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RNA Therapeutics Beyond mRNA- siRNA, Antisense Oligonucleotide and microRNA as next generation pharmaceutical modalities- formulation, delivery and clinical progress

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Abstract:
With highly targeted methods for controlling gene expression and treating a wide range of genetic, metabolic, viral, and cancerous disorders, RNA therapies have become a revolutionary technique in contemporary medicine. The main kinds of RNA (ribonucleic acid) treatments, such as antisense oligonucleotides (ASOs), small interfering RNA (siRNA), and microRNA (miRNA), are thoroughly reviewed in this study along with their methods of action, therapeutic uses, and most recent clinical developments. With a focus on viral and non-viral delivery methods such as lipid nanoparticles, liposomes, polymeric carriers, and viral vectors, this review also covers the formulation and delivery strategies used to enhance the stability, cellular uptake, and target specificity of these compounds. A comprehensive analysis is conducted of current issues, such as nuclease degradation, immune recognition, off-target effects, restricted extrahepatic distribution, and formulation-related hurdles. Furthermore, the expanding clinical value of these technologies is demonstrated by current clinical trials and recent advancements in FDA-approved RNA-based therapies. Promising approaches to circumvent current constraints are also highlighted, such as chemical changes, ligand-targeted delivery systems, formulations based on nanotechnology, and artificial intelligence-assisted therapeutic design. All things considered, RNA therapeutics are a quickly developing discipline with enormous promise to improve precision medicine and offer efficient treatment choices for illnesses that are still challenging to treat with traditional medicines.

Keywords- miRNA, siRNA, antisense oligonucleotide (ASO)

1. INTRODUCTION

Genetics accounts for most cases of human diseases. Therefore, gene therapy is one of the types of treatment involving the replacement of a disease-associated gene with a "healthy" gene or gene products. In 1990, for instance, the first-ever human gene therapy involving correction of adenosine deaminase deficiency was approved. The positive feature of gene therapy is that it successfully treats genetic diseases, as demonstrated by patients with severe combined immunodeficiency (SCID) in 2002. Presently, gene therapies are designed for such complicated conditions as polygenic tumours and degenerative neurologic diseases such as Alzheimer's disease.

At the same time, gene therapy does not involve only replacement. Genetic immunisation uses genes extensively. At present, only veterinary clinics can conduct DNA vaccination procedures. Human clinical trials, nevertheless, are underway to protect against such diseases as Ebola virus disease, HIV, influenza, malaria, and tuberculosis.[1]

Oligonucleotides comprise a class of small synthesised polynucleic acids (≈20-mer) with single or double strands that can be used for controlling gene expression. In the current review, oligonucleotides that have been employed through Watson-Crick base pairing for binding DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) will be considered. Gene expression can be affected by oligonucleotides through different means. For inducing degradation, altering splicing, or preventing protein translation, oligonucleotides may target pre-mRNA, mRNA, and non-coding RNA. Alternatively, transcriptional activation can be induced by binding the promoter regions of genes using a unique form of oligonucleotides called saRNAs. [2,3]

Fomivirsen (Vitravene) was the first nucleic acid drug to be approved by the FDA of the USA. It was designed specifically for immunocompromised people suffering from cytomegalovirus (CMV). Since then, several DNA-based drugs using DNA have been licensed, and studies related to DNA continue to be carried out. But because of the medicinal value of RNA, it too has become an interesting subject for study by biotech companies. [1]

2. TYPES OF RNA THERAPEUTICS

2.1. Antisense oligonucleotide

The use of antisense oligonucleotides (ASOs) can be considered another innovative development in RNA therapy. These molecules represent a very advanced tool of RNA regulation that enables the manipulation of gene expression in a highly specific way. These molecules are synthesized small, single-stranded DNAs or RNAs that specifically bind to certain regions of RNAs and affect processes related to RNA metabolism and functions. Taking into consideration the particular design of these molecules as well as their chemical modification, several different mechanisms of ASO action are known. The most widely used mechanism of action of ASOs consists of interaction of ASOs with RNase H, leading to its activation and cleavage of the RNA strand interacting with the DNA strand of the complex. ASOs using this mechanism possess a specific structure, called "gapmers", including chemically modified nucleotides located on both sides of the DNA strand.[1]

To ensure that splicing does not occur, the splicing modulators will be made such that they bind to the junctions of introns and exons in the pre-mRNA and thereby create steric hindrance. While most of the splicing modulators have a structure similar to that of DNA, certain modifications were made to prevent the recognition of duplexes formed by pre-mRNAs with splicing inhibitors by RNase H. The structure of some splicing inhibitors is similar to that of RNA.[4,5]

ASOs will be able to stimulate the action of RNase H and enhance the degradation of the RNA through such positioning. The control of RNA splicing regulation is another critical process through which ASOs act. Through such a mechanism, ASOs influence the splicing pattern of target genes by targeting specific RNA sequence regions involved in splicing, such as splice sites and regulators. Nusinersen, popularly known as Spinraza, is an FDA-approved drug used to treat spinal muscular atrophy and stands out as one of the best examples of success achieved through this modality of treatment in genetic diseases. This technology offers a versatile platform for regulation of gene expression via a variety of mechanisms like blocking translation initiation, regulating polyadenylation or interfering with miRNA functions.[3]

The incorporation of AI methodologies in designing and optimising ASOs is increasingly becoming beneficial. It is expected that such innovative computational methodologies will facilitate the rapid development of better ASOs having minimal side effects. Furthermore, new strategies utilising a combination of ASOs with CRISPR-Cas9 technologies are under investigation and can potentially contribute to more precise gene correction procedures. Delivery of ASOs has been a matter of concern until now, but modern research efforts focus on the fabrication of nanoparticles and targeted exosome delivery methods.[2]

2.2. siRNA

The siRNAs represent a form of double-stranded RNA capable of hybridising with their target RNA with exact Watson-Crick complementarity [7]. All siRNAs serve as gene silencers since they inhibit the process of RNA transcription or protein translation through the miRNA pathway. Twenty years after the discovery of RNA interference in 1998 [8,9], the siRNA drug patisiran/Onpattro appeared as the first-ever marketed siRNA-based medication in 2018 [9].

One such mechanism that can be used for the suppression of the expression of the desired genes is RNA interference (RNAi), which occurs via the utilisation of siRNAs. These molecules are usually composed of 20-25 base pairs of RNA, which exhibit the unique ability to destroy the mRNAs. Double-stranded RNAs are first introduced into the cellular environment as a starting point for the siRNA-mediated gene silencing process. Dicer enzymes hydrolyse the double-stranded RNAs into smaller siRNAs. The RNA-induced silencing complex (RISC) will then incorporate these newly formed siRNAs. The siRNAs will then recognise the target mRNA based on their sequence specificity. The protein Argonaute 2 (Ago2), a key component of the RISC complex, performs mRNA cleavage due to this interaction process. Following that, cellular mechanisms degrade the cleaved mRNA pieces, resulting in gene expression inhibition. One should emphasise that this mechanism provides a very high level of specificity since the target identification and RNA interference efficiency might significantly decrease due to just one nucleotide mismatch within the sequence of the siRNA molecule.[7]

A proof-of-concept demonstration of the effectiveness of siRNA in inhibiting gene expression came about in 2001, when Elbashir et al. discovered that artificial RNA duplexes made up of 21 base-pairs were effective in inhibiting gene expression in mammals [6]. This discovery led to the idea of using siRNAs in collaboration with ASOs in targeting protein expression linked to disease pathways, leading to the emergence of a new category of drugs. However, three critical problems – delivery, stability, and specificity – hampered the progress of siRNA in drug development [7].

Various siRNA drugs have already been introduced into clinical practice, and their potential as treatment tools for different diseases has been demonstrated. Thus, hereditary transthyretin-mediated amyloidosis may now be managed using patisiran (Onpattro). The use of inclisiran (Leqvio) to reduce low-density lipoprotein (LDL) cholesterol in patients suffering from atherosclerotic cardiovascular disease and the use of givosiran (Givlaari) to treat acute hepatic porphyria indicate the flexibility and effectiveness of siRNA technology in the treatment of numerous conditions.[1] Due to the impressive specificity of siRNAs that allows targeting almost any gene, these molecules are indispensable instruments for genomic studies and clinical applications.[2]

2.3. Microrna

One of the vital tools used in RNA therapies is miRNAs. miRNAs are small RNA molecules that are about 21 to 25 nucleotides in length, and their primary function is controlling genes without translation into proteins.[1]

The first miRNA discovered is called Lin-4, which contains sequences that complement the repeat region of the 3′ UTR of the lin-14 messenger RNA of Caenorhabditis elegans.[10] This was initially seen as an achievement in worm genetics. The discovery of miRNAs as being highly conserved in all animals, including humans, however, happened when a second miRNA, let-7, was discovered.[11,12]
First of all, the miRNAs are considered a type of small molecule known as nucleotides (nts). In other words, these are single-stranded sets of RNAs that do not code for proteins. This type of RNA is used as a gene regulator, and its size is within 21-25 nucleotides. The processing from pre-miRNA to mature miRNA is done through miRNA biogenesis. Details about miRNA biogenesis are found in Bhattacharya et al. [13] and Chakraborty et al. [14].
While both siRNAs and miRNAs bind to the complementary strands of their target mRNAs, miRNAs predominantly bind to the 3′-untranslated regions (3′-UTRs) of several targets[1]
Notably, miRNAs may regulate important signalling pathways in cancers, where miRNAs exert their influence on the signalling pathways through the regulation of key signalling proteins. In such instances, miR-200 is regulated by TGF-β in the malignant tissues, since TGF-β acts to suppress miR-200 production. MiR-200 is responsible for the regulation of several downstream signaling factors of the TGF-β signalling pathway, including Smad2, TβR-I, and TGF-β. Therefore, there exists a negative feedback mechanism between TGF-β and miR-200, where TGF-β inhibits malignancies.[15]
This review is aimed at discussing miRNA therapeutics in clinical trials and the current challenges of miRNA therapeutics.

3. FORMULATION OF siRNA

3.1. Barriers to siRNA delivery

The RNA interference modality (siRNAs and miRNAs) demands the utilisation of cytoplasmic proteins such as Argonaute and Tar RNA binding protein. A series of problems must first be addressed for the systematic administration of external siRNAs before they achieve gene silencing, and these include susceptibility to nucleases, circulating for a temporary period, immune system detection while in circulation, localisation into target cells, transmembrane transport, and escape from endosomes and lysosomes into the cytoplasm. Problems of stability and evasion of immune system detection have largely been solved with the development of chemical modifications. Yet other problems remain. [16,17,18]
However, both glomerular filtration and nonspecific binding protect against an accumulation of siRNAs within the desired tissue through the bloodstream. The surface neutralisation of the nanovehicles loaded with siRNAs ensures that they do not bind to proteins in the bloodstream. [19]
Protein decoration or protein corona formation would be required, on the other hand, for certain delivery systems that require targeted binding of the nanoformulation.[20,21] Examples include iLNPs, whose accumulation in the liver occurs via their interaction with serum lipoproteins, such as Apolipoprotein E3 (ApoE3), leading to targeted binding by the receptor, such as low-density lipoprotein receptor (LDLR).[17]

On the contrary, because of specific binding and interaction between the ligand—e.g., carbohydrate, peptide, antibody, aptamer, chemical compound, and so forth—and the receptor present on the cell surface, conjugates of ligand and siRNA would be able to deliver siRNA to the desired tissue and cell. The gene can be silenced powerfully using a lower dose of siRNA, while minimising the undesired effects and toxicity caused by siRNA in the wrong sites.[16]

The diameter of siRNA ranges between 2-3 nm while its length is approximately 7-8 nm. Since molecules less than 8 nm[22] in size can filter into the urine easily[22,23], this means the siRNA molecule is too large to be filtered out of the cells yet small enough to be freely filtered by glomeruli. For this reason, siRNAs will quickly accumulate in the bladder once out of the blood and then leave the body within minutes.[24]

3.2 Barriers to miRNA delivery

i.Instability of miRNAs and reproducibility of results in miRNA preparations

Nowadays, isolation of RNA is carried out through two methods:

the silica matrix/glass fibre filter method and the Trizol/TRI-Reagent method of isolation, the latter being the most common method used for isolation of total RNA (Bernardo et al. 2012). The stability of miRNAs and reproducibility of results have remained the major challenges in the manufacture and storage of miRNAs. As reported by one study, miRNAs and cDNA derived from these miRNAs were found to be highly unstable in RNA prepared by the mirVana Isolation Kit or TRI-Reagent method. When stored at -80 °C, they were observed to degrade slowly after a few days (Bravo et al. 2007). It is important to note that the purity of the extracted RNA differs based on how the same kind of tissues are isolated and preserved (Bernardo et al. 2012). TRIZOL/ TRI Reagent, which is free from any genomic DNA contamination, should be considered a "gold standard" method of miRNA isolation for standardising the process of miRNA isolation. In addition to that, it is necessary to preserve the integrity of miRNAs by providing them with an RNase-free environment, like RNase-free solutions, tubes, tips, and gloves.[25]

ii.Barriers to antisense oligonucleotide delivery

Transport of the drug to the target site constitutes one of the major challenges in ASO therapies. The drugs are supposed to enter the body through injection, cross biological barriers, and reach the cells where they will be absorbed. Upon entry into the cell, ASOs have to evade capture within the secretory vesicles and their lysosomal degradation. Several ways have been developed and are currently being investigated to improve the ASOs' stability, targeting, and trafficking. These involve the use of delivery systems, bioconjugation to different groups, and chemical modification.[26]

4. FORMULATION AND DELIVERY OF RNA THERAPEUTICS

4.1 Virus-Based miRNA and Anti-miRNA Oligonucleotide Delivery Systems

Oligonucleotides needed for effective gene transfer to different tissues could be delivered by genetic engineering of viruses, capable of producing high, sustained levels of gene expression. To reduce toxicity and provide space for the delivery of transgene(s), disease-causing elements are deleted from the virus genome in eukaryotic viruses. Different viral vectors, which are designed for the delivery of certain transgenes for therapeutic applications and target cell types, have emerged in the past few decades. Retroviral, lentiviral, adenoviral, and adeno-associated virus (AAV) vectors (reviewed in [27])

and bacteriophage-derived virus-like particle (VLP) vectors [28,29] are the commonly used viral vectors for miRNA or anti-mRNA (or antagomir) delivery.

4.1.1 Retroviral and Lentiviral Vectors

Consistent transport of therapy-gene-containing material into proliferating cells has been achieved through the use of retroviral vectors (RVs) that are formed from lipid-enveloped RNA viruses [30]. Moloney murine leukaemia viruses (MoMLVs) that have relatively simple genome structures encoding Gag, Pol, and Env proteins surrounded by LTRs constitute the basis for most of the retroviral vectors [31]. Viral RNA is transported to the cytoplasm, where it is converted to dsDNA, and then randomly integrated into any of the host chromosomes after it is identified and attaches to the receptors on the cell surfaces. Retroviruses have a "Janus face" because of their ability to integrate foreign DNA into the host chromosome. It may prove detrimental to the cell as insertional inactivation of essential genes or their regulatory elements occurs, despite being beneficial for transgenic expression [32,33].

However, there has been evidence that miRNA delivery through RV shows potential for use in regenerative medicine. For example, the up-regulation of miR-138 in mouse embryonic fibroblasts led to the formation of induced pluripotent stem cells, which has implications in regenerative medicine by inhibiting p53 signalling pathways [34].

The immunodeficiency viruses of cow (BIV), cat (FIV), horse (EIAV), simian (SIV), and human (HIV-2) are just some of the species from the lentivirus genus belonging to the family Retroviridae that have been altered to develop lentiviral vectors (LVs) [35,36]. LVs have the capacity to passively move across the nuclear membrane through the nuclear pore complex in an active manner, thereby making both quiescent and non-quiescent cells their targets, unlike RVs, which have to penetrate the nuclear membrane during mitosis [37].

Moreover, another critical issue linked to retroviruses is the greater possibility of triggering the process of oncogenesis through insertional mutagenesis [38,39]. As opposed to RVs, LVs tend to prefer integration into transcriptionally active sites, which decreases the probability of inducing insertional oncogenesis [40,41,42]. There are many cases where LVs were successfully used for the delivery of miRNA mimics or anti-miRs. In a mouse model of chronic lymphocytic leukaemia (CLL), the delivery of LVs and the subsequent increase in the expression of microRNAs miR-15a and miR-16 caused depletion of cancerous B cells and alleviation of the disease [43]. Another study tested the use of lentiviral miR-494 sponges and found that these anti-miRNAs can decrease tumour formation and metastasis by preventing miR-494 access to its targets [44].

4.1.2 Adenovirus and Adeno-Associated Virus Vectors

Adenovirus (Ad) and adeno-associated virus (AAV) were produced by the modification of non-enveloped viruses with double-stranded DNA and single-stranded DNA, respectively. Evolutionarily speaking, AAV was developed to be a potent delivery vector due to its non-pathogenicity, wide range of target tissues, and persistence within the body [45]. Moreover, two features of these viruses make them potent tools for medical purposes. As in the case with LVs, both Ad and AAV can infect resting or dividing cells. Contrary to RLVs and LVs, Ad and AAV will not integrate themselves into the host genome, hence will not become agents for the insertion of oncogenes. In contrast to RLVs and LVs, which can accommodate up to 8 kilobases (kB) of foreign sequences of nucleotides, Ad can contain 38 kB of foreign DNA [30].

AAVS can accommodate most miRNAs in cassettes, although it has a limited cargo capacity of ~4.8 Kb [46]. In order to deliver miRNAs, several viral vectors have been established. Moreover, there is the silencing of Elav-like family member 2 (CELF2) using the miR-196a, which is delivered by the AAV vector, and this leads to instability of androgen receptor (AR) mRNA and hence reduces the SBMA symptoms [47]. Recently, Tang et al. found that artificial miRNAs against hemagglutinin, when introduced using recombinant adenovirus, could be protective against lethal strains of influenza virus [48].

4.2 Non-Viral-Based miRNA and Anti-miRNA Oligonucleotide Delivery Systems

Even though virus-based miRNA delivery systems are highly efficient, they are associated with a high level of immunogenicity, toxicity, and size limitations. Non-viral miRNA delivery systems, which are less dangerous and more biocompatible, have been developed to overcome these problems. The non-viral delivery systems ensure that miRNA or miRNA-expressing vectors enter the cell without being degraded by nucleases. Among the various chemical methods for non-viral miRNA delivery systems are the following: lipid, polymer, inorganic, and extracellular vesicle carrier-based ways.

4.3 Formulation and delivery of siRNA

All the siRNAs were synthesised by Alnylam, and anion exchange HPLC and ESMS were employed to characterise them. The sequences of the sense and antisense strands of siRNAs are as follows:5′-GGAucAucucAAGucuuAcT*T-3′ corresponds to siFVII sense, whereas 5′-GuAAGAcuuGAGAuGAuccT*T-3′ corresponds to antisense.siCont sense: 5′-cuuAcGcuGAGuAcuucGAT*T-3′, antisense: 5′-UCGAAGuACUcAGCGuA

4.3.1. Lipid–siRNA Formulation

The lipid-siRNA formulation consisted of siRNA, PEG-lipid, cholesterol, and 98N12-5(1). Briefly, cholesterol (Sigma-Aldrich, St. Louis, MO), mPEG2000-lipid, and 98N12-5(1) stock solutions were prepared in ethanol to achieve appropriate molar ratios. Empty lipidoid nanoparticles self-assembled when the mixture of lipids was dissolved in 125 mmol/l sodium acetate buffer (pH 5.2) to obtain a solution with 35% ethanol content.[50] Lipidoids were then extruded via LIPEX Extruder (Northern Lipids, Burnaby, British Columbia, Canada) using a 0.08 μm membrane (Sterlitech, Kent, WA) to prepare particles with 50-60 nm in length. Bigger formulations were formed by initially allowing the empty lipidoid nanoparticle formulation to incubate for approximately 24 hours at 4 °C to explore the effect of size on activity. 0.4, 0.2, and 0.1 μm membranes were then employed for extrusion to create larger-sized formulations.

Lipidoid nanoparticles were incubated for 30 minutes at 37 °C once they were introduced to siRNA at an appropriate total lipid: siRNA ratio in 50 mmol/l sodium acetate (pH 5.2) and 35% ethanol. Dialysis with phosphate-buffered saline was used to remove the ethanol and change the buffer of siRNA-containing lipidoid nanoparticles. Finally, a 0.2 μm sterile filter was employed for filtration of the formulation. Particle size and zeta potential measurements were made using a Malvern Zetasizer NanoZS (Malvern, UK).

Measurements of siRNA content were carried out via UV absorbance at 260 nm, while measurements of siRNA entrapment efficiency were carried out using the Quant-iT RiboGreen RNA assay (Invitrogen, Carlsbad, CA).[51] In brief, the fluorescence of the RNA-binding dye, RiboGreen, in samples of

formulations with and without the addition of detergent Triton-X100 is compared. The signal in the absence of detergent comes only from unentrapped siRNA. The signal in the presence of detergent comes from totally entrapped siRNA.

4.4. Delivery of antisense oligonucleotide

Several types of drug delivery nanoparticles such as DNA nanostructures, exosome-like nano-carriers, and spherical nucleic acids have been developed along with the rapid evolution of the field of nanotechnology [52,53,54]. In this paper, we will consider liposomal drug delivery.

Liposomes represent lipid vesicles containing an internal aqueous space surrounded by a natural or synthetic lipid bilayer. Liposomes were discovered in the 1960s and since that time, have become a centre of attention in nanoparticle treatments due to the number of advantages of those, including stability, targeted delivery, minimal toxicity, and biocompatibility. Various kinds of liposomes are undergoing clinical trials nowadays. Different properties of liposomes, including their charge, size, and effectiveness, are determined by the components of liposomes, which are lipids, synthetic amphiphiles, and sterols [55,56].

Liposomes are susceptible to destabilisation by serum proteins, such as opsonins and lipoproteins (HDL, LDL), despite protecting ASOs from nucleases. Opsonins in blood recognise and bind liposomes, marking them for phagocytosis [57,58,59]. By removing lipids from liposomes, lipoproteins structurally destabilise them, causing their release [60]. Hence, PEGylated liposomes are protected against recognition by the serum components that bind to them. However, even though it is an advanced technique and quite promising, PEGylation has some limitations. In particular, due to its non-biodegradability, PEG may lead to adverse effects and hypersensitivity as a result of accumulation in the body [62]. Moreover, the efficacy of repetitive administration of PEGylated liposomal drugs can be hampered due to the formation of antibodies against PEG (anti-PEG IgM) after the first drug injection [59]. The recognition of PEGylated liposomes by the mononuclear phagocytic system and increased elimination rate from the bloodstream occurs if there are anti-PEG IgM antibodies [59,63].

Cholesterol, an essential component of the liposomal bilayer, fulfils several functions within the liposome. It increases the stability and circulation of the liposome by preventing any action of HDL on the liposome due to the dense packing of phospholipids in the bilayer [64]. Further, lipid density protects the liposomes from ion, molecule, and drug entry into the liposome through decreased liposome permeability [65]. Finally, cholesterol helps the liposome evade the immune system and increases its circulation through reduction in binding of Na+ ions to the liposome [61,64,65].

For prostate cancer treatment, a new iLi carrying ASO targeting Translationally Controlled Tumour Protein and decorated with the inclusion of Her2 antibody has been developed in a concept proof study [66]. From the results, ASO-iLi has been found to have more antiproliferative effects on PC-3 cell spheroids than ASO liposomes [66]. This is worth noting since the effect is achieved through prolonged times due to the delay in ASO release as a result of steric hindrance brought about by the antibody inclusion [66]. These liposomes respond to certain physiological stimuli by releasing their payload. The stimuli-sensitive liposomes take advantage of the artificial lipids that induce alterations in the structure of liposomes, leading to disruption of the liposomal barrier. These stimuli may arise externally, such as thermal, ultrasound, and magnetic stimuli, or they may be internal, including changes in pH and redox potential [67,68]. For example, when endocytosis occurs, pH stimuli cause liposomes to fuse with the endosomal membrane and release their payload into the cytoplasm when the endosomal pH decreases

[69]. Numerous clinical trials use liposomal drugs due to their increased stability, biocompatibility, long circulation time, targeted drug release, and efficiency [71]. Cancer is one of the diseases for which the treatment is based. The understanding of cancer genomes has greatly advanced ever since the oncogenes were discovered. Oligonucleotide therapy has been considerably facilitated due to the Cancer Gene Atlas [72].

5. CLINICAL PROGRESS OF RNA THERAPEUTICS

5.1. Clinical progress of siRNA

Through various mixed trials and many approvals, there is no doubt that siRNA-based therapies have made significant clinical advances; however, all these clinical advancements remain centred around liver-targeting drugs.[73]Further advancements have been witnessed in the field due to better chemistry and delivery systems; however, extrahepatic delivery remains the biggest hurdle to greater therapeutic efficacy.siRNA technology has evolved to become what it is today over the course of three stages.[74]

With approvals beginning in 2018 and extending into 2022, siRNA-based therapeutics have cemented themselves as clinical tools, primarily through the use of liver-targeting delivery systems[73]. Chemical modifications and GalNAc or nanoparticle delivery systems have taken the siRNAs to new heights of stability, specificity, and dosage efficacy, making them commercially viable treatments.[75]Post-approval, review articles always confirm that siRNA drugs are now part of mainstream pharmacotherapy, specifically for rare liver disease conditions. Some candidates that are in their final stages of development include vutrisiran, nedosiran, inclisiran, fitusiran, teprasiran, cosdosiran, and tivanisiran.

The focus of clinical trials is shifting from rare genetic diseases to a broader population base, like hypercholesterolemia and other chronic conditions. The targeting of organs is uneven because the brain, kidney, and lungs remain relatively under-targeted in experiments, while the organs that are favoured include the liver, eye, and skin.[76]

5.2. Clinical progress of antisense oligonucleotides

Success has been sporadic at best, but the field of antisense oligonucleotides has made definite strides in moving on from a period of extensive research work to obtaining approval, increased trial work, and disease-specific delivery systems. Genetic and neurodegenerative diseases have been the most successful in the industry so far, even though the cancer and some ALS work trails behind because of delivery problems.

The development of ASO therapy has undergone various stages from conception to actual use.[77]

As can be witnessed in various studies, ASOs have evolved from concept to being used in clinics through evidence of the existence of six approved drugs in 2019, nine FDA-approved medicines in 2024, and an enormous pipeline of drugs [77]. Efficacy depends on disease, as is witnessed in retinal and liver research for initial efficacy and target engagement, neurological applications for nusinersen and tofersen, and C9orf72-ALS, which failed to improve clinical outcomes and was discontinued.[78]

5.3. Clinical progress of miRNA

Developing biomarkers is widespread, therapeutic translation has been successful in reaching human studies, while no miRNA drug has entered phase III or received any approval as yet, which suggests that clinical progress of miRNA is real but at an early stage [79]. Standardisation, delivery, and toxicity remain major challenges, although circulating miRNA diagnostics and some therapeutic candidates such

as miravirsen, MRG-110, and newer anti-cancer drugs have made the most progress. Standardisation and validation problems prevent the use of circulating miRNAs as biomarkers in the prognostics and diagnostics of cancer, cardiovascular diseases, immunological diseases, and infectious diseases. Hepatitis C, cardiovascular repair, cancer, ADPKD, and cutaneous T-cell lymphoma are some diseases that have seen the development of miRNA-based therapies begin early human studies.[80]
Indeed, in cancers such as breast cancer, where panels are profiled with respect to diagnosis, prognosis, and treatment response, the use of multiple-miRNA panels is superior to that of single miRNAs.
Variability in measurement remains a challenge for the clinical application of biomarkers. Issues include inconsistent RNA extraction, non-standardised samples, non-standardised controls, and lack of panel validation.[79]

6. CONCLUSION

The other area that has emerged in the field of precision medicine and offers the possibility for extremely precise treatment options for diseases such as infections, metabolic conditions, genetic disorders, and cancers is RNA therapies. With the help of FDA-approved drugs as well as numerous drugs undergoing clinical trials, antisense oligonucleotides (ASOs), siRNA, and miRNA-based therapies offer different ways to regulate the activity of genes. However, the application of these therapies faces several problems and challenges like instability, degradation by nucleases, immune response, off-target effects, limited ability to deliver drugs to specific tissues, and complex production process. RNA-based drugs have become safer and more effective thanks to the latest advances in chemical modifications, lipid nanoparticles, delivery systems based on viruses and non-viruses, ligand-targeting approaches, and nanotechnology. Moreover, the integration of artificial intelligence into the development of delivery methods will likely speed up the development of new drugs. Collaborative research and well-planned clinical trials will be needed to address current limitations and expand the range of diseases to treat. In conclusion, RNA therapies are a rapidly developing field with great promise for personalising treatments for specific diseases.

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