Review



# Affordable mRNA Novel Proteins, Recombinant Protein Conversions and “Biosimilars”—Advice to Developers and Regulatory Agencies

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*Review*

# Affordable mRNA Novel Proteins, Recombinant Protein Conversions and "Biosimilars"—Advice to Developers and Regulatory Agencies

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**Abstract:** The mRNA technology can replace the expensive recombinant technology for every type of protein, making biological drugs more affordable. It can also expedite the entry of new biological drugs, and the copies of approved mRNA products can be treated as generic or biosimilar products due to their chemical nature. Hundreds of new protein drugs have been blocked due to the high cost of recombinant development. The low CAPEX and OPEX associated with mRNA technology bring it within the reach of developing countries currently deprived of lifesaving biological drugs. In this paper, we advise developers to introduce novel proteins, switch recombinant manufacturing to mRNA delivery, and further advise the regulatory authorities to allow approval of copies of mRNA products with less testing. We anticipate that mRNA technology will make protein drugs like natural and engineered proteins, monoclonal antibodies, and vaccines accessible to billions of patients worldwide.

**Keywords:** mRNA; recombinant; new drugs; approved functional proteins; mAbs; protein vaccines; biosimilars

## 1. Introduction

Protein drugs comprise therapeutic proteins, functional and engineered monoclonal antibodies, vaccines, and modifications manufactured by a recombinant engineering process that is the most expensive, blocking the availability of hundreds of new protein drugs and keeping the cost of protein drugs high. The mRNA technology was validated by the success of mRNA COVID-19 vaccines; mRNA is a chemical entity of nucleic acids that can be well characterized, making their development and manufacturing costs much lower. While mRNA can encode short interfering RNAs (siRNAs) or microRNAs (miRNAs) that participate in RNA interference (RNAi) pathways, as well (Table 1), it is their role in expressing proteins that are the focus of this paper. Table 1 lists the types of mRNAs that can provide these applications.

**Table 1.** Types of mRNAs.

| mRNA Type | Applications | Properties | Regulatory Status |
|---|---|---|---|
| Conventional mRNA [1] | Vaccines | Basic structure with 5' cap, UTRs, poly(A) tail | Approved for COVID-19 vaccines |
| Modified mRNA [2] | Protein replacement, cancer vaccines | Chemically modified nucleosides | Clinical trials for genetic diseases, COVID-19 vaccines |
| Self-amplifying mRNA (saRNA) [3] | Vaccines, gene therapies | Self-replicating sequences for dose-sparing | Early clinical trials |
| Circular RNA (circRNA) [4] | Long-term protein replacement therapies | Circular structure for enhanced stability | Preclinical and early clinical stages |
| Therapeutic Guide RNA [5] | Gene editing for genetic disorders | Guide sequence directs gene editing | Clinical trials under ATMP designation |

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Note: As of November 2024, there were 785 clinical trials listed in clinicaltrials.gov [6].

## 2. Protein Drugs

The discovery of proteins and understanding their structures and functions in the human body emerged gradually over centuries, marked by transformative scientific breakthroughs. The term "protein" itself derives from the Greek word "proteios," meaning "primary" or "of first importance," reflecting the early perception that these molecules were fundamental to life. Dutch chemist Gerardus Johannes Mulder is credited with coining the term in 1838 after analyzing organic molecules rich in nitrogen, including substances found in blood and egg whites [7]. This analysis pointed to a new class of compounds distinct from other known organic materials, yet it would take more than a century to uncover proteins' true complexity and significance.

The structure of proteins was first revealed through pioneering work in the 20th century. The linear sequence of amino acids within a protein, known as its primary structure, was first determined for insulin by Frederick Sanger in 1951 [8]. Sanger's work was groundbreaking because it demonstrated that proteins are not just amorphous organic compounds but are composed of specific sequences of amino acids that dictate their function. For this discovery, Sanger was awarded the Nobel Prize in Chemistry, marking a turning point in protein chemistry. This initial success led to further structural discoveries. Linus Pauling and Robert Corey, in the early 1950s, elucidated key structural motifs like the alpha-helix and beta-sheet [9]. These secondary structures revealed that proteins fold into specific configurations driven by hydrogen bonds, a crucial insight into how proteins achieve their functional forms. In 1958, John Kendrew took the understanding of protein structure even further by using X-ray crystallography to solve the three-dimensional structure of myoglobin [10]. This was the first time a protein's tertiary structure was visualized at an atomic level, showcasing the intricate folding patterns essential to biological function.

Simultaneously, the role of proteins in bodily functions began to unfold. By the mid-20th century, it was well understood that enzymes—specialized proteins—act as catalysts for metabolic reactions, facilitating nearly all chemical processes in the body [11]. The discovery that hormones, antibodies, and transport proteins also consist of proteins underscored their versatility, as these molecules were shown to be integral to signaling pathways, immune responses, and transport of essential molecules like oxygen. Through these advancements, proteins came to be viewed as central to nearly every cellular process, and their roles in health and disease became a focal point in medical research [12]. The advent of molecular biology, genomic sequencing, and proteomics in the late 20th and early 21st centuries further solidified proteins as the primary agents of biological function, offering a profound understanding of life at the molecular level and shaping modern medical and biological research.

While significant progress has been made in identifying human proteins, understanding their functions, and linking deficiencies to diseases, we do not yet have a complete and detailed understanding of all human proteins. The Human Genome Project [13] provided the complete sequence of human DNA, allowing scientists to predict approximately 20,000–25,000 protein-coding genes, laying the foundation for protein research. The Human Proteome Project (HPP) [14], an international initiative, aims to map all human proteins, identifying their structures and expression in various body parts. Although we have cataloged most human proteins, databases like UniProt (https://www.uniprot.org/) and the Human Protein Atlas (https://www.proteinatlas.org/ ) continue to expand our knowledge, offering detailed information on many proteins' functions, structures, and interactions.

However, not all essential proteins are "druggable," meaning they may lack characteristics that allow effective modulation by small molecules or biologics. Factors influencing druggability include protein structure, function, and accessibility within the body. The presence of well-defined binding pockets or cavities that can accommodate drug molecules is crucial; proteins lacking such features are challenging to target with small molecules. Additionally, proteins involved in complex interactions or those without a clear active site may be less amenable to drug targeting. For instance,

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proteins that function through protein-protein interactions often present flat surfaces, making it difficult for small molecules to bind effectively [15].

## 3. Recombinant Technology

Until the arrival of recombinant technology, the only supply of several critical therapeutic proteins was through natural sources. For example, insulin was initially extracted from the pancreas of pigs and cows. It is widely used for diabetes management despite causing immune reactions in some patients due to its animal origin [16]. Human growth hormone (hGH) was sourced from cadaver pituitary glands for growth disorders. However, this posed a risk of transmitting prion diseases, such as Creutzfeldt-Jakob disease, due to the sourcing from human tissues [17].

Similarly, Factor VIII and Factor IX, crucial for treating hemophilia A and B, were derived from pooled human plasma, which presented a significant risk of contamination with bloodborne viruses like HIV and hepatitis C [18]. Albumin, a blood volume expander used in cases of hypovolemia and shock, was extracted from pooled human plasma, leading to similar contamination concerns and supply limitations [19]. Immunoglobulins, particularly intravenous immunoglobulin (IVIG), were obtained from large pools of human plasma and are still often sourced this way, as recombinant versions remain difficult and complex. Interferons, such as interferon-alpha and Interferon-beta, were initially isolated from human leukocytes and fibroblasts for treating viral infections and multiple sclerosis, though in limited quantities due to sourcing challenges [20]. Additionally, Follicle-Stimulating Hormone (FSH), used for fertility treatments, was initially purified from the urine of postmenopausal women (urinary FSH) before recombinant FSH became available, which ensured a more stable supply [21]. The Hepatitis B vaccine was another key therapeutic originally plasma-derived from infected individuals, posing a risk of contamination and supply shortages until recombinant versions became available in the early 1980s [22].

Many recombinant proteins are copies of natural functional proteins designed to mimic natural proteins; others are novel or engineered molecules with unique functions distinct from naturally occurring proteins, highlighting the diversity and broad therapeutic applications of biological products. Many therapeutic proteins are constructed, designed, or modified using biotechnological methods to achieve targeted therapeutic effects. Engineered cytokines and growth factors are modified for enhanced stability or extended half-life, such as pegylated interferons used in hepatitis treatment, which are attached to polyethylene glycol (PEG) to extend their half-life.

Antibody fragments like Fab or scFv are engineered for specific applications, often with smaller sizes for better tissue penetration. Certolizumab pegol, a PEG-modified antibody fragment against TNF, improves pharmacokinetics [23], and Blinatumomab (Blincyto) is a bispecific T-cell engager using two scFvs to bridge T cells and cancer cells, facilitating targeted cell lysis in B-cell malignancies [24]. Nanobodies, single-domain antibodies derived from camelids, are engineered to have only the minimal binding domain, providing high tissue penetration, as seen in Caplacizumab, used to treat thrombotic thrombocytopenic purpura [25].

## 4. mRNA Technology

The discovery that ribosomes synthesize proteins evolved through critical research efforts in the mid-20th century, particularly in the 1950s and 1960s. Early work on cell biology had identified ribosomes as small, dense structures within the cytoplasm, yet their function remained unclear. The concept of ribosomes as the site of protein synthesis began to crystallize in the 1950s, as researchers linked ribosomes with cell protein production [26].

In 1955, cell biologist George Emil Palade observed ribosomes in the rough endoplasmic reticulum through electron microscopy. He noted that ribosomes were frequently associated with newly synthesized proteins, suggesting a possible role in protein assembly. Palade's observations marked the first significant indication that ribosomes could be involved in protein synthesis. However, it was not until subsequent research, particularly in the early 1960s, that this role was definitively established [27].

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The work of François Jacob and Jacques Monod in 1961 supported this by proposing the "messenger RNA hypothesis." They hypothesized that messenger RNA (mRNA) carried genetic information from DNA to ribosomes, where proteins were synthesized according to this genetic code. Their model predicted that ribosomes and mRNA translate genetic information into functional proteins [28]. Following this, in the early 1960s, studies by Matthew Meselson and Franklin Stahl helped clarify the mechanics of ribosomal protein synthesis, illustrating the critical role of mRNA as a template [29].

Finally, groundbreaking experiments by researchers like Marshall Nirenberg and Heinrich Matthaei, who cracked the genetic code in 1961, provided the first concrete experimental evidence that ribosomes use mRNA to assemble amino acids into proteins. By the mid-1960s, these discoveries firmly established ribosomes as the "machines" of protein synthesis, with their role in translating genetic information into proteins widely accepted in the scientific community [30].

Palade was eventually awarded the Nobel Prize in Physiology or Medicine in 1974 for his contributions to understanding cellular structures and functions, including his discoveries on ribosome involvement in protein synthesis [31]. Together, these efforts cemented the role of ribosomes as the sites of protein synthesis, a fundamental insight that revolutionized molecular biology and our understanding of cellular function.

### *4.1. Developing mRNA Protein Delivery*

A complete mRNA structure consists of several components, including a 5′ cap, 5′ untranslated region (UTR), coding sequence (CDS), 3′ UTR, and poly(A) tail. Capping is an essential process for creating functional mRNAs, as it modifies the ability of an mRNA to undergo processing and translation. Four main endogenous cap structures are known: cap0, cap1, cap2, and m6Am cap. In the cell, cap2-containing mRNAs account for about 50%. Meanwhile, the m6Am cap is formed by N6 methylation and is found on about 30–40% of mRNAs [32]. While the molecular function of cap2 is unclear, it is known that the m6Am cap contributes to increased mRNA stability in cells [33] (Figure 1).

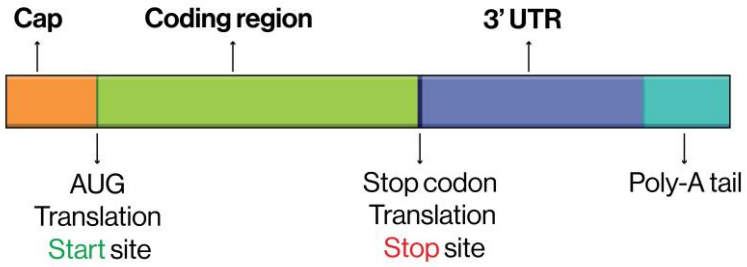


**Figure 1.** mRNA Structure (Shutterstock Image).

For mRNAs produced by in vitro transcription (IVT), two primary methods are used to cap the molecules. First, an ARCA (anti-reverse cap analog) may be added by replacing the 3′ hydroxyl group of m7G with a methoxy group. ARCA-capped mRNAs generally have relatively high translation efficiencies and long half-lives [34]. The co-transcriptional trimeric cap analog is the primary capping option, successfully applied in SARS-CoV-2 mRNA vaccines [35].

Modified bases have been utilized in mRNA production to reduce the immunogenicity of the mRNA. For instance, 5-methylcytidine (m5C), pseudouridine (Ψ), and N1-methyl pseudouridine (m1Ψ) have all been used for this purpose [36]. Among these modifications, m1Ψ-containing mRNAs were shown to induce more protein production than m5C- and Ψ-containing mRNAs [37].

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Other characteristics of the mRNA have also been shown to affect protein production. For instance, codon usage is essential during mRNA design, as synonymous codons can contribute to different protein production levels or affect protein folding and function [38]. In addition, more upstream open reading frames within an mRNA might titrate the translation initiation complex and affect protein translation [39].

Improvements to mRNA stability and efficiency can be made by several different approaches, including base modifications or the use of saRNAs and circRNAs. CircRNAs are more resistant to exonuclease degradation than linear RNAs due to their lack of 5′ and 3′ ends [40]. Noncanonical RNA splicing events endogenously produce this type of RNA, and some endogenous circRNAs are known to function as sponges for miRNAs or templates for stress-responsive peptides in mammalian cells [41].

While the use of both saRNAs and circRNAs seems to be growing, both types of RNA have key disadvantages. For example, saRNAs are limited by a potential safety concern that the alphavirus element may induce unwanted immune responses; this concern will require careful attention in clinical studies. Although circRNAs show excellent inherent stability, which can support a longer mRNA half-life and more sustained protein expression, a significant limitation of this approach is the complex manufacturing process. Besides selecting the most desirable characteristics of the mRNA, developing high-efficiency, low-toxicity mRNA vaccines, and drugs also requires optimizing a production process that can reliably generate pure mRNAs.

### *4.2. mRNA Purification and Quality Control*

After synthesis by IVT, an mRNA product may contain many impurities, which can promote mRNA degradation. As such, different regulatory agencies have generated quality guides for mRNA vaccines. For example, the US Pharmacopeia (USP) released the "Analytical Procedures of mRNA Vaccine Quality" for Quality by Design (QbD) of mRNA manufacturing standards and analytic methods in April 2023. Removing impurities is a critical step for mRNA drug development, and mRNA has many physicochemical properties that can be utilized for purification. For instance, mRNA is a giant molecule with a molecular weight often exceeding 300 kDa and a more than 50 nm physical size. These features make the molecule amenable to purification by size exclusion chromatography (SEC). Previously, mRNAs produced by IVT were separated from the DNA template, enzymes, and excess nucleoside triphosphates (NTPs) using a Superdex-75 column or other SEC columns [42]. Notably, the RNA conformation will affect SEC resolution, and double-stranded RNA (dsRNA) byproducts may not be separable due to their similarity in size to the single-stranded mRNA (ssRNA).

Additionally, SEC is often not appropriate for scale-up in large-scale manufacturing. Another property that can be exploited for mRNA purification is the high hydrophobicity of the molecule [43]. Hydrophobic interaction chromatography (HIC) with suitable binding salts has proven to be an effective means of separating mRNAs from proteins, dsRNAs, and short RNAs. A commonly used separation method in mRNA vaccine production is reverse-phase HPLC (RP-HPLC). Several studies have shown that RP-HPLC purification can eliminate dsRNA-induced immunity and increase translatability by 10- to 1000-fold compared to non-HPLC-purified mRNAs [44]. However, HPLC still has limitations, such as the potential use of toxic organic solvents.

Another approach for mRNA purification is affinity columns, such as oligo-dT columns that can effectively remove impurities without poly(A) tails. This method has been applied for SARS-CoV-2 and anti-influenza immunoglobulin G (IgG) mRNAs but cannot separate ssRNA and dsRNA well [45]. An alternative strategy to remove precursor and intron RNAs is affinity purification with highly selective affinity ligands [46]. Since dsRNA is difficult to remove by separation technologies, enzyme digestion with RNAIII may be needed, as this enzyme can digest dsRNA without affecting mRNA integrity [47]. Alternatively, cellulose fibers may remove dsRNAs in an ethanol-containing buffer due to specific binding by 2-hydroxyl residues in the dsRNA. This method is scalable and achieved 90% removal of dsRNA with more than 65% mRNA recovery [48].

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While impurities must be removed from IVT-generated mRNA, it is possible that chromatographic purification will affect mRNA structure and biological function. Therefore, further development of high-efficiency purification technologies is an ongoing pursuit. In this regard, using specific ligands for combination or sequential purification strategies might offer a new pathway to improve the purity and yield of IVT-generated mRNAs [49].

### *4.3. Storage and Cold Chain Management*

The instability of mRNA-LNPs during storage is primarily attributable to chemical degradation by hydrolysis and oxidation reactions. Hydrolysis can lead to the cleavage of phosphodiester bonds in the mRNA backbone, while oxidation may result in base cleavage and alterations to the mRNA secondary structure [50].

During the lyophilization process, cryoprotective reagents are critical for preventing mechanical disruption of the mRNA-LNPs due to ice crystals. The most common cryoprotectants encountered in the literature for freeze-drying microparticles are sugars, such as trehalose, sucrose, glucose, and mannitol [51]. Notably, different compositions and ratios of LNP components can greatly affect the physical and chemical properties of an mRNA-LNP product.

### *4.4. Lipid Compositions and Ligand Targeting*

A major challenge in ensuring the safe and efficient delivery of bioactive mRNA that can be easily degraded in the body has brought significant intellectual property. The delivery systems should ensure that the mRNA efficiently reaches the target cells and is taken up to produce the desired therapeutic effects. Moreover, modifying the surface of LNP carriers may allow the drugs to evade the immune system and improve circulation time. For instance, PEGylation can be used to enhance the stability of nanoparticles. Limited immune system activation is also crucial for the success of mRNA therapies, as unwanted immune responses can lead to adverse effects and reduce treatment effectiveness. Current efforts are focused on designing less immunogenic mRNA sequences and developing optimized LNP delivery systems to minimize immune reactions and increase cargo expression. The successful development of safe and effective delivery methods for mRNA-based drugs will require multidisciplinary studies, combining expertise in molecular biology, chemistry, materials science, and immunology. Ongoing research aims to address the remaining challenges related to mRNA-based drugs to unlock the full potential of this emerging class of therapeutics.

Currently, all FDA-approved LNPs are composed of four types of lipids: ionizable lipids, phospholipids, cholesterol, and PEG lipids [52]. Among these components, PEG lipids are of concern because they may induce the production of anti-PEG antibodies. Upon repeated injection of PEG-containing mRNA-LNPs, the anti-PEG antibodies will target PEG-coated mRNA-LNPs and reduce the delivery efficiency [53]. Although significant safety concerns have not been raised about mRNA vaccines, it is essential to remember that their clinical use is still relatively new, and the side effects and other limitations of mRNA medicines still need to be thoroughly studied.

The rapid development and high efficacy of mRNA-based COVID-19 vaccines stimulated great interest in mRNA-based drugs or vaccines against various infectious and immunological diseases. In addition, chemically synthesizing stable mRNA was a breakthrough that expanded the drug development potential for mRNA technology. Since IVT-generated mRNAs can be directly translated into therapeutic proteins, mRNA is widely considered a highly promising therapeutic modality in the pharmaceutical industry[54]. The potential of mRNA-based medicines is not only limited to vaccines for infectious diseases, but the technology may also prove to be an excellent medium for gene and protein therapies. An advantage of mRNA-based drugs is that they are not subject to the high production costs associated with antibody-based drugs. Another benefit is that optimized delivery strategies may improve therapeutic efficacy by allowing specific delivery of therapeutic nucleic acids to target cells [55].

LNPs are efficiently endocytosed into cells and transported to endosomal compartments. To further deliver the mRNA cargo into the cytosolic region, LNPs must escape from endosomes. Within

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the acidic endosomal environment, the ionizable lipids in LNPs are protonated and become positively charged, which allows the lipids to bind negatively charged lipid molecules on the endosomal membrane. This interaction triggers phase transition and fusion of the LNP with the endosomal membrane, releasing the nucleic acid cargo into the cytosol. A significant limitation of this approach is that most of the endocytosed LNPs are eventually guided to lysosomes for degradation, and only a tiny portion (about 2%) successfully escape from endosomes to deliver the nucleic acid cargo. Several strategies have the potential to overcome this limitation, including the development of novel ionizable lipids, incorporation of helper lipids, and inclusion of other new materials. With these approaches, researchers expect to enhance the endosomal escape of LNPs and increase the efficiency of nucleic acid delivery by LNPs.

**5. Recombinant vs Ribosomal**

The FDA's drug approval process is comprehensive and can span several years. In total, bringing a new drug to market can take approximately 10 to 15 years. Using mRNA technology instead of recombinant protein methods can significantly reduce drug development timelines for several reasons:

- Eliminating Protein Production: Traditional recombinant protein production requires creating, optimizing, and scaling up cell lines to produce the protein. mRNA eliminates this process, as mRNA directly encodes the protein of interest, which is then made within the patient's cells. This reduces both preclinical and manufacturing timelines.
- Simplified Manufacturing: mRNA synthesis is more straightforward and faster than protein production, as it involves in vitro transcription, which can be completed within weeks. In contrast, recombinant proteins require complex bioreactors, extensive purification, and scaling-up processes, which can take months or even years.
- Rapid Design and Iteration: mRNA sequences can be quickly modified if adjustments are needed, such as changes in dose or specific protein regions. This adaptability is especially valuable in early development, allowing researchers to iterate quickly without lengthy cell line adjustments.
- Streamlined Preclinical Testing: mRNA drugs, mainly vaccines, may bypass some traditional preclinical studies, as they have a different safety profile than recombinant proteins. The risk of toxicity is generally lower, as mRNA does not integrate into the genome and is rapidly degraded after the protein is expressed.
- Potential for Faster Clinical Trials: mRNA's faster production enables quicker scale-up for clinical trials, reducing the wait time between phases. mRNA therapies also tend to elicit a robust immune response, which may shorten dose-ranging and efficacy assessment stages, especially in vaccines.
- Regulatory Pathways and Accelerated Approval: The success of COVID-19 mRNA vaccines has led to new regulatory insights and expedited review pathways, potentially offering shorter approval timelines for future mRNA-based therapeutics.
- Estimated Time Reduction: MRNA technology can potentially reduce the typical drug development timeline from around 10–15 years to as short as 5–8 years in some cases, depending on the disease and the regulatory pathway chosen.

mRNA technology can significantly reduce costs in several areas, but the reduction is often not strictly proportional to the time savings. Here’s how mRNA can impact expenses compared to traditional recombinant protein-based drugs:

- Lower Manufacturing Costs: mRNA production uses more straightforward and more scalable processes. Traditional recombinant proteins require costly mammalian or microbial cell culture systems, which are resource-intensive and involve complex purification steps. In contrast, mRNA synthesis can be done rapidly in vitro, cutting manufacturing costs by approximately 30-40%.
- Reduced Infrastructure and Facility Requirements: Recombinant protein production often demands specialized bioreactors, sterile environments, and stringent quality controls. In contrast, mRNA manufacturing can use more straightforward equipment and smaller facilities, reducing fixed costs and the capital investment needed for facilities.

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- Streamlined Preclinical and Clinical Phases: mRNA's flexibility reduces the need for extended preclinical testing and enables faster transition through clinical trials. This can lower costs associated with maintaining and managing trials, reducing expenses for staffing, data monitoring, and patient recruitment.
- Less Batch Variability and Simplified Quality Control: Each batch is less variable with mRNA since it's made through a predictable in vitro transcription process. This contrasts with protein products, which vary depending on cell line behavior and production conditions. Reduced variability translates to less waste and fewer quality control expenditures.
- Reduced R&D Costs Through Rapid Iteration: Because mRNA sequences are easily adjustable, early-stage development costs drop as researchers can adjust sequences without creating new cell lines. This makes R&D less expensive, especially in preclinical phases, as modifications to dose or protein regions don't involve extensive re-optimization.

Overall, these factors can reduce the cost of developing an mRNA-based therapeutic to potentially half or less of a traditional protein-based therapeutic's cost, depending on the product's complexity and regulatory requirements. While some estimates suggest around a 50% reduction in expenses, the savings will depend on the production scale and regulatory complexities for each specific mRNA application.

Using mRNA for novel monoclonal antibodies (mAbs) can potentially be even faster and more cost effective than traditional recombinant methods for mAb development and production, though there are specific considerations:

- Faster Development Timeline: Producing mAbs typically involves generating stable cell lines, optimizing them, and scaling production. This process can take years. With mRNA, the sequence coding for the mAb can be synthesized and delivered directly to the body's cells, which then produce the antibody. This approach skips cell line development, reducing early-stage development by up to 50%. Additionally, mRNA-encoding mAbs can be optimized and modified more rapidly than protein-based mAbs, allowing faster adjustments and fewer delays if dose modifications or structural changes are needed.
- Lower Production Costs: Traditional mAb production is complex, requiring costly cell culture facilities, extensive purification processes, and quality control. mRNA bypasses these since manufacturing involves producing mRNA, which is more straightforward and can be done in smaller facilities. mRNA is highly scalable, allowing manufacturers to adjust production quickly to meet demand without needing major infrastructure expansion, which is costly in conventional mAb production. This reduces batch-production costs significantly. Producing mRNA with consistent quality is often simpler than ensuring consistency in mAb production across batches, especially in complex systems like CHO cells.
- Reduced R&D Costs: The mRNA platform is highly adaptable, allowing rapid iteration with lower costs if changes are required. For mAbs, adjustments to the mRNA sequence do not require creating new cell lines, thus eliminating associated time and expense. Since mRNA allows in vivo expression of mAbs, early testing can begin faster and at potentially lower costs.
- Speed of Regulatory Approval: The FDA has become increasingly familiar with mRNA technology thanks to recent approvals and accelerated pathways. For mAb-based treatments, this familiarity could result in quicker regulatory processes if safety profiles are favorable, though this is still a developing area. For novel mAbs, the use of mRNA can cut development costs and timelines even more than typical mRNA-based proteins. Costs could be reduced by over half compared to conventional mAb production methods, with timelines potentially shortened from 8–12 years to as little as 4–7 years for specific applications, especially if regulatory bodies offer expedited paths for mRNA-based mAbs.

**6. New Protein Drugs**

While the knowledge about endogenous proteins is well-developed, only a tiny fraction of these proteins has been developed to fulfill their deficiency or use them for a defined novel treatment due to the high cost of recombinant manufacturing that does not justify their development due to their smaller market. These constraints can be removed if the primary method of developing proteins shifts to mRNA delivery, which will allow these remarkable opportunities as examples:

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- Adipocyte Fatty Acid-Binding Protein (AFABP) transports fatty acids within adipose tissue. It may help regulate fat metabolism, with targeting AFABP proposed as a potential strategy to reduce fat storage and enhance fat mobilization [56].
- AMP-Activated Protein Kinase (AMPK) is an energy-sensing enzyme that promotes autophagy, reduces inflammation, and prevents age-related diseases; it can be activated through diet, exercise, or compounds like metformin [57].
- Amyloid Precursor Protein (APP) is essential for neuron health, though improper processing can lead to Alzheimer's; maintaining normal APP levels while preventing harmful byproducts is a research focus [58].
- Brain-derived neurotrophic Factor (BDNF) is crucial for synaptic plasticity, and its deficiency is linked to learning deficits and cognitive decline, with therapeutic approaches aimed at increasing BDNF levels showing promise. Nerve Growth Factor (NGF) supports neuron survival, with NGF gene therapy being explored for Alzheimer's disease [59].
- Calcium/Calmodulin-Dependent Protein Kinase II (CaMKII) is essential for memory processes, and targeting CaMKII signaling through therapy may support cognition. CREB (cAMP Response Element-Binding Protein) regulates memory consolidation, and therapies enhancing CREB may benefit learning in neurodegenerative conditions [60].
- Collagen: Collagen is a structural protein in connective tissues. Its extensive post-translational modifications, such as hydroxylation and glycosylation, are challenging to replicate in recombinant systems. While recombinant collagen-like proteins are produced, they may not fully mimic the properties of native collagen [61].
- Elastin: Elastin provides elasticity to tissues like skin and blood vessels. Recombinant production is complicated by its repetitive sequences and cross-linking requirements. Some elastin-like polypeptides are synthesized recombinantly but may not fully replicate native elastin's properties [62].
- Fibroblast Growth Factor (FGF) supports tissue repair, blood vessel formation, and cell survival in heart tissue [63].
- Forkhead Box O (FOXO) proteins, such as FOXO3, regulate cell survival, stress resistance, and metabolism, enhancing antioxidant defenses and DNA repair, with FOXO3 associated with longer lifespans [64].
- Fragile X Mental Retardation Protein (FMRP) is essential for cognitive development, with therapies targeting its affected pathways in intellectual disabilities [65].
- Heat Shock Proteins (HSPs), particularly HSP70, protect other proteins from damage by refolding misfolded proteins, preventing aggregation associated with Alzheimer's [66].
- Irisin, a protein produced by muscles during exercise, is believed to help convert white fat, which stores energy, into brown fat, which burns energy in a process called "browning," potentially increasing energy expenditure and reducing fat stores [67].
- Keratin is a structural protein in hair, nails, and skin. Its insolubility and tendency to form strong disulfide bonds make recombinant production challenging. Recombinant keratin-like proteins have been developed, but they may not fully replicate the characteristics of native keratin [68].
- Klotho, a protein associated with lifespan extension, regulates calcium and phosphate metabolism, supports kidney and cardiovascular health, and reduces inflammation, with higher levels linked to improved brain function [69].
- Leptin is a hormone that regulates energy balance by signaling the brain to reduce appetite when fat stores are adequate, potentially promoting fat burning in individuals with leptin sensitivity [70].
- Lipoprotein Lipase (LPL) breaks down triglycerides in the bloodstream into free fatty acids, which can be used for energy or stored in fat cells. [71].
- Myosin: Myosin is a motor protein complex essential for muscle contraction. Its large size and complex assembly make recombinant production challenging. While some subunits or fragments are available, full-length functional myosin is less commonly produced recombinantly [72].
- NAD+ is used for cellular energy production and repair, with NAD+ levels declining with age [73].

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- Neuregulin-1, essential for brain development, supports cognitive health, particularly schizophrenia [74].
- Oxytocin receptors influence social learning, and oxytocin treatments are explored for autism [75].
- p53, known as the "guardian of the genome," prevents DNA damage by regulating cell cycle and apoptosis, reducing cancer risk and supporting longevity [76].
- Platelet-derived growth Factor (PDGF), which recruits fibroblasts and smooth muscle cells for wound healing and scar formation [77];
- Reelin, vital for brain development, is linked to autism and schizophrenia, with signaling modulation as a potential treatment [78].
- SIRT1 activates pathways for stress protection, DNA repair, and antioxidant defenses, while SIRT3, located in mitochondria, regulates energy production and protects against oxidative stress [79].
- Synaptic proteins like PSD-95 and Synapsin are fundamental to memory, while GABA and glutamate receptor imbalances relate to intellectual disabilities and neurological disorders [80].
- Tubulin: Tubulin forms microtubules, crucial for cell structure and division. Recombinant expression of tubulin is complex due to its tendency to form aggregates and the need for specific chaperones for proper folding. Native tubulin is often purified from natural sources for research purposes [81].
- Vascular endothelial growth factor (VEGF) promotes nutrient and oxygen supply, which is crucial for healing, especially in muscle, bone, or skin injuries [82].

*Regulatory Guidelines*

The FDA includes certain RNA-based products under its gene therapy regulatory framework, mainly when they modify or regulate gene expression within cells to produce a therapeutic effect. Here are some RNA products the FDA considers gene therapies. How the FDA and EMA classify mRNA is a complex topic [83] that is evolving as many mRNA products besides the vaccines are emerging, such as to express therapeutic proteins; early clinical trials of mRNA therapeutics include studies of paracrine vascular endothelial growth factor (VEGF) mRNA for heart failure and of CRISPR–Cas9 mRNA for a congenital liver-specific storage disease. However, many challenges remain to be addressed before mRNA can be established as a general therapeutic modality with broad relevance to rare and common diseases. Various new technologies are being developed to surmount these challenges, including approaches to optimize mRNA cargos, lipid carriers with inherent tissue tropism, and in vivo percutaneous delivery systems. The judicious integration of these advances may unlock the promise of biologically targeted mRNA therapeutics, beyond vaccines and other immunostimulatory agents, for treating diverse clinical indications [84]. In this paper, we describe the projections of new mRNA product types,

Initially, neither the FDA nor the EMA had specific regulations for mRNA-based therapeutics as vaccines. Consequently, during the COVID-19 pandemic, regulatory agencies resorted to an emergency "rolling review" process that allowed data submission and review in real time, expediting vaccine approval. This emergency review, while responsive, omitted some standard tests required for GTPs, raising questions about long-term safety. This rapid approval process has left substantial gaps in the study of pharmacokinetics, toxicology, and long-term biodistribution of these vaccines—areas typically scrutinized in gene therapy evaluations. These gaps are relevant as mRNA vaccine developers aim to broaden applications to non-pandemic uses, such as influenza vaccines.

## 7. Recombinant Switchover

Using mRNA to deliver a protein that has been previously approved as a recombinant protein involves several key steps and considerations to ensure effective translation and bioavailability.

- Designing the mRNA Sequence for the Biosimilar Protein: Sequence Fidelity: The mRNA sequence should encode the exact amino acid sequence of the approved biosimilar protein. This requires designing the mRNA with codon optimization for efficient translation in the target cells (e.g., human cells), which helps achieve high expression levels [85].

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- 5′ and 3′ Untranslated Regions (UTRs): Optimized UTRs can enhance mRNA stability and translational efficiency. Choosing UTRs that increase translation in specific tissues or cells can help achieve targeted protein delivery. Codon-optimized mRNA constructs have significantly improved translation rates for proteins like erythropoietin, commonly delivered as a recombinant protein.
- Modified Nucleosides: Incorporating modified nucleosides such as pseudouridine and 1-methyl pseudouridine into the mRNA can reduce immune recognition while increasing stability and translational efficiency [2].
- Poly(A) Tail and Cap Structure: A cap structure (e.g., Cap 1) and a poly(A) tail increase mRNA stability and translation, enhancing the duration and consistency of protein production.
- LNP Formulation: Encapsulating mRNA in lipid nanoparticles (LNPs) protects it from degradation in the bloodstream, facilitates cellular uptake, and promotes endosomal escape, enabling the mRNA to reach the cytoplasm, which can be translated into protein [86].
- Demonstrating Biosimilarity: To meet biosimilar standards, the mRNA-produced protein must demonstrate similarity in structure, function, and therapeutic efficacy to the original recombinant protein. This often involves comparative testing, including in vitro and in vivo assays for pharmacokinetics, pharmacodynamics, immunogenicity, and stability.
- FDA and EMA Requirements: Regulatory agencies require evidence that the mRNA-delivered protein matches the reference product regarding potency and safety, which may include animal studies and clinical trials.
- Applications: mRNA delivery is under investigation for therapeutic proteins that traditionally require regular injections, such as monoclonal antibodies, clotting factors, and enzymes for genetic diseases. This approach aims to enable endogenous protein production, potentially reducing dosing frequency.
- Clinical Example: mRNA-based delivery of Factor VIII for hemophilia is being studied as a long-lasting alternative to recombinant protein infusions (87, 88). This approach to delivering biosimilar proteins via mRNA is in its early stages, but it holds promise for making therapeutic protein delivery more effective and patient-friendly. The development process includes complex regulatory and manufacturing considerations to ensure that the therapeutic effect and safety of the mRNA-delivered protein match those of the approved recombinant versions.

This structured, multi-faceted approach ensures that an mRNA product is rigorously evaluated, combining relevant aspects from biosimilar guidelines with additional mRNA-specific testing. By confirming equivalency in sequence, structure, stability, function, and immunogenicity, this guideline ensures that an mRNA product can be reliably positioned as a biosimilar.

The FDA has approved 100 therapeutic proteins [89], 144 monoclonal antibodies, and 29 recombinant protein vaccines [89]. As of 2024, the therapeutic proteins global market is estimated to increase from $132.4 billion in 2023 to r203.6 billion by 2029 at a compound annual growth rate (CAGR) of 7.5% from 2024 through 2029 [90]. Their affordability is best viewed by the reimbursements made by CMS for Medicare patients that are the lowest price paid for drugs; per gram cost of over $25 million for Inotuzumab ozogamicin, and the same drug with a different antibody, $2.7 Million. The lowest cost antibody is about $350,000 per gram, while the manufacturing cost for these products is less than $100 per gram [91].

Given below are the recombinant protein drugs approved by the FDA and the parenthesis showing when they were approved:

**Recombinant Proteins:** Insulin human (1982 - 2019); Somatropin (1976 - 2008); Epoetin (1989 - 2018); Antihemophilic factor viii (Recombinant) (1992 - 2019); Insulin lispro (1996 - 2020); Sargramostim (1991 - 2015); Filgrastim (2002 - 2024); Interferon alpha-2b (1986 - 2008); Coagulation factor viia (1999 - 2020); Etanercept (1998 - 2019); Insulin glargine (2000 - 2021); Coagulation factor ix (Recombinant) (1997 - 2017); Lutropin alpha (1994 - 2014); Hyaluronidase (human recombinant) (2005 - 2023); Interferon alpha-2a (1986 - 2004); Imiglucerase (1994 - 2010); Interferon beta-1b (1993 - 2009); Aflibercept (2011 - 2024); Follitropin beta (1997 - 2005); Follitropin alfa (1997 - 2004); Drotrecogin alfa, activated (2001 - 2007); Interferon beta-1a (1996 - 2002); Alglucosidase alfa (2006 - 2010); Efgartigimod (2021 - 2023); Insulin degludec (2015 - 2016); Alteplase (1987); Aldesleukin (1992); Dornase alfa (1993); Reteplase (1996); Becaplermin (1997); Interferon alfacon-1 (1997); Oprelvekin (1997); Thyrotropin alfa

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(1998); Denileukin diftitox (1999); Interferon gamma-1b (1999); Tenecteplase (2000); Anakinra (2001); Darbepoetin alfa (2001); Rasburicase (2002); Agalsidase beta (2003); Alefacept (2003); Laronidase (2003); Insulin glulisine (2004); Palifermin (2004); Abatacept (2005); Galsulfase (2005); Insulin detemir (2005); Mecasermin (2005); Idursulfase (2006); Methoxy polyethylene glycol-epoetin beta (2007); Insulin aspart (2008); Rilonacept (2008); Antithrombin (Recombinant) (2009); Belatacept (2011); Ocriplasmin (2012); Taliglucerase alfa (2012); Coagulation factor xiii a-subunit (2013); Tc99m-tilmanocept (2013); Antihemophilic factor viii (Recombinant), Fc-fusion protein (2014); Antihemophilic factor viii (Recombinant), porcine sequence (2014); Conestat alfa (2014); Elosulfase alfa (2014); Asfotase alfa (2015); Sebelipase alfa (2015); Antihemophilic factor viii (Recombinant), single chain (2016); Factor ix albumin fusion protein (2016); Cerliponase alfa (2017); Calaspargase pegol (2018); Cenegermin-bkbj (2018); Elapegademase-lvlr (2018); Tagraxofusp-erzs (2018); Luspatercept (2019); Avalglucosidase alfa (2021); Lonapegsomatropin (2021); Ropeginterferon alfa-2b-njft (2021); Daxibotulinumtoxina (2022); Eflapegrastim (2022); Olipudase alfa (2022); Tebentafusp (2022); Antihemophilic factor , fc-vwf-xten fusion protein-ehtl (2023); Cipaglucosidase alfa (2023); Efbemalenograstim alpha (2023); Somatrogon (2023); Velmanase alfa (2023); Nogapendekin alfa inbakicept (2024); Sotatercept (2024);

**Recombinant Engineered and Monoclonal Antibodies**: Active ingredient (Approval Years); Clobetasol (1985 - 2024); Muromonab cd3 (1992); Abciximab (1994); Capromab pendetide (1996); Imciromab pentetate (1996); Daclizumab (1997 - 2016); Rituximab (1997 - 2020); Basiliximab (1998); Infliximab (1998 - 2023); Palivizumab (1998); TC99M nofetumomab merpentan (1998); Trastuzumab (1998 - 2024); Gemtuzumab ozogamicin (2000 - 2017); Alemtuzumab (2001); Adalimumab (2002 - 2024); Ibritumomab tiuxetan (2002); Efalizumab (2003); Omalizumab (2003); Tositumomab (2003); Bevacizumab (2004 - 2023); Cetuximab (2004); Natalizumab (2004 - 2023); Technetium (99m tc) fanolesomab (2004); Panitumumab (2006); Ranibizumab (2006 - 2022); Eculizumab (2007 - 2024); Certolizumab pegol (2008); Canakinumab (2009); Golimumab (2009 - 2013); Ofatumumab (2009); Ustekinumab (2009 - 2024); Denosumab (2010 - 2024); Tocilizumab (2010 - 2024); Belimumab (2011 - 2017); Brentuximab vedotin (2011); Ipilimumab (2011); Pertuzumab (2012 - 2020); Raxibacumab (2012); Obinutuzumab (2013); Trastuzumab emtansine (2013); Blinatumomab (2014); Nivolumab (2014 - 2022); Pembrolizumab (2014); Ramucirumab (2014); Siltuximab (2014); Vedolizumab (2014 - 2024); Alirocumab (2015); Daratumumab (2015 - 2020); Dinutuximab (2015); Elotuzumab (2015); Evolocumab (2015); Idarucizumab (2015); Mepolizumab (2015 - 2019); Necitumumab (2015); Secukinumab (2015 - 2023); Atezolizumab (2016); Bezlotoxumab (2016); Ixekizumab (2016); Obiltoxaximab (2016); Olaratumab (2016); Reslizumab (2016); Avelumab (2017); Benralizumab (2017); Brodalumab (2017); Dupilumab (2017); Durvalumab (2017); Emicizumab (2017); Guselkumab (2017); Inotuzumab ozogamicin (2017); Ocrelizumab (2017); Sarilumab (2017); Burosumab-twza (2018); Cemiplimab (2018); Emapalumab-lzsg (2018); Erenumab (2018); Fremanezumab (2018); Galcanezumab-gnlm (2018); Ibalizumab-uiyk (2018); Lanadelumab (2018); Mogamulizumab (2018); Moxetumomab pasudotox (2018); Ravulizumab-cwvz (2018); Tildrakizumab (2018); Brolucizumab-dbll (2019); Caplacizumab (2019); Crizanlizumab (2019); Enfortumab vedotin (2019); Fam-trastuzumab deruxtecan-nxki (2019); Polatuzumab vedotin (2019); Risankizumab (2019 - 2022); Romosozumab (2019); Ansuvimab (2020); Atoltivimab (2020); Belantamab mafodotin (2020); Eptinezumab (2020); Inebilizumab (2020); Isatuximab (2020); Maftivimab (2020); Margetuximab (2020); Naxitamab (2020); Odesivimab (2020); Sacituzumab govitecan (2020); Satralizumab (2020); Tafasitamab (2020); Teprotumumab (2020); Aducanumab (2021); Amivantamab-vmjw (2021); Anifrolumab (2021); Dostarlimab (2021); Evinacumab (2021); Loncastuximab (2021); Tezepelumab (2021); Tisotumab vedotin (2021); Tralokinumab (2021); Faricimab (2022); Mirvetuximab soravtansine (2022); Mosunetuzumab (2022); Relatlimab (2022); Spesolimab (2022); Sutimlimab (2022); Teclistamab (2022); Teplizumab (2022); Tremelimumab (2022); Ublituximab (2022); Bimekizumab (2023); Elranatamab (2023); Epcoritamab (2023); Glofitamab (2023); Lecanemab (2023); Mirikizumab (2023); Nirsevimab (2023); Pozelimab (2023); Retifanlimab (2023); Rozanolixizumab (2023); Talquetamab (2023); Toripalimab (2023); Crovalimab (2024); Donanemab (2024); Tarlatamab (2024); Tislelizumab (2024);

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**Recombinant Protein Vaccines:** Vaccine Name (Approval Date); Measles, Mumps, and Rubella Virus Vaccine Live (1978); Rabies Vaccine (1980); Hepatitis B Vaccine (1986); Haemophilus b Conjugate Vaccine (1989); Typhoid Vi Polysaccharide Vaccine (1994); Varicella Virus Vaccine Live (1995); Anthrax Vaccine Adsorbed (2002); Tetanus and Diphtheria Toxoids Adsorbed (2003); Tetanus Toxoid, Reduced Diphtheria Toxoid, and Acellular Pertussis Vaccine, Adsorbed (2005); Measles, Mumps, Rubella, and Varicella Virus Vaccine Live (2005); Human Papillomavirus Vaccine (2006); Smallpox (Vaccinia) Vaccine, Live (2007); Rotavirus Vaccine, Live, Oral (2008); Japanese Encephalitis Vaccine, Inactivated (2009); Pneumococcal 13-valent Conjugate Vaccine (2010); Adenovirus Type 4 and Type 7 Vaccine, Live, Oral (2011); Influenza Vaccine (2013); Meningococcal Group B Vaccine (2015); Cholera Vaccine, Live, Oral (2016); Zoster Vaccine Recombinant, Adjuvanted (2017); Dengue Tetravalent Vaccine, Live (2019); Smallpox and Monkeypox Vaccine, Live, Non-Replicating (2019); Ebola Zaire Vaccine, Live (2019); Pneumococcal 20-valent Conjugate Vaccine (2021); Pneumococcal 15-valent Conjugate Vaccine (2021); Tick-Borne Encephalitis Vaccine (2021); COVID-19 Vaccine (Recombinant, Adjuvanted) (2022); Respiratory Syncytial Virus (RSV) Vaccine (2023); Respiratory Syncytial Virus (RSV) Vaccine (2023).

*Regulatory Guideline*

There are no regulatory guidelines to address the development of new mRNA products that are copies of approved recombinant drugs. The FDA and EMA will treat it as a new drug or biologic. However, several options are available to developers to reduce the testing burden under the FDA's GASK guideline [92] which allows the developers to present scientific arguments to resolve issues with better efficiency and rationality.

- Animal toxicology studies will be required to establish the product's safety due to its LNP formulation; if the developer uses a composition that the FDA has approved, the extent of nonclinical studies will be reduced but not removed as proof of safety is required. Regardless of whether only the subcutaneous route administers the product, intravenous research will also be necessary. Table 2 lists a proposed animal toxicology plan.

**Table 2.** Animal Toxicology testing plan.

| **Animal Species** | **Mouse** |
|---|---|
| Duration in-life | 28 days to 3 months |
| Administration | Repeated, subcutaneous |
| Dosage level | 2 |
| Groups | 2: saline control, dose group |
| Group size | 20 |
| Total Animals | 40 |
| Monitoring | Mortality, body weight, clinical observation, food consumption, local reaction, body temperature, FOB (mod. Irwin), hematology, clinical chemistry, urinalysis, bone marrow smear, blood coagulation parameters, cytokines TNF-alpha, IFN-gamma, IL-10, etc. |
| Post mortem | Necropsy and weight of selected organs; histopathological evaluation, RNA biodistribution |
| Duration | 3 months |

- Protein yield calculations will be made from animal expression studies that can be satisfied with rodent species since their expression is higher than in humans. Notably, a single mRNA molecule can yield hundreds or even thousands of protein molecules depending on mRNA stability and functionality. Based on animal conversion data, an early phase 0 study in humans will be required to confirm the mRNA dose-protein yield profile.
- A short phase II study with 4-6 subjects will establish a dose-response relationship and the pharmacokinetic disposition profile. These profiles are expected to not match well due to the inevitable difference in the absorption half-life and yield appearance. However, the FDA will at

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least require a comparison of the total AUC to relate it to the total dose reaching the body. Notably, the developer requests the same indications, requiring this equivalence to be established.

- A phase III or comparative efficacy study will likely not be required, but this waiver will require the developer to present a scientific argument to the FDA.
- The FDA will approve the plasmid design and construction, and its tests may include, as shown in Table 3)

**Table 3.** Plasmid Tests Analytical Methods.

| Test | Method | Specification |
|---|---|---|
| Identity | Restriction Enzyme Analysis with Agarose Gel Electrophoresis | Consistent with the reference standard |
| Plasmid Identity | Sanger Sequencing | Matches reference standard |
| Purity | A260/A280 | 1.8 – 2.0 |
| Concentration | A260 | Default 1 mg/mL |
| Residual Host Cell Protein | HCP ELISA | ≤ 1% |
| Residual Host Genomic DNA | Quantitative PCR | ≤ 5% |
| Residual Host RNA | Agarose Gel Electrophoresis | Non-detectable at 200 ng |
| Endotoxin | Quantitative LAL assay | ≤ 0.01 EU/μg |
| Bioburden | USP <61> | No growth after 48 hours |

The CMC data requirements are well-defined and should be complied with. Notably, the mRNA is a chemical entity; therefore, little batch-to-batch variability will be allowed. Also, unlike proteins, there are no post-translational concerns. Tables 4 and 5 list the release specifications of DS and DP that will be acceptable to the FDA.

**Table 4.** Characterization and Release Testing for mRNA Drug Substance.

| Quality | Attribute | Method (s) |
|---|---|---|
| Identity | mRNA sequence identity confirmation | Sanger sequencing |
| Content | RNA concentration | Ultraviolet Spectroscopy (UV) |
| Integrity | mRNA intactness | Capillary electrophoresis |
| Purity | 5′ capping efficiency | Reverse-phase liquid chromatography-mass spectroscopy (RP-LC-MS-MS) |
| | 3′ poly (A) tail length | Capillary Electrophoresis |
| | Product-related impurities - dsRNA | Slot-blot |
| | Product-related impurities -aggregate quantitation | Size exclusion-high-performance liquid chromatography (SE-HPLC) |
| | Process-related impurities - residual DNA template | Quantitative PCR (qPCR) |
| | Process-related impurities-quantitation of free/nonincorporated nucleosides | Reverse-phase liquid chromatography-mass spectroscopy (RP-LC-MS-MS) |
| | Process-related impurities - residual T7 RNA polymerase content | Enzyme-linked immunosorbent assay (ELISA) |
| Potency | Expression of target protein | Cell-based assay |
| Safety | Endotoxin | USP<85> |
| | Bioburden | USP <61> |

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| | | |
|---|---|---|
| Other | Appearance | USP <790> |
| | Residual solvents | USP <467> |
| | pH | USP <791> |

**Table 5.** Drug Product Release Specification.

| Quality | Attribute | Method (s) |
|---|---|---|
| Identity | RNA identification | Sanger sequencing |
| | Identity of lipids | Reversed-phase high-performance liquid chromatography with charged aerosol detector (RP-HPLC-CAD) |
| Content | RNA concentration/RNA encapsulation efficiency | Ribogreen Assay |
| | Lipid content | Reversed-phase high-performance liquid chromatography with charged aerosol detector (RP-HPLC-CAD) |
| Integrity | LNP size and polydispersity | Dynamic light scattering (DLS) |
| | RNA size and integrity | Capillary electrophoresis (CE) |
| Potency | Expression of target protein | Cell-based assay |
| Purity | Product-related impurities - aggregate quantitation | Size exclusion-high-performance liquid chromatography (SE-HPLC) |
| | Product-related impurities – the percentage of fragment mRNA | Ion pair reversed-phase high-performance liquid chromatography (IP-RP-HPLC) |
| Safety | Endotoxin | USP<85> |
| | Sterility | USP <71> |
| Other | Appearance | USP <790> |
| | pH | USP <791)> |
| | Subvisible particles | USP <787)> |
| | Osmolality | USP <785)> |
| | Residual solvents | USP <467> |
| | Extractable volume | USP <1>, USP <698> |
| | Container closure integrity | USP <1207> |

**8. "Biosimilar" mRNA**

The biological drug category of biosimilars involves only therapeutic proteins and not recombinant vaccines approved as biological products. The mRNA product will not qualify as a biosimilar in the current definition of biosimilars. On the other hand, an mRNA product is a chemical product that can be copied exactly as a reference product, better than what is possible for proteins with many structural variabilities, such as post-translational modifications, none of which apply to mRNA products. No guidelines are available from the FDA or EMA for developing these products. However, a developer can take advantage of scientific arguments as presented below to secure many concessions in the testing, even though the title of "biosimilar" is not likely to be given, neither would be the title of generic drug.

As of November 2024, the FDA has approved the following mRNA products:

- Comirnaty (BNT162b2), developed by Pfizer-BioNTech, became the first mRNA vaccine approved for COVID-19 prevention in August 2021, marking a significant milestone in mRNA vaccine technology with FDA approval. Shortly after, Spikevax (mRNA-1273) by Moderna was approved in December 2020 as the second mRNA vaccine for COVID-19 prevention, further solidifying the role of mRNA vaccines in the pandemic response. In May 2024, Moderna also introduced mRESVIA (mRNA-1345), the first mRNA vaccine approved specifically for preventing Respiratory Syncytial Virus (RSV) in adults aged 60 and over, expanding mRNA applications beyond COVID-19 [93].

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Several biotechnology companies have publicly disclosed their mRNA-based products, which are currently under development. These products span various therapeutic areas, including infectious diseases, oncology, and rare genetic disorders. Below is an overview of some essential mRNA products in development:

- Moderna, Inc. has a robust pipeline of mRNA-based vaccines and therapeutics across multiple areas. For infectious diseases, it includes the approved COVID-19 Vaccine (mRNA-1273), marketed as Spikevax®, a next-generation COVID-19 Vaccine (mRNA-1283) designed for more accessible storage and administration, and seasonal influenza vaccines (mRNA-1010, mRNA-1020, mRNA-1030) targeting multiple influenza strains. Moderna is also developing an RSV Vaccine (mRNA-1345) for older adults and combination vaccines, such as the flu and COVID-19 vaccine (mRNA-1083) and a triple vaccine targeting flu, COVID-19, and RSV (mRNA-1230). In oncology, Moderna collaborates with Merck on Individualized Neoantigen Therapy (INT) (mRNA-4157), a personalized cancer vaccine targeting melanoma and other cancers. Additionally, for rare diseases, Moderna is developing therapies for Propionic Acidemia (mRNA-3927) and Methylmalonic Acidemia (mRNA-3705) (wwww.moderna.com).
- BioNTech SE, widely recognized for its COVID-19 vaccine developed with Pfizer, is advancing its pipeline with mRNA therapies for infectious diseases, such as the influenza vaccine BNT161 and a shingles vaccine (BNT163), also developed with Pfizer. BioNTech's oncology portfolio includes FixVac (BNT111), an mRNA vaccine targeting advanced melanoma, and the Individualized Neoantigen Specific Immunotherapy (iNeST) (BNT122), developed in collaboration with Genentech (www.biointech.com).
- CureVac N.V. is developing mRNA-based vaccines and therapeutics, including a second-generation COVID-19 vaccine (CVnCoV) and a rabies vaccine (CV7202) for infectious diseases. It also explores mRNA-based cancer vaccines targeting various tumors (www.curevac.com).
- Translate Bio, now part of Sanofi, focuses on mRNA therapeutics, including influenza and COVID-19 vaccines and an inhaled mRNA therapy for cystic fibrosis (MRT5005) (www.translatebio.com).
- Arcturus Therapeutics is developing self-amplifying mRNA vaccines, such as a COVID-19 vaccine (ARCT-021) and an influenza vaccine, as well as mRNA therapy for Ornithine Transcarbamylase (OTC) Deficiency (ARCT-810), a urea cycle disorder (https://arcturusrx.com).
- Gritstone bio is working on mRNA-based cancer immunotherapies, including Granite (SLATE), personalized immunotherapy for solid tumors, and the CORAL program, which develops mRNA vaccines for infectious diseases, including COVID-19 (https://gritstonebio.com)
- eTheRNA Immunotherapies focuses on mRNA-based immunotherapies, specifically TriMix-Based Cancer Vaccines designed to stimulate immune responses against tumors (https://www.etherna.be).
- Imperial College London is advancing a self-amplifying RNA (saRNA) vaccine platform, including a saRNA COVID-19 vaccine targeting SARS-CoV-2 https://www.imperial.ac.uk).
- Chimeron Bio is developing mRNA therapies with OncoRNA to target solid tumors and various infectious disease vaccines. Providence Therapeutics is advancing mRNA vaccines, including PTX-COVID19-B, an mRNA COVID-19 vaccine candidate targeting SARS-CoV-2, and personalized cancer vaccines (https://www.chimeron.com).

### *8.1. Scenario 1*

If the mRNA sequence of a new product is identical to that of an already approved mRNA product—meaning it has the same nucleotide sequence, codon optimization, and untranslated regions (UTRs)—then it is expected to yield the same protein with identical primary and secondary structures. This is because the mRNA's codon sequence and subsequent protein synthesis dictate the primary structure (amino acid sequence) and secondary structure (local folding, such as alpha helices and beta sheets).

Furthermore, if both products use an identical lipid nanoparticle (LNP) formulation, the pharmacokinetics and biodistribution are expected to be similar, assuming similar administration routes and dosages. The LNPs, being the delivery vehicles, are critical to protecting the mRNA, aiding

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cellular uptake, and modulating the immune response. When LNP composition, size, and charge are identical, they should theoretically present similar distribution and cell targeting profiles.

The main difference between the two products would likely be yield variations, potentially due to slight differences in the manufacturing process or conditions, which could impact the efficiency of mRNA encapsulation or stability. These differences might affect the dose required to achieve a therapeutic level. Still, they should not lead to differences in the protein's structure or the safety profile, assuming the mRNA and LNP are identical.

However, if a pharmacokinetic (PK) comparison between the original and the new mRNA product demonstrates an identical profile, then any concerns about differences in yield effectively become irrelevant. Identical PK profiles suggest that both products achieve comparable concentrations, distribution, metabolism, and elimination in the body, leading to similar therapeutic levels over time.

This alignment in PK would indicate that any variations in manufacturing yields do not impact the ultimate bioavailability or effectiveness of the product. Therefore, with identical mRNA sequences and LNP formulations yielding the same PK profiles, there would be no reason to expect differences in safety, efficacy, or dosing requirements between the original product and the new one. Thus, the dosing of the biosimilar product can be the same as its reference products.

### *8.2. Scenario 2*

If the mRNA products have identical PK profiles but differ in untranslated regions (UTRs) or LNP formulations, they could be considered functionally equivalent concerning the therapeutic protein’s bioavailability and overall exposure. However, these differences could still influence immunogenicity or other biological interactions:

mRNA Sequence Variations (in UTRs): Changes in UTRs can affect translation efficiency and stability, potentially leading to different protein expression rates or half-life at the cellular level. Despite an identical PK profile at the systemic level, these differences could subtly influence intracellular dynamics, possibly impacting protein synthesis in specific tissues.

LNP Formulation Differences: LNPs can influence immune response, biodistribution to specific tissues, and the cellular uptake of mRNA. Different LNP compositions may lead to distinct immunogenic profiles even with matching PK profiles. Immune activation varies based on LNP characteristics such as lipid types, particle size, and charge, which could elicit varied innate or adaptive immune responses. This may affect tolerability, mainly if the immune system responds differently to the LNP components.

In regulatory terms, if two products have identical PK profiles and demonstrate no significant immunogenic differences in clinical studies, they might be considered equivalent from a therapeutic standpoint. However, the evaluation would still require careful assessment of potential immunogenicity, as this aspect could affect safety, especially with repeated dosing.

### *8.3. Scenario 3*

if the pharmacokinetic (PK) profile differs due to sequence variations, then the PK data can guide dose adjustments to achieve the desired clinical response. Sequence differences, especially in untranslated regions (UTRs) or codon optimization, can affect mRNA stability, translation efficiency, and thus protein expression levels. Consequently, these variations can lead to differences in bioavailability and the duration of therapeutic protein exposure.

To achieve the same clinical response as the original product, the dose of the new mRNA product can be calculated by analyzing the PK parameters, such as peak concentration (Cmax), time to peak concentration (Tmax), area under the curve (AUC), and half-life. By understanding these parameters, dosing can be optimized to match the original product's target therapeutic window and exposure profile. Key considerations include:

- Dose Adjustment Based on AUC: If the AUC differs, adjusting the dose to match the original product’s AUC can help achieve comparable exposure over time, potentially leading to similar clinical efficacy.

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- Peak and Duration Adjustments: Differences in C_max or half-life may require adjusting the frequency or amount of dosing. For instance, if the modified mRNA degrades faster, more frequent dosing or higher doses might be necessary to maintain therapeutic levels.
- Fine-tuning with Therapeutic Drug Monitoring: If achieving an exact match is challenging, therapeutic drug monitoring (TDM) could be employed to individualize dosing in response to observed PK variability, ensuring patients reach the intended therapeutic range.

A PK-based dose adjustment can help compensate for sequence-related differences in mRNA stability or translation efficiency, ultimately enabling the new product to achieve a clinical effect comparable to the original. However, clinical response monitoring would still be necessary, mainly if the modified mRNA sequence introduces unforeseen differences in protein expression dynamics.

### *8.4. Scenario 4*

The approach to comparing the toxicology of two mRNA products with similar or identical sequences depends on whether the LNP formulation is the same or different between the two products. Here's an optimal strategy for both cases:

### *8.5. Toxicology Comparison with Identical LNP Formulation*

- In Vitro Assays: Start with in vitro toxicity assays to assess cellular toxicity and immune responses. Cytotoxicity, cellular uptake, and inflammatory marker assays (e.g., cytokine release profiles) can highlight any subtle differences in toxicity due to minor sequence variations.
- Acute and Chronic Toxicity Studies: Conduct acute and chronic toxicity studies in animal models to observe any short-term or long-term toxicological impacts. Monitor biomarkers, organ weights, and histopathology to identify potential differences.
- Biodistribution and Target Organ Toxicity: With identical LNPs, differences in biodistribution are less likely; however, conducting biodistribution studies using labeled mRNA or LNPs can confirm this. Focus mainly on target organ toxicity (such as liver and spleen, where LNPs tend to accumulate) to assess any subtle toxicological effects.
- Comparative Immune Response: After administration, assess immune activation markers (e.g., interferons, cytokines). Even with identical LNPs, slight mRNA sequence differences can alter immune response intensity or duration.

### *8.6. Toxicology Comparison with Different LNP Formulations*

- Comparative PK and PD Studies: Begin with PK and pharmacodynamic (PD) comparisons to understand how differences in LNP formulations affect mRNA biodistribution and protein expression. Different LNP formulations may alter tissue targeting, clearance rates, and protein exposure levels.
- Immune Response Profiling: Different LNP formulations will likely elicit varied immune responses. If applicable, use immune profiling assays to measure systemic immune markers, such as cytokines (IL-6, TNF-$\alpha$) and adaptive immune responses. This will help assess any LNP-induced immunogenicity.
- In Vivo Toxicology Studies with Emphasis on LNP-Sensitive Organs: In vivo studies should assess organs where LNPs commonly accumulate (e.g., liver, spleen, lymph nodes). Detailed histopathological analysis and liver enzyme assays (e.g., ALT, AST) are critical to detect potential hepatotoxicity or immune cell infiltration in these organs.
- Comparative Inflammatory and Complement Activation Studies: Different LNP formulations can varying degrees, activate the complement system or inflammatory pathways. In vivo and ex vivo assays, such as the hemolysis assay for complement activation and inflammatory biomarker panels, will help detect LNP-specific immune and inflammatory responses.
- Repeat-Dose Toxicity Studies: Conduct repeat-dose toxicity studies in relevant animal models to evaluate cumulative toxic effects, which may be more pronounced with different LNP formulations. Monitoring clinical signs, body weight, hematology, and organ function over time will help identify chronic toxicity risks.

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Regulatory agencies will likely require comprehensive in vivo studies in both scenarios, even if the sequences are identical, due to possible differences in LNP behavior and immune responses. Moreover, any observed immunogenicity or toxicity differences must be carefully interpreted, especially for products intended for repeated administration. A stepwise approach, from in vitro to advanced in vivo studies, offers the most robust comparison for assessing each product's safety and potential risks.

To develop a comprehensive guideline for evaluating the similarity of mRNA products as biosimilars, it is essential to incorporate standards from biosimilar guidelines while addressing the unique structural, functional, and stability requirements specific to mRNA therapeutics. Here, the guideline must integrate aspects of molecular structure, stability, functionality, and immunogenicity to confirm equivalency with a reference mRNA product. A coherent, stepwise approach is outlined as follows.

### *8.7. Manufacturing Quality and Consistency*

The foundation of comparability begins with stringent control over the manufacturing process. Quality by Design (QbD) principles, as defined in biosimilar guidelines, are fundamental to ensure that the manufacturing process yields consistent, high-quality mRNA with precisely controlled sequence, structure, and nucleotide modifications. This process includes stringent monitoring of critical process parameters, such as enzyme concentrations and nucleotide levels, during the in vitro transcription (IVT) phase, as this phase determines sequence fidelity and modification consistency. The DNA template must be verified for quality, as even slight variations in the template could introduce sequence inconsistencies. When using modified nucleotides (e.g., pseudouridine), it is critical to validate their incorporation accurately, as modifications influence stability and immunogenicity (94, 95).

For mRNA products encapsulated in lipid nanoparticles (LNPs), maintaining consistency in particle size, encapsulation efficiency, and uniformity is essential, as these factors directly impact delivery efficiency. Good Manufacturing Practices (GMP) are also mandatory to prevent contamination and degradation, especially given RNA's susceptibility to hydrolysis and oxidation [96].

### *8.8. Structural Characterization*

Comparative structural analysis is fundamental to demonstrate that an mRNA biosimilar is identical to the reference product. Sequence identity, verified through high-throughput RNA sequencing, ensures that the nucleotide composition is exact, encompassing the coding sequence, untranslated regions (UTRs), and the poly(A) tail. Secondary structures formed by intramolecular base pairing, such as hairpins, must also be consistent, as these structures affect translation efficiency. Structural analysis using Selective 2'-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) or other probing techniques can help map these secondary structures.

In addition, chemical characterization must confirm the incorporation of modified nucleotides, as these affect stability and immunogenicity. Mass spectrometry and high-performance liquid chromatography (HPLC) can quantify modified nucleotide ratios and their precise positioning within the mRNA. This step is necessary because slight modification differences could lead to immune recognition or altered translation dynamics [95](Kowalski et al., 2019).

### *8.9. Stability Testing*

Stability assessments provide insights into the durability and resilience of the mRNA product under various storage and handling conditions. Both accelerated and real-time stability tests, derived from biosimilar standards, should be conducted to simulate long-term storage (e.g., -20°C) and accelerated conditions (e.g., 40°C with elevated humidity). Periodic sampling allows the detection of degradation products, loss of potency, and other changes in product integrity over time.

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Forced degradation studies under extreme pH, oxidative, and thermal conditions offer a comparative view of degradation pathways. Analytical tools such as HPLC, capillary electrophoresis, and UV spectroscopy can reveal physical and chemical degradation profiles. If the mRNA is encapsulated in LNPs, dynamic light scattering (DLS) and electron microscopy can track particle size changes and LNP stability under stress conditions.

Additionally, handling stability tests, including repeated freezing and thawing cycles, assess whether the product retains stability during routine clinical handling. This testing is especially relevant for mRNA, as freeze-thaw cycles can disrupt RNA integrity and LNP structure, impacting delivery efficiency and therapeutic effectiveness.

### *8.10. Functional Testing*

Functional testing is critical in establishing that the mRNA product translates into the intended protein at levels consistent with the reference. In vitro translation assays evaluate the protein yield, confirming that the mRNA efficiently directs protein synthesis in a controlled system. For more biologically relevant data, in vivo pharmacokinetic (PK) studies assess protein expression over time in an animal model, providing data on absorption, distribution, metabolism, and excretion (ADME) profiles . These PK studies reveal whether the mRNA product maintains bioavailability comparable to the reference mRNA.

Bioactivity assays further assess the functionality of the expressed protein. Cell-based or biochemical assays ensure the protein maintains the intended biological activity, which is particularly important for therapeutic applications requiring specific binding or enzymatic action. This functional confirmation is necessary to ensure that the translated protein mirrors the reference product's therapeutic effects.

### *8.11. Immunogenicity Assessment*

Immunogenicity concerns are central to evaluating the safety of mRNA therapeutics. Since immune responses can stem from both the mRNA and the LNPs used for encapsulation, a dual approach to immunogenicity testing is necessary. Assessing innate immune activation through cytokine release or toll-like receptor (TLR) assays provides insights into the initial immune response triggered by the mRNA productThis is especially pertinent if the mRNA contains unmodified nucleotides, which could be recognized as foreign by the immune system.

The adaptive immune response, including the potential for anti-drug antibodies (ADA) against the expressed protein, requires testing in preclinical models and, ultimately, human studies. These evaluations determine if repeated administrations will lead to neutralizing antibodies that could reduce efficacy or cause adverse effects [97].

### *8.12. Comparative Analytical Testing and Documentation*

Comprehensive analytical testing must compare critical attributes of the mRNA product and the reference, ensuring equivalency across purity, structural integrity, and bioactivity. Both capillary and gel electrophoresis can assess RNA integrity, while HPLC and mass spectrometry quantify impurities, such as truncated or double-stranded RNA byproducts.

The 5' cap structure and poly(A) tail length are essential for stability and translation initiation. Reverse transcription-polymerase chain reaction (RT-PCR) and enzymatic assays confirm these features align with the reference product. Furthermore, mass and charge distribution analyses through advanced analytical methods ensure that the mRNA's molecular profile is consistent, eliminating structural discrepancies.

Documentation consolidates all data from each phase of testing. This includes process validation records, stability data, bioactivity results, and immunogenicity profiles. Detailed documentation provides a comprehensive reference for regulatory submission, allowing agencies to verify the equivalency claims.

## 9. Proposed Guideline

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Creating a comprehensive guideline for mRNA products by integrating relevant elements from biosimilar guidelines involves several key areas: manufacturing quality, stability, structural characterization, functional assessment, and immunogenicity. Below is an outline of guidelines for mRNA therapeutics, tailored to meet their unique characteristics while drawing on established biosimilar principles.

*9.1. Quality by Design (QbD) and Manufacturing Controls*

- Process Development and Control: Following a QbD approach, ensure the manufacturing process is consistent and capable of producing high-quality mRNA with controlled sequence, structure, and modifications. Key aspects include:
- Template DNA Quality: DNA template purity is essential as it determines mRNA sequence fidelity.
- In Vitro Transcription (IVT) Consistency: Controls on enzyme concentrations, nucleotide substrates, and buffer systems to achieve consistent transcription.
- Modified Nucleotides: Validation is required to confirm their precise incorporation if modified nucleotides (e.g., pseudouridine) are used.
- Good Manufacturing Practices (GMP): Ensure GMP compliance, including aseptic processing for sterility and safety. This encompasses monitoring environmental conditions to protect RNA from degradation.
- Lipid Nanoparticle (LNP) Encapsulation: If using LNPs, specify particle size, encapsulation efficiency, and homogeneity.

*9.2. Structural Characterization*

- Sequence Identity: Use high-throughput RNA sequencing to verify that the mRNA sequence matches the reference sequence.
- Secondary Structure Verification: Secondary structures like hairpins can influence translation efficiency and stability. Techniques such as Selective 2′-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) or cryo-electron microscopy can validate these structures.
- Modified Nucleotide Analysis: Confirm the presence, location, and ratio of modified nucleotides using mass spectrometry or high-performance liquid chromatography (HPLC) to match the reference profile.

*9.3. Stability Testing*

- Accelerated and Real-Time Stability: Conduct stability studies under accelerated (e.g., 40°C, 75% RH) and real-time storage conditions to define shelf life. Real-time studies are essential to simulate actual storage conditions (e.g., -20°C).
- Thermal Cycling Studies: Test stability under repeated freezing and thawing cycles to mimic standard handling practices.
- Forced Degradation Studies: Subject the mRNA to stress conditions such as extreme pH, oxidative stress, and light exposure to identify degradation pathways and establish degradation products.
- LNP Stability: For mRNA in LNPs, monitor particle size and zeta potential over time. Use dynamic light scattering (DLS) or transmission electron microscopy (TEM) for LNP integrity analysis.

*9.4. Functional Testing*

- In Vitro Translation Efficiency: Conduct in vitro translation assays to ensure the mRNA product consistently translates into the desired protein with expected yield and activity.
- In Vivo Protein Expression and PK Studies: Perform animal studies to compare in vivo protein expression levels and pharmacokinetics (PK) with the reference mRNA product. This should include protein quantification and assessment of distribution, metabolism, and excretion.

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- Bioactivity of Expressed Protein: Confirm that the protein expressed from the mRNA has comparable bioactivity to the reference product, using cell-based or biochemical assays to match therapeutic efficacy.

*9.5. Immunogenicity Assessment*

- Innate Immune Activation: Assess immune activation potential, especially if using unmodified mRNA. Testing can involve cytokine release assays or toll-like receptor (TLR) assays to evaluate the activation of the innate immune response.
- Adaptive Immune Response: Evaluate the immunogenicity of the expressed protein, primarily if the therapeutic mRNA is intended for repeated administration. Animal studies and in vitro assays can provide preliminary data, though clinical testing is ultimately required.
- Anti-mRNA Antibody Formation: If patients receive multiple doses, assess the risk of anti-drug antibodies (ADA) against the mRNA or the lipid nanoparticles, as these could impact product efficacy or safety.

*9.6. Comparative Analytical Testing (Drawing from Biosimilar Guidelines)*

- Comparability Protocol: Create a side-by-side analytical comparison with the reference product, covering:
- Purity and Impurities: Use capillary electrophoresis, HPLC, and other analytical methods to quantify impurities, such as truncated RNA and double-stranded RNA by-products.
- 5′ Cap and 3′ Poly(A) Tail Length: Confirm that the mRNA's 5′ cap structure and poly(A) tail length are consistent with the reference, as these impact translation initiation and stability.
- Mass and Charge Profile: Analyze the molecular mass and charge distribution to detect any variations in mRNA composition.

*9.7. Additional Clinical Studies for Safety and Efficacy*

- Pharmacodynamics (PD) and PK Matching: Conduct clinical PK and PD studies in human volunteers to confirm that the mRNA product has bioavailability and therapeutic efficacy similar to the reference.
- Comparative Safety Assessment: Include safety assessments for single-dose and repeated administrations, especially monitoring for differences in immune response.

*9.8. Documentation and Regulatory Compliance*

- Detailed Documentation: Compile all testing data, including batch records, stability reports, and clinical trial data, by regulatory requirements.
- Risk Management: Follow risk assessment protocols to identify and mitigate potential risks unique to mRNAs, such as enzymatic degradation or immune activation.

**Table 4.** Summary of Key Comparability Requirements (Adapted from Biosimilar Guidelines).

| Testing Category | mRNA-Specific Considerations | Biosimilar Guideline Parallels |
|---|---|---|
| Sequence Identity | Complete sequence and modified nucleotides | Amino acid sequence identity |
| Secondary Structure | SHAPE and cryo-EM for folding patterns | Protein folding and glycosylation |
| Stability Testing | Thermal cycling, forced degradation | Real-time, accelerated, forced degradation |
| Functional Testing | In vitro translation, in vivo protein expression | Cell-based assays for protein activity |
| Immunogenicity | Innate and adaptive response assessments | Anti-drug antibody and immune response testing |
| PK/PD Clinical Testing | Protein expression PK and therapeutic bioactivity | PK/PD comparison in clinical studies |

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This guideline structure provides a comprehensive approach to establishing mRNA product equivalency, drawing on biosimilar principles while addressing the unique aspects of mRNA stability, structure, and function.

**10. Naming**

The regulatory agencies will face difficulty naming products that are biologically like reference mRNA products; the biosimilars must meet the following criteria: same route of administration, same active ingredient, and exact dosing. This definition will be met for a product that is a copy of an approved mRNA product, except for the requirement of the same active ingredient. However, the active ingredient is delivered through a middle-delivery scheme in this case.

However, if a mRNA product is designed to replace a recombinant product, the definition of biosimilar is not met. This will require creating another category, likely a “hybrid biosimilar,” where equivalence is established from PK profile similarity since the active molecule measured is the same. In many cases, such PK profile similarity will not be able to match, so a pharmacodynamic marker will be helpful. Still, if there is no such definitive marker, clinical efficacy testing may be required. It is worth noting that biological drugs have broad dose-response relationships, so the clinical responses will likely be matched. These concerns need to be discussed and brought under the FDA GASK guideline that promotes application. Scientific knowledge allows the developers to suggest regulatory compliance regardless of existing guidelines [92].

However, the FDA has confirmed to the author that if the end-product of an mRNA product is well-defined, it can be named as follows: Name of biological entity, mRNA (xxxx), Injection, as the FDA has confirmed it to the author.

**11. Intellectual Property**

According to the Derwent Global Patent Data (Clarivate), a total of 1213 patents in the field of mRNA-based medicines (including therapy, vaccine, and delivery systems) have been filed worldwide from 2020 to April 2024 [98]. This activity represents an almost 5.4-fold increase compared to the period spanning from 2011 to December 2019, before the outbreak of the COVID-19 pandemic.

While natural proteins are not patentable, modified or engineered protein variants that enhance specific properties, such as stability or function, can be patented. For example, modified versions of sirtuins or Klotho proteins with amino acid changes could qualify for patent protection. Moreover, the therapeutic use of mRNA—for instance, using mRNA to produce proteins with anti-aging or health-promoting benefits—can be patented as a treatment method. A method-of-use patent could cover the application of mRNA-encoding proteins like sirtuin or Klotho to combat age-related cellular damage or improve metabolic health. New combinations of mRNA encoding multiple proteins (such as an anti-aging "cocktail" delivering SIRT1, Klotho, and AMPK) may also be patentable as a novel therapeutic approach. Moderna and BioNTech's success with mRNA-based COVID-19 vaccines illustrates how patents protect unique mRNA sequences, formulations, and delivery systems, not the proteins themselves.

Similarly, patents cover the mRNA and delivery methods for anti-aging or other therapeutic proteins rather than the natural proteins they produce. Thus, mRNA designed to deliver therapeutic proteins and specific delivery technologies and therapeutic applications are patentable. It allows companies to secure intellectual property rights over innovative mRNA-based therapies, even if the proteins they encode occur naturally.

The landscape of patents on the products proposed is likely to expand fast; an understanding of the laws is essential for developers. Natural proteins, as they exist in the body, cannot be patented under U.S. patent law and similar laws worldwide because they are considered "products of nature." However, mRNA sequences designed to deliver or produce these proteins in the body can be patented for several reasons.

First, synthetic mRNA is regarded as an invention; designing, synthesizing, and modifying mRNA to deliver specific protein instructions safely and effectively in the body requires human

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ingenuity, making it a patentable invention. Modifications such as stabilization, codon optimization, or lipid nanoparticle encapsulation represent unique technical advances eligible for patents.

Second, delivery technology itself is patentable, including methods for delivering mRNA to specific tissues or cells and the carriers like lipid nanoparticles that transport the mRNA, which are essential for therapeutic effectiveness, especially in applications where targeted delivery is critical.

One primary intellectual property consideration is the LNP's composition; only three LNP-based products are approved by the FDA.

- Patisiran (Onpattro) Approval Year: 2018. Treatment of polyneuropathy caused by hereditary transthyretin-mediated amyloidosis (hATTR). Utilizes LNPs to deliver small interfering RNA (siRNA) targeting the transthyretin (TTR) gene, reducing the production of the TTR protein.
- Pfizer-BioNTech's Comirnaty and Moderna's Spikevax: Approval Year: 2020. Indication: Prevention of COVID-19. Employ LNPs to encapsulate messenger RNA (mRNA) encoding the SARS-CoV-2 spike protein, facilitating cellular uptake and subsequent immune response.
- Moderna's RSV Vaccine (mRESVIA): Approval Year: 202. Prevention of respiratory syncytial virus (RSV) in adults aged 60 and older. Uses LNPs to deliver mRNA encoding RSV antigens, stimulating an immune response against the virus.
- Lipid nanoparticle (LNP) formulations are widely used for delivering therapeutic agents, particularly nucleic acids like mRNA and siRNA. While patents protect many LNP technologies, some formulations are no longer under patent protection and are considered patent-free. These include early-generation LNPs and particular naturally occurring lipid-based delivery systems.
- Early LNP formulations, developed in the 1990s and early 2000s, often utilized cationic lipids such as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N, N,N-trimethylammonium chloride) and DOPE (dioleoylphosphatidylethanolamine). Many of these early patents have expired, rendering these formulations patent-free.

Certain lipid-based delivery systems, like those using naturally occurring lipids without proprietary modifications, may not be covered by active patents. However, their efficacy and stability might be limited compared to more advanced, patented LNP technologies. This lower efficiency is significant for vaccines but not as much when used to express functional proteins. Other formulations with expired patents utilized in FDA-approved products make a great choice. For example, the original lipid nanoparticle (LNP) formulation used by Onpattro (patisiran) during its development and early stages involved a specific combination of lipids optimized to deliver small interfering RNA (siRNA) efficiently. The primary components included:

- Ionizable Lipid: DLin-MC3-DMA: DLin-MC3-DMA was the critical ionizable lipid in Onpattro's formulation, specifically designed to become positively charged in the acidic environment of endosomes, which aids in the release of the siRNA into the cell's cytoplasm.
- Phospholipid: DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine). DSPC is a phospholipid that provides structural stability to the lipid nanoparticle and supports its interaction with cell membranes.
- Cholesterol: Cholesterol enhances the fluidity and stability of the LNP, improving its integrity and ability to circulate in the bloodstream.
- Polyethylene Glycol (PEG)-Lipid: DMG-PEG2000 (1,2-dimyristoyl-rac-glycero-3-methoxy polyethylene glycol-2000)

The U.S. Patent No. 8,450,239, granted on May 28, 2013, covers the current composition of lipids used in Onpattro, outlining the ratios and characteristics of lipids like DLin-MC3-DMA, DSPC, cholesterol, and PEG-lipids that allow for efficient nucleic acid delivery. This patent is expected to expire on August 5, 2028.

## 12. Conclusions

Recombinant technology made it possible to have access to proteins that control most body functions. Still, it comes at a high cost, making protein drugs, including functional proteins, engineered proteins, monoclonal antibodies, and protein vaccines out of the reach of most patients worldwide. The chemical-based mRNA technology is substantially less expensive to develop and manufacture and has fewer risks of side effects. mRNA technology will allow entry of hundreds of protein drugs that could not be brought to patients because of the high development cost and lower

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market potential; secondly, the hundreds of approved recombinant proteins could be transformed into mRNA technology to broaden their accessibility. Finally, approved mRNA products can be copied with much better assurance than the biosimilar copies of recombinant proteins because of the chemical nature of the mRNA products. However, acceptance of mRNA for protein drugs will require a significant shift in the thinking of regulatory agencies that are always slow to adopt novel pathways. However, this paper's technical details and arguments should create a foundation for this planning. The mRNA is here to stay, and in many ways, recombinant will slowly wane out; how fast it happens will depend on developers as well, who should capitalize on the FDA GASK guideline [92] to make proposals to the agencies for novel procedures to establish comparable clinical efficacy of their products with approved mRNA products or new mRNA delivery systems of novel proteins.

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