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mRNA Therapeutics Beyond COVID-19 Vaccines: Expanding Horizons in Precision Medicine and Disease Modulation

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Abstract

The BioNTech/Pfizer and Moderna COVID-19 mRNA vaccines achieved success has never been so successful before, and the messenger RNA (mRNA) has proven itself to be a highly versatile and powerful platform in modern medicine. This breakthrough established the safety, efficacy and scalability of the mRNA-based interventions and the intensive research on their possible use beyond infectious diseases. Recent advances in mRNA engineering, nucleoside modification, and lipid nanoparticle (LNP) delivery systems have transformed stability of mRNA, translation activity and local delivery on which the foundation of future generation therapeutic is based. The technology of mRNA is now applied to the clinical uses of precise medicine through mRNA vaccines in order to induce tumor specific anticancer immunotherapy; genetic disease, where mRNA vaccine is used to induce tissue regeneration and angiogenesis; and autoimmune regulation where mRNA technology is used to regenerate the immune tolerance. Also, mRNA is necessary to execute the CRISPR-based gene editing and enables the delivery of gene-editing elements to cells in a temporary and controllable manner to repair pathogenic mutations.

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All these developments lead to the future of mRNA as a therapy and preventive and control agent against complicated diseases, which are too specific and adaptable. The review will look at the revolutionary process of mRNA therapeutics beyond COVID 19 vaccines, the revolutionary change in technology, therapeutic use and future opportunities that lead to the centre of cancer precision and targeted therapy with mRNA.

Keywords: - mRNA therapeutics, lipid nanoparticles, precision medicine, immunotherapy, genetic disorders, gene editing.

1. Introduction

Messenger RNA (mRNA) therapeutics Are one of the most groundbreaking inventions in the field of modern medicine that have given a new meaning to the definition of the disease prevention, it's control, and perhaps the cure. The success of the COVID-19 mRNA vaccines, created by BioNTech/Pfizer (BNT162b2) and Moderna (mRNA-1273) had made the utilisation of mRNA as a therapeutic agent a clinic and commercial reality, even though the idea of using MRNA as a therapeutic agent was introduced several decades ago. These vaccines were not only successful in curbing the pandemic in the world, but also provided evidence of concept beyond any doubt to prove that synthetic mRNA Can safely be delivered into the human body where it can stimulate the body to produce some proteins which can ultimately produce protective immunity. This confirmation triggered the global excitement of the pursuit of mRNA beyond vaccines to an expansive disease arsenal **[1]**.

The basic idea of mRNA therapeutics is insanely simple: inject a synthetic mRNA sequence with a therapeutic protein into the cells of the host, and the cellular machinery translates the sequence into the required protein **[2]**. Compared to traditional drugs which work by altering natural biochemical pathways or therapeutic proteins produced by complex production systems, mRNA allows the body to synthesize its therapeutic proteins temporarily **[3]**. The mechanism offers the ability to treat disease at a molecular level in a direct, flexible and programmable manner. Speed is one of the best mRNA therapeutics **[4]**. After knowing the genetic sequence of a desired antigen or a protein, mRNA constructs can be prepared and expressed in only days **[5]**. This quick response has been important in the event of the COVID-19 pandemic where effective vaccines have been found in record time **[6]**. The process of production is cell-free, and no cell cultures or development of pathogens are required, which makes the process less complex and enables production to be scaled **[7]**.

Another significant strength of the mRNA technology is safety. Because the mRNA is non-integrative and exclusive to the cytoplasm, it is not subject to the risk of insertional mutagenesis that may be ligated with the DNA-based or viral vectors. Additionally, the mRNA molecules break

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down naturally after translation essentially reducing toxicity on long term basis. Nucleoside replacements and other chemical modifications coupled with sophisticated lipid nanoparticle (LNP) delivery platforms, have since improved stability and decreased undesired immune responses to provide accurate and transient therapeutic expression **[8].**

It is also crucial that mRNA therapeutics can be adapted. Due to its modular property, the mRNA can be rapidly reprogrammed to encode nearly any protein of interest making it applicable in a wide range of disease applications. Cancer immunotherapy involves mRNA that is expressed to produce tumor-associated antigens which teach the immune system to destroy the malignant cells **[9]**. It offers a temporary procedure in genetic diseases that compensates the absence or defectiveness of specific genes **[10]**. mRNA-based constructs enhance angiogenesis and tissue regeneration after the ischemic injury in cardiovascular disease **[11]**. Also, in autoimmune diseases, mRNA has the potential of balancing immune tolerance to restore normal immune regulation **[12]**.Of great potential is the integration of mRNA delivery with the CRISPR-Cas gene-editing technology **[13]**. The expression of CRISPR parts by means of mRNA makes it possible to provide temporary gene editing at lower risk of off-target effects and irreversible changes in the genome **[14]**. This combination has a great potential in regards to the accurate correction of genetic mutations responsible of inherited diseases **[15]**.

The move to a wider approach to disease management by mRNA development is a turning point in the therapeutic approach of the genome **[16]**. It is a representation of the trend of precision and personalized medicine where treatments can be designed to individual patients or individual molecular profiles **[17]**. The safety and efficacy of the mRNA-based therapies should also be improved by continuous advancements of the delivery system, formulation chemistry, and immune modulation. Simply put, the breakthrough of the COVID-19 vaccine turned the mRNA therapeutics into a hypothetical possibility to a real clinical option. Having unprecedented benefits of high-speed production, high safety levels, and unprecedented flexibility, mRNA technology is now ready to transform medicine beyond infectious diseases- introducing a new phase of molecular specificity and individualized medicine **[18]**.

The mRNA therapeutic system consists of fast production, lipid nanoparticle development, target delivery, and translation into the host cell to generate functional therapeutic proteins **(Figure 1).** This workflow takes advantage of the engineered mRNA structural elements such as 5' m7G cap, optimized untranslated regions, codon-optimized coding sequence and poly(A) tail to achieve the maximum stability and translational efficiency. Lipid nanoparticles mediate endosomal avoidance and cytoplasmic delivery, which allow the target cells to translate the encoded protein by ribosomes **[19].** This simplified mechanism is the foundation of the flexibility of mRNA therapeutics to a wide variety of disease applications in oncology to regenerative medicine.

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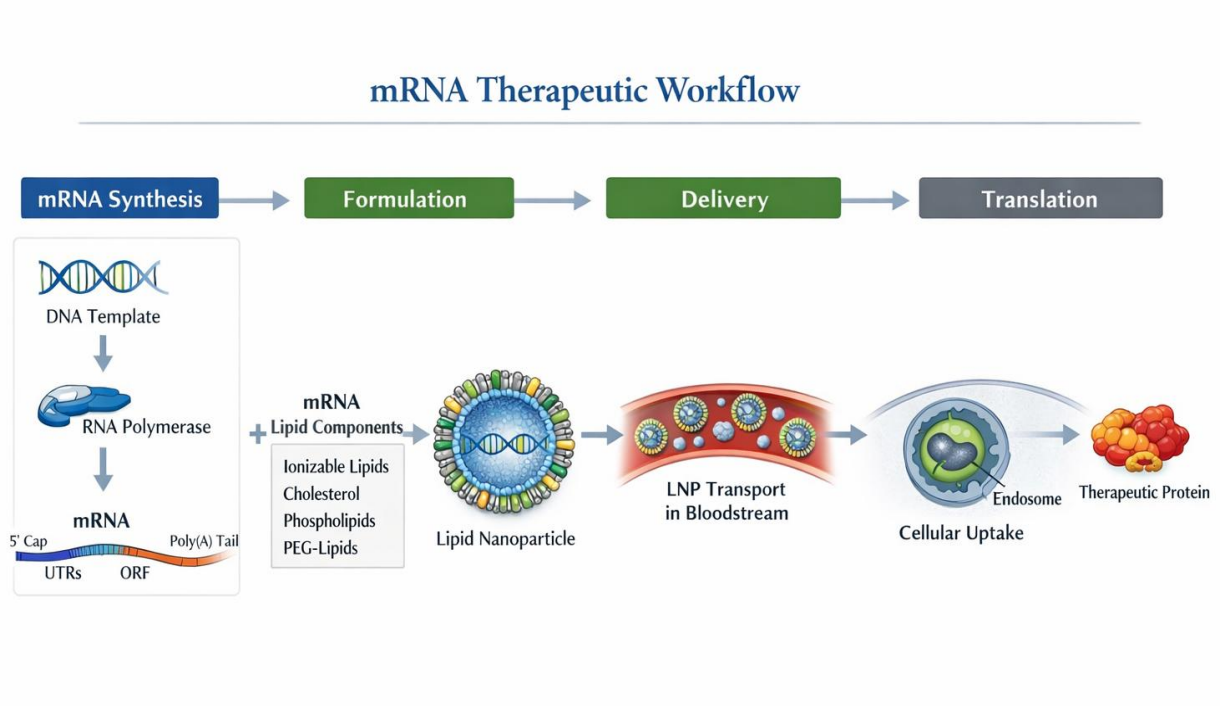

Figure 1: The mRNA Therapeutic Workflow: From Synthesis to Protein Translation

Flowchart of the entire mRNA therapeutic process. It has four consecutive steps, which include: (1) the synthesis of mRNA with modified structural elements such as 5′ cap (m7G), untranslated regions (UTRs), open reading frame (ORF) and poly(A) tail; (2) formulation into lipid nanoparticles (LNPs) consisting of ionizable lipids, cholesterol, phospholipids, and PEG-lipids; (3) systemic administration through intramuscular/ intravenous injection followed by cellular uptake by endocytosis (4) translation in the host cell cytoplasm resulting in therapeutic protein synthesis.

2. Fundamentals of mRNA Therapeutics

Messenger RNA (mRNA) therapeutics are a novel development in the medical field of molecular medicine, which allows cells to temporarily express particular proteins as therapeutic agents **[20]**. In contrast to the traditional small molecule drugs or engineered proteins, the mRNA therapies make use of the body's own translational machinery to translate functional proteins in vivo using a synthetic mRNA template **[21]**. The success of such types of therapies is conditional upon two important factors: the structural optimization of the mRNA molecule and the effectiveness of its delivery system to the core elements of synthetic mRNA and the processes that stabilize mRNA, translate into proteins, and deliver them is significant problem in the rational development of mRNA-based therapeutics

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Table 1 outlines the structural elements of synthetic mRNA and their biological roles that are imperative to the therapeutic effectiveness of the mRNA. All of the elements, 5' m7G cap, untranslated regions (UTRs), coding sequence, poly(A) tail, and modified nucleosides, play a unique role in mRNA stability, translation efficiency and immune evasion (Table 1). All of these engineered features make it possible to achieve high-fidelity protein expression and reduce innate immune recognition.

A. Synthetic mRNA, synthetic mRNA Structural components

A synthetic mRNA molecule is designed in such a way so that it resembles natural eukaryotic mRNA with engineered changes that enhance the stability of the molecule, its ability to be translated, and its safety. It comprises 4 key components which include the 5' cap, Untranslated regions (UTRs), open reading frame (ORF) and poly(A) tail. All these components have a different role on the overall performance of the therapeutic mRNA **[22].**

a. 5' Cap

The 5' cap system is a modified guanosine nucleotide (m7G) that is conjugated to the position of the first nucleotide in the mRNA by a 5'-5' triphosphate bond. It plays many essential roles, the most critical ones being the mRNA defence against exonuclease destruction, granting ribosome recruitment, and starting translation **[23]**. In artificial mRNA, a level of cap analogs like Anti-Reverse Cap Analog (ARCA) and CleanCap are utilized in order to obtain proper orientation and to promote stability **[24]**. The 5' cap also acts with the eukaryotic initiation factor eIF4E that is required in binding with the ribosome, which has a direct effect on translation efficiency **[25]**.

b. Untranslated Regions (UTRs)

UTRs in the 5' and 3' positions adjacent to the start of the open reading frame have regulatory roles in the stability of the mRNA and translation **[26]**. The 5' UTR modulates ribosome scanning and translation initiation whereas the 3' UTR plays a role in the mRNA localization, stability and translational regulation **[27]**. UTR sequences are usually based on endogenous genes that are highly stable like a- and b-globin mRNAs in order to maximize expression **[28]**. Additionally, elimination of destabilizing motifs such as AU-rich motif in 3' UTR can be used to increase the half-life of synthetic mRNAs **[29]**. UTRs are therefore important to the success rate of sustained and controlled protein expression in therapeutic use, which can be attained only through rational design **[30]**.

c. Open Reading Frame (ORF)

The open reading frame which is the middle functional part of the mRNA molecule codes the protein of interest. A key factor to consider in ORF design is codon optimization, which is the replacement of rare

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codons by synonymous codons by other codons which are represented by common tRNAs; it can be used to substantially increase translation efficiency without changing the amino acid sequence **[31].** Also modified nucleosides are employed including pseudouridine(ps) and 5-methylcytidine(m5C) that help to prevent recognition by intake immune sensor like Toll- like receptors (TLRs) and RNA-dependent protein kinase (PKR) inhibiting undesirable immune stimulation and degradation. The changes cause increased translation and reduced immunogenicity that result in increased therapeutic performance **[32]**.

d. Poly(A) Tail

The polyadenylated [poly (A)] tail (usually between 100 and 250 adenosine residues) is attached to the 3' end of the mRNA **[33]**. This formation shields the transcript against exonuclease decay and assists with the initiation of translation by forming circularization of the mRNA molecule by interactions with poly(A)-binding proteins (PABPs) and translation initiation factors **[34]**. The length of the poly(A) tail is also very important to optimize: shorter tail will result in premature degradation whereas longer tails will result in reduced translation efficiency **[35]**. Also, in combination with the 5' cap and UTRs, the poly(A) tail, is necessary in stabilizing a translational competent mRNA molecule that can efficiently translate into protein **[36]**.

B. Delivery Systems of mRNA Therapeutics

Although the potential of mRNA is enormous, its efficient utilisation in the body only works well with efficient delivery systems. Naked mRNA is extremely fragile and vulnerable to ubiquitous RNases degradation and its massive, negative charged size doesn't allow it to passively diffuse across the cell membranes. Hence, it is necessary to provide protective and efficient delivery systems to facilitate the cellular uptake, endosomal escape and translation in the cytoplasm **[37]**.

a. Lipid Nanoparticles (LNPs)

The current most sophisticated and used delivery systems of mRNA Therapeutics are lipid nanoparticles (LNPs). Lipid nanoparticles are made up of ionizable lipids, cholesterol, phospholipids, and PEG-lipids and surround and protect the mRNA against enzyme degradation and aid its entry into the cells **[38]**. The positively charged ionizable lipids are incorporated during the formulation when they are at low pH, and as a consequence of this low pH, the lipids are able to bind the negatively charged mRNA and enclose it **[39]**. They become neutral at physiological pH and this reduces toxicity. After administration- normally through intramuscular or intravenous injection- LNPs are absorbed into the cells by endocytosis. The ionizable lipids become positively charged again in the acidic environment, inside the endosomes, and facilitate fusion with the endosomal membrane and release of mRNA into the cytoplasm **[40]**.

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It is a process that results in efficient delivery and expression of the encoded protein.LNP composition optimization has helped COVID-19 mRNA vaccines to be a success with the accurate control of lipid ratios and the size of particles to lead to high delivery efficiency as well as robust immune reactions. The ongoing studies are on the enhancement of tissue-specific targeting, immunogenicity reduction, and repeat dosing with no side effects **[41]**.

b. **New Delivery strategies**

In addition to LNPs, other delivery systems (polymer-based nanoparticles, cationic nanoemulsions, and lipid-polymer hybrids) are currently being explored. The goal of these systems is to enhance stability, biocompatibility and cell-type-specific targeting of organs or cells to increase the therapeutic potential of mRNA to diseases that are not necessarily in vaccination, such as cancer and rare inherited diseases **[42].**

c. Therapeutic Design Significance

All mRNA-based therapeutics rely on the expertise of the engineering of mRNA structure and delivery system. The different parts, such as the molecule cap and the nanoparticle shell, are synergistic to protect the molecule, allow efficient translation and result in the required biological effect. The constant focusing of these design features will also boost the stability, tissue targeting, and therapeutic safety of mRNA, to new levels beyond vaccines into the domain of disease modulation as a whole and precision medicine **[43].**

Table 1:-Structural components of synthetic mRNA and their biological functions.

S.No.	Component	Structural Description	Biological Function/Significance	Refrences
1.	5' cap(m^7 G Cap)	A 7-methylguanosine linked to the 5' end via a 5' triphosphate bridge	Enhances mRNA stability by preventing exonuclease degradation; facilitate ribosomes recognition and initiation of translation; mimics natural eukaryotic mRNA structure.	**[38]**
2.	5' Untranslated regions (5' UTR)	Non-coding regions upstream of the start codon(Aug); sequence varies among constructs.	Regulates translation efficiency and scanning and binding; can be engineered to optimise protein expression.	**[40]**

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3.	Coding sequence(CDS/Open Reading Frame)	The mRNA region encoding the target protein or therapeutic peptide.	Determines the amino acid sequence of the translated protein; optimized for codon usage, GC content, and removal of inhibitory motifs to enhance translation and stability.	**[41]**
4.	3'. Untranslated regions (3' UTR)	Non-coding sequence downstream of the stop codon; derived from stable natural mRNAs.	Regulates mRNA half-life, Localisation, and translation efficiency; interacts with RNA-binding proteins and microRNAs.	**[39]**
5.	Poly(A) Tail	A stretch of adenosine residues (typically 100-150 bases) added at the 3' end	Protects mRNA from enzymatic degradation; promotes nuclear export and translation; interacts with poly(A)-binding proteins to form a closed loop structure with the 5' cap.	**[38]**
6.	Modified Nucleosides (e.g., N^1-methyl-pseudouridine)	Chemically modified based replacing uridine or cytidine residues.	Reduces innate immune recognition and inflammatory response; enhances translational capacity and mRNA stability.	**[32]**
7.	Optimized sequence Elements	Engineered motifs or synthetic elements within UTRs and CDS.	Improve translation yield, prevent secondary structure formation, and fine-tune gene expression kinetics.	**[42]**

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3. Evolution of mRNA Technology and the Impact of COVID-19.

The creation of messenger RNA (mRNA) therapeutics is 3-plus decades-long story that began with skepticism, continued with challenges, and has ended with something the scientific community has never witnessed in its history: a scientific success story. Initial studies of mRNA as a therapeutic modality had been carried out in the late 1980s, when researchers were able to demonstrate that in vitro-transcribed mRNA could be transferred into cells to express functional proteins. The first attempts at this promising idea were negated by severe shortcomings: mRNA was a fragile target by nature, rapidly destroyed by ubiquitous ribonucleases (RNases), and immunogenic beyond all reason, provoking responses to inflammation and rendering it not usable in therapeutic applications. These issues have kept the mRNA technology a theoretical interest but not a working mechanism in the medical field over the years **[44].**

Another breakthrough was made in the 2000s when KatalinKariko and Drew Weissman found that that incorporation of chemically modified nucleosides into synthetic mRNA, including pseudouridine, would reduce undesirable immune activation but not affect translational efficiency. This advancement offered a base on the construction of tolerable, stable and therapeutically feasible mRNA constructs. At the same time, the development of lipid nanoparticle (LNP) delivery systems transformed the delivery of mRNA by protecting the molecule against the effect of enzymes and allowing cells to effectively take up the molecule. All these innovations overcame the two key barriers of instability and immunogenicity which had long been a drag on the clinical use of mRNA **[45]**.

In the 2010s, a number of biotechnology firms identified the promise of mRNA and made huge investments in the optimization of its therapeutic uses. In the United States, Moderna (established in 2010) and Germany, BioNTech (established in 2008) became the most active mRNA technology movers and shakers. The early specializations of both companies were cancer immunotherapy and treatment of rare diseases, and both companies had mastered the art of mRNA design and codon optimization and delivery **[46]**. At the time when the COVID-19 pandemic broke out in late 2019, their platforms were in good place. In several weeks after the publication of the SARS-CoV-2 genome, Moderna and BioNTech, which collaborated with Pfizer, designed and manufactured mRNA vaccine candidates expressing the viral spike protein.

The mRNA vaccines against COVID-19 were a phenomenal synthesis of decades of study in nanotechnology, RNA biology, and chemistry. These vaccines showed excellent efficacy and safety in mass clinical trials, and emergency use authorizations were approved in the shortest possible period. The speed of designing and scalability of production along with flexibility of the mRNA platform enabled international implementation in months, something which could not have

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been possible with traditional vaccine technology. The Moderna vaccine and BioNTech/Pfizer vaccines not only reduced the pandemic but also demonstrated the effectiveness of mRNA as a strong and versatile and clinically tested therapeutic platform **[47]**.A success case of the pandemic served as a catalyst, making the global view of mRNA therapies to shift towards being considered as an experiment or necessity. After 2021, the development and research around mRNA technology has grown exponentially to hasten its application in oncology, heart repair, autoimmune regulation, and protein-replacement therapy **[48]**. Firms like CureVac, Arcturus Therapeutics, and Translate Bio entered the growing field of use, and diluted the therapeutic uses of mRNA.Factually, the COVID-19 crisis was a challenge and a breakthrough of mRNA technology. It had put decades of theoretical and preclinical experience into practical application under the greatest pressure in the world and the outcome is more than satisfactory. As the success of Moderna and BioNTech not only changed the entire concept of vaccines but also created a window to another world of accuracy and personalized medicine, mRNA has become a universal platform and can be adjusted to almost every therapeutic target **[49]**.

4. mRNA Therapeutics in Cancer Treatment

Cancer is also one of the most complicated and difficult diseases to be treated because of its heterogeneity, immune resistance systems, and genetic diversity of patients. The conventional forms of cancer treatment, including chemotherapy, radiotherapy, and targeted drugs are not very specific and may be highly toxic **[50]**. Immunotherapy has reshaped the world of oncology in the last ten years through the power of the immune system of the body to identify and destroy cancer cells. MRNA-based cancer vaccines have received tremendous interest as one of the new immunotherapy approaches that can provide highly personalized and tumor-targeted treatment approaches that are safe and versatile **[51]**.

A. Idea behind the use of Personalized mRNA Cancer Vaccines

Personalized cancer vaccines are the basis of mRNA cancer therapeutics, which activate an immune response to tumor-specific neoantigens. Neoantigens are products of somatic mutations specific to the tumor cells of an individual, thus making them the best targets, as they are not expressed in normal tissue, and therefore there is minimal chances of autoimmunity. This starts with the tumor genomic sequencing and bioinformatic analysis to determine patient specific mutations that create neoantigens. After a selection of the most immunogenic neoantigens has been carried out, synthetic mRNA is designed to encode these targets **[52].** When administered mRNA is usually delivered through intramuscular or intradermal injection, and it is endocytosed into antigen-presenting cells (APCs), including dendritic cells, which convert the mRNA into neoantigenic proteins. They are then processed and displayed on major histocompatibility complex

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(MHC) molecules which attracts cytotoxic CD8+ T cells and helper CD4+ T cells that specifically identify and kill tumor cells expressing the said neoantigens.This strategy is effective as it can be used to convert the tumor profile of every patient into a personalized vaccine. The mRNA synthesis process is flexible, which enables the rapid development and production of individual patient-based vaccines, which is not afforded by the traditional vaccine platforms. Also, mRNA has a transient expression and non-integrating characteristic, which guarantees a good safety profile **[53].**

B. Mechanism of Immune Recognition and Activation

The treatment effectiveness of the mRNA cancer vaccines depends on their capacity to induce both adaptive and innate immune responses. The mRNA is transduced into the cytoplasm of APCs when presented in lipid nanoparticles (LNPs), by a dendritic cell-based system, and translated into tumor-specific antigens. These antigens are broken down into peptide fragments and complexed with MHC class I and II and presented to T cells.

MHC Class I Presentation: This activates cytotoxic T lymphocytes that are CD8+ and kills tumor cells that express the same antigenic peptides.

MHC Class II Presentation: Stimulates the work of the CD4+ helper T cells, which contribute to the necessary cytokine production and increases the cytotoxic effect **[54]**.

Also, mRNA itself has the potential to serve as an immune adjuvant, which activates pattern recognition receptors (PRRs), including Toll-like receptors (TLR3, TLR7, and TLR8), and this leads to dendritic cell maturation and the release of cytokines. In order to prevent the high levels of inflammation, modified nucleosides (e.g., pseudouridine) are included into the mRNA sequence to achieve a balance between the immunostimulation and translation efficiency**.** This tailored immunogenic is ideal in antigen presentation without compromising the safety and tolerability **[55]**.

C. Clinical Progress and Key Trials

Groundbreaking discoveries in genomics, bioinformatics, and delivery systems have enhanced the process of the translation of mRNA cancer vaccines into the clinic. A number of mRNA-based cancer vaccines are in phase I and II clinical trials with an encouraging immunogenicity and safety profile across many cancer types. Among the most interesting programs is BioNTech BNT122 (RO7198457), which was created with the help of Roche/Genentech **[56]**. It is a personalized mRNA vaccine which encodes as many as 20 patient-specific neoantigens, and has been tested in patients with advanced melanoma, colorectal cancer, and non-small cell lung cancer. Other companies involved in the mRNA field, such as Moderna have created mRNA-4157 (V940) that

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has been developed in collaboration with Merck.Early clinical results have shown that V940 has strong T-cell responses to a variety of encoded neoantigens and leads to long-term clinical benefit and tumor regression in some patients. It is a personalized cancer vaccine which encodes up to 34

neoantigens and is used in combination with the PD-1 check point inhibitor, pembrolizumab (Keytruda) **[57].** One of the largest clinical milestones made on mRNA cancer vaccines so far is in a recent phase IIb trial in high-risk melanoma, where the combination of mRNA-4157 and pembrolizumab prolonged the risk of recurrence or death by 44 percent compared to pembrolizumab alone. Other programs that have been highlighted with such programmes are CureVac CV9202 against non-small cell lung cancer and BioNTech BNT111 which encode tumor-associated antigens common to all patients (instead of specific) in treatment of melanoma. Preliminary findings are that the antigen-specific CD4+ and CD8+ T-cell responses are highly induced, which confirms the platform potential **[58]**.

D. Delivery Platforms and New Technology

To achieve the success of mRNA cancer therapeutics, efficient delivery continues to be important. The lipid nanoparticles (LNPs), which are a prevalent delivery system in covid-19 vaccines, are also most frequently used in the oncology setting. They contain mRNA, preserve it against degradation and enable its cellular uptake, by endocytosis, and endosomal escape into the cytoplasm **[59]**. LNP modifications can be used to tune tissue targeting, biodistribution and immune activationAlternatively, ex vivo-loaded dendritic cell (DC) vaccines have been investigated where patient-derived dendritic cells are transfected with mRNA expressing tumor antigens and reinvented into the patient to directly present antigens to T cells. This is the most accurate method of controlling immune activation but is more complex and difficult to scale up as compared to LNP-based vaccines **[60]**. New methods of delivery involve polymeric nanoparticles, lipoplexes, and hybridizing materials that seek to enhance stability, biocompatibility, and targeted delivery to tumor-draining lymph nodes. Delivery systems should be optimized continuously to increase the amount of antigen produced and minimise an off-target effect **[61]**.

E. Challenges and Future Prospects

In spite of an impressive advancement, the challenges that can be faced before mRNA cancer vaccines can become a standard treatment option are still numerous. Determining the most immunogenic neoantigens will necessitate high-tech computational algorithms and high throughput sequencing procedures. Besides, immune checkpoint suppressions by the tumor microenvironment (TME) frequently require the use of combination therapies with immune checkpoint inhibitors, cytokines, or oncolytic viruses to improve their efficacy **[62]**.

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However, the achievement of personalized mRNA vaccines in melanoma and other cancers has confirmed the potential of the technology in the clinic and given serious interest to the market and academia. The trials that are still in progress are broadening the therapeutic use of mRNA to cover prostate and pancreatic cancer, as well as head-and-neck cancer **[63]**. With the ongoing

development of bioinformatics, delivery systems, and immunoengineering, mRNA-based cancer vaccines will be a base of precision oncology, which will provide a new dimension of personalized immune-based cancer treatment **[64].**

Table 2 presents significant ongoing clinical trials of mRNA cancer vaccines that have been proven to show clinical efficacy in various cancers. The most promising vaccines such as mRNA-4157 (44% reduction in recurrence risk) by Moderna and BNT122 by BioNTech have strong T-cell responses in combination with checkpoint inhibitors These phase II/III clinical trials confirm the potential of mRNA vaccines as a new precision oncology therapy, which targets a wide range of tumor neoantigens, in a personalized and tailored way.

Table 2:- Major ongoing clinical trials of mRNA cancer vaccines.

S.NO.	Vaccine/candidate	TargetAntigen / Cancer Type	Sponsor/Developer	Clinical Phase	Key Outcomes/ Findings	References
1.	mRNA-4157(V940)	Personalized Neoantigens – Melanoma, NSCLC	Moderna & Merck(MSD)	Phase IIb / III	Showed 44% Reduction in melanoma recurrence risk when combined with pembrolizumab (Keytruda); ongoing Phase III for advanced melanoma.	**[60]**
2.	BNT122 (RO7198457)	Individualized neoantigens –	BioNTech&Genentech (Roche)	Phase II	Demonstrated strong antigen-specific $CD8^+$ T-	**[59]**

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		Multiple solid tumors.			cell responses; combination with atezolizumab improved tumor control.	
3.	BNT111	Fixed tumour antigens (CLDN6, MAGE-C3, PRAME, NU-ESO-1) -Advanced melanoma	BioNTech SE	Phase II	Durable immune activation and partial tumor regression; well tolerated with cemiplimab.	**[61]**
4.	BNT113	HPV16 E6/E7 oncoproteins – HPV-positive head&neck squamous cell carcinoma.	BioNTech SE	Phase II	Enhanced antigen-specific T-cell response and measurable tumor shrinkage; low-grade toxicity profile.	**[62]**
5.	CV9202 (BI 1361849)	Multiple lung cancer-associated antigens (MUC1, NY-ESO-1, survivin, 5T4).	CureVac AG&Boehringer Ingelheim	PhaseI/II	Safe, immunogenic, and synergistic when combined with immune checkpoint inhibitors.	**[63]**
6.	mRNA-5671(V941)	KRAS mutations (G12C, G12D, G12V, G13D) – Pancreatic&colorectal cancers.	Moderna & Merck (MSD)	Phase I	Induced KRAS-specific T-cell immunity across multiple tumor types; well tolerated.	**[62]**

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7.	BNT115	Ovarian cancer antigens (MUC16, NY-ESO-1, etc.).	BioNTech SE	Phase I/II	Strong immunogenicity with minimal adverse effects; ongoing for advanced ovarian cancer.	**[60]**
8.	Lipo-mRNA Vaccine (CV9201)	Five tumor-associated antigens – NSCLC.	CureVac AG	Phase I	Demonstrated safety and antigen-specific immune activation; supported future vaccine combinations.	**[61]**
9.	mRNA-2752	OX40L, IL-23, IL-36γ – intratumoral mRNA immune modulator.	Moderna Therapeutics	Phase I	Increased cytotoxic T-cell infiltration; enhanced efficacy with immune checkpoint blockade.	**[60]**
10.	BNT114	Neoantigens – Triple-negative breast cancer (TNBC)	BioNTech SE	Phase I/II	Elicited specific T-cell responses; good safety profile and early signs of efficacy.	**[63]**

5. mRNA Therapy in Genetic and Metabolic Disorders.

The messenger RNA (mRNA) therapeutics have shown exceptional prospects in treating genetic and metabolic disorders since they allow the in situ expression of functional proteins that are lacking or malfunctioning because of an inherited mutation. In contrast to conventional gene therapy where DNA

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is introduced into the nucleus where there is a risk of genomic integration, mRNA therapy transacts directly into the cytoplasm where transient controlled protein expression has a better safety profile. This is an effective disease treatment approach to diseases due to loss-of-function mutation including cystic fibrosis, Duchenne muscular dystrophy (DMD), and enzyme-deficiency diseases like methylmalonicacidemia and propionic acidemia **[64]**.

The unique principle of the mRNA-based protein replacement therapy is based on the transfer of codon-optimized synthetic mRNA encoding the functional variant of the lost or dysfunctional protein into the cells of the patient. The host ribosomes then translate this mRNA into the therapeutic protein once internalized and basically suppress the underlying genetic defect. Due to the short-term action of mRNA and its natural destruction in the post-translation process, it is possible to repeat the treatment again without introducing irreversible changes to the genome **[65]**.

MRNA therapy in cystic fibrosis (CF) is expected to repair the malfunctioning production of the cystic fibrosis transmembrane conductance regulator (CFTR) protein that regulates the passage of chloride ions in epithelial cells. Preclinical experiments of lipid nanoparticle (LNP)-encapsulated CFTR mRNA have demonstrated re-establishment of ion channel activity in lung epithelial cells and enhanced mucus clearance which forms the basis of clinical development. On the same note, in the case of Duchenne muscular dystrophy (DMD), the mRNA therapy aims at the re-introduction of functional dystrophin or micro-dystrophin proteins in the muscular dystrophy cell to enhance muscle integrity and activity **[66]**.

mRNA therapy in enzyme-deficiency metabolic inborn errors, including methylmalonicacidemia (MMA) and propionic acidemia (PA), is an intravenous delivery of mRNA that codes the missing enzyme of metabolism directly to the liver, where the enzyme is produced and normal metabolic flux is restored **[67]**. Moderna and Translate Bio have presented preclinical data with encouraging potential results of the dramatic decrease in the toxic metabolite accumulation and the extension of the survival time of animal models **[68]**.

Nonetheless, significant issues in delivery have not been fully addressed yet, especially regarding effective and tissue-specific uptake of mRNA as well as reduction of immunogenicity. All in all, the development of mRNA-based protein replacement therapy has created a new frontier to treat rare genetic and metabolic pathologies that were previously deemed incurable because of their ability to shield the mRNA and deliver it to specific organs, particularly the liver and lungs. Subsequent improvements in delivery technology, stability of mRNA, and immunomodulation will be critical in converting the clinical research findings on these therapies into safe and effective clinical therapy **[69]**.

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The mRNA therapeutic system consists of fast production, lipid nanoparticle development, target delivery, and translation into the host cell to generate functional therapeutic proteins **(Figure 2).** This workflow takes advantage of the engineered mRNA structural elements such as 5' m7G cap, optimized untranslated regions, codon-optimized coding sequence and poly(A) tail to achieve the maximum stability and translational efficiency. Lipid nanoparticles mediate endosomal avoidance and cytoplasmic delivery, which allow the target cells to translate the encoded protein by ribosomes. This simplified mechanism is the foundation of the flexibility of mRNA therapeutics to a wide variety of disease applications in oncology to regenerative medicine **[70]**.

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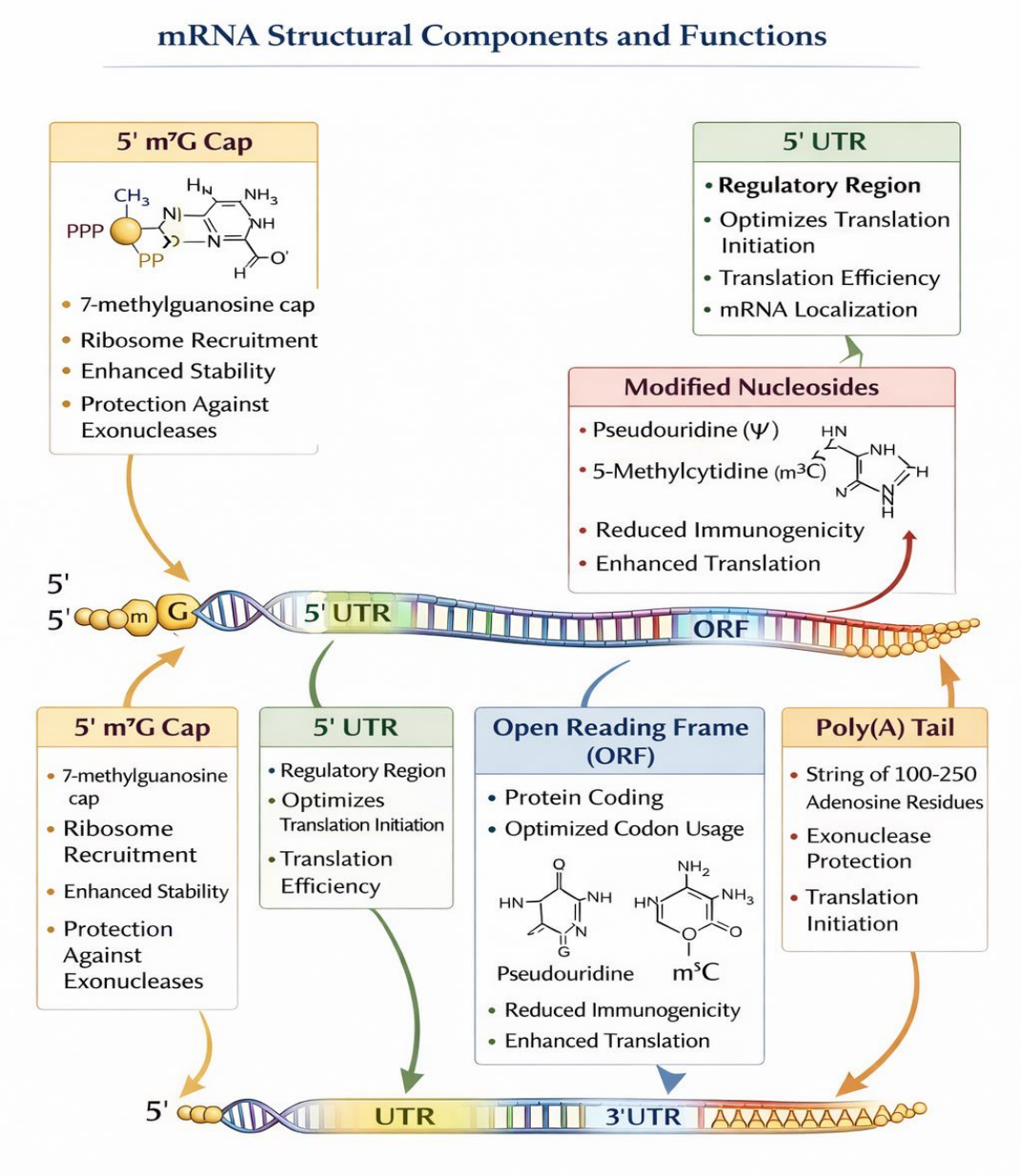

Figure 2: Mechanism of mRNA-based protein replacement therapy — showing translation of therapeutic protein inside cells.

Diagrammatic illustration of engineered mRNA architecture with increased stability, translation efficiency and decreased immunogenicity. m7G cap of 5′ protection against exonucleases and ribosomal recruitment, untranslated region (UTR) regulation of translation pace and mRNA half-life, codon-optimized open reading frame (ORF) production of the therapeutic protein with altered nucleosides (pseudouridine, m5C), and poly(A) tail cytoplasmic stability by PABP interactions.

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The chemical modifications reduce the innate immune recognition but preserve high translational capacities.

6. mRNA Applications in Cardiovascular and Autoimmune Diseases

mRNA therapeutics have lately expanded their range into cardiovascular and autoimmune diseases, two of the key disease areas that current therapies have frequently been poorly effective in or with non-curative symptoms. The exclusivity of mRNA to instruct the body cells to manufacture a therapeutic protein in a controlled and temporal way presents a novel method of controlling disease processes on the molecular basis. Restoring immune tolerance and preventing aberrant immune activation mRNA-based therapies have the goal of regenerating damaged heart tissue and stimulating angiogenesis in cardiovascular medicine, and restoring immune tolerance and preventing aberrant immune activation in autoimmune diseases **[71].**

In cardiovascular diseases, the leading mRNA-based therapeutic approach deals with ischemic heart disease, myocardial infarction and heart failure-diseases that are associated with functional cardiac tissue loss and insufficient blood supply. Among the most developed strategies is the expression of mRNA of vascular endothelial growth factor (VEGF-A) which promotes new blood vessels creation and improves the blood flow through the tissue **[72].** Higher preclinical and early clinical trials, including the Moderna AZD8601 (VEGF-A mRNA), have shown promising results with an improvement in myocardial activity and vascularization after the ischemic injury The angiogenic activity of VEGF is transiently expressed by mRNA, reducing the chance of uncontrolled angiogenesis, which is the main safety issue with previous gene therapy methods [**73]**.

In addition to angiogenesis, mRNA therapeutics are also potentially investigated in cardiac tissue regeneration. The restoration of cardiac contraction and fibrosis in preclinical models with the delivery of mRNA encoding cardioprotective or regenerative factors- including fibroblast growth factor (FGF), stromal cell-derived factor-1 (SDF-1) or transcription factors that stimulate cardiomyocyte proliferation has been demonstrated. Its non-integrating quality ensures minimum oncogenic risk of permanent gene modification and allows mRNA to be used in the transient expression of such potent biological agents **[74]**.

In autoimmune diseases, mRNA technology is not being used to provoke immunity, but to cause immune tolerance- an immune technology paradigm shift of its past vaccine use. Expressing particular autoantigens in tolerogenic conforms by encoding can also remodel the immune system to perceive these proteins as its own instead of foreign. As an illustration, there are preclinical studies in models of multiple sclerosis (MS) and type 1 diabetes (T1D) that indicate that mRNA expression of disease-relevant antigens, when delivered in immune suppressive lipid

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nanoparticles, can inhibit autoreactive T-cell responses and inhibit disease progression **[75].**

Table 3 enumerates mRNA therapeutic candidates under development for cardiovascular and autoimmune diseases, highlighting their transition beyond oncology applications. Key programs include VEGF-encoding mRNA for myocardial ischemia (AZD8601, EPICCURE trial) promoting angiogenesis and PSAT1-modRNA for heart regeneration post-infarction. These candidates demonstrate mRNA's potential to address unmet needs in heart failure, hypertension, and autoimmune dysregulation through targeted protein replacement and immunomodulation.

Table 3: List of mRNA candidates under development for cardiovascular and autoimmune diseases

S.NO.	mRNA Candidate / Vaccine	Therapeutic Target/ Mechanism	Developer/Institution	Clinical Trials Phase	References
1.	AZD8601 (VEGF-A mRNA)	Encodes vascular endothelial growth factor A (VEGF-A) to promote cardiac tissue regeneration and angiogenesis after myocardial ischemia.	Moderna Therapeutics&AstraZeneca	Completed Phase IIa (EPICCURE trial) – demonstrated improved cardiac perfusion and safety.	**[76]**
2.	mRNA-0184	Encodes relaxin protein to enhance cardiac remodeling and vascular compliance in heart failure.	Moderna Therapeutics	Preclinical / early Phase I – showing promising cardiac functional recovery in models.	**[77]**
3.	VEGF-A LNP-	Synthetic mRNA encoding VEGF-A for peripheral	CureVac AG	Preclinical – shown to induce	**[78]**

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	mRNA (CV0201)	artery disease (PAD).		angiogenesis and improved perfusion in animal models.	
4.	mRNA-6231	Encodes IL-2 mutein designed for selective expansion of regulatory T cells (Tregs) to suppress autoimmune activity.	Moderna Therapeutics	Phase I – demonstrated tolerability and potential immune-modulatory effect in autoimmune conditions.	**[79]**
5.	BNT151	Encodes IL-2 variant to stimulate cytotoxic T-cell responses in autoimmune disorders and oncology overlap indications.	BioNTech SE	Phase I/II – ongoing safety and immune activation evaluation.	**[80]**
6.	mRNA-6981	Encodes PD-L1 fusion protein to restore immune tolerance in autoimmune diseases.	Moderna Therapeutics	Preclinical – effective in restoring immune balance in lupus and type 1 diabetes animal models.	**[75]**
7.	CV7202 (Modified	Encodes IL-10 for systemic	CureVac AG	Preclinical – significant	**[77]**

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	IL-10 mRNA)	inflammation reduction in rheumatoid arthritis and Crohn's disease.		suppression of inflammatory cytokine release.	
8.	BNT162b2 (Repurposed platform study)	Using optimized lipid nanoparticle platform for mRNA delivery targeting autoimmune myocarditis.	BioNTech SE	Platform under preclinical evaluation for cardiovascular inflammation modulation.	**[79]**
9.	mRNA-TREM2	Encodes TREM2 protein to regulate microglial activity for neuroinflammatory cardiovascular cross-talk.	Moderna Therapeutics	Discovery / Preclinical – under investigation for Alzheimer's-associated vascular inflammation.	**[80]**
10.	VEGF-A Isoform mRNA (BNT161)	Designed to enhance endothelial regeneration and repair post-myocardial infarction.	BioNTech SE	Preclinical – promising angiogenic and cardioprotective properties.	**[81]**

7. mRNA for CRISPR-Cas9 and Gene Editing Delivery

The mRNA technology has become quite a prominent and safer mechanism of delivery of CRISPR-Cas9-based systems to genome editing, which is a solution to most of the constraints of

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the existing viral and plasmid DNA delivery methods. With artificial mRNA-mediated transient expression of CRISPR-associated (Cas9) nuclease and guide RNA (gRNA) elements, investigators have the ability to perform targeted, regulated and efficient genomic modification without the potential of long-term genetic integration into the genome**.** The approach has dramatically developed the gene-editing technology and enhanced safety profiles and therapeutic applicability of CRISPR technologies **[82].**

In traditional gene editing, the Cas9 and gRNA sequences are delivered using viral vectors e.g. lentiviruses or adeno-associated viruses (AAVs). Although these vectors are useful in high transfection efficiency, they have significant limitations such as the induction of gene insertion, extended Cas9 expression, off-target gene mutation, and immune stimulation. Continuous Cas9 activation enhances the risk of non-intentional double strand breakages, and this is a matter of safety concern particularly in therapeutic application.

In comparison, mRNA-based CRISPR delivery offers a short-term system of expression, the Cas9 mRNA and gRNA are directly transfected into the cytoplasm and translated into active Cas9 protein before being rapidly degraded **[83]**. This provisional association of Cas9 helps greatly to diminish the danger of off-target effects and decrease the genotoxicity. In addition, mRNA does not require nuclear delivery, thus eliminating the threat of insertional mutagenesis- which is one of the significant setbacks of viral delivery systems. The other advantage of mRNA-mediated CRISPR delivery is that the expression of Cas9 can be tightly controlled in time, and can be delivered in brief bursts of editing activity, which can be fine-tuned to particular therapeutic requirements. Lipid nanoparticles (LNPs) that have already been proven in mRNA vaccines are popular in CRISPR mRNA delivery because of their high biocompatibility and capacity to entrap both mRNA and gRNA components **[84]**. CRISPR systems based on LNPs have been shown to have strong genome editing in liver, muscle and hematopoietic cells in preclinical and early clinical applications.

As an example, other firms like Intellia Therapeutics and Moderna are leading the way in systemic in vivo CRISPR editing by formulations based on mRNA-LNPs. The NTLA-2001, an Intellia treatment for transthyretin amyloidosis (ATTR), was the first clinical demonstration of CRISPR gene editing performed with the use of LNP-mRNA directly to human cells in which the pathogenic protein levels remained reduced **[85].**

8. Monoclonal Antibody and Regenerative Medicine via mRNA

mRNA technology is not limited to use in vaccines and a gene therapy- the technology is also revolutionizing the monoclonal antibody manufacturing and regenerative medicine fields. Traditionally, the bulk bioreactors produce monoclonal antibodies (mAbs) using the complicated

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and time-intensive cell culture processes. In comparison, monoclonal antibodies that are synthesized in vivo in a patient can be synthesized using mRNA-based methods. The introduction of synthetic mRNA coding the heavy and light chains of an antibody into host cells can convert them into sort of temporary bioreactors where antibodies are synthesized and ultimately this will allow the cell to recognize and neutralize specific targets **[86].**

There are several advantages to this method: it can be prepared fast; its dosage is adjustable and it may be able to generate antibodies because of new pathogens or treatment needs without having to be produced in cells. Preclinical expression of neutralizing antibodies against infectious diseases such as rabies, influenza and SARS-CoV-2 has been successful upon delivery of the respective mRNA **[87]**.

Figure 3 shows mRNA in regenerative medicine to stimulate tissue repair and regeneration by expressing growth factors, signalling molecules and structural proteins involved in the coordination of tissue repair. The angiogenesis and cardiac tissue regeneration and after infarction myocardial recovery, like mRNA encoding vascular endothelial growth factor (VEGF) or insulin-like growth factor -1 (IGF-1), have been demonstrated to be stimulated. Similarly, the mRNAs of the bone morphogenetic proteins (BMPs) or fibroblast growth factors (FGFs) are used to enhance the regeneration of bones and muscles in preclinical studies **[88].**

They are not associated with risks of fibrosis and tumorigenesis since mRNA is temporary and permits the protein expression to be localized and managed. Together, these applications point to the promise of mRNA to transform antibody therapy and regenerative medicine with rapid, programmable and now patient-specific therapeutic applications which are no longer constrained by the traditional generation of biologic therapies **[89]**.

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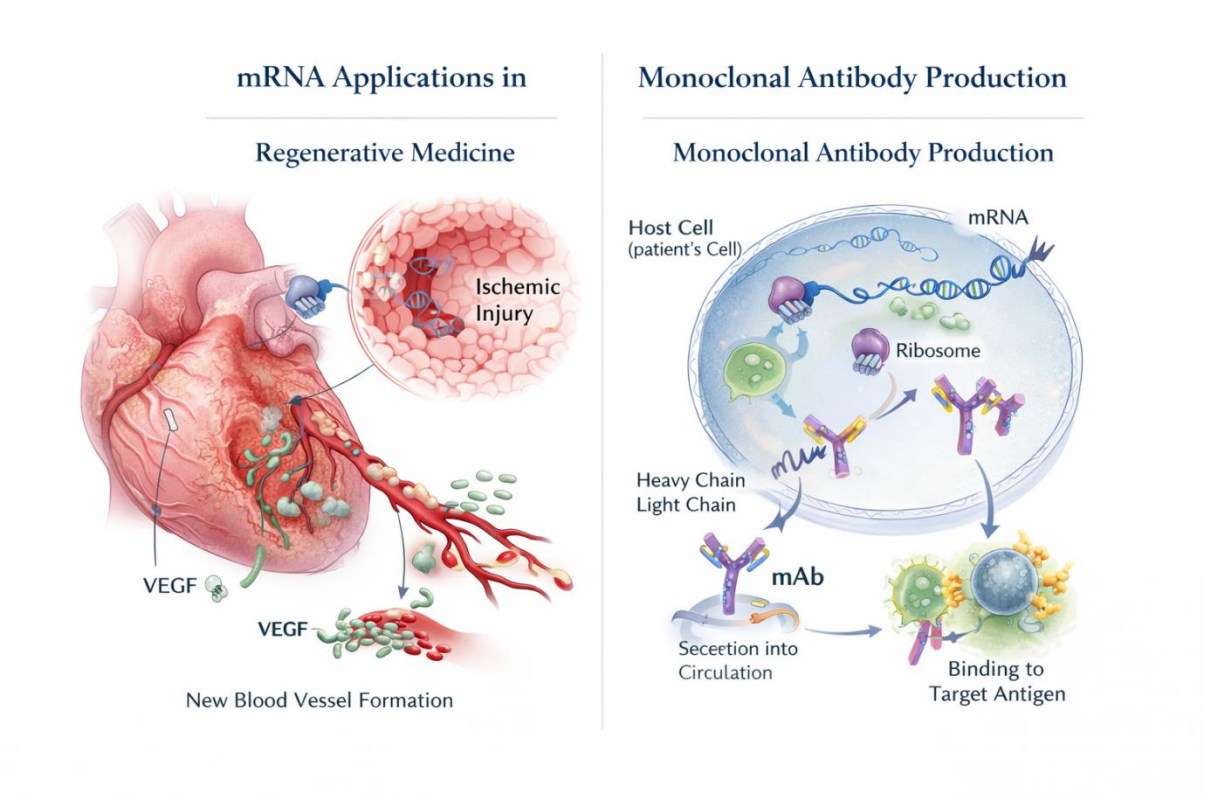

Figure 3:- Conceptual illustration showing mRNA use in regenerative medicine and monoclonal antibody production.

Dual Panel schematic indicating mRNA applications in the absence of vaccination. Left: mRNA-LNPs transfer VEGF to ischemic cardiac tissue that stimulates angiogenesis and myocardium regeneration by forming new vessels. Right Host cells encode mRNA into production of functional monoclonal antibodies to neutralize their target or to regulate immunity. This technology allows the quick initial transient generation of therapeutic proteins in the tissues of the patient.

9. Challenges and Limitations

Although mRNA therapeutics have the potential to transform the medical landscape, a number of scientific, technical, and regulatory factors have remained to curb the widespread clinical application of these therapeutics. Among the main obstacles is mRNA instability because naked mRNA molecules are vulnerable to quick degradation by extracellular ribonucleases (RNases). Despite the enhancement of lipid nanoparticles (LNPs) stability by chemical modifications and optimized formulations, a consistent expression level has been considered a major issue **[90]**. In addition, the need of ultra-cold storage and transportation required as with first-generation

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COVID-19 mRNA vaccines places a strain on logistics which inhibits large-scale global allocation,especially in low-resource environments **[91]**.

The other significant weakness is accidental immune activation. Artificial mRNA may activate the innate immune sensors like Toll-like receptors (TLRs), with the result of an excess of inflammation or low levels of protein translation. Although the effects have been alleviated by the incorporation of modified nucleosides (e.g., pseudouridine) and removal of contaminants of double-stranded RNA, fine-tuning of immune tolerance without affecting therapeutic efficacy is complicated **[92]**. Scalability and the cost of production is also a major challenge. Production of high-purity mRNA and LNPs is a demanding technology and quality management, which is expensive relative to traditional biologics. The three points of streamlining production pipelines, enhancing ambient temperature stability, and scalable production are important in the future of mRNA therapeutics **[93].**

On top of technical obstacles, there are also issues of ethics and regulation, which are also challenges. The speed of development of mRNA-based interventions also puts the safety of these interventions in the long term, balanced access, and transparency of data in question. In personalized mRNA vaccines and gene-editing drugs, regulatory authorities continue to establish systems to assess personalized formulations, and existing regulatory and approval systems are not designed to support personalized formulations **[94]**.

10. Future Prospects and Innovations

The future of mRNA therapeutics has impressive evolution, as it is based on groundbreaking advances that are targeted at eliminating the current shortcomings and broadening the therapeutic scope to other diseases other than infectious diseases. The creation of circular mRNA (cmRNA) one of the most promising achievements as it removes free ends that can be degraded by exonucleases and leads to a large increase in stability and extended protein expression. Compared to traditional linear mRNA, cmRNA has better translational activity and lower immunogenicity which makes cmRNA an advanced platform of long-term therapeutic solutions.

The second important area of research development is the development of thermostable mRNA formulations. Researchers are now moving toward the development of vaccines and therapeutics that are capable of being stored at room temperature, and so not rely on ultra-cold storage, as well as making these accessible in more parts of the world, especially in low-resource areas. The ever-improving of lipid nanoparticles (LNPs) delivery systems is also redefining the environment. Advanced LNPs are being designed with detailed targeting of delivery to specific tissues, i.e. liver, heart, or immune cells with minimal off-target effects. New options of polymer-based nanoparticles and lipid-polymer hybrids are also available with tunable release profiles and

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increased biocompatibility. Other than technological innovation, the incorporation of personalized medicine platforms will make strides in the design of therapeutics. With the integration of mRNA production with AI-mediated genomics, it is possible to develop patient-specific applications in a short period of time, including personalized cancer vaccines or enzyme-replacement therapies, in a safe way. Pharmacists and pharmaceutical scientists will be playing critical roles in this change. Their knowledge on formulation science, stability evaluation and regulatory requirement will be essential in the process of applying laboratory innovations into clinical products. Moreover, they will be at the forefront of pharmacovigilance, counseling of patients and optimization of therapy, which guarantees safe and effective interventions based on mRNA.

11. Conclusion

Messenger RNA (mRNA) therapeutics have already moved beyond a hypothetical technology to become a viable and transformative medical platform and change the nature of precision and personalized medicine. The effectiveness of COVID-19 mRNA vaccinations by BioNTech/Pfizer and Moderna was evidence that synthetic mRNA can be safely, quickly and efficiently administered to humans, and served as a basis to explore more significant therapeutic uses. After that, mRNA has been developed to be applied in cancer immunotherapy, genetic disease repair, cardiovascular regeneration, autoimmune changes, and CRISPR-based gene editing. The improvement in technological systems has significantly improved mRNA stability, translation efficiency, and targeted delivery, especially using lipid nanoparticles (LNPs) to control protein expression with precision because of technological growth, specifically the nucleoside modification, codon optimization, and lipid nanoparticle (LNP) delivery. The mRNA has expanded the scope of preventive vaccines with these innovations to active disease treatment and modulation on a molecular level. Also, there are novel approaches like the use of circular mRNA, thermostable preparations, as well as organ-targeted delivery systems, which would help address the existing constraints of stability, immunogenicity, and storage.

Regardless of this development, there are a number of issues that are yet to be solved. Such concerns as lack of longevity of transient expression, immune response, the cost of production, and cold-chain support have to be resolved before mRNA therapeutics will be clinically fully matured. Ethical and regulatory guidelines should also change to take into account individualized and fast-adjustable treatments. Still, there is no denying the fact that the future of mRNA therapeutics looks very bright. The capacity to program cellular machinery in order to produce customized proteins provides the platform with a distinctive advantage compared to the traditional drugs and biologics. With the wide-ranging convergence of interdisciplinary activity in nanotechnology, immunology, and bioengineering, mRNA-based therapeutics will become the

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new center of interest in future medicine delivering safer, quicker, and precise therapies to otherwise untreatable complex diseases. The post-COVID-19 period in a way will not be the

conclusion of the revolution of mRNA in medicine, but rather a start thereof, i.e. turning it into a universal system of treatment, which would be able to transform the future of the entire global healthcare.

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