the potency and tolerability of antisense drug but also have enhanced the drug distribution to the targeted RNA inside the cells [5, 6]. The clinical application of antisense drugs requires safe and efficient carrier system, and currently, the viral and non-viral vectors are the most common methods used to deliver the antisense drugs specifically to the target tissues and cells. The viral vector-based delivery is most advantageous due to their high transfection efficiency [7]. Also, the new chemistries and better antisense oligonucleotide designs further improve the unwanted side effects, safety, and tolerability. From the last three decades, several antisense drugs have entered

into clinical trials and market for the treatment of a broad variety of diseases, and numerous oligonucleotides are under clinical development [6, 8–10]. The first-generation antisense drug, fomivirsen, targeting cytomegalovirus, was approved for the treatment of cytomegalovirus retinitis [11]. Many second-generation drugs are under development and are showing encouraging activity in the clinic. Now oligonucleotide therapy has come a long way and has been established as promising therapeutic tool. During this period, several clinical trials have been performed on thousands of participants for several diseases and only six molecules provided the clear clinical benefit in rigorously controlled trials [10]. As of now, there are six FDA-approved drugs based on oligonucleotide therapy: (1) fomivirsen for treatment of CMV retinitis in AIDS patients, (2) mipomersen for treatment of familial hypercholesterolemia, (3) defibrotide for treatment of veno-occlusive disease in the liver, (4) eteplirsen for the treatment of Duchenne muscular dystrophy, (5) pegaptanib for the treatment of neovascular age-related macular degeneration, and (6) nusinersen for the management of spinal muscular atrophy [10, 12]. In conclusion oligonucleotide-based antisense therapy has provided solutions to untreatable diseases. Future inventions in this technology will help in establishing the better and affordable cure to many more diseases.

3. Antisense therapeutic interventions in various diseases

The antisense technology is well placed to influence the developments in human genetics and genomics to generate drugs for the treatment of monogenic and polygenic diseases. The "antisense" are the oligodeoxyribonucleotide molecules complementary to the DNA or RNA sequence of the target gene designed to hybridize specific mRNA. By capitalizing "antisense" DNA approach, the overexpressed proteins can be blocked in several diseases such as cancer, neurological diseases, cardiovascular diseases, inflammation and autoimmune diseases, infectious diseases, etc. [6].

3.1 Antisense therapy for cancer

The beauty of antisense technology is that it can precisely recognize the DNA location in a gene, a single mRNA class, and can distinguish between the normal and mutated oncogenes in cancer cells. Several studies have confirmed that in cancer patients, this can be used as an inhibitor of gene expression, which will decrease the tumor growth by manipulating the important cellular functions and protein production. By decreasing the specific gene expression, inducing the degradation of target mRNA, and initiating the premature termination of transcription, the antisense therapy can correct the abnormal expression of cellular genes and mutations in tumor cells. One of the major limitations of this approach is nuclease degradation. For this, several strategies are under development. Delivery of antisense oligo or drug to distant as well target tumors is a major hurdle. The development of suitable delivery systems for targeting therapeutic genes and antisense agents into targeted tumor cells and tissues is one of the potential approaches that needs to be further developed and explored. In order to enhance the gene therapy and antisense therapy against a wide variety of cancers and cancer types in future, the development of next generation of carriers will a remarkable progress in the field of gene therapy and could serve as a promising technology for systemic cancer gene therapy and antisense therapy. As we know, cancer has been a major area of therapeutic investigation for antisense technology. Currently, custirsen, a chimeric 2′MOE-modified antisense drug targeting clusterin, is being evaluated in phase III clinical

trials for the treatment of prostate and lung cancers [13]. Also, the antisense drug, AZD9150, which targets signal transducer and activates the transcription 3 (STAT3) in several types of cancers [14] has shown encouraging activity as a single agent in several cancer types. The modified oligonucleotide targeting androgen receptor is in the clinical trial as a possible treatment for prostate cancer [15]. Several additional antisense drugs, including microRNAs and siRNAs, are in early-stage clinical trials.

3.2 Antisense therapy for cardiovascular diseases

The first antisense drug, mipomersen, is approved by the FDA as an adjunct therapy for homozygous familial hypercholesterolemia, which reduces apolipoprotein B mRNA levels [16]. Apolipoprotein C III (apoCIII) plays a critical role in the metabolism of triglyceride-rich lipoproteins, and decreased expression is associated with a lower risk of cardiovascular disease [16, 17]. The clinical trial of volanesorsen, an antisense drug, is designed to reduce apoCIII mRNA levels, and the drug is currently being investigated in placebo-controlled phase III clinical trials for the treatment of familial chylomicronemia syndrome and familial partial lipodystrophy. It has been shown that severe factor XI deficiency provides protection against deep vein thrombosis and therefore cardiovascular morbidity and mortality [6]. The antisense drug IONIS-FXIRx can lower the factor XI levels and has the potential to be more effective than conventional anti-thrombotics. A phase II study with IONIS-FXIRx/BAY 2306001 is ongoing to investigate the drug's effects in patients with end-stage renal disease on hemodialysis [6, 18, 19].

3.3 Antisense therapy for inflammation and autoimmune diseases

The antisense drugs have been and are currently being evaluated for multiple inflammatory diseases, such as inflammatory bowel disease. The oral drug, mongersen targeting the SMAD7 mRNA, showed the promising effects on patients with ulcerative colitis. Also, alicaforsen drug, targeting intercellular adhesion molecule 1 (CD54), has been tested for its effects by systemic delivery in patients with Crohn's disease [20] as well as in the rectal enema patients with ulcerative colitis (or active unremitting pouchitis) [21–23]. This drug is currently being developed for the treatment of chronic refractory pouchitis [21, 24].

3.4 Antisense therapy in neurological disorders

Antisense drugs are being evaluated for multiple neurological diseases and are administered systemically into the cerebrospinal fluid (CSF) that surrounds the brain. Antisense oligonucleotides cannot cross the intact blood–brain barrier efficiently; therefore, they are directly introduced into the CSF or parenchyma to treat brain or spinal cord diseases. Thus, neurological diseases can be approached using different antisense mechanisms and oligonucleotide designs, with single-stranded antisense oligonucleotides and siRNAs used for local therapy [6]. **Duchenne muscular dystrophy** is a progressive, severely disabling, and ultimately lethal neuromuscular disease caused by point mutations, insertions, or chromosomal rearrangements in the dystrophin gene resulting in truncated protein or loss of transcript through nonsense-mediated decay [25]. Because of multiple genomic alterations in Duchenne muscular dystrophy, no single oligonucleotide will address all forms of the disease [6, 25]. Antisense oligonucleotides designed to promote skipping of exon 51 are the most advanced in clinical trials, and the modified phosphorothioate oligonucleotide drug, eteplirsen, is under regulatory review for marketing approval. Additional antisense drugs are currently under development

for targeting other exons, which will broaden the treatment of the patient population. **Myotonic dystrophy type 1 (DM1)** is a multisystemic disease caused by a triplet repeat expansion (CTG) in the 3′ untranslated region of myotonic dystrophy protein kinase (DMPK) gene [26]. IONIS-DMPK-2.5Rx is a chimeric antisense oligonucleotide and is currently under a randomized controlled study trail in DM1 patients. **Transthyretin amyloidosis** is a form of systemic amyloidosis caused by misfolded transthyretin protein (TTR), in multiple tissues, including peripheral nerves, the gastrointestinal tract, and the heart [27]. Three different antisense drugs, IONIS-TTRRx, RNase H–dependent, and patisiran, are currently in development for the treatment of TTR amyloidosis, as well as for both familial amyloid polyneuropathy and cardiomyopathy [28]. **Spinal muscular atrophy (SMA)**, a progressive motor neuron disease, usually occurs in infancy or childhood caused by deletions or mutations in the survival of motor neuron 1 (SMN1) gene [29]. Nusinersen drug is a fully modified oligonucleotide designed to bind to a specific sequence in intron 7 of the SMN1 and 2 pre-mRNAs, enhancing exon 7 inclusion and increasing the production of SMN protein [30, 31] which is under review for market authorization. **Huntington's disease (HD)** is an autosomal dominant neurodegenerative disorder resulting from an expanded CAG repeat in the huntingtin (HTT) gene, which causes a toxic gain of function due to an expanded polyglutamine tract in the resulting protein. Antisense oligonucleotide designed to lower total HTT has been shown to provide a prolonged improvement in HD, and the drug is in clinical trial phase [30, 31].

3.5 Antisense therapy for infectious diseases

Various antisense mechanisms can be utilized to inhibit viral replication—for example, by binding to viral mRNA to block the translation of the protein or to degrade the viral RNA through an RNase mechanism or by blocking host microRNAs that support viral replication [6]. Several antisense therapies are currently undergoing clinical trials for various infectious diseases. MicroRNA-122 (miR-122) is highly abundant in the liver and is essential to the stability and propagation of hepatitis C virus (HCV) [32]. This mRNA binds to a highly conserved 5′ untranslated region of the HCV genome, protecting it from degradation and host innate immune responses [32]. Additionally, miR-122 is also believed to play a major role in inflammatory activity in the liver [33], and RG-101, a GalNAc-conjugated oligonucleotide drug, is designed to inhibit miR-122 and HCV replication. The results from this clinical trial were very encouraging and support continued study of the drug.

4. The past, present, and future

The discovery of DNA as hereditary material and the helical structure base paring of DNA have opened the new avenue toward the current understanding and use of nucleic acids for the development of oligonucleotide-based therapies [6, 34]. The antisense oligonucleotides bind to RNA through Watson-Crick base pairing theory and modulate the function of the targeted RNA. This leads to the key discovery and direct importance of development of oligonucleotide-based drugs and medicine [6, 34]. The initial discovery of antisense technology is to enhance or improve the protein and small molecule-based technology by targeting RNA instead of protein. As a therapeutic strategy, it effects the RNA processing and modulates protein expression by binding to RNAs encoding difficult-to-target proteins. However, translating this technology into the clinic had some disadvantage such as inadequate target

arrangement, insufficient biological activity, and off-target toxic effects over two decades, but this strategy significantly develops the numbers and types of targets that can be approached for therapy. Further, this technology has a great advantage to develop drugs for the treatment of both monogenic and polygenic diseases as well as influence the human genetics and genomics [12, 34]. The novel chemical modifications of antisense oligonucleotides have been engaged to address these limitations over the years. Both antisense alterations and their mechanism of their action have not only improved the clinical trial design but also provide the breakthrough toward the translation of antisense oligonucleotide-based strategies into therapies [6, 34]. Additionally, multiple clinical trials have clearly confirmed the clinical uses of antisense pharmacology in humans and demonstrated their safety by using various mechanisms. The current antisense drugs, which are under clinical development phase, target different tissues both systemically and locally. The advanced oligonucleotide chemistry further enhances the antisense drug properties by increasing potency, safety, and broader tissue distribution [6]. The advancement of oligonucleotide designs had improved the antisense mechanism of binding to RNA to inhibit their function with and without out RNA degradation. Recently, several researchers demonstrated that antisense oligonucleotides can also be utilized to overexpress or suppress the protein production [34, 35]. Many nonpathogenic disease conditions lack an effective treatment; the rapid development of new improved next-generation antisense oligonucleotide-based drugs bear the potential of intense clinical application and therapeutic impact on the treatment soon, demonstrating that this new class of molecular medicines/drugs has several potential and advantageous applications in the clinic. There was always a lag period from the early discoveries till the therapy enters into the clinical trials and further in the market [34]. RNA-based precision medicine and gene therapy still need the improvement because of their off-target effects. The future applications of antisense technology will solely depend on the performance of novel molecules in the clinical trials. Till date tremendous progress has been made toward the antisense technology, despite the fact this has yet to deliver its full potential [36]. There are still more unanswered questions which need further improvement of the technology. The establishment of first-generation phosphorothioate oligodeoxynucleotides is a great asset and valuable pharmacological tool, which has shown the promise of new therapies to the patient. Further development of new improved second- and third-generation antisense oligonucleotides with novel formulation will result in better therapies for patients. Even though the tremendous progress has been made in antisense technology, there are still more questions that remain unanswered for the technology and opportunities to further improve upon the platform [36]. This technology has provided solutions and confidence to diseases which were earlier considered untreatable. The high-cost factor has prevented this treatment mode inaccessible for the general masses. To fulfill the clinical need, future innovations to this technology might help in finding better and affordable cure to many more diseases which is available to all. Furthermore, the development of CRISPR, an RNA-guided gene-editing technology, and the delivery of mRNA transcribed in vitro are a major development of the RNA therapeutics. The clinical applications and validations of RNA-based antisense drugs for modulation of gene/protein expression and genome editing are currently being investigated both in the laboratory and in the clinic [1]. The CRISPR-Cas genome editing has transformed the field and impacted the biomedical science field which has stimulated the development of RNA-based antisense delivery approaches to facilitate clinical translation of CRISPR-Cas technology [1]. For cancer therapy, the first US-based human trial using CRISPR-Cas9 ex vivo is isolated from the T cells of cancer patients by knocking out the genes encoding PD1 and T-cell receptor alpha/beta [1]. Unquestionably, the field of antisense RNA

therapeutics is presently undertaking foremost development, and the potential for using RNA antisense drugs for personalized medicine and immunotherapy as well as to address genetic, infectious, and chronic diseases will ensure the continued development of antisense RNA therapeutics for years to come [1].

5. Summary and conclusion

In summary the antisense oligonucleotides are short, synthetic, single-stranded oligodeoxynucleotides that can alter RNA and reduce, restore, or modify protein expression through several distinct mechanisms by targeting the source of the pathogenesis; antisense-mediated therapies have a higher chance of success than therapies targeting downstream pathways. The advancement in the understanding of antisense pharmacology has provided new energy to translate these therapeutics into the clinic. Further advancement of antisense technology in the clinical settings requires more optimization of antisense delivery, target engagement, and safety profile. This technology holds the potential to change the therapeutic landscape for many disease conditions in near future. Most recently, the first gene therapy-based product, Gendicine (Ad-p53), got approved to treat head and neck squamous cell carcinoma in combination with radiotherapy. Also, the drug, Vitravene (known as fomivirsen), was approved for cytomegalovirus retinitis, Macugen (known as pegaptanib) for age-related macular degeneration, Kynamro (known as mipomersen) for homozygous familial hypercholesterolemia, Exondys 51 (known as eteplirsen) for Duchenne muscular dystrophy, Defitelio (known as defibrotide) for severe hepatic veno-occlusive disease, and Spinraza (known as nusinersen) for spinal muscular atrophy by the FDA. The development of antisense therapeutics has now become a clinical reality. The true advancement in the antisense design, chemistries, synthesis, and delivery technologies has been made for adequate stability, efficacy, specificity, and immune evasion. Finally, antisense technology is beginning to bear fruit.

Author details

Shashwat Sharad[1,2]

1 Center for Prostate Disease Research, Department of Surgery, Uniformed Services University and the Walter Reed National Military Medical Center, Bethesda, Maryland, USA

2 Henry Jackson Foundation for the Advancement of Military Medicine, Bethesda, Maryland, USA

*Address all correspondence to: sharadshashwat@gmail.com

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