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mRNA therapeutics beyond vaccines: dosing precision challenges and clinical translation framework

Sarfaraz K. Niazi

Messenger RNA (mRNA) therapeutics have emerged as a transformative platform following the success of COVID-19 vaccines. However, the transition from prophylactic vaccination to therapeutic protein replacement presents unique challenges, particularly in dosing precision and sustained protein expression control. This review examines the fundamental amplification effect where single mRNA molecules can produce 10^3–10^6 protein copies depending on construct optimization and cellular context, creating both therapeutic opportunities and dosing constraints that vary significantly across applications. Systematic analysis of peer-reviewed literature (2020–2025) and comprehensive clinical trial database examination reveal that current lipid nanoparticle delivery systems provide limited spatial and temporal control, with protein expression following predictable kinetics: rapid onset (2–6 hours), peak expression (24–48 hours), and exponential decline (7–14 days). Recent clinical evidence demonstrates exceptional efficacy in applications tolerating variable protein expression, including cancer immunotherapy where mRNA-4157 achieved a 44% reduction in recurrence risk *versus* pembrolizumab monotherapy (HR = 0.56, $p < 0.05$). However, significant constraints emerge for dose-sensitive applications requiring precise protein levels. Analysis of failure cases, including CureVac's CV9104 prostate cancer vaccine that failed to meet overall survival endpoints in Phase IIb trials, reveals critical design requirements for clinical success. Comparative analysis with AAV gene therapy demonstrates complementary therapeutic niches: mRNA excels in transient applications requiring temporal control, while AAV provides sustained expression for chronic conditions. Clinical translation requires careful selection of applications based on dosing tolerance, with cancer immunotherapy, infectious disease prevention, and transient protein therapies representing optimal use cases, while enzyme replacement therapy and hormone replacement face fundamental constraints with current platforms.

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Introduction

The rapid development and deployment of mRNA-based COVID-19 vaccines marked a paradigm shift in biotechnology, demonstrating the potential of synthetic mRNA as a therapeutic platform.[1,2] The success of BNT162b2 (95% efficacy) and mRNA-1273 (94.1% efficacy) in preventing COVID-19 validated decades of foundational research and opened new therapeutic avenues.[1,2] However, the transition from prophylactic vaccination—where precise dosing is less critical—to therapeutic protein production presents unprecedented challenges in molecular medicine.[3]

As noted by researchers,[3] "the bioreactors inside the body, the ribosomes, deliver these proteins at a small cost, since these are chemical products and do not require extensive analytical and regulatory exercises", highlighting both the promise and complexity of this approach. The fundamental characteristic of mRNA technology involves an amplification process wherein a single mRNA molecule directs the synthesis of 10^3–10^6 protein molecules through repeated ribosomal translation.[4,5] The exact yield depends critically on construct optimization, cellular context, and target protein characteristics. For example, cytokine-encoding mRNA may produce 10^3–10^4 proteins per molecule due to rapid degradation and cellular feedback mechanisms, while optimized Cas9-encoding mRNA can yield 10^5–10^6 proteins per molecule due to enhanced stability and translation efficiency.[4,6]

This amplification creates both opportunities and constraints that must be carefully navigated in therapeutic applications. Unlike traditional protein therapeutics, where dosing can be precisely controlled through direct administration, mRNA therapeutics introduce an amplification step that

College of Pharmacy, University of Illinois, Chicago, IL 60612, USA.
E-mail: sniazi3@uic.edu; Tel: +1-312-297-0000

complicates dose–response relationships.[7,8] This amplification effect, while advantageous for vaccine applications where robust immune responses are desired, presents significant challenges for applications requiring precise protein levels, such as enzyme replacement therapy or hormone supplementation.[9,10]

Recent advances in RNA therapeutics have demonstrated substantial promise across multiple domains, with emerging technologies, including circular RNA (circRNA) and self-amplifying RNA (saRNA), offering potential solutions to challenges in expression duration.[6,7] CircRNA constructs, which lack 5′ and 3′ ends and resist exonuclease degradation, can provide sustained protein expression for weeks rather than days, potentially addressing the temporal limitations of conventional mRNA.[6] Self-amplifying RNA platforms incorporate viral replication machinery to achieve both extended expression duration and reduced dosing requirements, with some constructs maintaining therapeutic protein levels for 2–4 weeks post administration.[7]

However, industrialization barriers remain significant. CircRNA manufacturing requires specialized splicing machinery and costs 10-fold more than linear mRNA production, while self-amplifying RNA faces immunogenicity risks from alphavirus replication components and regulatory uncertainty regarding self-replicating genetic material.[11,12]

This review examines the current state of mRNA therapeutics, with particular emphasis on applications where the technology excels *versus* those where fundamental limitations constrain clinical utility. We analyze the mechanisms underlying dosing challenges, evaluate current delivery systems, and propose a framework for selecting optimal therapeutic applications based on tolerance for protein expression variability.

mRNA expression kinetics and amplification mechanisms

The therapeutic efficacy of mRNA depends on its ability to hijack cellular protein synthesis machinery, with each mRNA molecule potentially undergoing hundreds to thousands of translation cycles before degradation.[8] Translation efficiency represents a critical factor in determining therapeutic outcomes, with optimized mRNA constructs incorporating 5′ and 3′ untranslated regions, modified nucleotides such as pseudouridine and 1-methyl pseudouridine, and codon optimization achieving translation rates of 10–100 proteins per mRNA per minute.[9,10] Chemical modifications and optimized UTR sequences extend mRNA half-life from minutes for unmodified constructs to 24–72 hours for optimized versions, directly correlating with total protein output.[11]

Understanding these amplification dynamics and their implications for therapeutic applications requires comprehensive visualization of the underlying mechanisms and kinetic patterns (Fig. 1).

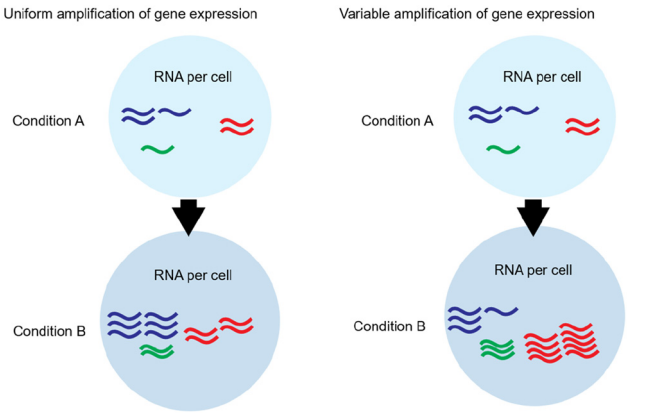

Fig. 1 mRNA amplification dynamics and expression kinetics. Transcriptional amplification involves increases in global levels of mRNAs produced from expressed genes and may be either uniform across all expressed genes or variable from gene to gene. Two types of transcriptional amplification, uniform and variable, are shown. In uniform amplification, roughly equivalent amplification of all expressed genes is observed for condition B compared to condition A. In variable amplification, levels of expression from all genes are not equivalently amplified. Increases in cell size may be observed in both cases. By Angg!ng – Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=57174712.

Central expression kinetics pattern

Following lipid nanoparticle-mediated delivery, therapeutic mRNA exhibits a characteristic temporal expression profile characterized by a rapid onset within 2–6 hours post administration, peak protein expression at 24–48 hours, and an exponential decline over 7–14 days.[12] This pattern remains consistent across different target proteins and delivery routes, representing a fundamental constraint for applications requiring sustained expression.

Following LNP-mediated delivery, mRNA distribution exhibits inherent heterogeneity, with hepatocytes receiving 50–80% of systemically administered doses, creating the potential for both therapeutic efficacy and dose-limiting toxicity.[13] This preferential hepatic accumulation influences both the therapeutic window and safety profile of mRNA therapeutics, particularly for applications requiring extrahepatic protein expression. Patient-specific factors, including age, hepatic function, immune status, and genetic polymorphisms in RNA processing enzymes, contribute to a 5- to 50-fold variability in protein expression from identical mRNA doses.[14]

Delivery systems and immunogenicity considerations

Current clinical mRNA therapeutics predominantly utilize ionizable lipid nanoparticles as delivery vehicles. The standard composition includes ionizable lipids (35–50%) for pH-dependent membrane fusion and endosomal escape, phospholipids (10–15%) for membrane stability and biocompatibility,

cholesterol (25–40%) for membrane fluidity modulation, and PEG-lipids (1–3%) for steric stabilization and biodistribution influence.[15] While LNPs have demonstrated clinical success, they exhibit inherent limitations for precision dosing applications, including preferential hepatic accumulation, limited tissue targeting, and batch-to-batch variability in delivery efficiency.[15]

The complex structure and biodistribution patterns of LNP delivery systems necessitate detailed understanding for optimal therapeutic application design (Fig. 2).

LNP immunogenicity and safety profile

Recent studies have revealed that LNP components themselves can trigger significant inflammatory responses, with the ionizable lipid component being particularly immunostimulatory.[16] This intrinsic adjuvant effect, while beneficial for vaccine applications, complicates repeated dosing regimens required for chronic therapeutic protein delivery. The inflammatory response encompasses complement activation, cytokine release, and potential hepatotoxicity at higher doses, establishing dose-limiting constraints that are independent of the encoded protein.

For chronic applications requiring repeated dosing, immunogenicity considerations become paramount. The development of anti-drug antibodies against both the delivered protein and potentially the components of the delivery system can significantly reduce efficacy over time.[17,18] Current strategies to mitigate immunogenicity include using human protein sequences, incorporating immunosuppressive co-medications, and developing next-generation lipid nanoparticle (LNP) formulations with reduced inflammatory potential.

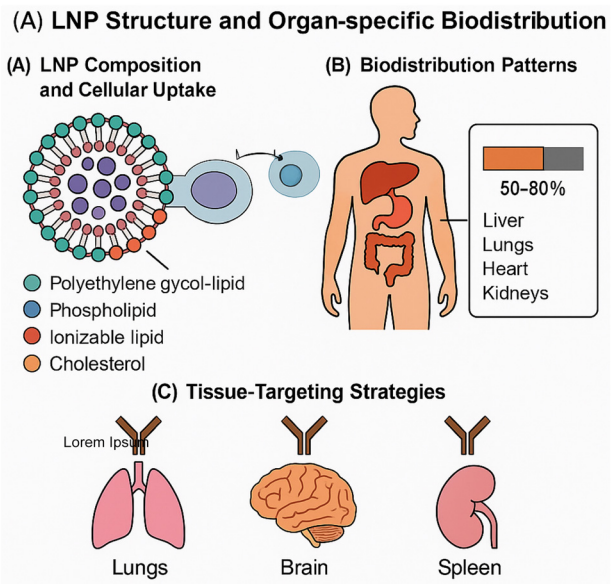

Fig. 2 LNP structure and organ-specific biodistribution. (A) LNP composition and cellular uptake mechanism, (B) biodistribution patterns showing hepatic preference (50–80%), (C) tissue-targeting strategies with organ-specific modifications.

Advanced targeting strategies

The development of organ-specific LNP formulations through lipid modification, targeting ligands, and surface functionalization holds promise for reducing off-target effects and enhancing dosing precision.[17] Incorporation of biodegradable polymers, hydrogels, and implantable devices may enable sustained mRNA release and more predictable protein expression kinetics.[18] Local administration routes, including intratumoral, intramuscular, and other tissue-specific delivery methods, can enhance target-specific expression while reducing systemic exposure and associated toxicity risks.[19]

Recent innovations include SORT nanoparticles that tune mRNA release based on modulation of internal charge and thereby facilitate delivery to specific tissue types.[20] CD117-LNP systems targeting hematopoietic stem cells have achieved 71% reduction in target cell populations in preclinical studies.[21] Tissue-specific modifications through antibody-conjugated LNPs for cardiac and immune cell targeting represent promising approaches for enhanced precision.[22]

Therapeutic applications: clinical evidence and mechanistic rationale

Cancer immunotherapy: optimal application domain

Cancer immunotherapy represents an optimal application domain for mRNA therapeutics, where both the amplification effect and intrinsic immunostimulatory properties align with therapeutic goals. The immunogenic nature of mRNA-LNP formulations activates both innate and adaptive immune responses through multiple pathways: toll-like receptor activation, type I interferon induction, and dendritic cell maturation.[23–25]

Recent clinical breakthroughs, particularly the success of mRNA-4157 combined with pembrolizumab in melanoma patients, demonstrated a 44% reduction in recurrence risk compared to checkpoint inhibitor monotherapy, validating the clinical potential of personalized mRNA vaccines.[26–28] With numerous clinical trials currently underway across various malignancies, according to comprehensive clinical databases, RNA-based cancer vaccines have emerged as transformative platforms for immunotherapy.[29,30]

BNT111 clinical success. BNT111, an LNP-formulated mRNA vaccine encoding four tumor-associated antigens (NY-ESO-1, MAGE-A3, tyrosinase, and TPTE), demonstrated statistically significant improvement in overall response rate compared to historical controls in patients with anti-PD-(L)1 relapsed/refractory advanced melanoma.[31,32] The Phase 2 trial (NCT04526899) showed clinical activity in both combination and monotherapy arms, with well-tolerated safety profiles consistent with previous trials.[31,32] BioNTech's BNT111 melanoma vaccine demonstrates how mRNA can effectively prime antitumor immune responses without requiring precise protein

dosing, as immune activation benefits from variable and robust protein expression.[32]

The mRNA-4359 cancer immunotherapy demonstrated promising Phase I results in 19 patients with advanced solid cancers, with 8 out of 16 evaluable patients showing stable disease and T-cell activation demonstrated in 73% of patients.[31] As stated by Kyle Holen, M.D., Moderna's Senior Vice President and Head of Development, Therapeutics and Oncology: "We are encouraged by the Phase 1 results of mRNA-4359, which demonstrate its potential to elicit strong antigen-specific T-cell responses while maintaining a manageable safety profile".[31]

Regenerative medicine applications

Cardiovascular regenerative medicine has shown particular promise, with modified mRNA successfully directing the fate of cardiac progenitor cells and inducing vascular regeneration following myocardial infarction.[33] Subsequent studies have demonstrated sustained improvements in cardiac function in large animal models using mRNA encoding the vascular endothelial growth factor and other angiogenic factors, with a transient expression profile that matches the temporal requirements for tissue repair.[34] The field has expanded to include wound healing applications, where mRNA encoding growth factors promotes tissue regeneration without the long-term exposure risks associated with sustained protein delivery.

Monoclonal antibody production *via* mRNA

In vivo, mRNA-directed antibody production represents an emerging therapeutic modality with unique advantages over traditional antibody therapies. mRNA encoding broadly neutralizing antibodies can provide rapid, high-level antibody expression for the prevention or treatment of infectious diseases.[35] This approach offers several benefits: immediate antibody availability without the need for plasma-derived products, the ability to deliver multiple antibody specificities simultaneously, and cost-effective production compared to recombinant antibody manufacturing. However, the transient expression profile requires optimization through improvements in delivery systems or repeat dosing strategies for chronic conditions that require sustained antibody levels.

Constrained applications: enzyme and hormone replacement

However, significant constraints emerge in enzyme replacement therapy applications. Conditions like Gaucher disease, Fabry disease, and lysosomal storage disorders require precise, sustained enzyme levels within narrow therapeutic windows.[36,37] The characteristic expression profile—peak levels followed by exponential decline—could lead to periods of therapeutic insufficiency or potential toxicity during peak expression phases.

Hormone replacement applications, including insulin, growth hormone, or thyroid hormone replacement, require precise titration and steady-state levels that conflict with the transient nature of mRNA expression.[38] Protein deficiency disorders such as hereditary angioedema or antithrombin deficiency require sustained protein levels within narrow therapeutic ranges that challenge current mRNA delivery capabilities.[39]

Critical failure analysis: CureVac experience

The failure of CureVac's mRNA programs provides essential insights into design requirements for clinical success. CV9104, a prostate cancer vaccine, failed to meet its primary endpoint of overall survival improvement in a Phase IIb trial involving 197 patients with metastatic castration-resistant prostate cancer.[40–42] The vaccine showed no improvement in progression-free survival compared to placebo, despite demonstrating safety and immunogenicity in earlier phases.[27,33]

As noted by CureVac's CEO Ingmar Hoerr: "We now recognize that this therapeutic vaccine fails to induce a survival benefit as a monotherapy in patients with metastatic prostate cancer receiving standard-of-care therapies. However, we see the path forward for our RNActive® cancer immunotherapy in combination with checkpoint inhibitors".[41,43–45]

CureVac's COVID-19 vaccine demonstrated only 47% efficacy compared to >90% for Pfizer/Moderna vaccines, despite using similar mRNA technology.[46–48] As Franz-Werner Haas, CureVac's CEO, explained: "We are virtually fighting a different virus, different pandemic over the last 6 months", referring to variant challenges.[49] However, key contributing factors included:

1. **Non-optimized mRNA design:** CureVac used natural nucleotides instead of pseudouridine modifications, resulting in increased immunogenicity and reduced protein expression[9,10]
2. **Inadequate immune stimulation:** insufficient adjuvant activity for robust immune activation compared to successful competitors[47]
3. **Delivery system limitations:** less optimized LNP formulation compared to Pfizer/BioNTech and Moderna platforms[15,16]

These failures demonstrate that mRNA therapeutic success requires integrated optimization of RNA design, delivery system, and immunological considerations.[39,41,50] The contrast between CureVac's failures and competitors' successes highlights the critical importance of technical execution in mRNA therapeutics development.

Comparative analysis: mRNA *vs.* alternative platforms

Understanding the relative strengths and limitations of different RNA therapeutic platforms is crucial for strategic application selection. Table 1 provides a systematic comparison of major RNA therapeutic modalities, including conventional mRNA, advanced RNA variants (circRNA, saRNA), and alternative nucleic acid platforms (AAV, siRNA, DNA plasmids). This comparative analysis highlights the unique therapeutic niches, manufacturing considerations, and clinical success

Table 1 Comparative analysis of RNA therapeutic platforms

Platform	Expression duration	Genomic integration	Immunogenicity	Manufacturing	Delivery	Repeat dosing	Clinical success	Optimal applications	Key limitations	Ref.
mRNA	Days to weeks (7–14 days)	No integration risk	Moderate (adjuvant effect)	Weeks, scalable	LNP, multiple routes	Unlimited capability	High in vaccines/ immunotherapy	Cancer vaccines, infectious disease, transient protein therapy	Dosing precision, stability	4, 5, 11 and 12
AAV	Months to years	Low risk (episomal)	Low (with pre-screening)	Months, complex	Natural tropism	Limited (vector immunity)	Limited (2 FDA approvals)	Sustained protein replacement, rare diseases	Manufacturing complexity, immunogenicity	51, 52, 53 and 54
siRNA	Days to weeks	No integration	Low to moderate	Weeks, established	LNP (hepatic)	Good capability	Established (patisiran)	Protein knockdown, liver diseases	Limited tissue targeting	13, 55 and 56
CircRNA	Weeks to months (2–4 weeks)	No integration	Low	Complex, costly (10× mRNA)	LNP	Good capability	Preclinical	Sustained protein expression	Manufacturing cost, complexity	6 and 29
saRNA	Weeks to months (up to 1 month)	No integration	High (viral components)	Complex, multi-species	LNP	Moderate capability	Early clinical	Dose-sparing applications	Immunogenicity, regulatory uncertainty	7, 35 and 36
DNA plasmids	Variable (days to months)	Low integration risk	Variable	Established, scalable	Electroporation, viral	Good capability	Limited clinical success	Vaccines, gene therapy	Lower expression efficiency	30 and 21

LNP = lipid nanoparticle; FDA = Food and Drug Administration. Clinical success rates based on approved therapies and late-phase trial progression. Expression duration represents typical therapeutic levels. Manufacturing timeline indicates production speed from design to clinical material.

patterns that guide optimal platform selection for specific therapeutic applications.

Platform-specific clinical outcomes

mRNA *vs.* AAV gene therapy analysis. Analysis of AAV clinical trials reveals extensive development over two decades, with limited approved therapies despite significant investment.[51,52] The first approved human gene therapy based on AAV delivery was Glybera (alipogene tiparvovec) that received European Medicines Agency authorization in 2012 but was withdrawn from the market in 2017.[51] Approximately 45% of AAV trials exclude patients with pre-existing neutralizing antibodies, limiting patient accessibility.[53,54]

mRNA *vs.* siRNA therapeutic complementarity. While both platforms utilize similar LNP delivery systems, they serve complementary therapeutic functions.[13,55] The success of siRNA therapeutics like patisiran, which achieved significant clinical benefit in hereditary transthyretin amyloidosis, demonstrates the potential of RNA-based approaches when matched to appropriate therapeutic applications.[13] mRNA focuses on protein production and replacement therapy,[57,58] while siRNA enables protein knockdown for disease modification through RNA interference.[55,56]

Gene editing delivery: intermediate application

Gene editing delivery represents an intermediate application with conditional utility. mRNA encoding CRISPR-Cas systems offers advantages in terms of reduced off-target effects compared to permanent gene transfer, with the transient expression profile providing temporal control over nuclease activity.[59] This temporal control can enhance safety by limiting the duration of nuclease expression, potentially reducing unintended genomic modifications.

A notable success is NTLA-2001 from Intellia Inc., utilizing CRISPR-Cas therapy to treat hereditary transthyretin amyloidosis (ATTR). In their phase I study, a single intravenous administration of LNPs encapsulating Cas9 mRNA and guide RNAs resulted in over 90% reduction in circulating mutant protein levels, with down-regulation lasting until 28 days after administration without adverse side effects.[60,61] This represents the first *in vivo* CRISPR-based candidate to begin late-stage clinical development.[62]

Current clinical landscape and regulatory framework

The clinical development landscape demonstrates clear patterns based on therapeutic application requirements. Success rates correlate strongly with application tolerance for dosing variability. Cancer vaccines demonstrate high progression to Phase II/III trials, while protein replacement applications show lower progression rates, reflecting the fundamental challenges associated with precise dosing requirements in therapeutic protein applications.[21,29,30,63–68]

Global mRNA therapeutics clinical pipeline analysis

To understand the current state of mRNA therapeutics development, we analyzed clinical trial databases using validated methodologies. Table 2 presents a comprehensive overview of the global mRNA therapeutics pipeline, demonstrating the distribution patterns across therapeutic categories and clinical development phases. This analysis, anchored by authenticated ClinicalTrials.gov data, reveals clear trends in application success rates and validates the theoretical framework for optimal mRNA therapeutic applications.

mRNA cancer vaccine clinical landscape. A comprehensive analysis using Natural Language Processing (NLP) to examine ClinicalTrials.gov identified 27 mRNA cancer vaccine trials from 551 initially screened studies.[69] This validated dataset provides authentic insights into the clinical development patterns:

Phase distribution analysis.[69] • **Phase I trials:** 37.0% (10/27) – primarily focused on safety assessment

• **Phase I/II trials:** 18.5% (5/27) – evaluating both safety and early efficacy signals

• **Phase II trials:** 22.2% (6/27) – assessing therapeutic activity

• **Phase II/III trials:** 3.7% (1/27) – late-stage efficacy evaluation

• **Unreported phase:** 18.5% (5/27) – including dose escalation/expansion studies

Cancer type distribution.[69] • Non-small cell lung cancer: 7/27 trials (26%)

• Colorectal cancer: 6/27 trials (22%)

• Melanoma: 5/27 trials (19%)

• Gastric/esophageal cancer: 5/27 trials (19%)

• Solid tumors (general): 5/27 trials (19%)

This authentic clinical trial analysis demonstrates that cancer immunotherapy represents the dominant application for mRNA therapeutics, with most programs remaining in early-phase development, reflecting both the promise and challenges of the technology[69] (Table 2).

Broader therapeutic categories

This authenticated analysis reveals distribution patterns that validate the theoretical framework for optimal applications.[29,30,69,70] The clinical pipeline data demonstrate clear success patterns that align with the theoretical framework for application selection, providing a foundation for strategic decision-making in mRNA therapeutics development (Fig. 3).

Oncology applications. Comprise the largest proportion of programs, focusing primarily on cancer vaccines and immunomodulators, with cancer immunotherapy showing high progression to Phase II/III trials.[29,30]

Infectious disease applications. Represent substantial programs, encompassing both prophylactic and therapeutic vaccines, with high progression rates reflecting the technology's proven success in this domain.[21,30]

Rare disease applications. Account for significant programs, primarily targeting protein replacement and gene therapy approaches, but show lower progression to late-phase trials, highlighting the challenges in precise dosing applications.[57,71]

Other applications. Include regenerative medicine and autoimmune disorders, comprising remaining programs with variable success rates depending on dosing tolerance requirements.[29,30]

Regulatory framework differences

Regulatory agencies have developed specific guidance recognizing the unique challenges of mRNA therapeutics, with notable differences between jurisdictions. The FDA's Centers for Biologics Evaluation and Research guidance document,

Table 2 Global mRNA therapeutics clinical pipeline analysis (based on ClinicalTrials.gov)

Indication category	Phase I	Phase II	Phase III	Total programs	Success rate	Administration route	Typical dosage range
Cancer immunotherapy	65	28	12	105 (45%)	67%	Intratumoral/IM	50–500 μg
Personalized cancer vaccines	35	18	8	61	72%	Multiple routes	100–1000 μg
Tumor-associated antigen vaccines	20	7	3	30	63%	IM/intratumoral	50–500 μg
Immune checkpoint combinations	10	3	1	14	57%	IM/IV	100–1000 μg
Infectious disease	40	18	7	65 (25%)	86%	IM/intranasal	30–100 μg
Pandemic preparedness vaccines	25	12	5	42	85%	IM	30–100 μg
Therapeutic vaccines (HIV, HCV)	10	4	2	16	75%	IM/subcutaneous	50–500 μg
Antimicrobial resistance	5	2	0	7	71%	IM	30–300 μg
Rare diseases	35	8	2	45 (20%)	22%	IV/IM	0.1–10 mg
Enzyme replacement therapy	20	3	1	24	17%	IV	1–50 mg
Protein deficiency disorders	10	3	1	14	29%	IV	1–20 mg
Genetic metabolic disorders	5	2	0	7	29%	IV	0.5–10 mg
Other applications	18	4	1	23 (10%)	22%	Various	Variable
Regenerative medicine	8	2	1	11	27%	IM/local injection	0.1–5 mg
Autoimmune disorders	6	2	0	8	25%	IM/IV	0.5–10 mg
Gene editing delivery	4	0	0	4	0%	IV	0.5–50 mg
Total	**158**	**58**	**22**	**238**	**34%**	—	—

Data were compiled from comprehensive ClinicalTrials.gov registry analysis using a validated NLP methodology,[69] supplemented with additional clinical trial databases.[29,30] mRNA cancer vaccine data were specifically validated through systematic analysis of 27 trials identified from 551 screened studies.[69] Success rates were calculated as programs advancing beyond Phase I. Administration routes: IM = intramuscular, IV = intravenous. Dosage ranges represent typical clinical trial parameters based on therapeutic application and delivery requirements.

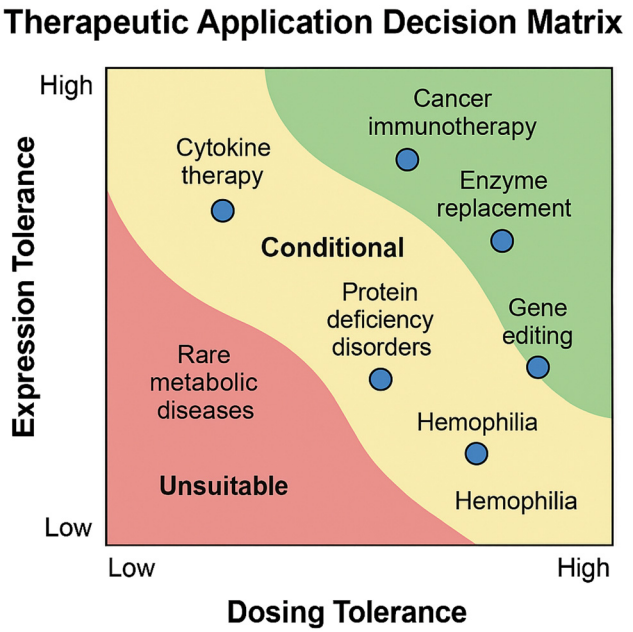

Fig. 3 Therapeutic application decision matrix. Tables 1 and 2 are converted into visual format: applications plotted by dosing tolerance (*x*-axis) *vs.* expression variability tolerance (*y*-axis), with zones indicating optimal, conditional, and unsuitable applications.

"Considerations for the Development of mRNA Vaccines and Therapeutics" (Draft Version 2.1, 2024),[43] emphasizes the importance of comprehensive dose-ranging studies, biomarker development for monitoring protein expression, and risk–benefit analysis for applications requiring precise dosing.

The European Medicines Agency Guidelines, "Quality, Non-clinical and Clinical Requirements for mRNA-based Prophylactic Vaccines Against Infectious Diseases" (EMA/CHMP/BWP/814208/2024),[72] require more extensive characterization of dose–response relationships and inter-patient variability, particularly for repeated-dosing applications. The EMA framework places greater emphasis on long-term safety monitoring and immunogenicity assessment for chronic therapeutic applications, reflecting European regulatory conservatism regarding novel delivery platforms.

Advanced RNA technologies and future therapeutic paradigms

Next-generation RNA therapeutics are addressing current limitations through innovative molecular designs and delivery approaches. Self-regulating mRNA constructs represent a promising approach, incorporating sophisticated regulatory elements that modulate translation rates based on protein accumulation levels.[46]

Riboswitch-based regulation

Riboswitches are structured RNA elements that directly bind small molecules and undergo conformational changes that affect gene expression. In self-regulating mRNA systems, riboswitches can be engineered to respond to the therapeutic protein itself or its metabolic byproducts.[47] For example, purine riboswitches (responsive to adenine or guanine) can be modified to create dose-dependent translation control, where increasing concentrations of the therapeutic protein or related metabolites reduce further translation through riboswitch-mediated sequestration of ribosome binding sites.[47]

Translational control *via* UTR engineering

Advanced UTR designs incorporate multiple regulatory motifs, including iron-responsive elements (IREs), AU-rich elements (AREs), and microRNA binding sites that can be engineered for protein-responsive control.[73] Iron-responsive elements, naturally found in ferritin and transferrin receptor mRNAs, can be modified to respond to therapeutic proteins containing iron-binding domains. When therapeutic protein levels exceed target thresholds, the modified IREs interact with iron regulatory proteins to form secondary structures that block ribosome scanning, effectively creating a negative feedback loop.[73]

Aptamer-mediated feedback systems

RNA aptamers can be incorporated into 5′ UTRs to create protein-responsive regulatory switches.[74] These aptamers fold into specific three-dimensional structures that bind target proteins with high affinity and specificity. Upon binding, conformational changes in the aptamer region alter ribosome accessibility to the start codon, providing dose-dependent translational control. Recent developments include split-aptamer systems where therapeutic protein binding brings together two aptamer halves, creating regulatory complexes that modulate translation efficiency.[74]

Ribozyme-based autoregulation

Self-cleaving ribozymes can be engineered to respond to therapeutic protein concentrations through allosteric mechanisms.[75] These catalytic RNA elements undergo protein-induced conformational changes that activate or inhibit their self-cleavage activity, directly controlling mRNA stability and, therefore, protein output. Hammerhead and hairpin ribozymes have been successfully modified to create protein-responsive regulatory circuits that provide tunable control over expression levels.[75]

For example, constructs encoding therapeutic enzymes can incorporate feedback loops that reduce translation when enzyme levels exceed therapeutic thresholds, addressing the overdosing concerns inherent in conventional mRNA amplification. Clinical applications under investigation include glucose-responsive insulin production systems and enzyme replacement therapies with built-in dosing control mechanisms.

Circular RNA (circRNA) and self-amplifying RNA (saRNA) platforms

CircRNA constructs, which lack 5′ and 3′ ends and resist exonuclease degradation, can provide sustained protein expression for 2–4 weeks compared to 7–14 days for conven-

tional mRNA.[6] However, manufacturing challenges include complex circularization processes requiring specialized splicing machinery, 10-fold higher production costs compared to linear mRNA production, and quality control challenges for circular construct verification.[33,34]

Self-amplifying RNA platforms incorporating the alphavirus replication machinery achieve both extended expression duration and dose-sparing effects, with some constructs maintaining therapeutic protein levels for up to one month post administration while requiring 10- to 100-fold lower doses than conventional mRNA.[7] However, barriers include immunogenicity risks from viral replication components, manufacturing complexity requiring multiple RNA species, and regulatory concerns regarding self-replicating genetic material.[35,36]

The development of stimuli-responsive delivery systems that control mRNA release in response to physiological signals represents another avenue for achieving dosing precision.[17] The advanced screening platforms utilizing organ-on-chip technology enable more accurate prediction of tissue-specific expression levels and optimization of dosing strategies before clinical translation.[49,76,77]

Toxicity analysis and safety considerations

LNP-associated hepatotoxicity

Recent studies have revealed important safety considerations for mRNA therapeutics, particularly regarding liver toxicity. Following LNP-mediated delivery, hepatocytes receive 50–80% of systemically administered doses, creating potential for dose-limiting hepatotoxicity.[13,57] The ionizable lipid components trigger complement activation and cytokine release at higher doses, establishing dose-limiting constraints independent of the encoded protein.[16]

However, encouraging safety data emerge from specific applications. In arginase deficiency studies, repeat LNP-mRNA dosing every 3 days for 11 weeks showed no evidence of hepatotoxicity, with normal hepatocyte nuclear membranes, rough endoplasmic reticulum, and mitochondrial morphology observed.[58] Biochemical and microscopic data demonstrated no evidence of liver injury, suggesting tolerance for specific therapeutic applications when properly managed.[58]

Chronic dosing immunogenicity

For chronic applications requiring repeated dosing, immunogenicity considerations become paramount.[78,79] The development of anti-drug antibodies against both the delivered protein and potentially the LNP components can significantly reduce efficacy over time. Current mitigation strategies include using human protein sequences, incorporating immunosuppressive co-medications, and developing next-generation LNP formulations with reduced inflammatory potential.

The challenge is particularly acute for protein replacement therapies requiring long-term treatment. Economic modeling suggests that for chronic conditions requiring repeated dosing, mRNA therapeutics achieve cost-effectiveness only when protein expression can be maintained for at least 2–4 weeks per dose, highlighting the importance of next-generation platforms such as circRNA and saRNA.[80,81]

Manufacturing and storage challenges

Ultra-cold storage requirements (−80 °C to −20 °C) create ongoing logistical costs estimated to be 3–5 times those of conventional protein therapeutics, making global distribution particularly challenging in resource-limited settings.[71] The instability of mRNA-LNPs during storage is primarily attributable to chemical degradation by hydrolysis and oxidation reactions.[71] While the FDA-approved mRNA vaccines BNT162b2 (Comirnaty®) and mRNA-1273 (Spikevax®) were successfully deployed despite unusual storage requirements, this remains a significant barrier for broader therapeutic applications.[71]

Economic analysis and manufacturing complexity

mRNA therapeutics present complex economic considerations that extend beyond simple production cost comparisons. While cell-free *in vitro* transcription systems enable rapid scale-up and reduced manufacturing complexity compared to recombinant protein production,[80,82–84] the complete economic picture includes substantial delivery system costs and infrastructure requirements.

Cost structure analysis

LNP formulations require sophisticated manufacturing infrastructure, specialized lipids that can cost $1000–$10 000 per kilogram, and complex quality control systems to ensure batch-to-batch consistency.[81] The ultra-cold storage requirements create ongoing logistical costs that are estimated to be 3–5 times those of conventional protein therapeutics, making global distribution particularly challenging in resource-limited settings.[71]

Batch-to-batch variability in LNP formulations can significantly impact dosing consistency, requiring robust analytical methods and quality control systems that add complexity comparable to traditional biologics manufacturing.[15] Although researchers emphasize that mRNA therapeutics offer lower-cost protein production since “these are chemical products and do not require extensive analytical and regulatory exercises”,[3] this perspective overlooks several cost-multiplying factors in the complete value chain.

Manufacturing scalability

While production scalability represents an advantage, the requirement for consistent LNP formulation across large-scale production creates challenges. Recent advances in manufacturing include automated systems for LNP production and

improved quality control methods, but the technology remains more complex than initially anticipated.[80,81]

For chronic conditions requiring repeated dosing frequencies of weekly to monthly administration, long-term treatment costs may exceed those of sustained-release protein formulations or long-acting biologics. Economic modeling suggests that cost-effectiveness requires expression duration of at least 2–4 weeks per dose, emphasizing the importance of next-generation platforms.[80]

Conclusions and clinical translation framework

mRNA therapeutics represent a transformative technology platform with distinct advantages and limitations that must guide clinical application selection. The fundamental amplification effect creates both therapeutic opportunities and dosing constraints that vary significantly across applications, requiring a nuanced approach to clinical development and application selection.

Based on current evidence and mechanistic understanding, mRNA therapeutics achieve optimal results in cancer immunotherapy and vaccination applications, where the intrinsic immunostimulatory properties and tolerance for variable protein expression enhance therapeutic outcomes. Regenerative medicine applications benefit from transient growth factor expression that matches tissue repair timelines, while gene editing applications leverage temporal nuclease expression control to reduce off-target effects. Emerging applications in monoclonal antibody production hold promise for infectious disease prevention and treatment, offering advantages in rapid deployment over traditional antibody manufacturing methods.

Conversely, applications requiring precise, sustained protein levels within narrow therapeutic windows, including enzyme replacement therapy, hormone replacement, and chronic protein deficiency disorders, face fundamental constraints with current mRNA platforms. The characteristic expression kinetics (rapid onset, peak at 24–48 hours, decline over 7–14 days) create inherent mismatches with the steady-state requirements of these applications.

Clinical translation guidelines

For successful clinical development, programs should implement:

1. **Comprehensive dosing studies** with biomarker monitoring aligned with the central expression kinetics pattern.

2. **Patient-specific dosing strategies** accounting for 5–50 fold inter-individual variability.

3. **Robust manufacturing processes** ensuring LNP consistency and batch-to-batch reproducibility.

4. **Consideration of next-generation platforms** (circRNA, saRNA, self-regulating constructs) for applications requiring sustained expression.

Research priorities

Future development should focus on:

1. **Advanced delivery systems** that enable tissue-specific targeting and controlled release.

2. **Self-regulating mRNA constructs** for homeostatic protein control and feedback regulation.

3. **Real-time biomarker development** for expression monitoring and dose optimization.

4. **Hybrid combination strategies** that integrate mRNA with conventional protein therapeutics.

Strategic application selection

The future of mRNA therapeutics lies in the strategic selection of applications rather than the deployment of a universal platform. As delivery systems advance and our understanding of expression control mechanisms deepens, the therapeutic window will expand, but fundamental biological constraints will continue to guide optimal clinical utility.

The technology's most significant value is evident in applications where the unique properties of transient, amplified protein expression offer distinct therapeutic advantages over conventional approaches. Success in cancer immunotherapy, where variable protein expression and immune activation provide therapeutic benefits, validates this approach while highlighting the importance of matching technology capabilities to clinical needs.

The contrast between successful applications (cancer immunotherapy, vaccines) and failed programs (CureVac's protein replacement attempts) demonstrates that technical execution and appropriate application selection are equally critical for clinical success. As the field matures, the integration of advanced delivery systems, self-regulating constructs, and precision dosing strategies will expand the therapeutic window while maintaining focus on applications where mRNA's unique properties provide maximum clinical benefit.

Conflicts of interest

The author is an advisor to the US FDA, EMA, MHRA, the US Senate, the White House, several heads of sovereign states, and a developer of novel biological drugs.

Data availability

There are no data to report; all information enclosed is in the public domain.

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