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Review Article

RNA-Based Therapeutics: From mRNA Medicine to siRNA and Antisense Therapies

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ABSTRACT

RNA-based therapeutics have revolutionized medicinal chemistry. They include multiple techniques such as antisense oligonucleotides, RNA interference and messenger RNA, which regulate gene expression and provide innovative treatment strategies. These effects can be achieved by enhancing gene expression, suppressing gene expression or through self-replication of RNA.

RNA-based therapeutics can be categorized into three groups according to their mechanism of action. The first group targets nucleic acids using, for example, antisense oligonucleotides (AONs) or the RNA interference (RNAi) pathway. The second group of RNA drugs targets proteins using RNA aptamers. The third group comprises mRNA drugs that encode proteins. These innovative successes, along with the ever-expanding trials of RNA-based drugs, vaccines and cellular therapies, suggest that RNA-based therapies may soon become part of routine clinical practice.[1]

Keywords: RNA therapeutics, RNA-targeting therapeutics, RNA (ribonucleic acid), Small interfering RNA (siRNA), Oligonucleotides, Antisense RNA/antisense oligonucleotides (ASOs), Messenger RNA (mRNA), RNA interference, Lipid nanoparticles, Targeted drug delivery, Gene therapy, Drug development.

1. INTRODUCTION:

Traditional drug therapies (such as small molecules or protein based) have been used to help prevent and treat many diseases. However, not all diseases can be solved using traditional medicines. In some cases it can be difficult to target certain disease associated proteins. Other diseases are caused due to incorrect gene expression. Traditional Medicines may have multiple targets, can have undesired side effects or need to be administered multiple times. Due to these issues scientists have turned to looking at drugs that can target the genetic information.[2]

Nucleic acid therapeutics have been developed to do this. Rather than targeting a protein through binding with a drug, they target or use DNA/RNA to manipulate a biological pathway. RNA is a very useful target as it naturally transports genetic messages, is involved in protein synthesis and is involved in regulating gene activity. By designing a specific RNA molecule researchers can get cells to express a required protein, decrease the expression of a disease associated protein or alter the processing of the genetic message. This means RNA can be used in a multitude of ways.

There are three main types of RNA therapeutics: messenger RNA (mRNA), small interfering RNA (siRNA) and antisense oligonucleotides. Therapeutic mRNA provides a genetic message that can be translated within cells to create a desired protein. This is the principle behind mRNA vaccines, but is also being explored as a treatment for replacing proteins. siRNA works by using a naturally occurring pathway within cells known as RNA interference to reduce expression of a specific mRNA thus reducing production of the corresponding protein. ASOs are short, designed strands of nucleic acids which bind to complementary RNA. They may cause degradation of the RNA or change how it is processed.[3]

With improvements in RNA chemistry and delivery systems, it is becoming clear how useful these different types of RNA can be. mRNA vaccines have shown how genetic information can be delivered to cells to produce antigen, to induce an immune response. Inclisiran is an example of siRNA being used clinically in cardiology to reduce PCSK9 production in the liver. RNA-based therapies are being studied for a variety of diseases such as genetic disorders, neurology, cancer, infectious diseases and other health issues. It is not always the case that all RNA therapies will be appropriate for all diseases. The type of tissue, mechanism of action and evidence will dictate which type of RNA will be most appropriate.[4]

However key issues remain to be addressed. RNA may be labile to enzymatic degradation, have poor membrane penetration properties and may not be delivered to the right cells in high enough quantities. Certain RNA species may cause off-target immune activation. Other factors such as tissue targeting, durability of action, quality of manufacture, cost of treatment and long term safety considerations need to be considered. Researchers are looking at ways to chemically modify, improve formulations and increase targeting specificity to overcome these issues.[5]

2. METHODOLOGY/LITERATURE SEARCH STRATEGY:

2.1 Databases and Information Sources

These were used as initial core literature. Searches can be performed on databases such as PubMed/MEDLINE, Scopus, Web of Science and Embase to increase the amount of evidence available on the topic. Google Scholar, Clinical trial registries and reference list from

relevant reviews and research papers could also be useful. A record of the dates of search, what database was used and how many articles were found should be noted.

2.2 Search Strategy

The search should be based around the general topic, key therapeutics, delivery methods and safety of the topic. This will depend on the database searched, the search terms can vary depending on indexing terms and syntax. Additional search terms were "RNA interference", "chemical modification", "lipid nanoparticles", "gene silencing", "vaccine", "cardiovascular disease", "neurological disorders" and "genetic disorders". The Boolean operator AND narrows the search by requiring terms from both groups, whereas OR broadens it by including any of the related terms. Truncation symbols, inverted commas and controlled vocabulary should be used according to the rules of each database.

2.3 Inclusion Criteria

- Peer-reviewed primary research, review articles and clinical articles.
- Articles published in English.
- Studies directly related to mRNA therapeutics, siRNA, ASOs or RNA delivery systems.
- Pre-clinical and clinical studies of mechanism, formulation, delivery, safety and efficacy, and pivotal studies on the history of antisense technology, RNA interference and modified RNA.

2.4 Exclusion Criteria

- Duplicate records.
- Studies that mention RNA but are not relevant to therapeutic nucleic acids.
- Studies with too little information to determine relevance.
- Promotional material (unless useful as additional context).
- Retracted publications (unless discussing the retraction).

2.5 Study Selection

Once the search was completed, all records from the databases were combined and duplicates were removed. Titles and abstracts were screened against the inclusion and exclusion criteria, and the full text of relevant articles was assessed. Reasons for excluding full-text articles should be documented. The final report should record how many records were identified, how many duplicates were removed, and how many records were screened, assessed in full text and included. If the work is designed as a systematic review, the PRISMA guidelines should be followed and a flow diagram included; all numbers must reflect the real screening process.

3. BACKGROUND AND EVOLUTION OF RNA BASED THERAPEUTICS:

3.1 RNA as a Therapeutic Molecule

RNA is composed of smaller units called nucleotides. Each nucleotide consists of a ribose sugar, phosphate and one nitrogenous base (adenine, uracil, cytosine and guanine). DNA is responsible for storing genetic information while RNA plays a rolein regulating information. Many RNA molecules are single stranded however, they can form structures which interact with proteins and other nucleic acids.

There are different types of RNA with various functions. For example, messenger RNA has coding instructions that are read by ribosomes to create proteins. MicroRNA helps regulate gene expression. RNA based drugs need careful designing as their sequence and chemical structure will affect how they act within the body. RNA can easily be damaged by nucleases and may not be taken up efficiently by cells.[6]

3.2 Evolution of RNA Therapeutics

The first development of RNA therapeutics was based on a designed nucleic acid sequence that can specifically target complementary genetic sequences. In 1978, Zamecnik and Stephenson showed that a specific oligodeoxynucleotide could inhibit Rous sarcoma virus replication and transformation. This study helped develop the antisense principle where a short man-made nucleic acid sequence can target and interact with a complementary sequence to block activity.

A further milestone was achieved in 1998 where Fire and colleagues showed double stranded RNA could produce potent specific genetic interference in Caenorhabditis elegans.[7]

Researchers also started developing mRNAs to deliver messenger to cells to make a specific protein. The Further development of the design and formulation of mRNAs have allowed them to become a potential drug platform. Delivery technologies have also been vital in allowing these to move from research to clinical applications. Lipid nanoparticles can be used to protect nucleic acids and help them enter cells. Other methods including chemical modifications or attaching oligonucleotides to targeting molecules can also help improve stability or delivery to specific tissues. However the strategy must be tailored to the therapeutic molecule. An mRNA medicinal product that is being used to make a protein will have different requirements to an siRNA or ASO that is targeting existing RNA.[8]

RNA therapeutics are being developed to improve selectivity, longevity, safety and availability. Key developments include improving delivery to tissues outside the liver, decreasing immune stimulation, understanding of off-target effects an robust manufacturing methods.[9]

3.3 Major Classes of RNA Based Therapeutics

3.3.1 Messenger RNA (mRNA)

mRNA therapeutics deliver a coded message to the cells so that it can be translated to a specific protein. Depending on what the protein is required for it can beused as an antigen in a vaccine formulation, replace a protein not created properly bythe body orhave another desired therapeutic effect. A benefit of using mRNA is that the coding sequence can be altered to target different molecules however we must consider the stability, translation efficiency, immune stimulation and delivery to the correct cells. Modifications to nucleosides and lipid nanoparticle formulations aresome methods used to improve the performance of the drug.

3.3.2 Small Interfering RNA (siRNA)

Small interfering RNA therapeutics aim to reduce expression of a specific gene. When entering a cell, the guide strand of siRNA binds to the RISC complex, where the complex can identify the target mRNA by sequence complementarity. The RISC complex can cause cleavage of the mRNA resulting in reduction of protein expression. Inclisiran is an example of this, targeting the production of PCSK9 in the liver, therefore reducing the levels of LDL cholesterol. Targeted therapy, efficient delivery, longer lasting and lack of off target effects/immune response should be considered.

3.3.3 Antisense Oligonucleotides (ASOs)

Antisense oligonucleotides are short synthetic nucleic acid sequences that bind tocomplementary RNA sequences. Depending on the chemistry used and design, the antisense oligonucleotide can cause recruitment of RNase H which will cause degradation of the target RNA. Alternatively, they can inhibit a particular interaction or affect splice sites of pre-mRNA. Antisense oligonucleotides are a useful choice for treating certain genetic and neurological conditions. The efficiency of antisense oligonucleotides depend on the chemical stability, targeting the right tissue, entering the cell and targeting the correct area. ASO chemistry and delivery are an active areaof research to improve therapeutic effects.

Overall, these three technologies have similar aims but different functions. mRNA contains coding information for a protein to be expressed. siRNA utilise the RNA interference pathway within a cell to reduce expression of a target gene. ASOs bind to RNA to affect stability and processing. This means they will be suitable for different diseases depending on their differences, delivery requirements and safety considerations.

4. mRNA:

The term mRNA means messenger RNA, which is a ribonucleic acid that carries genetic instructions to cells to make proteins. Our cells reserve genetic information inDNA, but to produce proteins our body cannot directly use DNA so the information from the DNA is transcribed into messenger RNA that is used as template for protein synthesis in the process of translation. Scientists use this natural process to prepare an mRNA molecule that transmits instructions for production of particular protein inside a targeted cell, then this mRNA is translated into protein to perform its desired biological function. For example, mRNA vaccines instruct cells to produce a selected antigen, which stimulates an immune response.[10]

4.1 Structure of mRNA

An mRNA molecule has five important structural components (Figure 1):

1. 5′ cap.
2. 5′ untranslated region (5′ UTR).
3. Coding sequence (CDS).
4. 3′ untranslated region (3′ UTR).
5. Poly(A) tail.

4.2 Synthetic and Modified mRNA

Synthetic mRNA is being designed for better therapeutic efficiency. Modifications reduce innate immune activation and improve stability, and codon optimization increases translation.[11]

4.3 Therapeutic Uses

Optimization and UTR modifications improve translation efficiency and protein production, making mRNA therapeutics more effective.

4.4 Delivery Systems

mRNA is unstable and cannot easily cross cell membranes, so adequate delivery systems must be used. Lipid nanoparticles (LNPs) are commonly used to protect mRNA and enable cellular uptake. Other delivery methods include liposomes, polymer-based carriers and emerging nanotechnology-based systems.

4.5 Applications

mRNA technology has a number of applications in medicine, including:

1. Vaccines: preventing infectious disease.
2. Protein replacement: making proteins that are missing or not working properly.
3. Cancer immunotherapy: boosting immune responses against cancer cells.
4. Gene editing: temporary production of gene-modification tools such as CRISPR-associated proteins.
5. Regenerative medicine: helping tissue repair and regeneration.
6. Inherited disorders: therapeutic protein production as a possible treatment of rare diseases.

4.6 Advantages and Limitations

mRNA therapeutics have the advantages of rapid development, flexible design, transient protein expression and no need for nuclear entry. However, they are also challenged by poor natural stability, temperature sensitive storage, delivery difficulties, high production costs and unwanted innate immune activation.

To summarize, mRNA-based therapeutics offer a promising platform for developing novel therapeutics for infectious diseases, cancer and genetic conditions.[12]

5. siRNA BASED THERAPEUTICS:

Small interfering RNAs (siRNA) form a novel class of nucleic acid based therapeutics which use RNA interference (RNAi) to modulate gene expression. These are short double stranded RNA molecules usually between 20 and 25 nucleotides in length. The RNAi process is illustrated in Figure 2.

They can selectively reduce the expression of disease associated genes. They differ from traditional drugs as they target mRNA rather than proteins. This can provide therapeutic potential for genetic, metabolic and cardiovascular and other diseases.[13]

5.1 Mechanism of Action

Once inside the cell the siRNA is loaded onto the RNA induced silencing complex (RISC). The passenger strand is digested and the guide strand leads the complex to complementary mRNA. A key element of the RISC complex is the Argonaute-2 (AGO2) which will cause cleavage of the target mRNA. This will lead to degradation and inhibition of protein synthesis. This is known as post transcription al gene silencing.[14]

5.2 siRNA Design and Chemical Modification

Careful design of the siRNA sequence is required to ensure high specificity and reduce any off target effects. The addition of chemical modifications such as 2′-O-methyl, 2′- fluoro and phosphorothioate groups increase nuclease resistance, stability and increase the longevity of the therapeutic. Chemical modification can help reduce unwanted immune stimulation.[15]]

5.3 Delivery Strategies

Delivery of siRNA is an issue due to its instability and inability to penetrate cell membranes. Lipid nanoparticles (LNPs) can be used to protect siRNA and aid cellular uptake. N-acetylgalactosamine (GalNAc) conjugation can deliver to liver cells via asialoglycoprotein receptors (ASGPR). Common siRNA delivery constructs are shown in Figure 3.

6. ANTISENSE OLIGONUCLEOTIDE (ASO) THERAPIES:

Antisense oligonucleotides (ASOs) are short synthetic nucleic acid strands that bind to complementary RNA sequences. They regulate gene expression by disrupting mRNA processing or protein synthesis.

6.1 Mechanisms of ASO Action

There are four main mechanisms of ASO action (Figure 4):

1. RNase H-mediated degradation: ASOs bind to the target mRNA and activate RNase H to cleave the RNA, preventing protein production.
2. Steric blocking: ASOs bind to specific RNA regions, blocking access to proteins or ribosomes.
3. Splicing Modulation: ASOs modulate pre-mRNA splicing, controlling exon inclusion or exclusion and helping to correct abnormal RNA processing.
4. Inhibiting translation: ASOs block ribosome binding or movement, decreasing the production of disease- associated proteins.[16]

6.2 Chemistry and Chemical Modifications

Natural ASOs are easily degraded by nucleases. Chemical modifications improve their stability, binding strength and therapeutic activity. The main modifications are:

- Phosphorothioate: Replacesan oxygen atom with sulfur, increasing resistance to degradation.
- 2′-O- methyl and2′- O-methoxyethyl: Improve stability and RNA-binding affinity.
- Locked Nucleic Acids (LNA): Improve binding strength to target RNA.
- Morpholino: Provides high resistance to enzymatic degradation and mainly works through steric blocking.[17]

6.3 Delivery and Tissue Distribution

ASOs are large and negatively charged, making it difficult for them to enter cells. Different delivery methods help overcome this problem.

- Lipid-based systems: liposomes and lipid nanoparticles protect ASOs and improve cellular uptake.
- GalNAc conjugation: helps deliver ASOs specifically to liver cells.
- Other conjugates (for example peptide conjugates) can improve cellular uptake.

ASOs are administered by subcutaneous, intravenous or intrathecal routes, depending on the disease and target tissue.

7. A COMPARISON OF ASO, siRNA AND mRNA THERAPIES:

Gene expression is modulated by RNA therapies through many ways. Therapeutic proteins are produced by mRNA, and certain genes' expression is down regulated or changed by siRNA and ASOs.[18] The main differences are summarized in Table 1.

8. CURRENT CLINICAL DATA:

RNA-based treatments have progressed from exploratory research to licensed medications for metabolic, genetic, and viral illnesses. Their clinical success serves as an example of what RNA technology may accomplish in contemporary medicine.[19]

8.1. Approved RNA-Based Drugs

A. mRNA treatments. Comirnaty and Spikevax are COVID-19 mRNA vaccines. They produce the SARS-CoV-2 spike protein and trigger an immunological reaction. They demonstrate the useof lipid nanoparticles for clinical mRNA delivery.[20]

B. siRNA treatments. Hereditary transthyretin amyloidosis is treated with siRNA therapy using patisiran. Acute hepatic porphyria is treated with givosiran. LDL cholesterol is reduced by inclisiran. Lumasiran is used to treat type 1 primary hyperoxaluria. The medications function by reducing the synthesis of specific proteins associated with illness.[21]

C. ASO treatments. Nusinersen, an ASO treatment for spinal muscular atrophy, modifies SMN2 splicing. Transthyretin production is decreased by inotersen. In amyotrophic lateral sclerosis, tofersen targets SOD1 mRNA. Eplontersen reduces the synthesis of transthyretin. The approved RNA-based therapeutics relevant to this review are listed in Table 4, and representative agents, targets and routes of administration in Table 2.

8.2. Clinical Research

Clinical studies are used to evaluate the efficacy and safety of RNA medications. They proceed through three phases:

- Phase I: determines dose, safety and tolerance.
- Phase II: examines appropriate dosage and early efficacy.
- Phase III: verifies the safety and effectiveness of the treatment in larger patient groups.[22]

8.3. Clinical Efficacy of RNA Therapies

mRNA: COVID-19 vaccines demonstrated the ability of mRNA to elicit protective immune responses. **siRNA:** inclisiran causes long-term decreases in LDL cholesterol, while patisiran reduces the synthesis of transthyretin. **ASO:** nusinersen increases the synthesis of functional SMN protein by altering RNA splicing. These findings

demonstrate that RNA-based medications may provide significant therapeutic benefits in a number of diseases.

8.4. Clinical Outcome Comparison

Although all three platforms have demonstrated clinical benefits, the mechanisms and treatment targets of each platform differ. The comparison is limited by variations in patient groups, treatment outcomes, and clinical trial design. For long-term safety and effectiveness, more research is required.[23]

9. RNA THERAPEUTICS DRUG DELIVERY METHODS:

RNA molecules have trouble passing through membranes and are readily broken down by enzymes. To safeguard RNA, boost cellular absorption, and ensure its release in the target cells, effective delivery methods are therefore required.

9.1. Lipid Nanoparticles (LNPs)

RNA therapies are often delivered non-virally using lipid nanoparticles. They contain lipids to help the cell absorb them and to shield the RNA from deterioration.

Uses: Patisiran and mRNA COVID-19 vaccines. **Benefits** include increased cellular transport, prevented degradation, and improved RNA stability. Limitations: Include buildup in the liver, ineffective endosomal escape, and possible immunological reaction.[24]

9.2 Liposomes

Liposomes are spherical vesicles composed of lipid bilayers that can transport medicinal compounds. They are investigated for mRNA and siRNA delivery.

Benefits: biocompatibility, versatility in formulation, and protection for medicinal compounds.

Limitations: Include stability issues, inconsistent cellular absorption, and poor intracellular release.[5]

9.3 Polymer-based Delivery

Polymer carriers are natural or manmade polymers that bind and transport RNA into cells. Examples include polyethylenimine (PEI) and poly(beta-aminoesters)(PBAEs).

Pros: Tunable characteristics, protect RNA, and probable biodegradability.

Limitations: Include potential toxicity, low RNA release, and restricted clinical applicability.[25]

9.4. N-Acetylgalactosamine (GalNAc) Conjugation

GalNAc conjugation is an effective method for delivering RNA therapies to liver cells. GalNAc binds to asialoglycoprotein receptors(ASGPR) on hepatocytes, resulting in receptor-dependent cellular absorption. Examples include Inclisiran, Givosiran, and Lumasiran. Advantages include efficient liver targeting, improved administration, and lower dose frequency.

Limitations: More effective for liver-targeted therapies, but less efficient for extra hepatic distribution.[26]

9.5. Viral and Non-viral Delivery Methods

Viral delivery is the process of delivering genetic material into cells using engineered viruses. Efficient delivery is achieved, although it may result in immunological reactions and is limited by payload size.

Non-viral delivery: LNPs, liposomes, polymers, and GalNAc conjugates are used to deliver RNA. Such systems offer the advantage of greater formulation freedom and are free of the constraints associated with viral vectors. Today's RNA therapies are primarily given by non-viral techniques.[27]

9.6. Tissue-specific Delivery

Targeted delivery is a way for delivering RNA directly to the organ or cell type ofinterest, hence reducing undesired effects.

- Liver: GalNAc conjugation and LNPs.
- Brain: ASOs are delivered intrathecally.
- Lungs: inhaled RNA formulations.
- Tumours: targeted nanoparticles and ligands.

Improving tissue selectivity is a significant hurdle in RNA therapies.

9.7. Emerging Technologies for Delivery

New technologies are being developed to increase RNA stability, cellular absorption, and tissue targeting capabilities.

1. Exosomes: Natural extracellular vesicles being studied for the transport of RNA.
2. Peptide-mediated delivery: Using cell penetrating peptides to increase cellular uptake.
3. Hybrid nanoparticles: These are a combination of multiple delivery mechanisms that improve targeting and delivery.
4. Chemical changes: increase RNA stability and resistance to enzymatic destruction.
5. AI-assisted delivery: Helps optimize RNA sequence design and delivery formulation.

These technologies indicate promising prospects for future RNA therapies, but many are still under development.[28] The main delivery strategies are compared in Table 3.

10. CLINICAL APPLICATIONS:

siRNA therapeutics have shown clinical benefits in many diseases for example patisiran for Hereditary Transthyretin Mediated Amyloidosis, givosiran for Acute Hepatic Porphyria, lumasiran for Primary Hyperoxaluria Type 1 and inclisiran for reducing LDL cholesterol. They are being researched for applications in viral infections, cancers and other genetic diseases.[29]

Advantages and limitations: siRNA provides high specificity, strong gene knockdown and long duration of effect, which could mean less frequent dosing. Disadvantages include difficulty targeting extrahepatic tissue, off target gene knockdown, immune responses, toxicity and complex production. With continual improvements in chemistry and targeting, siRNA may have a broader range of clinical applications [30]

10.1 Infectious Disease

RNA-based technologies can inhibit the activity of disease-causing target RNAs and proteins and are used to produce vaccines and to treat infectious agents. RNA therapy allows rapid development cycles, is highly specific to pathogens and can be adapted to changing circumstances. CRISPR nanocarriers can deliver components that target viral genomes.[31]

10.2 Cancer

Since its discovery, RNAi has contributed to a vast understanding of neoplasia and holds great promise for anticancer therapeutics. Cancer is one of the most challenging therapeutic conditions. RNAi acts through a sequence-specific mechanism in which siRNA duplexes target complementary mRNAs, eliminating transcripts that drive cancer growth.[32]

10.3 Genetic and Rare Diseases

RNA therapeutics that use complementary siRNA to inhibit the expression of specific proteins have been approved by the FDA for genetic diseases. Safe and effective delivery depends on systems such as lipid nanoparticles and extracellular vesicles, together with chemical modification. Monogenic diseases, which originate from DNA mutations, result in incorrect or deficient mRNA and the corresponding protein. At present, about 15–17 therapeutics for rare genetic diseases have been approved.[33]

10.4 Cardiovascular Diseases

RNA agents (siRNA, aptamers, microRNA) must overcome barriers such as target stability, immunogenicity and adequate binding. Because cardiovascular disease is common and a leading cause of mortality, efficacy is needed in conditions such as hypercholesterolemia, diabetes and cardiac amyloidosis. Aptamers bind specific target molecules and are selected through an iterative process, whereas mRNA directly encodes functional proteins. Risk factors such as hyperlipidemia and diabetes mellitus remain important considerations.[34]

10.5 Metabolic Diseases

RNA therapeutics are used to treat metabolic disorders; four such drugs are in clinical use. Several target hereditary transthyretin-mediated amyloidosis (hATTR) and acute hepatic porphyria, which are linked to liver proteins. Inclisiran is an siRNA-based therapy that reduces low-density lipoprotein (LDL) cholesterol.[35]

10.6 Neurological Disease

RNA therapeutics have made rapid progress in rare genetic diseases and neurodegenerative disorders, although safe and effective delivery to the nervous system remains a challenge. They are being explored for conditions such as Alzheimer's disease and ischaemic stroke. Administering mRNA as a therapeutic for neurological disorders has several advantages, such as producing proteins in their native form in mature, non-dividing cells.[36]

10.7 Liver Disease

With the rising prevalence of liver disease, the development of RNA therapeutics is expanding alongside traditional treatments such as antiviral drugs, immunoglobulins, corticosteroids and anti-rejection medications. A key advantage of mRNA is that it does not enter the cell nucleus and therefore does not integrate into the genome.[37]

10.8 Vaccination and Immunotherapy

The history of vaccination began with its first major milestone, when Edward Jenner discovered the smallpox vaccine. In later years, RNA-based therapy attracted growing interest in vaccination. These vaccines prevent infections and diseases by inducing a harmless piece of protein derived from a virus. A notable example is the COVID-19 vaccine, which uses RNA technology. Related immunotherapy approaches include:

- Utilizing T cells or immune cells: treatments that leverage the patient's own immune cells to target diseases.
- Peptide-based treatments: therapies using specific protein fragments to trigger a targeted immune response.
- Viral vectors: utilizing modified viruses to deliver therapeutic genes or antigens into cells.[39]

11. SAFETY AND LIMITATIONS:

11.1 Immunogenicity

As demonstrated by their recent use in SARS-CoV-2 vaccination, in vitro-transcribed (IVT) RNAs are emerging as novel biologics for therapeutic innovation. Compared with DNA- or virus-mediated gene delivery systems, IVT RNAs offer safety by enabling temporary expression of the desired proteins. However, because of their immunogenicity, which is partly caused by double-stranded RNA (dsRNA) by-products of IVT, in vitro-transcribed RNAs should be used cautiously: dsRNA triggers an innate immune response in cells.[39]

11.2 Off-target Effects

Off-target effects of short RNAs can cause adverse events in small RNA therapies and complicate the interpretation of preclinical results. Hybridization-dependent effects, particularly miRNA-like off-target effects, are of greatest concern. Strategies to reduce them, such as sequence design, chemical modification and target prediction, should be applied even in the early stages of small RNA therapeutic development.[3]

11.3 Innate Immune Activation

Due to their many potential uses, nucleic acid-based treatments have drawn more attention in recent years. Because of their low toxicity and ability to stimulate the body's own innate immunity by acting on the natural mechanisms of its activation, immunostimulatory nucleic acids represent a promising class of potential drugs for the treatment of viral and tumor al diseases. As analytical techniques and methods for synthesizing nucleic acids and their derivatives advance, so does the repertoire of nucleic acids that directly interact with immune system components.[40]

11.4 Hepatic and Renal Toxicity

About 2 million people die from chronic liver disorders each year, about half from complications of cirrhosis and the rest from viral hepatitis and hepatocellular cancer. Delivering nucleic acid medicines with nanoengineered materials that exploit the liver's distinct anatomical and physiological features is therapeutically promising, and the liver is a key target for gene editing, gene silencing and gene augmentation.[41]

11.5 Delivery and Biodistribution

For RNA-based gene therapy to work inside target cells without triggering undesirable immune reactions, therapeutic RNA must be delivered efficiently. Non-viral

delivery techniques, which avoid the drawbacks of viral vectors, can transport RNA into cells. Lipid-based and polymer-based strategies can be distinguished by whether they target particular cell types actively or passively, and the route from preclinical drug delivery research to clinical licensure offers opportunities to improve novel delivery systems.[42]

11.6 RNA Stability

Therapeutic mRNAs and vaccines are being developed for COVID-19 and many other human diseases, but mRNA instability and inefficient protein expression impede their optimization. Design principles can overcome these obstacles; for example, the sequencing-based platform PERSIST-seq systematically characterizes in-cell mRNA stability, ribosome load and in-solution stability across libraries of mRNAs.[43]

11.7 Endosomal Escape

Macromolecular RNA therapies are taken up into cells by endocytosis because they are too big, too charged, and/or too hydrophilic to passively diffuse across the cellular membrane, in contrast to small-molecule treatments. Even in semipermissive tissues- cell membrane, consist of a lipid bilayer that entraps 99% or more of RNA therapies. Therefore, the rate-limiting delivery issue of endosomal escape must be resolved in a clinically meaningful way before RNA therapies may reach their full clinical potential to treat widespread human disease.[44]

11.8 Manufacturing and Quality Control

RNA-based products are among the most promising technologies for future therapeutic development, infectious disease management and worldwide immunization. A strong quality-by-design (QbD) foundation for future manufacturing process development, design and control can be built from the evaluation of critical quality attributes (CQAs), product–process interactions, relevant process analytical technologies and process modelling capabilities. QbD adoption will be central to the development, pre-qualification and regulatory approval of rapid-response, disease-agnostic RNA platform manufacturing processes, allowing RNA technology to realize its full potential.[45]

11.9 Cost and Accessibility

The positive preliminary results with voretigene neparvovec demonstrate the promising therapeutic potential of gene therapy. However, widespread access is hampered by the related expenditures, especially in low-to-middle income nations.

Making use of developments in mRNA therapy could provide a more financially feasible option. Additionally, switching to non-viral distribution methods may offer the twin advantages of lower expenses and greater scalability. To fully utilize the potential of mRNA methods in equitable access to medicines to prevent vision loss, relevant parties must work together to develop and implement a research agenda.[46]

11.10 Long-term Safety and Considerations

Therapeutic medicines for a variety of diseases are being created quickly on the basis of decades of long-term study in the field of RNA therapy. Numerous clinical trials are under way for RNA-based treatments of different incurable diseases, which often cannot be treated with other conventional medication classes. The development of RNA therapy rests on a number of significant RNA biology discoveries, and RNA-based pharmaceuticals offer clear benefits.[47]

12. EMERGING ADVANCES:

12.1 Next-Generation mRNA Technologies

Improved formulations of modified mRNA, as used in COVID-19 vaccines, together with lipid, protein and polymer nanoparticles, enhance stability and delivery. Next-generation mRNA technologies now have a clear role in vaccines, cancer immunotherapy and protein replacement.

12.2 Self-amplifying RNA (saRNA)

saRNA can generate an immune response in vivo and replicates autonomously within the cell, producing antigen for longer than conventional mRNA. saRNA vaccines also permit smaller doses to be administered while inducing potent immune responses.[48]

12.3 Circular RNA Therapies

Circular RNA (circRNA) is a covalently closed RNA loop without free 5′ or 3′ ends or poly(A) tails. CircRNA can be used for gene regulation, disease research and therapy.

12.4 Enhanced Lipid Nanoparticles

Lipid nanoparticles (LNPs) are used as delivery vehicles for siRNA and mRNA. Patisiran (Onpattro) became the first siRNA-LNP drug in 2018. LNPs are now used to deliver vaccines, gene-editing components and cancer therapies.

12.5. Targeted RNA Delivery

RNA therapeutics incorporate mRNA, RNA interference (RNAi), and antisense oligonucleotides (ASOs). Next-generation delivery nanocarriers and CRISPR technology have been developed to deliver targeted RNA therapeutics for genetic and rare disease treatment.

12.6 RNA Editing

RNA editing provides the ability to modify RNA transiently without changing the underlying DNA. ADAR enzymes convert adenine (A) to inosine (I), which is interpreted as guanosine (G). RNA editing can be used for mutation correction in genetic disease.

12.7 CRISPR-associated RNA Delivery

CRISPR-Cas uses a guide RNA (gRNA) to direct the Cas enzyme to a target DNA sequence and can be used to edit the genome. It can be used to treat genetic disease, for example sickle cell disease.

12.8 Personalized RNA Therapeutics

Personalized RNA therapies include:

- mRNA vaccines: provide protection by triggering immune responses to various infections and cancers.
- siRNA: silences specific disease-causing genes.
- ASOs: regulate gene expression by altering RNA splicing.
- CRISPR-Cas13: targets RNA without changing DNA.

12.9 Combination RNA Therapies

Combination RNA therapies use mRNA, siRNA, self-amplifying RNA and ASOs together. Nanoparticles protect the RNA from degradation and improve delivery to the cell. Applications include vaccines, cancer immunotherapy and protein replacement.

12.10 Ai-enabled RNA Design

AI assists with design of RNA sequences, analysis of RNA structures, increased stability and delivery optimization, as well as personalized medicines and cancer immunotherapy.[49]

13. PHARMACIST ROLE:

Pharmacists are essential to the safe and efficient use of RNA-based treatments. They help choose the best treatments, ensure that they are administered correctly, monitor the effects of treatment and educate patients.

13.1. Mechanisms and Therapeutic Selection

To support proper drug selection based on the patient's disease and treatment needs, pharmacists should understand the mechanisms of mRNA, siRNA and ASO treatments.

13.2. Medication Use and Administration Considerations

Pharmacists ensure appropriate dosage, timing, route and administration technique.

13.3. Efficacy and Side-effect Monitoring

To ensure patient safety and therapeutic efficacy, pharmacists monitor the patient's response to therapy, laboratory results and side effects.

13.4. Patient Counselling

They educate patients on the advantages of treatment, how to take it, its adverse effects and the need to follow the regimen.

13.5. Storage and Handling

To preserve the stability and efficacy of RNA medications, pharmacists ensure proper storage, temperature control, preparation and handling.

13.6. Pharmacovigilance

They support long-term safety monitoring and identify, document and report adverse drug reactions.

13.7. Drug Interactions and Treatment Coordination

To help patients get the most from treatment, pharmacists check for additional medications and interactions. They also collaborate with other medical specialists.

13.8. Patient Access and Adherence

They help patients understand treatment plans, adhere to routine therapy and gain access to expensive RNA medications.[50]

14. RESEARCH GAPS:

Even though RNA therapies have advanced significantly, there are still many unresolved issues that keep them from being applied in clinical settings. It is very difficult to create efficient and targeted delivery systems that can deliver RNA molecules to specific tissues and cells, especially in organs other than the liver like the brain, lungs, and muscles. Comprehensive research is also required to examine off-target effects, unanticipated immune reactions, and potential long-term toxicity in order to ensure patient safety.[51]

Further research is needed on the effects of repeated dosing, individual variations in therapeutic response, and interactions between RNA molecules and biological systems. Another important area of research focus is the standardization of manufacturing processes, quality-control methods, and mass production. Furthermore, access to RNA-based drugs may be restricted due to their high cost, particularly in low- and middle-income countries. Closing these research gaps through better RNA design, long-term safety studies, economical manufacturing, and carefully planned clinical trials will be crucial to enhancing the safety, efficacy, and accessibility of RNA-based treatments.[52]

15. FUTURE PERSPECTIVES:

Potential future directions in the development of RNA drug vehicles include: 1) provide for tissue-specific delivery; 2) improve the molecular stability of the RNA drug vehicles; 3) minimize side effects; and 4) manufacture the drugs using inexpensive and scalable processes suitable for widespread clinical use. Advances in the application of personalized treatment for individual patients may also lead to improved patient outcomes. Eventually, application of RNA drug vehicles for a greater number of disease types will be possible.[53]

16. CONCLUSION:

Next-generation mRNA technologies, including self-amplifying RNA, circular RNA therapeutics, and improved lipid nanoparticles, represent major advancements in modern medicine. These technologies offer promising approaches for developing effective vaccines, treating cancer, delivering therapeutic proteins, and managing genetic disorders. With continuous research and improvements in safety, stability, and delivery systems, mRNA-based therapeutics have the potential to transform the future of healthcare.

Tables and Figures

Table 1: Comparison of mRNA, ASO and siRNA therapies

Characteristic	mRNA	ASO	siRNA
Mechanism	Produces therapeutic proteins through translation	Degrades RNA or modifies RNA splicing	Degrades target mRNA through the RISC pathway
Principal target	Protein-producing machinery	mRNA or pre-mRNA	Specific mRNA
Duration	Short-term expression	Variable, depending on chemical modification	Relatively prolonged gene silencing
Delivery	Mainly lipid nanoparticles (LNPs)	Chemical modifications and delivery carriers	LNPs and GalNAc conjugation
Applications	Vaccines, protein replacement and cancer research	Neurological and genetic disorders	Genetic and metabolic disorders
Limitations	RNA instability and immune reactions	Toxicity and limited tissue penetration	Off-target effects and delivery challenges

Table 2: Representative RNA therapeutics, targets and routes of administration

Therapeutic	Platform	Target	Disease	Route
Comirnaty	mRNA	Spike antigen	COVID-19	Intramuscular
Patisiran	siRNA	TTR	Amyloidosis	Intravenous
Inclisiran	siRNA	PCSK9	Hypercholesterolemia	Subcutaneous
Givosiran	siRNA	ALAS1	Acute hepatic porphyria	Subcutaneous
Nusinersen	ASO	SMN2	Spinal muscular atrophy	Intrathecal
Tofersen	ASO	SOD1	Amyotrophic lateral sclerosis (ALS)	Intrathecal

Table 3: Main advantages and limitations of RNA delivery strategies

Delivery strategy	Main advantage	Main limitation
LNPs	Protect RNA and improve cellular uptake	Endosomal escape
Liposomes	Flexible lipid-based delivery	Stability
Polymers	Tunable characteristics	Potential toxicity
GalNAc	Liver-specific targeting	Limited tissue distribution
Viral vectors	Efficient gene transfer	Immunogenicity
Exosomes	Potentially biocompatible delivery	Manufacturing challenges
Peptides	Improved cellular penetration	Stability and toxicity

Table 4: FDA-approved RNA-based therapeutics relevant to this review

S. No.	Drug name	Brand name	RNA type	Target disease / category	FDA approval year
1	Patisiran	Onpattro	siRNA	Hereditary transthyretin amyloidosis (hATTR) — Genetic / Liver	2018
2	Givosiran	Givlaari	siRNA	Acute hepatic porphyria — Metabolic / Liver	2019
3	Lumasiran	Oxlumo	siRNA	Primary hyperoxaluria type 1 — Metabolic / Liver	2020
4	Inclisiran	Leqvio	siRNA	Hypercholesterolemia — Cardiovascular / Metabolic	2021

5	Nusinersen	Spinraza	ASO	Spinal muscular atrophy (SMA) — Neurological	2016
6	Tofersen	Qalsody	ASO	SOD1-associated amyotrophic lateral sclerosis (ALS) — Neurological	2023
7	Eteplirsen	Exondys 51	ASO	Duchenne muscular dystrophy — Genetic	2016
8	Inotersen	Tegsedi	ASO	hATTR-associated polyneuropathy — Genetic / Neurological	2018
9	COVID-19 mRNA vaccine (BNT162b2)	Comirnaty	mRNA	Prevention of COVID-19 — Infectious disease / Vaccination	2021 (FDA approval; initially EUA in 2020)
10	COVID-19 mRNA vaccine (mRNA-1273)	Spikevax	mRNA	Prevention of COVID-19 — Infectious disease / Vaccination	2022 (FDA approval; initially EUA in 2020)

Figure 1: Structural elements of mRNA (5′ cap, untranslated regions, coding sequence and poly(A) tail)

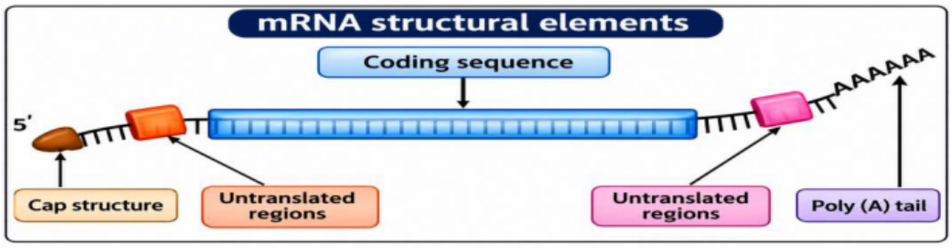


Figure 2: The RNA interference process

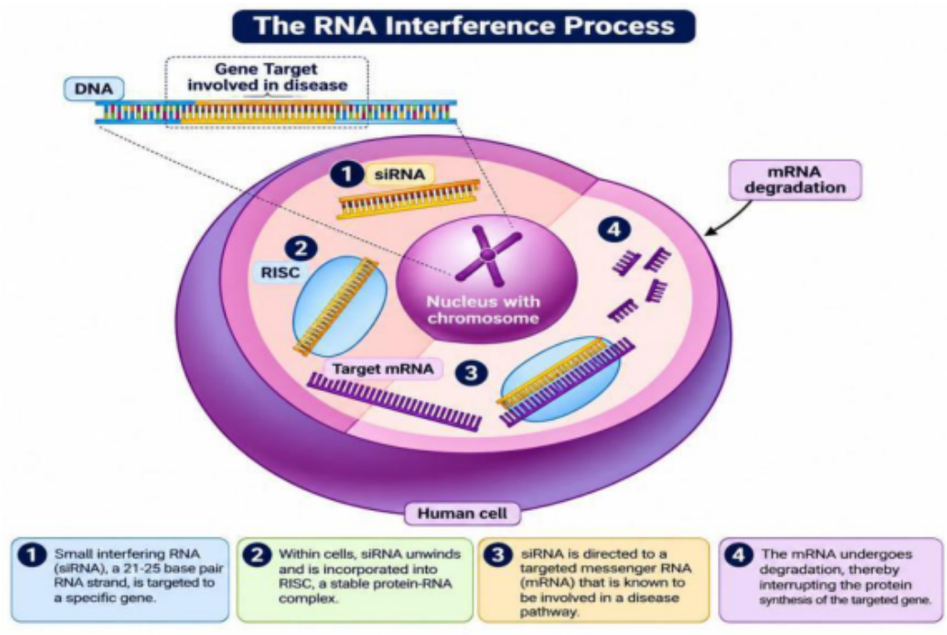


Figure 3: Delivery constructs for siRNA: (a) lipid nanoparticle, (b) PEG–ligand conjugate, (c) chemically stabilized, ligand-targeted siRNA and (d) GalNAc conjugate

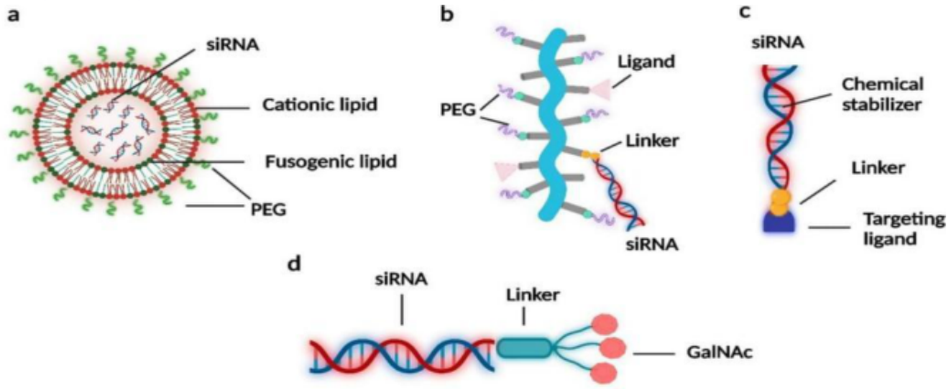
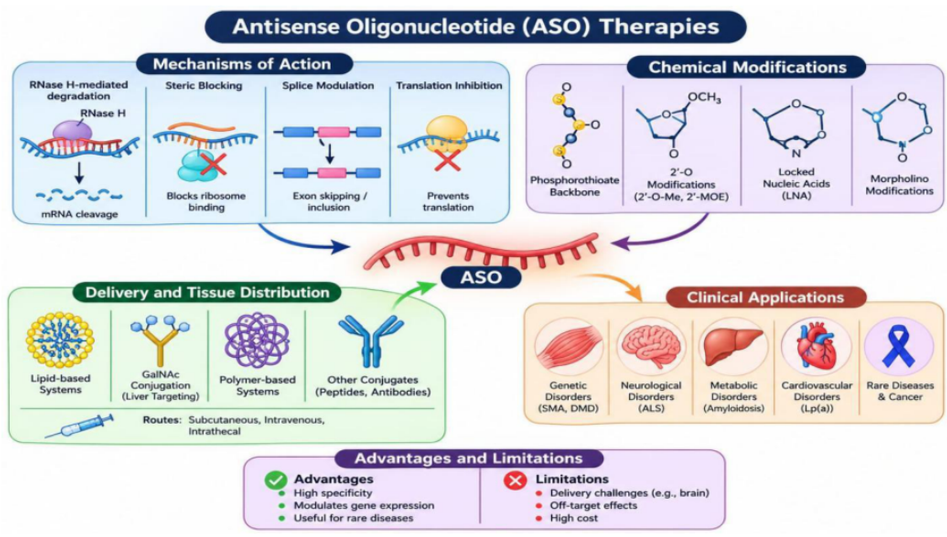


Figure 4: Antisense oligonucleotide therapies: mechanisms of action, chemical modifications, delivery and clinical applications



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