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Recent Developments in RNA Therapeutics for Humans Disorders

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Abstract:

Recent research has uncovered a wide range of RNAs, including noncoding RNAs, and have discovered their varied modes of action inside cells. These ribonucleic acids (RNAs) play a crucial role in controlling many cellular processes and are thus anticipated to be significant targets for the treatment of human disorders. In recent years, RNA-based medicinal approaches have made significant advancements alongside their comprehensive functional research. Following extensive study and experimentation, medications based on antisense RNAs and small interfering RNAs have been successfully created and are already being used in clinical settings. Furthermore, there is now ongoing research focused on the development of pharmaceuticals using RNA aptamers and messenger RNA. In addition to the advancement of RNA-based medications, many techniques have been devised to effectively deliver RNA drugs into cells. RNA treatment offers several benefits compared to current therapeutics based on small molecules or monoclonal antibodies, mostly due to its ability to selectively target all genes inside cells. The purpose of this article is to provide an overview of the introduction of various RNA-based technologies and the introduction of RNA-based drugs in the market. In addition, the future prospects of RNA therapy will be addressed.

INTRODUCTION

The use of antisense oligonucleotides (ASOs) to hinder protein synthesis in the early 1980s greatly facilitated the fast progress of RNA-based treatments. The idea of RNA interference (RNAi) and the use of small interfering RNA (siRNA) to suppress human genes resulted in a surge in financial support for RNA therapies throughout the 2000s (1). Additional RNA molecule regulators and associated processes have been thoroughly studied. Currently, many RNA-based drugs have been authorized and others undergoing phase III research. RNA-based treatments have promising potential in contrast to traditional protein-targeted and DNA-based medications, because of their unique physicochemical and physiological features. RNAs play a crucial role in three fundamental biological macromolecules: DNAs, RNAs, and proteins (2). RNA molecules, including ASOs, siRNAs, and miRNAs, can specifically bind to and target both mRNAs and ncRNAs via Watson-Crick base-pairing. Hence, RNA has the potential to selectively target any desired gene by precisely choosing the appropriate nucleotide sequence on the target RNA. In contrast, a mere 0.05% of the human genome has been affected by the presently authorized protein-targeted treatments, which include small-molecule drugs and antibodies (3). This is because the majority of DNA sequences in the human genome are translated into noncoding transcripts. In addition, about 85% of proteins do not possess particular clefts and pockets that may accommodate tiny molecules for binding. Furthermore, intracellular delivery of in vitro transcribed (IVT) mRNA may be

used for protein replacement therapy or vaccination purposes (3).

The majority of medications available today are either tiny molecules or proteins. Small molecule medications often act as competitive inhibitors of their target proteins, but protein-based drugs are often used to attach to target proteins, replace dysfunctional target proteins, or compensate for insufficient levels of a target protein. An inherent challenge associated with protein-based medications is their limited ability to penetrate target cells due to their huge size (4). Consequently, these treatments can only exert their therapeutic effects when the target molecule is located outside the cell or is expelled. Although small-molecule and protein-based medications have proven beneficial in several situations, there remains a multitude of disorders that cannot be addressed with either small molecules or proteins (5). For instance, a significant number of individuals with diabetes have insulin resistance, rendering the administration of more insulin ineffective in reducing their blood glucose levels. RNA-based medicines show promise as future therapies for disorders like diabetes, cancer, and Huntington's disease. RNA treatments provide improved therapy options for targeting the pathophysiological underpinnings of various illnesses, perhaps resulting in enhanced patient outcomes. Furthermore, the United States Food and Drug Administration (FDA) has previously approved several RNA treatments. Additionally, several other therapies are now undergoing clinical trials at different stages, providing further evidence of the effectiveness of RNA therapies in treating various disorders. This article offers a comprehensive summary of notable advancements in the field of RNA-based therapies. The categorization of RNA-based therapeutics and their mechanisms of action have been delineated. This study also addresses the key obstacles in implementing these RNA therapeutics and their remedies.

Different types of RNA-based therapies and mechanisms of action

Antisense oligonucleotides (ASOs)

In this review, antisense oligonucleotides (ASOs) refer to chemically manufactured oligonucleotides, typically 12-30 nucleotides long, which are specifically engineered to bind to RNA using Watson-Crick base pairing principles. The specificity of ASOs is partly determined by their length, since oligonucleotides that are 16–20 nucleotides in size may selectively attach to a single target RNA. After attaching to the specific RNA, the oligonucleotide alters RNA function in several ways (6). These processes may be roughly classified as either facilitating RNA breakage and destruction or as occupancy-only mechanisms, sometimes known as steric blocking. The modulation of RNA by the ASO is contingent upon the chemistry and design of the ASO, the specific location on the RNA where the ASO is intended to bind, and the role of the RNA. By considering the specific chemical and positional criteria for various pathways, it is feasible to deliberately create ASOs (antisense oligonucleotides) to regulate the target RNA. However, some screening is still necessary to get the most effective activity and tolerability (7).

During occupancy-mediated degradation, ASOs attach to and cut the target RNA at the ASO binding sites using natural enzymes, which increases the suppression of target transcripts. This process is sometimes referred to as enzymatic RNA breakdown due to its reliance on certain enzymes. The most well-defined mechanism for degradation is by RNase H1, which functions as a highly selective endonuclease to specifically break the RNA-DNA heteroduplex (8). RNase H1 efficiently and selectively targets both the cytoplasmic and nuclear transcripts owing to its widespread distribution. Ribozymes, along with other enzymes, may facilitate occupancy-mediated destruction. The ribozyme catalyzes the cleavage of target RNAs using either hammerhead or hairpin structures. Furthermore, the substrate recognition domain of the enzyme may be altered to enhance the cleavage at either the cis or trans site (9).

ASOs in occupancy-only methods regulate the up- or down-regulation of target transcripts by directly binding to them, independent of particular enzymes. Occasionally, this process is referred to as the steric block mechanism. The manipulation of RNA splicing is the most often-used approach (10). Splice-switching ASOs can modify the way genetic material is spliced by specifically targeting cis-elements that regulate splicing. Cis-acting elements exert their influence on nearby splice sites by attracting trans-splicing factors, which may either activate or stop the splicing process. They are composed of splicing enhancers and silencers. When ASOs form base pairs with a splicing enhancer sequence, they prevent the stimulatory splicing factor from binding to its specific enhancer-binding site. This inhibition of splicing leads to exon skipping. On the other hand, ASOs specifically aim at a splicing silencer sequence motif that prevents the inhibitory splicing factor from binding. The silencer element has a negative regulatory effect on splicing activation at the splice site, leading to the inclusion of the exon (11, 12).

Eteplirsen regulates the process of skipping exons, whereas nusinersen promotes the process of including exons. Regarding the applicability aspect, we analyze two splice-modulating ASO medicines, namely eteplirsen and nusinersen. The FDA approved to nusinersen in 2016 for the treatment of spinal muscular atrophy (SMA). This condition is the result of deletions or mutations in the survival motor neuron 1 (SMN1) gene. Insufficient production of SMN protein

results in the weakening and wasting away of skeletal and respiratory muscles (13). Nusinersen regulates the process of splicing in SMN2, which differs from SMN1 only in that it undergoes alternative splicing and excludes exon 7. This exclusion leads to the production of a truncated protein with a functionality that ranges from 5% to 10%. Nevertheless, nusinersen controls the process of alternative splicing in a manner that ensures the inclusion of exon 7. This inclusion leads to the production of fully functioning SMN, which ultimately improves motor function in individuals with SMA (14).

Eteplirsen received FDA approval in 2016 for the treatment of Duchenne muscular dystrophy (DMD). Alterations in the DMD gene, which encodes the dystrophin protein, result in the onset of DMD (15). The predominant mutation leading to DMD is situated in exon 51. Consequently, eteplirsen, a 30-mer ASO, specifically focuses on exon 51 of the DMD gene. This targeting results in the exclusion of exon 51 during the process of alternative splicing. This mechanism avoids the occurrence of frameshift mutations, which result in the synthesis of dysfunctional dystrophin. The resultant dystrophin protein is somewhat shorter than its wild-type cousin, however it retains its functioning (16).

Inotersen was granted FDA clearance in 2018 for the treatment of familial amyloid polyneuropathy. The etiology of this condition may be attributed to autosomal dominant mutations in the transthyretin (TTR) gene (17). These mutations cause the TTR tetramer to be disrupted, resulting in the aggregation of TTR monomers into amyloid deposits throughout the body. To counteract the accumulation of TTR, inotersen specifically acts on the 3′ UTR of the TTR mRNA, hence blocking the synthesis of TTR and impeding the advancement of the illness. Clinical investigations have shown that inotersen is effective and safe in reducing the levels of circulating TTR (18).

RNA interference (RNAi)

RNA interference (RNAi) is a regulatory mechanism found in most eukaryotic cells. It involves the use of tiny double-stranded RNA (dsRNA) molecules to limit gene activity via homology-dependent mechanisms. Small interfering RNAs (siRNA) are double-stranded RNA molecules that are around 21-22 base pairs long. They include distinct 2 nucleotide overhangs at the 3’ end, which enables them to be identified by the enzymatic machinery of RNAi (19). This recognition ultimately results in the targeted mRNA being broken down by a process that relies on homology. SiRNAs in mammalian cells are generated by the cleavage of longer dsRNA precursors by the RNase III endonuclease Dicer, or they may be created using chemical or biological techniques. Dicer forms complexes with RNA-binding proteins, including TAR-RNA-binding protein (TRBP), PACT, and Ago-2. These proteins play a role in transferring siRNAs to the RNA-induced silencing complex (RISC) (20). The fundamental elements of RISC consist of the members belonging to the Argonaute (Ago) family. Among humans, there are eight members in this family, but only Ago-2 exhibits a functional catalytic domain for cleavage action. When siRNAs are loaded into RISC, they exist as double-stranded molecules. However, Ago-2 enzyme cleaves and releases one of the strands, known as the “passenger” strand. This process results in an active version of RISC that contains a single-stranded molecule called the “guide” RNA. The guide RNA is responsible for directing the specificity of target identification via intermolecular base pairing. The selectivity of strand loading into RISC is determined by rules that rely on the differing thermodynamic stabilities of the ends of the siRNAs. The PIWI domain of Ago-2 prefers to bind to the less thermodynamically stable end (21).

MicroRNAs (miRNAs) play a crucial role in RNAi. These are naturally occurring double-stranded molecules that control gene expression after transcription by forming a complex with RISC and attaching to the 3’ untranslated regions (UTRs) of target sequences via small sections of similarity, known as “seed sequences”. The main mode of operation of miRNAs is to inhibit translation, however, this may also destroy the messenger RNA (22). The miRNA duplexes exhibit partial Watson-Crick base pairing, and unlike siRNAs, the antisense strand cannot be determined by cleaving the passenger strand. Consequently, another method must be used to choose the antisense strand. miRNAs serve as natural substrates for the RNAi machinery. The original transcripts of microRNAs (pri-miRNAs) are first produced as lengthy molecules. These pri-miRNAs are then processed in the nucleus by the Microprocessor complex, which is made up of Drosha and DGCR8. This processing results in the formation of hairpin structures that are around 60-70 base pairs in length, known as pre-miRNAs (23). The pre-miRNAs undergo further processing in the cytoplasm by an enzyme called Dicer. One of the two strands is then inserted into the RISC, likely by contact with one of the accessory proteins associated with Dicer. Crucially, it is feasible to use this inherent gene silencing process to control the expression of certain gene(s). When the siRNA effector is introduced into the cell, it will trigger the activation of RISC, leading to a powerful and focused suppression of the desired mRNA. Due to its high efficacy and specificity, RNAi has become the preferred approach for suppressing particular gene expression in mammalian cells (24).

RNA activation (RNAa) is a distinct mechanism from RNAi in which dsRNA stimulates gene expression by specifically targeting promoter regions. saRNAs, also

known as small activating RNAs, are produced by synthesizing homologous sequences that are located near or inside gene promoters. These saRNAs can induce RNAa, which is the process of activating gene expression. Like miRNA-like target recognition, the functions of saRNAs rely on the AGO2 protein (25). Within the nucleus, AGO2–saRNA utilizes the "seed" region to establish base pairing with sequences present in the chromatin-bound RNA transcripts or complementary DNA. In addition to saRNA and AGO2, new studies have shown that the RNA-induced transcriptional activation (RITA) complex also includes RHA and CTR9. saRNA has the potential to mitigate the decrease in expression of dormant tumor suppressors genes, such as p21, or other often disrupted genes, such as E-cadherin. Consequently, saRNA might facilitate the advancement of therapeutic approaches based on dsRNA for the treatment of cancer and other related conditions (26, 27).

Patisiran was the first siRNA therapeutic authorized for use by the FDA (28). In 2018, it received approval for treating polyneuropathy resulting from hereditary transthyretin-mediated (hATTR) amyloidosis (29). hATTR amyloidosis is an inherited condition characterized by the accumulation of aberrant TTR protein, mostly in the peripheral nervous system, leading to polyneuropathy. Patisiran is a small interfering RNA (siRNA) medication that specifically targets the mutant TTR mRNA, resulting in the breakdown of the mRNA and a reduction in the production of TTR protein. Studies have shown that this intervention significantly decreases the accumulation of TTR in individuals with polyneuropathy resulting from hATTR amyloidosis (28).

In 2020, the FDA approved lumasiran for the treatment of primary hyperoxaluria type 1 (PH1) (30). Different genetic alterations in the enzyme alanine-glyoxylate aminotransferase result in elevated levels of oxalate and the creation of calcium oxalate crystals, which ultimately leads to the development of PH1. Lumasiran specifically targets the mRNA responsible for encoding glycolate oxidase. This action results in a decrease in the availability of the substance needed for oxalate formation, thereby lowering the levels of oxalate (31).

The FDA approved inclisiran in December 2021 for the treatment of atherosclerotic cardiovascular disease (ASCVD) or heterozygous familial hypercholesterolemia (HeFH) (32). These disorders are distinguished by elevated levels of LDL-C. Inclisiran functions by specifically targeting the mRNA responsible for producing proprotein convertase subtilsin/kexin type 9 (PCSK9), a protein involved in lipid metabolism and the control of cholesterol levels (32). This action leads to a reduction in LDL-C levels. Inclisiran has shown efficacy in lowering LDL-C levels in individuals who have not achieved sufficient reduction with statin monotherapy. In addition, inclisiran has shown enhanced effectiveness in reducing LDL-C levels when used with statins in individuals who have only seen partial success with statins alone in decreasing LDL-C levels (32).

mRNAs and mRNA vaccine

mRNA is a kind of RNA that is synthesized by transcribing the genomic DNA and functions as a template for protein synthesis. Messenger RNAs (mRNAs) are generally around 2 kilobases (kb) long and are characterized by the presence of a 5′ cap, a 5′ untranslated region (UTR), a coding region, a 3′ UTR, and a poly(A) tail. Messenger RNAs (mRNAs) are very suitable options for addressing disorders that have a well-established genetic basis (33). Historically, messenger RNAs (mRNAs) have been used in replacement treatment to address disorders resulting from inadequate production of a certain protein. Furthermore, CRISPR–Cas-based mRNA treatments have the potential to rectify DNA mutations that result in non-functional downstream products. The mRNA vaccination is an innovative technique that combines principles from molecular biology and immunology (34). The technique is intricately linked to gene therapy. The exogenous mRNAs encoding antigens are delivered into somatic cells to facilitate the synthesis of antigens via the expression system. Synthetic antigens can elicit an immunological response. In 1990, scientists used mRNA expression vectors to directly introduce mRNAs into live mouse somatic cells to express luciferase, beta-galactosidase, and chloramphenicol acetyltransferase (35). In 1992, Jirikowski et al. discovered that the introduction of mRNAs encoding oxytocin and vasopressin into genetically mutated mice with diabetes insipidus resulted in a temporary reversal of the condition within a few hours after injection (36). Despite the great results that were revealed thereafter, we did not make any significant advancements in mRNA investigations. The problems included mRNA instability, heightened immunogenicity, and the absence of an efficient mRNA delivery method (37).

Over the course of these decades, more investigations and advancements in experimental methodologies have led to advancements in the safety, effectiveness, and industrial-scale manufacturing of mRNA vaccines. The benefits of mRNA-based vaccinations make them a top priority for treating cancers and viral illnesses. Initially, mRNA vaccines are safe in stimulating the production of antibodies during phase I clinical trials in humans (38). The rationale for this is that mRNA does not possess the ability to replicate itself. The mRNA vector does not possess any traits associated with antibiotic resistance, genomic integration, or

significant immunogenic reactions. In addition, nucleases efficiently break down single-stranded RNA. While the degraded mRNA components stimulate an overactive immune system response, the development of a modified mRNA delivery mechanism may improve effectiveness and prevent any adverse effects (39).

Furthermore, enhanced treatment effectiveness is achieved by the use of modified mRNAs and mRNA carriers. The nucleoside-modified mRNA vaccination for HSV-2 (herpes simplex virus 2) reduced the amount of virus present in viral illnesses. Mannose-functionalized liposomes were used for the intracellular delivery of mRNA. The vector safeguarded the mRNA from degradation and enhanced mRNA overexpression by increasing the expression of mannose receptor (CD206) on the surfaces of cells (40). Currently, much research has been conducted on several types of delivery vectors and modified mRNAs to assess their therapeutic effectiveness, particularly in the context of the COVID-19 pandemic. Manufacturing mRNA vaccines on a big scale is often industrialized. Mass manufacturing depends on translational research, which is crucial for increasing production speed. In laboratory settings, translational technology efficiently chooses formulations and structures in preclinical and clinical investigations (41, 42).

Presently, two mRNA vaccines have received approval from the FDA, while one is currently undergoing clinical testing. BNT162b2 was the first mRNA vaccination authorized by the FDA. The partnership between Pfizer and BioNTech resulted in the development of this vaccine, which aims to induce a strong immune response and production of antibodies against SARS-CoV-2, the virus responsible for causing COVID-19. This vaccine candidate underwent clinical trials in both Germany and the US, and the results showed a considerable reduction in the likelihood of developing COVID-19. BNT162b2 codes for the complete membrane-anchored spike (S) protein, which has two minor changes to enhance its conformational stability (43). The second mRNA vaccine authorized by the FDA is mRNA-1273. This vaccine contains the perfusion-stabilized S protein of SARS-CoV-2, together with the S1-S2 cleavage site. Like BNT162b2, the mRNA-1273 vaccination was produced by Moderna to prevent the acquisition of COVID-19. Both FDA-approved mRNA COVID-19 vaccines include 1-methyl-pseudouridine, which serves to inhibit the detection of the innate immune system, while simultaneously enhancing the capacity of the mRNA to be translated. Furthermore, both of them are enclosed by lipid nanoparticles. Moreover, both vaccines have shown substantial efficacy in preventing SARS-CoV-2 infection, while also upholding rigorous safety protocols. It is important to acknowledge the remarkable pace at which both vaccinations were produced. Thus far, no other vaccine has been created this quickly while yet preserving effectiveness. This emphasizes the therapeutic benefit of using RNA-based vaccinations in the treatment of life-threatening illnesses (44).

Aptamer

Nucleic acid aptamers are a unique group of synthetic polymers or oligomers made up of single-stranded ssDNA or RNA molecules. They possess the ability to attach to a particular target by creating secondary and/or tertiary structures. The term “aptamer” is derived from the Latin word “aptus”, which means “to fit”, and the Greek word “meros”, which means “particle”. It was selected to depict the “lock and key model” that characterizes the interaction between aptamers and their binding targets. Aptamers have strong binding affinity and selectivity towards a diverse array of targets, including proteins, peptides, small compounds, metal ions, bacteria, viruses, and even living cells. The development of aptamers originated in 1990 via an experiment conducted by Tuerk and Gold, using the systematic evolution of ligands in an exponential enrichment (SELEX) technique. The aptamer selection technique used the SELEX approach, which included using purified target molecules and starting with a vast library of random oligonucleotides. The oligonucleotides with high affinity for the target molecule were chosen from the original library using a process that included repeated cycles of binding to the target, selection, and amplification.

Aptamers are a potentially valuable group of molecules that serve as the chemical counterparts of antibodies. Monoclonal antibodies (mAbs) are widely acknowledged as very effective instruments in contemporary medicine for both therapeutic and diagnostic purposes. Aptamers have many advantages over typical antibodies, including exceptional chemical stability and efficient large-scale chemical synthesis. Furthermore, they may be manufactured in huge quantities at a minimal expense, while maintaining a high level of consistency and dependability. Aptamers may exert their effects via three primary mechanisms: (1) Aptamers with cell-type specificity can transport therapeutic agents to the desired tissue or cells; (2) Aptamers can function as agonists, activating their target molecules; (3) Aptamers can act as antagonists, inhibiting the association of molecules involved in disease processes.

Pegaptanib is the first aptamer to get FDA approval and is used in the treatment of neovascular age-related macular degeneration. This condition is distinguished by the deterioration of the retina, resulting in a loss of eyesight. This disorder has been linked to elevated levels of vascular endothelial growth factor (VEGF). Thus, anti-VEGF therapy was considered a very effective

approach for managing this condition. Pegaptanib had a strong attraction to VEGF and effectively sequestered it, hence inhibiting its ability to attach to its receptor. Following successful clinical studies demonstrating the efficacy of pegaptanib in improving or halting eyesight loss, the FDA approved its usage in 2004.

In 2020, the FDA approved Defibrotide for the treatment of hepatic veno-occlusive disease/ sinusoidal obstruction syndrome. This is a potentially fatal complication that may occur as a result of chemotherapy and hematopoietic stem cell transplant (HSCT) conditioning. Defibrotide has been shown to enhance the stability of endothelial cells by decreasing endothelial cell activation. This protects endothelial cells, preventing any more harm and thereby reversing this situation. Both FDA-approved medications have negligible side effects and underscore the potential of aptamer-based therapies.

Ribozymes

Ribozymes, which are RNA molecules with catalytic properties, may selectively break down certain target mRNAs. They are also considered a kind of RNA therapy. Catalytic RNAs were first discovered in the early 1980s, which led to a surge of research into gene expression inhibitors based on nucleic acids. In the late 1980s and early 1990s, researchers established simplified catalytic motifs. This breakthrough allowed for the chemical synthesis of ribozymes, which are molecules capable of inhibiting gene expression in a precise manner. Ribozymes function as catalysts that facilitate the cleavage or synthesis of covalent bonds, enhancing certain processes with or without the involvement of proteins. Over 10 ribozymes primarily catalyze generic acid-base reactions, however the specific mechanisms differ. The general base causes deprotonation at the 2′-OH position to activate the 2′-O− group, whereas the general acid protonates the 5′-O of the leaving group. After the reaction concludes, the 3,5-phosphodiester link at the cutting site is cleaved, resulting in the formation of covalent bonds between 2′-O− and 3′-phosphoric acid. Instead of the mRNA being divided into two fragments, this process occurs. Translation is hindered due to mRNA degradation, resulting in the inhibition of gene expression.

Ribozymes occur naturally but may also be purposefully designed to selectively recognize and bind to certain sequences either on the same nucleic acid strand (cis) or on a noncovalently linked nucleic acid (trans). The phrase “hammerhead” ribozyme refers to a collection of tiny ribozymes that can cleave themselves, and they are generated from single-stranded plant viroid RNAs. The hammerhead ribozyme consists of 30 nucleotides and its simple structure makes it a very suitable option for the creation of trans-acting ribozymes. Through the use of Watson-Crick base pairing, it is possible to manipulate it to specifically cleave any desired RNA target. The “hairpin” or “paperclip” ribozyme is a ribozyme that is derived from plants and is capable of self-cleavage. It has been found in the negative strand of tobacco satellite RNA.34 The hairpin ribozyme catalyzes a reversible process that cleaves RNA substrates, resulting in the formation of 2′,3′-cyclic phosphate and 5′-hydroxyl termini. Through the process of engineering, it is capable of cleaving and converting multiple copies of various targets in the trans form, which refers to a noncovalently linked nucleic acid. Furthermore, the hairpin ribozyme has been used to facilitate ligation processes.

Ribozymes are molecular entities that possess the ability to catalyze chemical reactions. Like protein enzymes, they need precise folding into a well-organized tertiary structure to carry out their tasks. The nucleophilic attack group and the reactive phosphorus atom of the nucleoside hydrolase ribozyme are situated inside the same nucleotide, facilitating the creation of their active structure. In the case of big ribozymes, the nucleophilic attack group and the sensitive phosphorus atom are either located at a significant distance from each other or exist in separate molecules. Consequently, the identification of the cleavage site and the correct placement of the substrate in the active site are more intricate, sometimes necessitating the involvement of metal ions or protein components. For instance, the presence of the magnesium ion plays a crucial role in the interaction between the RNase P ribozyme and the substrate, as well as in the stability of its active center. Nevertheless, the precise targeting of ribozyme activity is a significant obstacle that restricts the practical use of ribozymes. Ribozymes typically identify their cleavage sites by base pairing, however, certain ribozymes may tolerate minor mismatches.

Final thoughts and prospective developments

RNA molecules are very versatile and have a wide range of activities. The use of RNA therapies in clinical settings for the treatment and prevention of human illnesses has shown substantial promise in cutting-edge research. In addition, the process of developing RNA therapies is comparatively easier, faster, and more cost-effective than the creation of standard pharmaceuticals based on proteins and small molecules. How RNA treatment techniques target different clinical problems might vary. For example, siRNAs have a high degree of specificity and selectively target a single mRNA molecule. As a result, they are very efficient in treating diseases that result from mutations in a single gene. However, miRNAs are well-suited for treating disorders that include many pathologies and/or gene alterations since they may target different mRNAs. The field of RNA therapies is now faced with the

following challenges: (i) Cell specificity refers to the ideal characteristic of an RNA therapeutic molecule that selectively targets certain cells without causing unintended effects on other cells or undesirable effects on the intended target cells. (ii) One of the major obstacles in RNA treatments is effectively and consistently delivering the molecule to the exact kind of cell it is intended for while ensuring that the delivery agent remains functional and capable of carrying out its intended purpose.

Emphasizing early study design in clinical trials is crucial to mitigate potential bad outcomes, such as acute toxicity. In addition, this first research design needs to prioritize in vivo functional assays over just relying on in vitro functional tests. Moreover, a crucial aspect of RNA therapy development is to evaluate the clinical results about their efficacy in addressing the mechanistic factors. To achieve this objective, it is necessary to thoroughly assess the RNA therapeutic candidates, specifically in terms of their immunological tolerance, pharmacokinetics, and pharmacodynamics. RNA therapeutic compounds are often designed using the cellular and molecular pathways that cause illnesses. As a result, these molecules are well-positioned for future clinical trials. The existing gaps in knowledge need the adoption of a contemporary strategy to comprehensively comprehend the cellular and molecular mechanisms behind diseases. This understanding will facilitate the development of treatment strategies aimed at not only ameliorating symptoms but also addressing the precise etiology of the illness. Despite the existing barriers in RNA therapy development, the combination of innovative multidisciplinary methods, advancements in contemporary science, and enhanced early study design for clinical trials may ultimately overcome these problems shortly. This will provide significant optimism for the practical use of RNA therapies in many disease states and result in an improved standard of living for millions of patients.

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Authors's Contribution

Farnaz Roshan Mehr: data curation; editing and review. Fatemeh Gabeleh and Roshanak Jazayeri: investigation and writing. All authors read and confirmed the final manuscript.

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