

# RNA Drug As Therapeutic Agents: A Review Based On Literature.

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**Received:** 05-April -2023

**Accepted:** 20 October 2023,

**Online:** 15 Feb 2024

## Abstract

Small RNA targeted drugs have received significant interest from the public and researchers in the last decade due to their capability in treating different illnesses. RNA drugs corresponds to the small molecules which interact with and regulate the expression of particular genes at the level of RNA. Unlike ordinary small molecular chemicals, RNA drugs act directly on the nucleus and gene to produce very efficient and effective curative functions. Some of the RNA drugs are siRNA, ASOs, and mRNA, although the last one is used in vaccines. Both compounds, SiRNA and ASOs, act by attaching to the target mRNA chain and, as a result, the latter can be degraded or prevented from being translated into a protein. On the other hand, mRNA vaccines involve introducing a particular mRNA sequence into cells, and the outcome is that cells synthesize a certain protein that can be a viral antigen to spark an immune response. The RNA drugs have demonstrated quite significant outcomes with initial trials for diseases like cancer, genetic disorders as well as certain infections. For instance, siRNA based drugs are introduced for the treatment of hereditary transthyretin amyloidosis which is a rare genetic disease and ASO based drugs for spinal muscular atrophy. mRNA vaccines have also been developed to fight against the epidemic such as COVID-19. However, there are few major issues that have to be considered with regard to RNA drugs These are: These are; problems of drug delivery and stability; and issues to do with side or off-target effects. Despite of these hurdles, RNA drugs are a promising new category of drugs for diseases that can be treated by this technique. As the research work in the field of RNA medicine grows, the public might observe more RNA-based drugs being approved for the clinical use in the future years.

### AUTHOR'S CONTRIBUTION:

Concept & Design of Study: MM,SR Drafting: RA ,Data Analysis::ZR, Critical Review: TAK

Final Approval of version: SR

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(2024). RNA Drug As Therapeutic Agents: A Review Based On Literature.: Review Article. Pakistan Journal of Advances in Medicine and Medical Research, 2(01), 93–101.

<https://doi.org/10.69837/pjammr.v2i01.28>

### Introduction

RNA therapeutics A substantial number of studies conducted over the last couple of decades, have clearly identified RNAs as drug targets and this consequently has contributed to our understanding that RNA-based drugs will be effective in treating a multitude of disease conditions. RNA drugs work by targeting the genetic material that encodes proteins, as opposed to protein-based traditional small molecule drugs which are designed based on known structure-function gradients. siRNA and mRNA are the two primary classes of RNA drugs. Functionally, siRNA binds to and destructs the matching messenger RNA (mRNA) type which encoded for a specific protein. Instead, the mRNA provides cells with directions for creating specific proteins that may help address diseases caused by protein shortages. RNA drugs have immense performance in the treatment of various diseases like cancer, virgin disorders and viral infection. The first RNA drug was approved by the US Food and Drug Administration (FDA), for Onpattro in 2018, which treat a rare genetic disorder called hereditary transthyretin-mediated amyloidosis. Since then, many more RNA drugs have been approved or are being studied in clinical trials including mRNA vaccines for COVID-19. RNA drugs come with great expectations and new challenges related to their development and delivery. RNA is a fragile molecule that can be easily broken down in the body, so However, delivering the protein to the appropriate cells or tissues is often a challenge. Furthermore, RNA drugs may also trigger off-target effects as well as immune responses in a subset of the patients. Among these yhup, the promise of RNA drugs to transform the treatment options for multiple diseases is spurring intensive research and development in this space. It seems likely that, as our knowledge

of RNA biology and the features supporting it being druggable expands, this class will still expand significantly in its reach within medicine.

### Ancient and Modern RNA Therapeutics Strategies:

RNA therapeutics have come a long way since then, and advances in technology and new methods all contribute to the development of RNA drugs as safer, more efficacious tools. Both traditional and new methods utilized in the development of RNA drugs will be explained fleshing it out further in this answer.

RNA Program Development initially targets identification - design and synthesis of RNA molecules. Antisense oligonucleotides (ASOs), designed short, synthetic RNA molecules that bind to messenger RNA(mRNA) and block production of disease-causing proteins were initial class of therapeutic agent developed. ASOs were initially made by chemically synthesizing them, via a process that took place in multiple steps as each nucleotide building block was added one at the time to obtain the desired sequence. It was a long difficult operation that required time and effort by thepound to give low or poor yield-sensitive pure products. In vitro transcription (IVT) was an alternative method of RNA synthesis in which large amounts of RNA were produced from a DNA template by using synthesizing enzymes, such as the ones found on T7 and SP6 bacteriophages. IVT is a straightforward, scalable approach, but the resulting RNA can be impure and trailed by undesired contaminants that impact their efficacy and safety. The Next Challenge: Drug Delivery Drug delivery was one of the biggest

hurdles when it came to RNA drug development. Previously, RNA molecules have been delivered using cationic lipids/polymer which were shown to be toxic and immunostimulatory.

### **Novel Strategies for RNA Drug Discovery:**

Rapid development of RNA synthesis, modification and delivery technologies has created new opportunities for the discovery of effective RNA drugs.

#### **RNA Synthesis:**

For example, solid-phase synthesis is a method where the RNA molecule is chemically synthesized on column using an automated synthesizer. It is a high-yield, top-quality RNA molecule synthesis method.

#### **RNA Modification:**

Since RNA molecules have an inherently short half-life, chemical modifications to these RNAs can also allow for greater product stability and possibly efficacy. One such change is replacing units with locked nucleic acids (LNAs) to modify human RNA for a better binding and more specific mRNA target.

#### **Delivery:**

In conclusion, and perhaps most importantly, new cutting-edge delivery systems have been developed as part of the latest breakthroughs in RNA drug delivery. One of these is by using lipid nanoparticles, which serve as a vehicle that stabilize RNA molecules from degradation and help ferry them into the cells you are trying to target. Lipid nanoparticles are highly versatile, can be readily manufactured and have demonstrated efficacy in both preclinical animal models as well as humans in clinical trials.

In conclusion, the history of RNA drug development is a story of advancement as new and improved formulations for synthesis, modification & delivering have shaped this field over time. These advances enhance the accuracy and safety profile of RNA-based medicines, making them highly promising for treating a wide variety of diseases.

### **RNA Drug Modifications**

RNA drugs, also called nucleic acid therapeutics, target the RNA of cells in order to address different diseases]. These drugs can be developed to silence, or tone up the expression of certain genes. There are many classes of structural modifications that can be introduced in RNA drugs to enhance their efficacy, stability and specificity. Notably, for 2'-O-methyl groups (position of remdesivir) an ethoxy acetal group was developed (~590), to enable a visible "kink" when in the conformation found in RNA and ~45% higher basicity at greater pH values than Ph; these modifications are also less luminescent with colloidal binding [64]. 2'-O-methyl modification adds a methyl group at the 2' position of ribose sugar in an RNA molecule. This alteration improves the stability and target specificity of RNA drugs, while protecting them from nuclease degradation. 2'-OMe-modified RNA is less immunogenic as well.

#### **Phosphorothioate Chemical Substitution:**

Phosphorothioate modification is replacement of one non-bridging oxygen atoms in the PO<sub>4</sub> internucleic linkage within an RNA molecule, phosphorous must be linked with sulfur. This modification provides greater stability and a reduced ability of the RNA drug to be digested by nucleases. Phosphorothioate modification provide enhanced cellular uptake and tissue distribution of RNA.

### **Locked Nucleic Acid (LNA) Modification**

A type of chemical modification to RNA is the locked nucleic acid (LNA) where some nucleotides are replaced by LNA during its synthesis. LNA nucleotides contain a methylene bridge connecting the 2' oxygen and 4' carbon atoms, which confines the sugar ring in an opposite predetermined conformation. These modifications also make the RNA drug more stable, allows it to be specific as well as increase affinity of the RNA drug for its target.

### **Peptide Nucleic Acid (PNA) modification:**

PNAs are a type of PNA oligomer which replaces the conventional RNA sugar-phosphate backbone with special peptide-like units. By introducing this modification the stability, specificity and binding affinity of the RNA drug to its target is enhanced along with improved resistance towards nuclease degradation. PNA modified RNA is also not likely to activate an immune response.

### **Methylation of 5-Methylcytosine (5-mC)**

This involves the addition of a methyl group to the 5 position in cytosine base within an RNA molecule and is referred as 5-mC modification. These changes provide greater stability and specificity for the RNA drug, while increasing resistance to nuclease degradation. Moreover, 5-mC-modified RNA is more readily taken up by cells in vivo and also displays improved tissue distribution.

### **Unnatural Base Pair (UBP) Edit:**

Modification of unnatural base pair by insertion into transcribed RNA Synthetic nucleotides that can base pair with one another as well as naturally occurring nucleotides, thus opening up possibilities for the synthesis of RNAs possessing unique properties. The modification can stabilize the structure and enhance specificity or affinity,

potentially allowing it to perform new functions. Therefore, these changes apart from increasing potency can combine to provide improved stability and specificity of RNA drugs that together make them important tools for the treatment of different diseases.

### **RNA Drug Delivery Methods**

**Chemical Approaches to RNA Delivery for Therapeutic Mechanisms** RNA drug delivery is a fast-growing area and many chemical strategies have been developed to transport RNA therapeutics into cells or tissues. There are two major groups of RNA molecules being used in the field: siRNA and mRNA.

#### **Chemical methods in RNA drug delivery Lipid-based delivery systems:**

These use cationic lipids to complex with the negatively charged RNA molecules [ 180] aiding in cellular uptake. RNA stability, cell type targeting and nuclease protection can be improved by designing these delivery systems.

#### **Polymersome Delivery systems;**

These include the encapsulation of RNAs within biodegradable or non-biodegradable polymers, enabling protection from degradation. Moreover, such delivery systems can be engineered to deliver RNAs that target specific types of cells and release RNA molecule under regulated conditions.

#### **Delivery systems based on peptides**

They include cationic peptides that are able to bind reversibly with RNA molecules, resulting in complex formation and an increase in their biological uptake. Furthermore, other peptide-based delivery systems can be formulated to increase RNA stability and cell-specific targeting.

### **Delivery systems based on aptamers:**

These included the use of aptamers-small strands of either RNA or DNA that can bind to targets. The structurally diverse nature of various aptamers allows for the possibility to use their specificity in targeting particular cell types or tissues by selecting appropriate aptamers that strongly bind to certain receptors located on cellular surface, membrane proteins and other biomolecules.

### **Inorganic nanoparticles:**

For example, the combination of nanoparticles and RNA encapsulation for cell uptake (materials here could be gold or silica as well as iron oxide) are being tested. Inorganic nanoparticles can also be functionalized with ligands to target specific cell types or tissues. The delivery of RNA can also be achieved by physical methods, such as electroporation using an electric field to transiently disrupt cell membranes and encourage the absorption of R-NA molecules. Other physical methods used such as ultrasound mediated delivery and microinjection. All three of these approaches have their merits and limitations, with the choice of delivery method being dependent on the RNA therapeutic intended for delivery as well as cell/tissue targets/therapeutic effect required. These are the critical points to be considered in the successful delivery of RNA drugs, along with safety and efficacy parameters of a given delivery system.

### **Physical methods of RNA drug delivery**

RNA therapeutics are potential compounds for treatment of a multitude of diseases such as cancer, viral infection and genetic disorders. Nevertheless, RNA drugs are very unstable and have shown poor cell penetration which is their biggest issue once delivered to target cells. Physical delivery methods have been developed to cope with these, and in this answer I will

discuss some of the most widely used physical delivery approach for RNA drugs.

### **Electroporation:**

It is a method where one applies short electric pulses to open the cell membrane and let RNA molecules in. In this manner, the target is suspended in an RNA solution and subsequently applied with a electric current. The electrical field transiently punches holes in the cell membrane, thus opening paths for RNA molecules to get inside of each cell. Among the other mechanisms used to deliver RNA drugs, electroporation is particularly useful for in vitro and ex vivo applications (to treat cells outside of a patient), such as gene editing with stem cells.

### **Microinjection:**

Thus microinjection enables blunt transfer of RNA molecules through a microneedle directly into the cytoplasm of target cells. This procedure is very accurate and can deliver a large number of RNA molecules to individual cells or tissues. However, this technique is invasive and requires specialized equipment with corresponding expertise.

### **Ultrasound:**

Thus, we developed ultrasound to penetrate the cell membrane and improve RNA uptake by target cells. In this technique, RNA is mixed with micro bubbles and then injected into the blood-stream. During the treatment, ultrasound waves act upon this tissue and cause themicrobubbles to oscillate which can breakthe cell membrane allowing entry of RNAmolecules into the cells. First of all, ultrasound is non-invasive and can be applied on both in vitro,(3) which makes it a very appealing therapeutic framework.

**Magnetofection:**

Magnetofection: Here, magnetic fields are applied to boost the penetration of RNA molecules into target cells. This method involves the mixing of RNA molecules with magnetic nanoparticles and addition to target cells. Next, a magnetic field is applied to the target cells which causes the nanoparticles of iron-oxide core and liposome shallot move towards them and release their RNA molecules. This non invasive technique is appropriate for in vitro and even, at least to some extent, in vivo delivery. Collectively, while physical delivery approaches can provide excellent means to deliver RNA drugs into target cells it has both its pros and cons for each one of them henceforth the selection would largely depend; which system (cell) is being targeted by (use-of-the-drug), also depending on type & other characteristics associated with the space-time complexity RNAs are favorable to be delivered.

**All the illnesses, these are RNA drugs**

Overview RNA drugs or RNA based therapeutics has been a buzzword for the past few years, and indeed represents an emerging class of therapeutic arsenal. Small but increasingly large RNA molecules are used to bring the efficacy of drugs home, and can enhance or suppress expression levels for a gene in your body... helping treat diseases.

**Below are examples of RNA drugs for various conditions,**

**Cancer:**

21) Drugs that silence cancer-causing genes, also known as oncogenes have been developed using RNA interference (RNAi) technology. One such drug is Patisiran, an RNAi therapy that goes after the gene for a protein called transthyretin (TTR), which can build up in people and give rise to an ultra-orphan disease

known as hereditary TTR-mediated amyloidosis. Patisiran - for the treatment of hATTR amyloidosis.b) A further RNA drug (mRNA vaccines) has been manufactured to prevent cancer. How do these mRNA vaccines work: Once the injected piece of mRNA inside our body that encodes a specific antigen gets into our circulation, this triggers immune response whereby target cells (cancer cell) get to be killed. Approval has been granted for using mRNA vaccines for the prevention of cervical cancer and they are in development stages to be used as a treatment option against other cancers.

**Genetic diseases:**

Antisense oligos (ASOs) represent one such class of RNA drugs that can be directed against particular genes involved in genetic diseases. ASOs work by binding to the messenger ribonucleic acid (mRNA) that shuttles genetic directions from DNA, which carries disease-causing mutations in this case, to a cell's protein-producing machinery and prevents production altogether or alters it in such manner as can treat the disease. Spinraza is an ASO drug which already exists and has been approved for treating spinal muscular atrophy, a genetic disease that causes muscle wasting.(b) As have single nucleotide genetic diseases that been treated with mRNA therapy as well. According to the company, mRNA therapy introduces a synthetic mRNA molecule into the body (in this case that codes for LCA10 gene GUCY2D) in order to generate enough functional protein missing or defective in patients. An example is the mRNA therapy candidate QR-110 for treating a rare genetic disease called Leber congenital amaurosis 10 (LCA10), also leading towards blindness.

### Infectious diseases:

A) mRNA vaccines for infectious diseases like Covid-19, Influenza and rabies. The mRNA vaccines work with the introduction of a small piece of messenger RNA (mRNA) in the body to encode for some antigen from pathogen, and elicit immune response on human immune system. (b) RNA aptamers are small pieces of RNA, which act like drugs and bind to select proteins on the surface of pathogens thereby blocking their entry into host cells or affecting function. For instance, an RNA aptamer (Nox-A12) is designed to bind a protein called CXCL12 and thereby prevent its functions in the growth and spreading of some cancers as well as maintaining life support for certain viruses such as HIV or SARS-CoV-2. To sum it up, RNA drugs have a bright future in treating many different diseases (e. g., cancer genetic disorders and infectious illnesses etc.). These drugs that work by targeting disease-specific genes or proteins in the body can up-regulate, down-regulate, and modify their expression such as to treat a certain type of disorder eventually even curing it.

### Conclusions and prospective for the development of RNA drugs.

RNA drugs, also called RNA-based therapeutics are an emerging class of therapeutic molecules for manipulating gene expression and protein production to treat a variety of diseases such as cancer, genetic disorders or infectious disease. There are two main classes of RNA drugs being developed to treat diseases-small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs). siRNA drugs degrade targeted messenger RNA (mRNA) molecules and block the production of specific proteins. In contrast, ASOs bind to particular mRNA molecules and obstruct their translation or splicing. Preclinical and clinical results of both types of therapeutic RNA drugs have been encouraging. Fomivirsen,

an antisense oligonucleotide (ASO) targeting CMV mRNAs is the first RNA therapeutic to receive marketing approval from regulatory authorities commencing with CV in 1998. These RNA drugs are subsequently from these medications till Phase III of a clinical trial and approved to enter the market: three hereditary transthyretin amyloidosis siRNA-based drugs, primary hyperoxaluria type 1. Although RNA drugs hold great promise there are still some hurdles that need to be tackled in terms of their development and clinical use. Delivery is a big hurdle since RNA drugs are large, negatively charged molecules that can be broken down by enzymes and quickly flushed from the body. However, changes in delivery technologies including lipid nanoparticles enabled better endosomal release and increased RNA drug stability. One of the most significant issues are off-target effects - where RNA drugs might interact with a different mRNA molecule and cause changes in protein expression. This would result in off-target toxicity and a narrow therapeutic window of RNA drugs. Common strategies to minimize off-target effects, such as RNA drug design for desired specificity and modified nucleotides which improve target selectivity are employed. Such future directions include the generation of new classes of RNA-based therapeutics, for instance - a circular RNAs and riboswitches as well as advancements in delivery technologies that can lower off-target effects through promoting specificity to increase therapeutic efficiency. The emerging field of personalized RNA drugs, designed through the sequence-based customization for specific genetic mutations and expression patterns in individual patients is also looking promising to lead proliferations thereafter (9). Overall, RNA drugs are an up-and-coming field with significant advancements ahead that completely change the way many diseases can be treated. There are still challenges but with the research and development

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under way, it seems promising that more advances will be made.

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**Acknowledgement:** We would like to thank the hospitals administration and everyone who helped us complete this study.

**Disclaimer:** Nil

**Conflict of Interest:** There is no conflict of interest.

**Funding Disclosure:** Nil



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