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Review Article

Next-Generation Therapy: mRNA Redefining Pharmacy Practice

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ABSTRACT

Next-generation medications using mRNA and biologics are revolutionizing pharmacy practice and providing accurate, adaptable, and rapid therapy options by using the body's own cellular machinery to produce therapeutic proteins. mRNA treatments, classified as biological pharmaceuticals, use in vitro transcribed mRNA to send genetic instructions for protein synthesis directly into patient cells, circumventing the complex production processes associated with conventional protein-based medications. These treatments have shown considerable promise in immunotherapy, vaccines, and protein replacement due to ongoing advancements in delivery technologies, mRNA design, and synthetic biology techniques that improve their efficacy, specificity, and safety.The implementation of synthetic biology expands the therapeutic potential beyond traditional applications by introducing novel modalities like logic gates on single mRNA molecules and modular self-assembled mRNA nanoparticles. As mRNA biologics move from clinical trials to commercialization, manufacturing advancements and exacting analytical techniques are essential for guaranteeing quality and scalability while meeting regulatory criteria. Additionally, mRNA systems' programmability and adaptability put them in a position to overcome obstacles that DNA and protein therapies must overcome, such as therapeutic index restrictions and targeted delivery.Overall, mRNA and biologics are redefining pharmacy by enabling personalized, on-demand therapies with broad applications across oncology, infectious diseases, and genetic disorders, marking a paradigm shift in drug development and clinical practice.

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INTRODUCTION:

Systems for delivering drugs are essential to customized therapy. These systems enable better treatment results, fewer side effects, and increased patient compliance by customizing drug administration to each patient's unique details. The goal of personalized medicine is to tailor medical treatments according to each patient's particular genetic composition, way of life, and medical background.Next-generation drug delivery systems have been made possible by the combination of cutting-edge technologies with medication delivery, especially in the treatment of chronic illnesses and cancer. By releasing tailored nanoparticles under regulated conditions, these systems seek to improve target efficiency at the tissue and cellular levels. These nanoparticles are made to react to particular stimuli, including reactive oxygen species (ROS), pH, or enzymes. This makes it possible to provide accurate, individualized treatments that are catered to each patient's particular need.

Numerous investigations on the potential of messenger RNAs (mRNAs) as a treatment for infectious diseases and cancer have been carried out throughout thirty years. The ineffective transport of naked modified mRNA, which leads to low amounts of protein production, has been a significant barrier in the development of this technology. Numerous delivery options have been assessed in order to tackle this difficulty.After decades of research and development, the first RNA-based medicine, patisiran (OnpattroTM), was released in 2018. The US Food and Drug Administration (FDA) licensed this lipid nanoparticle (LNP)-encapsulated small interfering RNA (siRNA) to treat inherited ATTR amyloidosis. This was a significant breakthrough in the area and opened the door for the widespread consumption of mRNA-based drugs [1-2].There are numerous disorders that can be treated with mRNA technology.Although the development of new mRNA products was rapid, the move from workspace to clinical implementation of mRNA took over ten years, and extensive work was necessary for generating stable mRNAs that could be translated in vivo [3-6].Because of the high immunogenicity, poor stability, and high cost of mRNA synthesis, pharmaceutical companies have generally been unwilling to invest in significant research and clinical trials on these products. Innovative approaches to mRNA modification, purification, and sequence design have reignited interest in mRNA as a therapeutic strategy [7]. Multiple studies are now exploring mRNA drugs and vaccines because of their vast variety of possible applications and ease of synthesis. Since mRNA medications and vaccines are

generally anticipated to be effective substitute treatments for malignancies, one of the main areas of ongoing research is cancer prevention and treatment [8].

Researchers first proposed the idea of nucleic acid-encoded medications more than 20 years ago when they showed that the encoded protein was expressed in the injected muscle when in vitro transcribed (IVT) mRNA or plasmid DNA (pDNA) was directly injected into the mice's skeletal muscle.In the first several decades after mRNA was discovered in eukaryotic cells, the primary focus was to understand its structural and functional properties, as well as its metabolism. Furthermore, a broader research community will have simpler access to mRNA recombinant engineering resources. Preclinical research on IVT mRNA began in the 1990s for a range of applications, including protein substitution and vaccination techniques for infectious and malignant illnesses [9-17].Thus, cumulative information enabled current scientific and technological breakthroughs to overcome some of the problems posed by mRNA, including its poor immunogenicity and short half-life [18].IVT mRNA-based therapy approaches differ from other nucleic acid-based treatments in several important aspects. IVT mRNA is promptly translated once it reaches the cytoplasm; it doesn't need to enter the nucleus to operate. In contrast, DNA therapies require access to the nucleus in order to be converted into RNA, and the disruption of the nuclear envelope during cell division is necessary for them to operate. By combining these cutting-edge strategies, biologic drug delivery systems present promising opportunities for better treatment outcomes in the fields of chronic illnesses and neoplasia. They also address the need for personalized therapies in drug delivery, patient-specific therapeutic and diagnostic maneuvers (theranostics), and precision medicine in the use of biologics [19-21].

Understanding mRNA Technology:

Ribonucleic acid (RNA) is a macromolecule that exists in all living cells. Cells use RNA for various activities, including protein synthesis. Messenger RNA (mRNA) transmits protein-encoding information from a cell's DNA (deoxyribonucleic acid) to its protein manufacturing machinery. Enzymes known as RNA polymerases convert DNA sequences into mRNA. Ribosomes make proteins by joining amino acids in the order specified by the mRNA sequence, known as "translation." mRNA, or messenger RNA, is a compound that contains the instructions for cells to produce a protein utilizing their natural machinery. Altering mRNA, which plays a crucial role in protein creation, is a viable alternative to altering the cell's DNA.

Adding the right mRNA to a cell can lead it to produce a certain protein [22].To travel through cells effectively, mRNA moves within a protective bubble known as a lipid nanoparticle.Lipid nanoparticles are tiny bubbles of lipid that can enclose mRNA. Encapsulation can preserve mRNA from destruction before it reaches the target cells. Controlling the lipids and chemicals used in nanoparticle production can enhance mRNA delivery to targeted cells, increasing the likelihood of protein production. Both successful mRNA-based COVID-19 vaccines used carefully designed lipid nanoparticles.Messenger RNA plays a crucial function in protein creation, making it a potential target for several medicinal therapies and vaccinations. COVID-19 vaccinations are the first FDA-approved mRNA-based vaccinestechnology. Clinical trials are underway for HIV, rabies, and influenza vaccines, as well as cancer and uncommon disease treatments [23]. However, there are challenges to mRNA research and its wider applications, such as potential immune responses, targeting the right tissue, and preventing premature degradation.

History of mRNA Technology:

Although mRNA technology has been considered since the 1960s, SARS-CoV-2 was the first to demonstrate its effectiveness. Its effectiveness in treating COVID-19 has increased interest in creating comparable methods for other illnesses. To put it briefly, messenger ribonucleic acids, or mRNAs, cause cells to mount an immune defense before being destroyed. They work by giving the body an outline for an antigen unique to the disease that is transcribed, causing the body to produce antibodies against it [24-25]. The immune system will identify this antigen once it has developed in the body and be prepared to fight the virus, bacteria, or parasite. Before the COVID-19 pandemic, scientists started developing mRNA vaccines to prevent influenza, cancer, and a number of other diseases, such as rabies, Zika, and Ebola. But in recent years, the region has grown significantly [26].

Mechanism of Action:

Essentially mRNA technology's mechanism of action is not complicated—once inside cells, it directs them to generate proteins—researchers have spent years creating methods which enable mRNA to function effectively in actual environments. To allow our own cells to conduct the arduous work of making proteins, mRNA has proved to be a superb platform for the development of vaccinations (and possibly medicines), which in turn produces an immune response that helps protect us from illnesses.

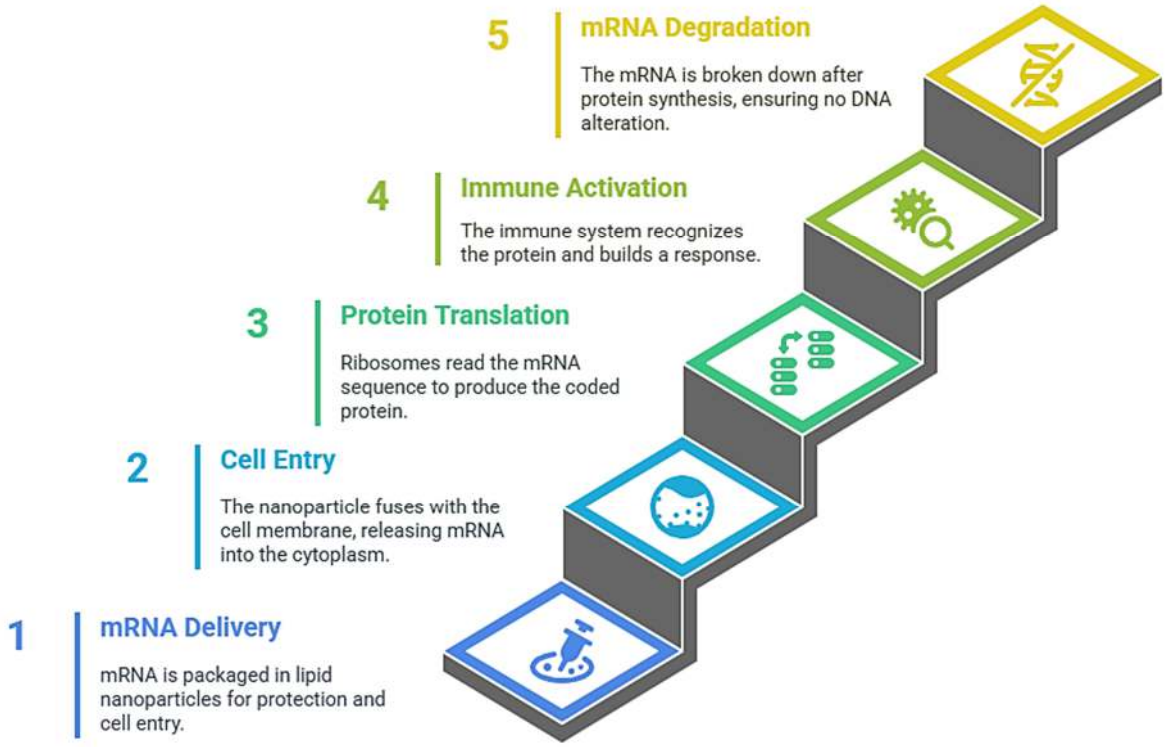

Figure 1. Mechanism of Action of mRNA Technology

Applications in therapeutics

mRNA-based medicines are a promising therapeutic class that might revolutionize cancer treatment. Alternatives include immunomodulatory drugs, monoclonal antibodies, therapeutic vaccines, and CAR (Chimeric Antigen Receptor) cell therapies [27-31].Unlike DNA-based medications, mRNAs can be used as templates to produce any protein or peptide through protein synthesis in transfected cells [32].Compared to DNA-based medications, mRNA-based therapies offer higher transfection efficiency, reduced toxicities, and do not require nucleus translocation for function [33].Furthermore, mRNA molecules are less susceptible to insertional mutagenesis, reducing the chance of unintentional infection [34].mRNAs have the ability to effectively cure disorders that require high protein levels due to their continuous translation and sustained production of encoded proteins and peptides [35].Although well-designed mRNA can boost translation efficiency, it is not feasible to administer naked mRNA due to its low cellular absorption efficiency [36].The anionic cell membrane repels negatively charged mRNA, leading to poor absorption. Moreover, mRNA medicines are typically larger than other compounds

that can easily penetrate into cells. Additionally, naked mRNA is susceptible to nuclease destruction [37].Multiple strategies have been explored to disseminate mRNA both in vivo and in vitro, addressing these hurdles.

Application of mRNA Vaccines:

mRNA vaccines have been found to be a reliable and efficient way of suppressing COVID-19 spread [38]. The first company to receive an emergency application for an mRNA vaccine was BNT/Pfizer (BNT162b2). The Moderna vaccine (mRNA-1273) received its license in a flash. Approximately 90% of fully vaccinated people and 80% of moderately vaccinated people were protected from wild-type SARS-CoV-2 infection. Prior to SARS-CoV-2, the quickest mumps vaccine was created utilizing an attenuated virus. The development of mRNA vaccines has accelerated, allowing emergency approval in just a few months[39].Recent studies using mRNA vaccines have examined the efficacy of a variety of infectious diseases, including Respiratory Syncytial Virus (RSV), influenza, Ebola virus, Zika virus (ZIKV), rabies virus, T. gondii, Streptococcus spp., and new COVID-19 variants. Because mucosal immunity is essential for preventing infectious illnesses, several vaccine delivery techniques have been developed [40-41].Two methods for optimizing COVID-19 mRNA vaccines include adjusting two proline codons to stabilize the translation product and employing a modified mRNA encoding the prefusion S protein (BNT162b2 and mRNA 1273). Numerous mRNA-based drugs, particularly those for illnesses involving immune cells, are being developed for clinical use.

Clinical Applications of mRNA Therapies:

Vaccines for Infectious Diseases:

Vaccination is a popular method for preventing several diseases. Vaccines have successfully averted and cured several life-threatening infections. Clinically approved vaccinations include pathogens that have been inactivated or attenuated, as well as components and transmissible viruses. Vaccines often take 15-40 years to progress from preclinical research to clinical testing. mRNA-based therapy aims to treat diseases that are resistant to traditional treatments, such as cancer, infectious diseases, cardiovascular disease, cerebrovascular disease, metabolic genetic diseases, and others. mRNA therapies provide a number of advantages, including high efficacy, minimal adverse effects, and ease of manufacture. The primary goal of this study is to evaluate new mRNA vaccine technology. New mRNA therapeutics, such as self-amplifying mRNA (saRNA) and circular mRNA

(cRNA), are being studied in preclinical and clinical settings and offer tremendous promise in comparison to FDA-approved non-replicative mRNA- From preclinical research to clinical trials, vaccines frequently take 15–40 years. mRNA drugs provide many benefits, such as high effectiveness, few adverse effects, and simplicity of manufacture. This study's main objective is to assess novel mRNA vaccination technology. Compared to FDA-approved non-replicative mRNA-based pharmaceuticals, novel mRNA treatments such as self-amplifying mRNA (saRNA) and circular mRNA (cRNA), which are being studied in preclinical and clinical contexts, have enormous potential. Compared to already licensed mRNA vaccines, SaRNA vaccines may require far lower doses and obviate requirement for many injections[42]. The decrease in efficacy across novel variations has been addressed via vaccine design. It is anticipated that a number of interventions, such as booster shots and bivalent vaccines, will improve vaccine effectiveness [43].

Influenza:

In 1918, the world experienced an influenza pandemic, and the virus remains a top cause of death today. Three of the four influenza virus types—A, B, C, and D—have the ability to infect humans[44]. While influenza C frequently causes minor illness in adults, influenza A and B are the primary illness of seasonal epidemics. After 10 years of virus epidemics, the World Health Organization (WHO) claims to have attenuated influenza. Immunization considerably lowered mortality rates [45]. Several authorized influenza vaccines include recombinant, inactivated split, live attenuated, inactivated subunit, and disabled whole virus. When influenza and SARS-CoV-2 co-infect, the risk of death and morbidity increases. Some research suggests that influenza may make SARS-CoV-2 infections more common. Researchers are creating combination vaccinations that offer simultaneous protection against both viruses in order to solve the problem of co-infection [46-47]. It is anticipated that combined mRNA immunizations will reduce the likelihood of pandemics and lethal diseases.

Flaviviruses:

The Dengue virus, Zika virus, Japanese encephalitis virus, and yellow fever virus are all members of the Flaviviridae family of viruses [48]. Because of the association to fetal death, the ZIKV epidemics in 2015 and 2016 produced a global health disaster [49]. Since this virus having only one serotype, developing vaccines against it is simpler than taking into account different strains [50]. Because of their comparable envelope proteins (about 50%), ZIKV

vaccines may cross-neutralize DENV. Neutralizing antibodies to DENV serotypes may be low in poorly designed Zika vaccinations. Serious symptoms might arise from recurrent infections with various DENV serotypes that become aggravated by antibodies [51].Concerned about ADE; Antibody Dependent enhancement (ADE) of dengue fever, the USFDA has only approved Dengyaxia (DENV Vaccine) [52]. Currently, there are no licensed Zika vaccinations. The Brazilian and Indonesian governments have licensed Qdenga (TAK-003) for dengue prevention [53]. Two Dengue DNA vaccines are undertaking phase I studies (NCT00290147 and NCT01502358), while one Zika DNA vaccine (VRC5283) has completed phase I investigations and is now in phase II clinical trials (NCT03110770).

Personalized Cancer Vaccines:

Cancer immunotherapies engage the immune system to inhibit tumor growth and possibly remove cancer from the body [54]. Cancer vaccines use tumor-specific antigens to enhance immune responses as well as activate T cells, which eradicate cancer [55]. In 2010, the US Food and Drug Administration authorized the first cancer vaccine. The phase III clinical study discovered that replacing GM-CSF-activated APCs in patients led to a 4.1 month improvement in their expected lifespan [56].Several strategies have been used to improve the efficacy of cancer vaccines, including combining immune stimulatory molecules with cancer antigens, promoting immune-activating conditions in the tumor microenvironment, and combining vaccines with traditional medical treatments such as radiation or chemotherapy. One viable next-generation cancer therapeutic option is mRNA vaccines.

Tumor antigens are categorized as tumour-associated (TAA), tumor-specific (TSA), or neoantigens. TAA expression differs between tumors and healthy tissues; malignancies show higher levels, whereas healthy tissues show lower levels. During clinical immunotherapy, TAA, a non-mutated self-antigen, leads to inadequate T-cell responses [57]. Dendritic cells (DCs), the main APCs that increase T cell immunity, are the target of the majority of cancer vaccines [58]. Without changing the content or adding surface ligands, RNA-LPX can adjust the net charge and distribute mRNAs to DCs and macrophages in lymphoid organs in a consistent manner. The mRNA-LPX vaccination was efficacious when injected in situ because it delivered IL-12 mRNA to modify the tumor microenvironment and rewire DCs to activate T cells [59]. Another approach is to create ex vivo DC vaccines by combining RNA-LPX with

iron oxide nanoparticles (IONPs) and giving them to patients. RNA-IONPs increased the effectiveness of DC transfection and made it possible to use magnetic resonance imaging (MRI) to track migration. RNA-IONP-treated DC injection dramatically reduced the growth of tumors [60].Bacterial-derived outermost membrane vesicles (OMV) have been studied as a possible mRNA delivery route for cancer vaccinations. The RNA binding protein L7Ae and the lysosomal escape protein listeriolysin O (OMV-LL) were genetically engineered to accumulate box C/D sequence-labeled mRNA antigens by binding to the OMV surface. The combination effectively transported mRNA to DCs in vivo, causing antigen cross-presentation and endosome escape. The novel delivery method showed remarkable therapeutic advantages in a colon cancer animal model [61].

Drugs in Clinical Trial:

During the COVID-19 pandemic, Moderna and BNT/Pfizer's mRNA vaccines introduced new disease-fighting capabilities, accelerating research and clinical trials using mRNA platforms[62].mRNA-based COVID-19 vaccines have been rapidly developed and introduced globally in recent years. These vaccines solve the issues of immunogenicity & mRNA stability, which were previously significant challenges to advancement. Scientists hope that new mRNA vaccines will effectively protect and treat persons afflicted with the virus. It is anticipated that the new COVID-19 vaccines will produce cross-neutralization antibodies against VOCs, especially Delta and Omicron variants, and provide safer, longer-lasting protection[63].mRNA vaccines are now being researched for a number of viral illnesses, including Zika (NCT04917861), RSV (NCT05127434), and EBV (NCT05164094) [64].Phase II/III trials are currently in progress for the mRNA-1647 cytomegalovirus vaccine (NCT05683457, NCT05085366), which contains the CMV pentamer including glycoprotein B antigens. Moderna's fourth mRNA vaccine, the seasonal qIRV influenza vaccine (mRNA-1010; against WHO-proposed strains), is in phase III trials (NCT04956575). Moderna is testing an mRNA vaccine (mRNA-1073) to prevent influenza and COVID-19 [65]. CRISPR-modified primary human T cells are currently being investigated in clinical trials as a therapy for metastatic gastrointestinal cancer (NCT04426669). This medicine is expected to block CISH, an intracellular checkpoint protein that was previously inaccessible, without interfering with cell viability or function [66–67]. Adenine base editor (ABE) therapy for

sickle cell anemia has begun a clinical trial (NCT05456880).

Future directions in Pharmacy Practice:

Within a year of the COVID-19 pandemic, mRNA vaccines for SARS-CoV-2 were developed and approved, demonstrating the enormous potential of this technology. COVID-19 mRNA vaccines were found to be more effective and safer than other vaccinations based on inactivated viruses, recombinant proteins, or viral vectors. The mRNA vaccines have a significantly shorter design-to-manufacture time (66 days), compared to the typical 10-15 year schedule for vaccine development [68-70]. Altered nucleotides, along with cationic liposomes, have solved the hurdles of poor stability and delivery into cells, making mRNA an extremely useful tool for disease prevention and treatment [70–73]. Future efforts to establish innovative mRNA uses in cancer vaccines and immunotherapy may benefit from the knowledge gained by carrying mRNA vaccines through all stages of pharmaceutical medicine development and commercialization [74-75].

Conclusion:

The field of RNA-based therapeutics is at the forefront of research. More academic and industrial researchers are working in this field as a result of the benefits of mRNA-based therapies over traditional biomedicines. mRNA has a variety of properties, including: Shorter, simpler, and less expensive development and production procedures compared to traditional biologics that rely on complicated systems alike; cell lines or E. coli. In vitro enzymatic mRNA synthesis offers multiple benefits which include a low risk of pathogenic infection, the ability to switch between different proteins by altering the mRNA sequence, the absence of mRNA integration inside the genome, along withcapability to target intracellular proteins that were previously inaccessible to anti-body and protein drugs. Potential uses include infectious disease vaccines, e.g., cell therapy, cancer immunotherapy, COVID-19, gene editing, and protein replacement treatment. Direct intervention in genetic illnesses can be accomplished by either suppressing the expression of harmful proteins or introducing functional proteins. MRNA vaccines elicit extensive and effective immune responses, leading to better protection rates compared to conventional vaccines. Examples are Moderna's and BNT/Pfizer's COVID-19 vaccine.

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