Abuhassan et al., ***BioImpacts.*** 2026;16:33056
doi:10.34172/bi.33056
https://bi.tbzmed.ac.ir/

RNA vaccines in motion: A multidisciplinary exploration of design, delivery, and deployment

Qamar Abuhassan[1*], Ahmed Aldulaimi[2], O. S. Waleed[3], Subbulakshmi Ganesan[4], V. Kavitha[5], Laxmidhar Maharana[6], Renu Sharma[7], Mutabar Latipova[8,9], Nazira Madatova[10], Doniyor Jumanazarov[11], Aseel Smerat[12,13]

[1]Department of Pharmaceutics and Pharmaceutical Technology, School of Pharmacy, University of Jordan, Amman, 11942, Jordan
[2]Department of Pharmacy, Al-Zahrawi University, Karbala, Iraq
[3]Medical Laboratory Technique College, The Islamic University, Najaf, Iraq
[4]Department of Chemistry and Biochemistry, JAIN (Deemed-to-be University), Bangalore, Karnataka, India
[5]Department of Chemistry, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India
[6]Department of Pharmaceutical Sciences, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India
[7]Department of Chemistry, University Institute of Sciences, Chandigarh University, Mohali, Punjab, India
[8]University of Tashkent for Applied Sciences, Str. Gavhar 1, Tashkent 100149, Uzbekistan
[9]National Research University TIIAME, Kori Niyoziy 39, Tashkent 100000, Uzbekistan
[10]The Department of Pharmaceutical and Chemistry, Alfraganus University, Tashkent, 100190, Uzbekistan
[11]Urgench State University, Kh. Alimdjan str. 14, Urgench 220100, Uzbekistan
[12]Faculty of Educational Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan
[13]Centre for Research Impact and Outcome, Chitkara University, Punjab, India

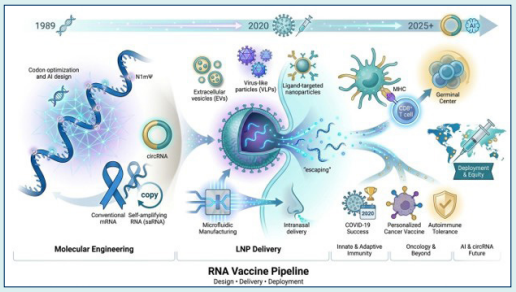
Article Info

Article Type:
Review

Article History:
Received: 26 Oct. 2025
Revised: 4 Jun. 2026
Accepted: 27 Jun. 2026
ePublished: 16 Aug. 2026

Keywords:
RNA vaccines, mRNA, Self amplifying RNA, Lipid nanoparticles, Codon optimization, Chemical modification

Abstract



RNA vaccines represent a major advance in prophylactic and therapeutic vaccinology, largely due to the success of nucleoside-modified mRNA vaccines encapsulated in lipid nanoparticles (LNPs) during the COVID-19 pandemic. These platforms build on long-standing progress in RNA chemistry, immunology, and nanotechnology. This review concentrates primarily on molecular design (codon/sequence optimization, chemical modifications, and data-driven antigen selection) and delivery optimization (LNPs and emerging alternatives like virus-like particles, extracellular vesicles, and ligand-targeted systems). Clinical progress is highlighted through examples from oncology, including personalized neoantigen approaches, combination therapies, and immunopeptidomics-guided validation, as well as early preclinical work on tolerance induction and localized protein replacement. Overall, continued refinement of RNA sequence engineering and delivery systems positions these platforms as flexible tools for rapid-response vaccination and therapy, especially where fast antigen adaptation and scalable production are required.

Introduction

This section outlines the historical development of RNA vaccines, from early molecular discoveries to their clinical deployment during the COVID-19 pandemic, with emphasis on nucleoside modifications and lipid nanoparticle (LNP)–based delivery. The conceptual framework for RNA vaccines emerged decades before their clinical debut, with key discoveries in synthetic oligonucleotides (1978) and RNA interference (1998) forming the molecular groundwork for later therapeutic applications.[1] Although messenger RNA was first identified in 1961, its use as a vaccine platform remained unrealized until nearly three decades later, when the first successful preclinical applications were reported.[2,3] More than three decades of research in RNA biology, immunology, chemical modification, and nanoparticle technology collectively enabled the rapid development of RNA vaccines against SARS-CoV-2.[2,4]

*Corresponding author: Qamar Abuhassan, Email: Singh.ann12031@gmail.com

Review Highlights

What is the current knowledge?

- Nucleoside-modified mRNA vaccines encapsulated in lipid nanoparticles (LNPs) demonstrated high efficacy and rapid deployment during the COVID-19 pandemic, overcoming prior barriers of RNA instability, inefficient delivery, and low translational efficiency through decades of advances in RNA chemistry, immunology, and nanotechnology.
- Compared with traditional vaccines, RNA platforms enable fast antigen redesign, standardized manufacturing without pathogen culture, and flexible use beyond infectious diseases, yet remain constrained by cold-chain requirements, off-target biodistribution, and variable immunogenicity across populations.

What is new here?

- This review delivers a multidisciplinary synthesis of molecular design (codon optimization, AI-driven antigen selection, nucleoside modifications including N1-methylpseudouridine) paired with next-generation delivery systems (LNPs plus emerging EVs, VLPs, ligand-targeted, and intranasal routes) to improve stability, tissue specificity, and controlled immune activation.
- It maps clinical progress with a focus on oncology—personalized neoantigen vaccines, immunopeptidomics validation, combination checkpoint therapies—and extends RNA applications to tolerance induction in autoimmunity and in-situ enzyme replacement for rare metabolic disorders.
- Forward-looking analysis integrates manufacturing scalability (microfluidic production, thermostable lyophilized formulations), global equity via WHO-supported mRNA technology-transfer hubs, regulatory/ethical frameworks, and disruptive innovations (circular RNA, self-boosting hydrogels, AI-optimized constructs) to address durability, access, and rapid-response readiness.

Prior to 2019, several technical hurdles including intrinsic mRNA instability, inefficient in vivo delivery, and limited translational efficiency restricted widespread clinical adoption.[5,6] Early strategies therefore relied on ex vivo loading of dendritic cells with antigen-encoding mRNA rather than direct systemic administration.[7] Subsequent breakthroughs, such as nucleoside modifications, optimized 5′ cap structures, extended poly(A) tails, and advanced delivery systems (e.g., LNPs, polymers, and peptide-based carriers), substantially mitigated these limitations by enhancing RNA stability, translational output, and cellular targeting.[8,9] Compared with traditional vaccine modalities, mRNA platforms enable rapid antigen redesign, standardized manufacturing pipelines, and a reduced reliance on pathogen culture, collectively supporting accelerated development timelines.[5,10] Notably, mRNA-1273 and BNT162b2 demonstrated high neutralizing antibody responses and robust clinical efficacy in pivotal trials, supporting the clinical viability of nucleoside-modified mRNA platforms.[11] The COVID-19 pandemic represented an inflection point, accelerating the clinical validation of nucleoside-modified mRNA vaccine platforms.[2] In parallel, next-generation architectures—including self-amplifying mRNA, trans-amplifying mRNA, and circular RNA have been developed to further extend durability, potency, and dose-sparing potential.[3] Fig. 1 illustrates the evolutionary timeline of RNA vaccine technology from 1989 to 2025.

The rapid maturation of RNA vaccine platforms has been driven by sustained integration across molecular biology, nanotechnology, immunology, and clinical medicine. Cross-disciplinary approaches have increasingly supported antigen discovery, mRNA sequence engineering, lipid component optimization, and stability prediction.[12,13] Computational and data-driven modeling has increasingly supported hypothesis generation and candidate prioritization, complementing experimental validation rather than replacing it. Collaborative networks linking chemists, engineers, biologists, and clinicians have therefore become essential for bridging basic discovery and translational application. Such interdisciplinary collaboration has become essential for bridging basic discovery and translational application.[14] Despite these advances, translation from laboratory systems to clinical settings remains constrained by incomplete understanding of nano–bio interface interactions, experimental artefacts, and data interpretation challenges that may compromise safety and efficacy predictions.[15] Addressing these limitations will require systematic validation strategies and improved alignment between preclinical models and human biology. Large-scale consortia and public–private partnerships have further accelerated innovation in the RNA vaccine field. The FDA's Critical Path Initiative modernized drug development pipelines through the integration of genomics and advanced imaging technologies, while the Innovative Medicines Initiative has coordinated more than 40 collaborative projects linking academia, industry, patients, and regulatory bodies.[16–18] More recently, the ATOM consortium has highlighted the potential of open-source, AI-assisted discovery frameworks for optimizing complex molecular design parameters.[19] The central challenge addressed in this review is how multidisciplinary innovations spanning RNA chemistry, immunology, delivery science, and computational design converge to improve the precision, stability, and global accessibility of next-generation RNA vaccines. Emphasis remains on molecular design and delivery, with other aspects included only to frame translational relevance.

Molecular foundations of RNA vaccine technology

The molecular foundations of RNA vaccine technology encompass the design principles, architectures, and chemical optimizations that transform RNA molecules into efficient and safe immunogens. These systems integrate molecular engineering with computational and data-driven design to enhance stability, potency,

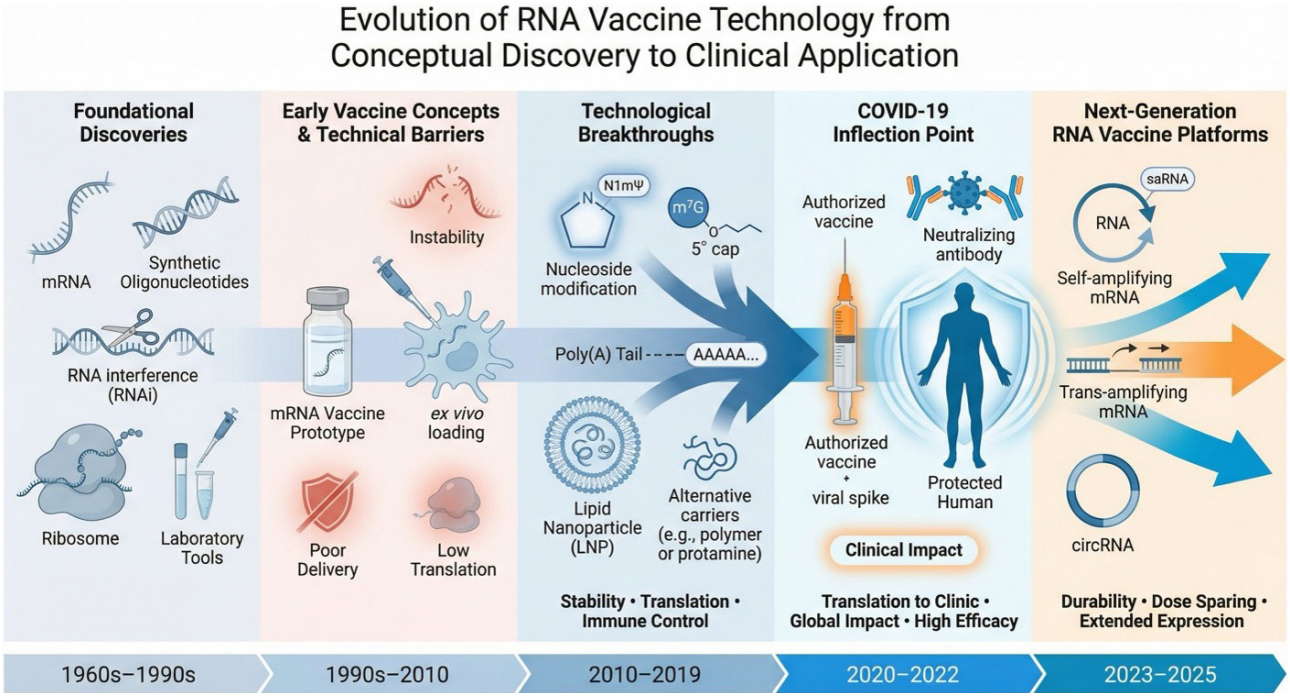


Fig. 1. Evolution of RNA vaccine technology from conceptual discovery to clinical application. *Notes:* Foundational Discoveries (1960s–1990s): Early milestones included the identification of mRNA, development of synthetic oligonucleotides, laboratory tools, ribosomal biology, and the advent of RNA interference (RNAi). Early Vaccine Concepts & Technical Barriers (1990s–2010): The prototype mRNA vaccine revealed critical limitations such as molecular instability, inefficient delivery, low translational efficacy, and technical hurdles involving ex vivo loading. Technological Breakthroughs (2010–2019): Advances such as nucleoside modification (e.g., N1mΨ, m7G cap), Poly(A) tail engineering, lipid nanoparticle (LNP) carriers, and alternative polymer vehicles enabled enhanced stability, translation, and immune control. COVID-19 Inflection Point (2020–2022): Accelerated development led to authorized mRNA vaccines targeting viral spike proteins, producing robust neutralizing antibody responses and demonstrating clinical efficacy at a global scale. Next-Generation RNA Vaccine Platforms (2023–2025): Emerging strategies encompass self-amplifying (saRNA) and trans-amplifying mRNA, as well as circular RNA (circRNA) modalities, offering improvements in durability, dose-sparing, and extended gene expression. This timeline is intended to provide contextual orientation rather than a comprehensive historical account.

and manufacturability. The following subsections outline the key differences between messenger RNA (mRNA) and self-amplifying RNA (saRNA) vaccines, antigen-sequence optimization strategies, and chemical modifications that collectively underpin next-generation vaccine development. This section focuses on molecular and sequence-level determinants of RNA vaccine performance, while delivery route–specific and immunological outcomes are addressed in subsequent sections.

mRNA and saRNA platforms

mRNA and saRNA formulations represent distinct yet complementary strategies in RNA vaccine design, each defined by unique molecular architectures and translational properties. Conventional non-replicating mRNA consists of a 5′ cap, optimized 5′ and 3′ untranslated regions (UTRs), an open reading frame (ORF) encoding the target antigen, and a polyadenylated tail. Once delivered into host cells, it directly engages the translational machinery, with protein yield proportional to the administered dose. By contrast, saRNA incorporates both the antigen-encoding ORF and engineered replicase genes derived from positive-sense RNA viruses such as alphaviruses. The replicase enables intracellular amplification of the RNA template, substantially increasing antigen expression per input molecule and reducing the required dose, thereby enhancing both immunogenicity and cost-effectiveness .[20] This property renders saRNA a valuable option for rapid response and dose-sparing vaccine strategies, particularly under pandemic conditions.[21]

Clinical assessments have compared three main categories: non-amplifying mRNA, base-modified mRNA (bmRNA), and saRNA. During the COVID-19 pandemic, nucleoside-modified mRNA vaccines demonstrated high clinical efficacy in specific indications, whereas saRNA platforms remain under active clinical evaluation with ongoing optimization of stability, manufacturing, and delivery.[20,22,23] Advances such as m5C incorporation, optimized 5′-cap analogs, tunable poly(A) length, polymerase fidelity improvement, and alphavirus replicon refinement have yielded multi-fold enhancements in antigen expression and reduced innate immune activation.[24-27] Key structural and mechanistic distinctions between conventional mRNA and saRNA vaccines are summarized in Table 1.

Antigen design and codon optimization

Rational antigen design is a critical determinant of RNA vaccine performance. Codon optimization aligns the antigen-coding sequence with host-preferred codon frequencies to maximize translational efficiency and structural fidelity. Established tools including JCat, OPTIMIZER, ATGme, and GeneOptimizer enhance the

Table 1. Comparative features, advantages, and limitations of non-replicating mRNA and saRNA vaccine platforms

Feature	Conventional mRNA vaccine	Self-amplifying RNA (saRNA vaccine)
Structure	Contains 5′ cap, UTRs, ORF, poly(A) tail[23,24]	Adds alphavirus replicase genes before ORF[22,23]
Length (bp)	~1–2 kb[5]	~9–12 kb due to replicase region[23,24]
Replication Mechanism	Direct translation; no intrinsic amplification[3,5]	Cytosolic self-amplification via RNA-dependent RNA polymerase[22,23]
Dosage Requirement	Typical clinical dose ranges reported in human studies (e.g., 25–100 µg)[5]	0.1–10 µg efficient expression[25,2]6
Protein Yield	Linear relation to dose[3]	Substantially higher protein yield per input dose[24,25]
Duration of Expression	12–24 hours[5]	Several days due to replicase activity[22,23,2]5
Innate Immune Activation	Reactogenic; requires modified bases[20,28]	Tuned activation from replicase-controlled kinetics[23]
RNA Modifications	N1-methyl-pseudouridine, 5′ mCAP[24]	Often unmodified; replicase substitutes for attenuation[23,24]
Thermostability	Sensitive to RNase; requires cryostorage[3,28]	Improved secondary structure enhances thermal resistance[22,24]
Encapsulation Efficiency	Readily incorporated in LNP (70–100 nm)[6]	Lower efficiency due to bulk RNA length[22,23]
Manufacturing Complexity	Short IVT template; high yield[29,30]	Large size → low transcription efficiency[22,23,25]
Scalability	Standardized production pipeline[22]	Emerging scale-up methods (Bioreactor IVT)[24,25]
Delivery Compatibility	Efficient with LNPs, polymers[6,26]	Requires modified microfluidic LNP assembly[25,26]
Immunogenicity Profile	Balanced innate/adaptive responses[3,5,8]	Enhanced both arms due to amplified translation[24-26]
Safety and Reactogenicity	Proven clinical safety (COVID-19 vaccines)[2,5]	Favorable animal data; human trials ongoing[23,24]
Storage Conditions	−20 °C (frozen) or 2–8 °C lyophilized[28]	Similar; stable under freeze-drying[22,24]
Regulatory Status	Approved Phase III and marketing[2,5]	Early phase I/II clinical testing[22,23,25]
Main Challenges	Limited translation efficiency & short expression[29-31]	Length affects yield, encapsulation uniformity, and error rate[22-25]
Future Potential	Rapid prototype for variant antigens[22,30,31]	Pandemic-scale vaccine production capacity with dose reduction[24,26]

codon adaptation index (CAI) across diverse expression systems.[30]

More recently, data-driven and machine-learning–based algorithms have enabled finer-grained control over RNA secondary structures, energy minimization, and translation kinetics. Machine learning frameworks trained on host genomic signatures have in several contexts matched or exceeded traditional heuristic approaches.[31] However, caution is required in interpreting performance metrics, as fold-change values depend strongly on experimental context—for instance, while the *LinearDesign* pipeline demonstrated notable improvements in translational efficiency, subsequent analyses indicated that the originally reported 128-fold increase was highly context-dependent and not generalizable across systems.[32] The iDRO framework further integrates deep learning for joint optimization of sequence composition and UTR motifs, producing improved expression efficiency across multiple experimental systems.[33] Refined strategies such as "codon harmonization," which reintroduces rare codons at domain boundaries to synchronize folding, can enhance co-translational protein assembly and boost antigen yield several-fold.[34,35] Structural studies confirm that codon usage modulates ribosomal pausing and folding dynamics.[36]

Contemporary antigen-engineering pipelines now employ machine-learning models that integrate multi-omic inputs—genomic, proteomic, and structural—to predict antigenicity, optimize epitope density, and minimize off-target reactivity. These AI-driven models accelerate iterative antigen screening, epitope discovery, and adjuvant pairing, compressing experimental timelines while maintaining interpretability and regulatory traceability.[37-40] In oncology, such approaches are particularly useful for prioritizing personalized neoantigens unique non-self epitopes arising from somatic mutations—within individualized vaccine constructs.

RNA modifications

Chemical modifications to RNA molecules enhance both translation efficiency and immune compatibility. Among these, N1-methyl-pseudouridine (N1mΨ) increases ribosome occupancy and diminishes eIF2α-mediated inhibition, promoting higher translational output.[41] The magnitude of this benefit depends on the lipid carrier system for example, MC3- and KC2-based LNPs synergize with N1mΨ to boost expression, whereas other lipid chemistries exert more modest effects. (N1mΨ) preferentially stabilizes transcripts with specific 5′-UTR contexts, and its immunomodulatory profile can vary across species.[42,43] Additional modifications such as m5C, m6A, m5U, s2U, and pseudouridine can reduce recognition by innate immune sensors including TLR3, TLR7, TLR8, and RIG-I. Mechanistic dissection shows that m6A reduces RIG-I binding affinity, while pseudouridine alters ligand conformation to prevent downstream signaling.[44,45] Combining nucleoside modification with optimized manufacturing that removes double-stranded RNA contaminants further minimizes unwanted immunogenicity.[46] Emerging strategies such as direct bioconjugation of small molecules to RNA offer an orthogonal route to conceal immunogenic motifs from toll-like receptors.[47] Together, these advances enable RNA constructs with high protein yield, controlled

innate activation, and stable manufacturability features essential for next generation vaccine and therapeutic RNA platforms.[39,41-43]

Immune mechanisms activated by RNA vaccines

RNA vaccines trigger a complex interplay between innate and adaptive immune responses, mediated by pattern recognition receptors (PRRs) that detect RNA molecules and by LNP components that influence downstream signaling. The combined action of modified RNA and tailored LNP chemistry orchestrates cytokine release, antigen presentation, and memory formation, providing a flexible immunological interface for precision vaccine design. This section emphasizes mechanistic principles of immune activation rather than exhaustive pathway cataloging, with translational and formulation-specific considerations discussed in later sections.

Innate immune activation and Toll-like receptors

PRRs are fundamental to RNA sensing and orchestrating early immune responses within specific cellular compartments. Endosomal Toll-like receptors (TLR3, TLR7, TLR8) recognize single- and double-stranded RNA upon endocytic internalization. In contrast, cytosolic receptors including RIG-I–like helicases, MDA5, and NLRP3 detect uncapped or long dsRNA in the cytoplasm. PRR expression profiles vary across cell types, enabling broad pathogen recognition while limiting inappropriate autoinflammatory activation.[48-50] In vaccinology, these sensors calibrate adaptive immunity by modulating antigen-presenting cell (APC) behavior. Live-attenuated vaccines achieve exceptional efficacy because their intrinsic RNA structures provide natural adjuvanticity.[51] RNA-derived interferon stimulation also directs T-cell differentiation through cytokines such as IL-12, IL-21, and type I IFNs, influencing both effector function and memory durability.[50] Deepening our mechanistic understanding of these pathways enables rational optimization of synthetic RNA adjuvants to achieve balanced immunogenic activation without excessive reactogenicity. LNP formulations profoundly shape how these innate receptors perceive RNA vaccines. Certain ionizable lipid formulations within LNPs have been reported in some systems to engage TLR4-dependent signaling pathways, although the magnitude and downstream effects may vary depending on formulation, species, and experimental context.[52] This TLR4-MyD88–associated pathway may contribute to adjuvant effects in certain formulations, with lipid headgroup geometry and protonation kinetics potentially influencing receptor engagement and downstream cytokine release. Amine-containing lipid headgroups can interact with receptors such as TLR4 and CD1d, promoting lipid raft assembly and enhancing antigen uptake and cross-presentation. promoting lipid-raft assembly and enhancing both antigen uptake and cross-presentation.[53] Certain lipid combinations preferentially engage TLR2-MyD88 signaling, augmenting antigen-specific responses and thus overall vaccine potency.[54] Consequently, while LNP-mediated innate activation is strongly influenced by ionizable-lipid chemistry and structural motifs, the nucleic acid payload and its structural features may also contribute to immune modulation.[55]

Adaptive immunity: T- and B-cell responses

The immunological mechanism underlying LNP-encapsulated mRNA vaccines is shown in Fig. 2. mRNA vaccines sustain prolonged germinal-center (GC) activity compared with conventional platforms. Following SARS-CoV-2 mRNA vaccination, GC B-cell responses persist in draining lymph nodes for several months in humans, with germinal center B cell responses detected in a substantial proportion of recipients. This persistent GC reaction supports continued affinity maturation and durable serological memory. Somatic hypermutation frequencies increase markedly over the first several months, peaking in bone marrow plasma cells and correlating with enhanced antibody avidity.[56,57] Likewise, mRNA-based influenza vaccines elicit GC reactivity for up to 26 weeks in half of participants, broadening recall memory beyond that induced by inactivated vaccines.[58] Sequential immunization on the same (ipsilateral) site further refines GC selection through local clonal expansion and plasma-cell differentiation.[59]

Antigen presentation kinetics critically influence $CD8^+$ T-cell responses. Studies reveal that a gradually increasing antigen load spanning several days yields stronger $CD8^+$ T-cell expansion and antiviral functionality than single-pulse dosing, highlighting quantitative kinetics as an independent determinant of cellular immunity.[60] Rapidly degraded cytosolic proteins produce epitopes that drive enhanced $CD8^+$ activity compared with slower processing routes.[61] Extended antigen availability over multiple days promotes long-lived $CD8^+$ memory formation promotes long-lived $CD8^+$ memory formation even without elevated effector counts, associated with down-regulation of Eomesodermin, Spi2A, and c-JUN phosphorylation.[62] Such temporal dynamics inform rational design strategies for achieving sustained $CD8^+$ T-cell protection through controlled antigen persistence and optimized delivery kinetics.[63]

Adjuvant effects of RNA and its delivery vehicle

Here, adjuvant effects are discussed at a mechanistic level, without detailing formulation-specific optimization strategies addressed elsewhere. LNP lipids serve as potent regulators of inflammasome activity. Ionizable-lipid composition modulates NLRP3 assembly under serum-free conditions, while protein-corona formation around LNPs attenuates activation by limiting lysosomal rupture and macrophage engulfment.[64] Particular lipid species dictate NLRP3 trafficking; for example, phosphatidylinositol-4-phosphate anchors inflammasome complexes to the trans-Golgi network, influencing cytokine secretion and metabolic coupling.[65] Both self-derived and pathogen lipids can stimulate these pathways—endogenous

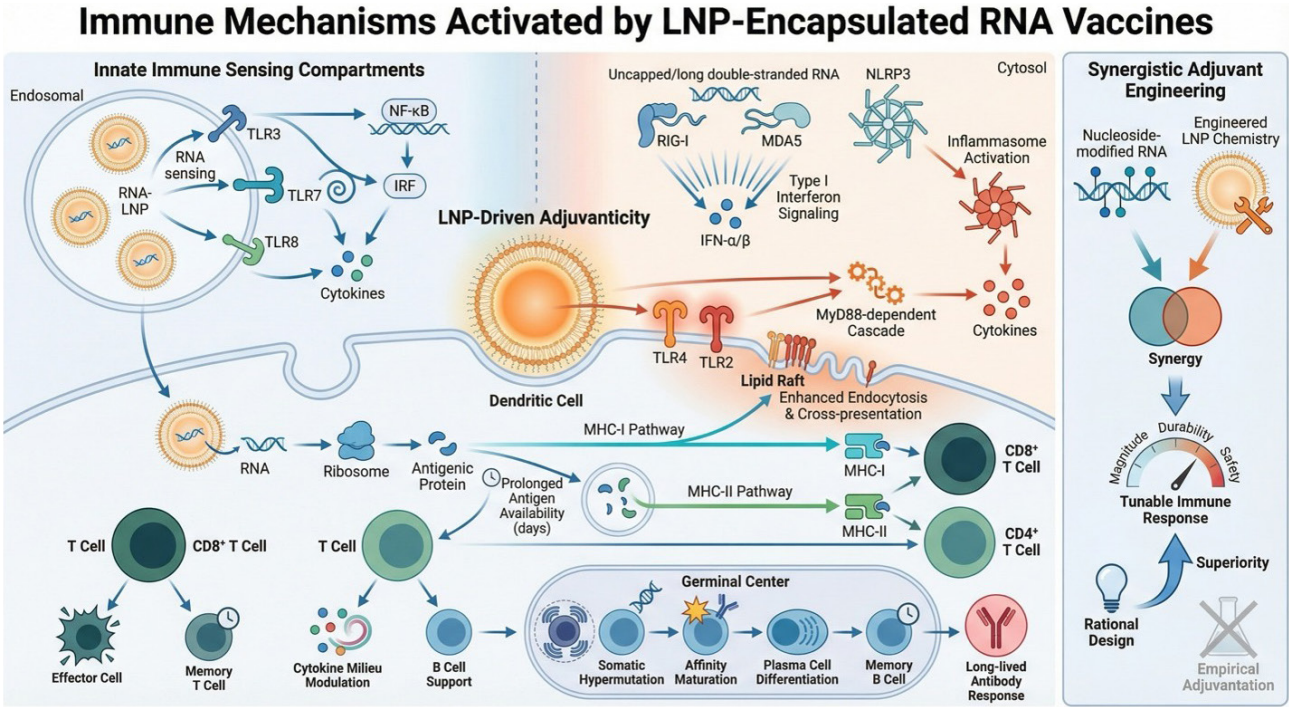


Fig. 2. Immune mechanisms activated by lipid nanoparticle (LNP)-encapsulated RNA vaccines. Following cellular uptake, LNP-encapsulated RNA is sensed by innate immune receptors within endosomal and cytosolic compartments. Endosomal Toll-like receptors (TLR3, TLR7, and TLR8) detect RNA species and initiate downstream NF-κB and interferon regulatory factor (IRF) signaling, resulting in pro-inflammatory cytokine production. In parallel, cytosolic RNA sensors including RIG-I and MDA5 recognize uncapped or double-stranded RNA, inducing type I interferon (IFN-α/β) responses, while NLRP3 inflammasome activation further amplifies innate immune signaling. LNP-driven adjuvanticity enhances dendritic cell activation via TLR2 and TLR4 signaling within lipid raft domains, promoting MyD88-dependent cascades, efficient endocytosis, and antigen cross-presentation. Translated antigenic proteins are processed through both MHC class I and class II pathways, leading to robust activation of $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells, respectively. Prolonged antigen availability supports germinal center formation, enabling somatic hypermutation, affinity maturation, plasma cell differentiation, and the generation of long-lived memory B cells and durable antibody responses. Synergistic adjuvant engineering: Rational integration of nucleoside-modified RNA and engineered LNP chemistry allows fine-tuning of immune magnitude and safety profiles, yielding superior and tunable immune responses compared with empirical adjuvantation strategies.

lipids are implicated in metabolic disease, whereas microbial variants trigger inflammasome activation during infection.[66] Synthetic adjuvant lipids exploit or temper these mechanisms, offering therapeutic leverage where dysregulated lipid metabolism drives pathology.[67] Recent data reveal synergistic adjuvant interactions between nucleoside-modified RNA and engineered LNP chemistries. Adjusting phospholipid and PEG-lipid ratios substantially alters cellular biodistribution and inflammatory signatures, correlating with immunogenic magnitude.[68] Novel "adjuvant lipidoids" partially substituting ionizable lipids can preserve delivery efficiency while providing intrinsic TLR7/8 agonism, thus reinforcing innate activation without compromising tolerability.[69] Furthermore, SDI RNA—a direct RIG-I agonist—loaded into LNPs of different ionizable-lipid compositions (K-Ac7-Dsa vs. S-Ac7-Dog) exhibits distinctive cytokine fingerprints coupling to divergent antibody and T-cell responses.[70] Co-formulation of SDI-RNA–LNPs with IMDQ-PEG-Chol intensifies influenza-challenge protection, demonstrating the principle of dual-agonist synergy between RNA cargo and lipid-based adjuvancy. Collectively, these findings suggest that engineering of both RNA modifications and LNP composition may influence immune amplitude and durability, although these effects remain formulation- and context-dependent.

Delivery strategies: vehicles and vectors

This section reviews delivery strategies for RNA therapeutics, emphasizing the diversity and mechanistic design of vehicles and vectors that determine pharmacokinetics, biodistribution, and immune performance. LNPs remain the clinical gold standard given their superior capability for RNA protection, cellular uptake, and efficient endosomal escape. However, emerging candidates—including extracellular vesicles (EVs), virus-like particles (VLPs), peptides, and polymeric nanocarriers—are being actively explored as next-generation alternatives, particularly for tissue-specific delivery and long-term immune programming. This comparative insight clarifies how physicochemical tuning, surface functionalization, and route-of-entry strategies collectively shape RNA vaccine efficacy and safety. This section focuses on mechanistic and design principles of delivery systems rather than exhaustive performance benchmarking across clinical indications.

Lipid nanoparticles

LNPs constitute the most clinically validated and scalable system for mRNA delivery, shielding RNA from degradation, promoting uptake, and facilitating cytoplasmic release. Each component—ionizable lipid,

helper lipids, cholesterol, and PEG-lipid—plays a precisely defined structural and immunological role. The choice and ratio of helper lipids (e.g., DSPC, DOPC, DOPE) significantly influence LNP biodistribution across preclinical models. The ionizable lipid is particularly critical; in a murine subcutaneous model, SM-102 has been reported to exhibit higher bioavailability than several other ionizable lipids.[71] Helper-lipid composition also affects stability: DSPC has been associated with improved particle stability and enhanced transfection performance in human skin explant models when paired with C12-200 ionizable lipid.[72] Molecular-dynamics simulations reveal that elevated ionizable-lipid content triggers formation of cholesterol-linked disordered aggregates, dynamically reshaping the nanoparticle core architecture.[73] Furthermore, even subtle linker variations such as amide versus urea linkers can result in dramatically altered biodistribution, including targeted pulmonary delivery relevant for metastatic tumors.[74] Manufacturing scalability remains a major focus. Manufacturing scalability remains a major focus. Microfluidic platforms with multiple mixing channels, including 128-channel silicon–glass array configurations in some reports, enable continuous large-scale production with potential yield improvements over single-channel systems while maintaining particle uniformity.[75,76] Downstream optimization, including tangential-flow filtration (TFF) and sterile filtration, supports highly concentrated formulations while mitigating fouling.[77] Continuous-flow T-shaped mixers remain the manufacturing standard bridging laboratory and industrial scales.[78] The Pfizer–BioNTech and Moderna COVID-19 vaccines illustrate the clinical feasibility of LNP-based RNA delivery, highlighting the importance of lipid charge balance and particle size control for efficient cytoplasmic release and antigen expression, and robust clinical immunogenicity. The structural and mechanistic foundations of LNPs—including pH-driven endosomal escape via protonatable lipids—are summarized in Fig. 3.

Novel delivery systems

Beyond LNPs, multiple classes of biomimetic or synthetic carriers are emerging. EVs, especially exosome-based systems, are being investigated as natural vesicular carriers with potential targeting capacity and potentially favorable immunogenicity profiles. In selected CRISPR/Cas9 reporter models, EV-based platforms have shown higher RNA delivery efficiency than benchmark DLin-MC3-DMA LNPs under specific experimental conditions.[79–82] VLPs, which mimic viral architecture without encoding genetic

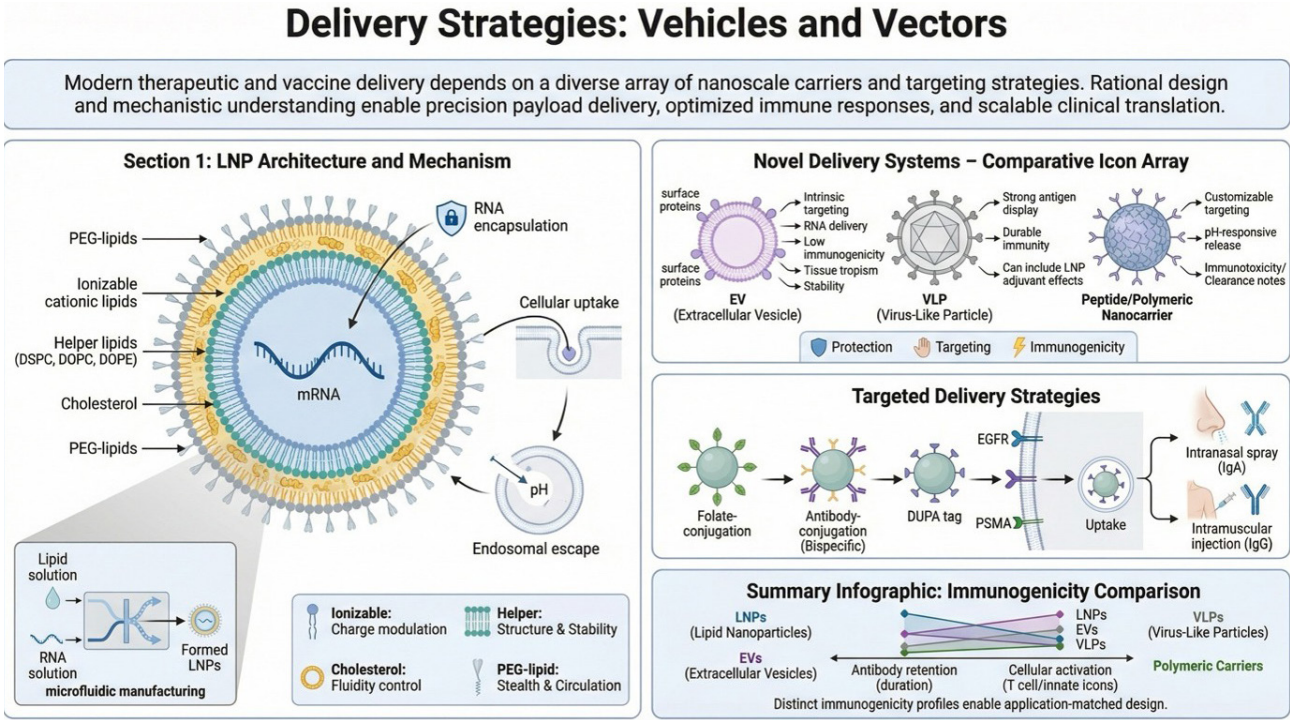


Fig. 3. Delivery strategies for nucleic acid vaccines and therapeutics: vehicles and targeting vectors. Modern therapeutic and vaccine delivery relies on a diverse array of nanoscale carriers and targeting strategies, where rational design and mechanistic understanding enable precise payload delivery, optimized immune responses, and scalable clinical translation. Section 1: Lipid Nanoparticle (LNP) Architecture and Mechanism. LNPs are composed of ionizable cationic lipids, helper phospholipids (e.g., DSPC, DOPE), cholesterol, and PEG-lipids, enabling efficient RNA encapsulation, cellular uptake, pH-triggered endosomal escape, and cytosolic release of mRNA. Microfluidic manufacturing ensures reproducible particle formation and scalable production. Novel Delivery Systems – Comparative Overview. Extracellular vesicles (EVs) offer intrinsic targeting and low immunogenicity; virus-like particles (VLPs) provide strong antigen display and durable immune activation; and peptide or polymeric nanocarriers allow customizable targeting, pH-responsive release, and tunable clearance profiles. Targeted Delivery Strategies. Surface functionalization through folate conjugation, bispecific antibody attachment, or small-molecule ligands (e.g., DUPA targeting PSMA or EGFR-directed strategies) enables receptor-mediated uptake and tissue-specific delivery. Administration routes including intranasal (IgA-biased) and intramuscular (IgG-biased) delivery further shape immune outcomes. Immunogenicity Comparison. Distinct delivery platforms exhibit differential profiles in antibody durability, cellular activation, and innate immune engagement, allowing application-matched selection of carriers for vaccines and therapeutic nucleic acids.

material, exhibit strong antigen display properties. In murine studies, Newcastle-disease VLPs encoding RSV glycoproteins sustained antibody titers over extended periods in murine models compared to the sharp decline seen in RSV-immunized controls after three months.[83] Likewise, influenza VLPs conferred protection over several months, accompanied by maintenance of bone marrow plasma cell populations.[84] Transferable memory B-cells further confirm their long-lived immunogenic potential.[83] Although LNPs themselves can serve as functional adjuvants in VLP formulations stimulating TLR9-dependent type I interferon responses—comprehensive, head-to-head analyses of long-term memory remain scarce.[85] Current evidence suggests that VLPs may preferentially support durable humoral memory, whereas LNP-based systems are often associated with rapid cellular response induction.[86] Peptide-based and polymeric nanocarriers additionally provide versatile delivery opportunities owing to their modular design and ease of chemical modification for ligand conjugation or pH-responsive release. Despite impressive preclinical versatility, translation barriers include immunotoxicity, systemic clearance, and industrial scalability constraints, requiring advanced bioprocess engineering before clinical adoption.

Targeted Delivery and tissue specificity

Therapeutic success of RNA vaccines and drugs depends on precise tissue targeting that expands beyond the liver's inherent tropism for unmodified LNPs. Recent innovations combine customized lipid backbones with covalent ligand conjugation and advanced formulation engineering to detour hepatic accumulation and achieve site-directed delivery.[87] Ligand-based strategies include folate-mediated targeting for folate-receptor–overexpressing tumors and DUPA-anchored systems for prostate-specific membrane antigen (PSMA).[88] Cutting-edge bispecific antibody tethering techniques enable unmodified LNPs to selectively deliver RNA to EGFR- or PSMA-expressing cells, yielding efficient receptor-mediated uptake in both in-vitro and in-vivo models.[89] For respiratory pathogens, intranasal RNA vaccination has been investigated as an approach to elicit both local mucosal IgA and systemic IgG responses.[90] In multiple preclinical models, optimized nanoparticle-encapsulated intranasal mRNA vaccines have been associated with enhanced systemic IgG and mucosal IgA responses.[91,92] Some comparative preclinical studies indicate that intranasal formulations may elicit stronger mucosal IgA responses than intramuscular mRNA delivery in specific models.[93] Collectively, these outcomes position intranasal administration as a strategic platform for respiratory-pathogen interception, combining localized protection with systemic defense. Lipid-based systems currently define the clinical standard, but the field is rapidly expanding toward biomimetic and multifunctional nanocarriers integrating smart-targeting, receptor logic, and route-specific adjuvanticity. Continuous advances in formulation chemistry and manufacturing scalability will determine how efficiently these next-generation carriers transition from bench to bedside.

Clinical translation and therapeutic areas

RNA vaccine platforms have rapidly progressed from conceptual innovation to widespread clinical implementation, fundamentally redefining translational timelines in vaccinology and nucleic-acid therapeutics. Catalyzed by the unprecedented global deployment during the SARS-CoV-2 pandemic, contemporary pipelines increasingly integrate advanced sequence engineering, rational delivery-chemistry optimization, and modular manufacturing infrastructures to address both classical and emerging disease targets. These advances position RNA platforms as adaptable therapeutic systems rather than pathogen-specific solutions. This section highlights representative translational trajectories and therapeutic use cases rather than providing an exhaustive clinical efficacy comparison across indications.

Infectious diseases

The rapid development and global deployment of SARS-CoV-2 mRNA vaccines established an important technological and regulatory milestone for modern vaccinology.[94] Extending this paradigm to highly mutable pathogens—including human immunodeficiency virus type 1 (HIV-1), respiratory syncytial virus (RSV), and seasonal or pandemic influenza—remains a central objective due to their antigenic diversity and immune-escape capacity.[95,96] For these viruses, a central immunological challenge is the induction of broadly neutralizing or cross-reactive antibody responses capable of recognizing conserved viral epitopes.

Reverse-vaccinology strategies therefore prioritize functionally constrained regions within viral fusion glycoproteins, such as HIV Env, RSV F, and the influenza hemagglutinin (HA) stem, to enable cross-strain protection.[96] In HIV-1, early-phase clinical programs emphasize sequential immunization regimens, germline-targeting immunogens, and structure-guided epitope scaffolding, often combined with nanoparticle display to enhance epitope stability and conformational fidelity.[97] Influenza efforts similarly explore mRNA-encoded chimeric HA constructs and consensus-sequence hemagglutinins to broaden serological coverage, while RSV vaccines focus on pre-fusion F-protein stabilization to maximize neutralization potency. Beyond antigen design, iterative mRNA optimization—including codon harmonization, nucleoside modification (N1-methyl-pseudouridine), untranslated-region tuning, and thermostable LNP formulations—has substantially expanded the feasible pathogen landscape.[94] A particularly promising frontier is neglected tropical diseases (NTDs), where limited commercial incentives and prolonged development cycles have historically constrained vaccine innovation.[98] In malaria, LNP-delivered mRNA encoding Plasmodium

berghei circumsporozoite antigens, combined with invariant NKT-cell agonists, induced robust liver-resident memory CD8+ T-cell responses and conferred protective immunity in murine models.[99]

Comparable mRNA-based approaches are under investigation for tuberculosis and HIV, although cost-of-goods, cold-chain logistics, and delivery scalability remain substantial barriers.[100] To mitigate these constraints, WHO-supported mRNA technology-transfer hubs in low- and middle-income countries (LMICs) aim to decentralize manufacturing capacity and facilitate region-specific vaccine production for endemic diseases, thereby narrowing global access gaps.[98]

Oncology

Oncologic mRNA vaccines predominantly leverage personalized neoantigen targeting, informed by tumor genomic and transcriptomic profiling. Next-generation sequencing data are processed through computational pipelines—including pVACtools, MuPeXI, TIminer, and OpenVax—that rank candidate neoepitopes based primarily on predicted MHC binding affinity. However, direct benchmarking reveals marked inter-algorithm variability when applied to identical patient datasets, underscoring limitations of affinity-only prediction models.[101] Emerging neoantigen prioritization strategies increasingly consider integrative biological features—such as variant allele fraction, transcript abundance, and clonal architecture—to improve predictive relevance.[102] For example, the TruNeo framework applies multi-parameter modeling and achieved substantially higher recall for experimentally validated immunogenic neoantigens compared with commonly used MHC-binding–focused predictors.[103] Similarly, proteogenomic approaches integrating mass spectrometry–based immunopeptidomics can experimentally validate epitope presentation and refine candidate prioritization.[104]

Beyond epitope ranking, modern mRNA vaccine development increasingly relies on integrated computational and structural design pipelines. Codon optimization algorithms, untranslated region (UTR) engineering, and RNA secondary structure modeling are routinely employed to enhance translational efficiency and transcript stability. In oncology settings, machine learning–assisted neoantigen prioritization frameworks integrate multi-omic features and immunogenicity datasets to refine candidate selection beyond affinity-based prediction alone. Moreover, in silico modeling contributes to rational construct design and sequence optimization, linking genomic discovery to manufacturable vaccine architectures. Such computational infrastructures now represent a foundational component of contemporary biopharmaceutical development.[105]

Therapeutic scheduling represents another critical determinant of efficacy. Preclinical and early clinical data indicate that mRNA vaccination may be most effective when administered during immunologically permissive windows following chemotherapy-induced nadir.[106] As monotherapies, mRNA vaccines rarely achieve durable tumor control; however, they may help convert immunologically "cold" tumors into more inflamed, T-cell-permissive microenvironments, thereby sensitizing tumors to immune checkpoint blockade.[107] Triple combination regimens comprising mRNA vaccination plus anti-PD-L1 and anti-TIGIT antibodies have been associated with enhanced CD8+ T-cell activation and improved survival outcomes in murine models.[108] saRNA vaccine platforms extend conventional mRNA approaches by incorporating viral replicase machinery—typically derived from alphavirus nonstructural proteins (nsP1–nsP4)—which enables intracellular RNA amplification following cytoplasmic delivery. This replication process can substantially enhance antigen expression while enabling dose-sparing vaccine strategies.[109] Efficient intracellular delivery of these relatively large RNA constructs depends on optimized LNP systems composed of ionizable lipids, helper phospholipids, cholesterol, and polyethylene glycol (PEG)–lipids, which together promote endosomal escape and cytosolic release of RNA cargo.[110] Rational engineering of these nanoformulations—including modulation of ionizable lipid pKa, lipid composition, and particle architecture—has emerged as a critical determinant of delivery efficiency, stability, and immunogenic performance in next-generation RNA vaccine platforms.[111]

Representative clinical investigations of oncologic mRNA and saRNA vaccines—including the phase II KEYNOTE-942 study evaluating personalized mRNA-4157 in combination with pembrolizumab in resected melanoma (NCT03897881)—are summarized in Table 2, with each entry linked to its corresponding clinical trial registry identifier.

Autoimmune and rare diseases

mRNA technology is also being adapted for antigen-specific immune tolerance and in situ protein replacement, addressing disease domains traditionally inaccessible to vaccine platforms. In experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis, non-inflammatory mRNA vaccines encoding myelin autoantigens and incorporating N1-methyl-pseudouridine induced regulatory T-cell populations and suppressed paralytic disease without systemic immunosuppression.[112] This tolerogenic outcome arises from targeted delivery to splenic dendritic cells in the absence of co-stimulatory signaling. Although no tolerogenic mRNA vaccines have yet entered clinical trials for autoimmune diseases, adjacent antigen-specific approaches—such as tolerogenic dendritic-cell therapies are under active investigation in type 1 diabetes, rheumatoid arthritis, and Crohn's disease.[113,114]

For rare metabolic disorders, mRNA has demonstrated strong preclinical performance as a transient yet highly efficient enzyme-replacement modality. In Fabry disease, a single dose of α-galactosidase A mRNA produced sustained supraphysiologic enzyme levels for up to six

Table 2. Representative clinical trials of oncologic mRNA and self-amplifying RNA (saRNA) vaccines

Trial ID/ Registry	Sponsor/ Candidate	Target antigen(s)	RNA platform/ formulation	Phase	Combination regimen	Route	Reported status/outcome
NCT04534205	BioNTech/BNT122 (Autogene cevumeran)	Personalized neoantigens	mRNA-LPX	Phase I	± anti–PD-1	Intravenous	Safety, tolerability, and induction of neoantigen-specific T-cell responses in advanced solid tumors.
NCT03313778	Moderna/mRNA-4157 (V940)	Personalized neoantigens (up to 34 patient-specific tumor neoantigens)	mRNA-LNP	Phase I	Pembrolizumab	Intramuscular	Safety, tolerability, and immunogenicity of individualized mRNA-4157, with clinical activity reported in combination with pembrolizumab.
NCT03480152	National Cancer Institute (NCI)/ CV9202 (CureVac)	Shared tumor antigens (e.g., MUC1, CEA)	RNActive® mRNA	Phase I	Monotherapy	Intradermal	Completed; demonstrated safety and induction of antigen-specific immune responses in NSCLC.
NCT03639714	Gritstone bio	Personalized and/or shared neoantigens	saRNA-LNP	Phase I/II	Anti–PD-1	Intramuscular	Phase I/II evaluation of GRANITE (personalized saRNA vaccine) in microsatellite-stable colorectal cancer.
NCT03897881	Moderna/Merck (KEYNOTE-942/ mRNA-4157)	Personalized melanoma neoantigens	mRNA-LNP	Phase II	Pembrolizumab	Intramuscular	Significant improvement in recurrence-free survival (RFS) compared to pembrolizumab monotherapy in adjuvant melanoma.
NCT06040125	Dana-Farber Cancer Institute/ CVX203 (CureVac)	Shared antigens (e.g., MUC1, NY-ESO-1)	mRNA-LNP	Phase II	Atezolizumab	Intramuscular	Safety and preliminary efficacy of CVX203 in combination with immune checkpoint inhibitors.

Abbreviations: LNP, lipid nanoparticle; saRNA, self-amplifying RNA; RFS, recurrence-free survival; PD-1, programmed cell death protein 1; NSCLC, non-small cell lung cancer; IM, intramuscular; IV, intravenous; ID, intradermal; MSS, microsatellite stable.

weeks in mice and exceeded normal serum enzyme activity levels by a substantial margin in non-human primates.[115,116] In Gaucher disease, mRNA and self-amplifying RNA encoding β-glucocerebrosidase are being investigated as potential approaches for neuronopathic variants, which remain poorly addressed by conventional enzyme therapies due to blood–brain-barrier exclusion.[117] Despite the need for repeat dosing owing to transient expression, the platform's modular design, rapid scalability, and reduced cold-chain dependency offer substantial translational advantages over recombinant protein therapeutics.[117,118] Clinical translation of RNA platforms is no longer limited to infectious disease containment but now spans precision oncology, immune reprogramming, and metabolic replacement therapy. Continued integration of computational design, delivery engineering, and decentralized manufacturing will determine how broadly these technologies reshape future therapeutic landscapes.

Manufacturing and scalability

The long-term success of RNA vaccine platforms depends not only on immunogenic performance but also on manufacturing robustness, regulatory harmonization, and scalable distribution infrastructures. While the COVID-19 pandemic demonstrated the feasibility of ultra-rapid RNA vaccine deployment, it simultaneously exposed vulnerabilities in global production capacity, cold-chain dependence, and technology access inequities. Consequently, contemporary development efforts increasingly emphasize platform standardization, continuous manufacturing, and geographically distributed production models to support sustainable global deployment. Regulatory agencies have responded by refining quality, safety, and clinical-evaluation guidelines for RNA-based products, with a growing emphasis on platform-level characterization rather than product-specific reassessment. Harmonization across regulatory jurisdictions is now recognized as a critical enabler for accelerated authorization during public-health emergencies, particularly for adaptable RNA platforms intended for rapid antigen exchange. Looking forward, thermostable formulations, modular manufacturing units, and pre-validated pan-pathogen sequence libraries are envisioned as foundational components of a rapid-response RNA ecosystem, positioning RNA vaccines as first-line tools against emerging infectious threats and beyond.

In vitro transcription and purification

In vitro transcription (IVT) remains the cornerstone of mRNA and saRNA manufacturing but is inherently challenged by the generation of double-stranded RNA (dsRNA) byproducts, which activate innate immune sensors and compromise translational efficiency. Traditional mitigation strategies rely heavily on downstream chromatographic purification, which increases cost, complexity, and scalability constraints. Recent advances focus on enzymatic control of dsRNA formation at the transcriptional stage, thereby reducing

reliance on intensive purification. The thermostable HiT7 RNA polymerase, operating at 50 °C rather than the conventional 37 °C, significantly reduces dsRNA accumulation and associated RNA-sensor activation compared with SP6 polymerase, while maintaining high transcript yields.[119] Complementarily, the psychrophilic VSW-3 RNA polymerase, active at 4–25 °C, markedly reduces both terminal loop-back and full-length dsRNA species, achieving transcript yields comparable to T7 polymerase.[120] When enzymatic optimization alone is insufficient, simple and scalable downstream approaches such as cellulose-based purification remove the majority of dsRNA contaminants while preserving a substantial proportion of mRNA recovery, offering cost-effective alternatives to complex chromatographic workflows.[121,122] In parallel, microfluidic manufacturing technologies have transformed nanoparticle and microparticle production by enabling precise control over size, morphology, and polydispersity, helping to address long-standing challenges related to batch-to-batch variability.[123,124] Empirical studies demonstrate that microfluidic liposome production consistently yields narrowly distributed nanoparticles within the optimal size range for RNA delivery, while protein-loaded PLGA microparticles produced via microfluidics exhibit markedly narrower size distributions and lower inter-batch variability than those generated by conventional homogenization.[125,126] Such reproducibility is increasingly viewed as indispensable for regulatory compliance and large-scale clinical translation.

Scale-up and cold-chain logistics

Here, extended storage refers to stability maintained over prolonged refrigerated or controlled ambient periods without significant loss of RNA integrity or translatability. Cold-chain dependence remains one of the most significant logistical barriers to global RNA vaccine deployment. Recent formulation research has therefore focused on enhancing RNA stability under refrigerated or carefully controlled ambient conditions without compromising delivery efficiency.[127] Nanostructured lipid carrier (NLC)–based RNA vaccines have demonstrated sustained physicochemical and biological stability following lyophilization under standard refrigerated storage conditions, while liquid NLC formulations similarly maintain stability during extended refrigerated storage.[128] Likewise, lipid nanoparticle formulations incorporating cyclic cationic lipids preserve high encapsulation efficiency and colloidal stability over long-term liquid storage, with no detectable particle size drift.[129] Lyophilization strategies have expanded beyond conventional cryoprotectants such as sucrose and trehalose to include advanced excipient systems that enhance long-term stability and reconstitution performance under refrigerated conditions. Anhydrobiosis-inspired preservation platforms, such as RNAstable, have further demonstrated the feasibility of maintaining RNA integrity over prolonged controlled ambient storage periods, highlighting emerging opportunities to reduce strict cold-chain requirements under defined conditions.[130] Disaccharides particularly sucrose consistently demonstrate strong protective effects during lyophilization, preserving transfection efficiency and nanoparticle integrity upon rehydration.[131–133] Low-molecular-weight dextrans, notably dextran 3000, provide comparable stabilization with approximately 40% lower osmolality, enabling higher post-rehydration vector concentrations.[134] In addition, semi-dendritic hydrophilic polymers with extended linear architectures exhibit exceptional preservation of both mRNA and saRNA lipid nanoparticles, likely through enhanced hydration and aggregation-prevention mechanisms.[133] Collectively, these findings suggest that cold-chain constraints are increasingly technical rather than fundamental limitations of RNA platforms.

Global access and equity

Post-pandemic restructuring of global vaccine manufacturing has positioned Africa as a strategic focal point for RNA production, driven by health-security priorities and efforts to strengthen regional manufacturing independence. The WHO-supported mRNA technology-transfer hub in Cape Town, led by Afrigen, successfully reverse-engineered the Moderna COVID-19 vaccine, marking a symbolic and technical milestone in African vaccine autonomy.[135] Despite representing a substantial share of global vaccine demand of global vaccine demand, Africa currently produces only a small fraction of its vaccines locally, with end-to-end manufacturing capacity limited primarily to South Africa and Senegal.[136] Recent initiatives signal gradual change. Rwanda has established the continent's first mRNA vaccine manufacturing facility, with Senegal, South Africa, and Kenya advancing parallel efforts.[137] Priority targets for regional production include measles–rubella, yellow fever, cholera, rotavirus, and meningococcal vaccines. Financial support mechanisms such as GAVI's $1 billion African Vaccine Manufacturing Accelerator, announced in December 2023, provide critical catalytic funding.[136]

Nevertheless, long-term sustainability remains uncertain, particularly in the context of fluctuating global demand and evolving technology platforms. Experiences from technology-transfer programs highlight mixed economic outcomes. In Tanzania, domestic vaccine manufacturing faced persistent barriers—including limited political support for TRIPS flexibilities, insufficient economies of scale, and constrained pricing competitiveness.[138] Conversely, technology transfer has contributed to a structural shift whereby the majority of global vaccine doses are now produced in emerging economies rather than industrialized nations.[139] Expanded licensing initiatives by the U.S. National Institutes of Health for neglected diseases further illustrate the potential of strategic intellectual-property sharing to improve access.[140] However, successful deployment requires evaluation beyond price-per-dose metrics, incorporating total immunization-system costs and targeted subsidies

to offset early adoption risks for manufacturers implementing advanced RNA technologies.[141] Manufacturing scalability, cold-chain flexibility, and equitable technology transfer are increasingly recognized as key determinants of RNA vaccine impact. Progress in enzymatic IVT control, microfluidic reproducibility, and formulation stabilization suggests that many previously perceived barriers are tractable. The remaining challenges are increasingly systemic—regulatory, economic, and geopolitical—rather than purely technological.

Regulatory, ethical, and societal considerations

The rapid clinical translation of mRNA vaccines has been accompanied by important regulatory, ethical, and societal considerations that extend beyond scientific performance metrics. During the COVID-19 pandemic, emergency regulatory mechanisms enabled unprecedented acceleration of vaccine development and deployment, reshaping public expectations of biomedical innovation while simultaneously exposing vulnerabilities in governance, communication, and global equity frameworks. As mRNA platforms transition from emergency countermeasures to routine biomedical tools, addressing regulatory legitimacy, public trust, and intellectual-property governance becomes essential for long-term sustainability. This section discusses regulatory pathways, public trust, and intellectual-property frameworks that may influence the long-term integration of mRNA vaccine technologies into routine biomedical practice.

Regulatory pathways and accelerated approvals

The COVID-19 pandemic fundamentally reconfigured regulatory pathways for mRNA vaccines, most notably through widespread reliance on Emergency Use Authorization (EUA) mechanisms. Although EUA provisions were originally established in 2004 to address bioterrorism threats, their large-scale deployment during a global pandemic represented a large-scale evaluation of regulatory responsiveness during a global public-health emergency.[142] Early emergency authorizations during the pandemic highlighted concerns regarding evidence thresholds, transparency of decision-making, and maintenance of public confidence, insufficient evidence thresholds, and potential erosion of public confidence, reinforcing the necessity of safeguarding scientific rigor even under emergency conditions.[143]

Several regulatory innovations emerged as defining features of the pandemic response, including rolling reviews, accelerated assessment pathways, reliance-based approvals, and fully digital regulatory submissions. At the global level, the WHO Emergency Use Listing (EUL) mechanism played a critical role in facilitating vaccine access for LMICs, underscoring the importance of supranational coordination during health emergencies.[144] However, these flexibilities also intensified scrutiny regarding the balance between speed and oversight. Despite the demonstrated clinical success of base-modified mRNA (bmRNA) vaccines during COVID-19, several surveys suggest that segments of the population remain hesitant toward mRNA-based technologies, highlighting persistent gaps in public understanding and acceptance.[145] The inherent modularity of lipid-nanoparticle-encapsulated, antigen-encoding mRNA constructs enabled vaccine development timelines to contract from years to months during the pandemic. This process culminated in the emergency authorization of two mRNA vaccines by the U.S. FDA and conditional approvals by the European Medicines Agency.[146] Beyond COVID-19, regulatory agencies are increasingly beginning to recognize the platform nature of mRNA technologies, which may enable more streamlined evaluation pathways for vaccines targeting emerging pathogens, Zika virus, influenza, and other emerging pathogens.[147,148] Nonetheless, the long-term legitimacy of accelerated pathways will depend on transparent post-authorization surveillance, consistent safety communication, and clearly defined transition criteria to full licensure.

Public trust, vaccine hesitancy, and misinformation

Since 2020, a growing body of research has examined the dynamics of misinformation surrounding mRNA vaccines and its impact on public trust. Accumulating evidence suggests that communication strategies emphasizing scientific consensus, evidentiary weight, and transparency outperform fear-based or overly assertive messaging, which may exacerbate skepticism.[149] Humor-based engagement and narrative framing can enhance message retention, but their effectiveness depends strongly on cultural context and audience segmentation. Prebunking strategies grounded in inoculation theory have emerged as strategies that have shown potential in experimental studies. Experimental studies suggest that alerting individuals to common misinformation techniques prior to exposure may help preserve pro vaccination attitudes, with especially pronounced effects among older adults.[150] Text-based refutations and factual corrections reduce short-term susceptibility to false claims circulating on social media platforms, although the durability and scalability of these interventions remain active areas of investigation.[151] A comprehensive response to vaccine misinformation therefore requires multilayered interventions, integrating health-literacy initiatives, proactive and credible communication by trusted institutions, real-time fact-checking infrastructures, and culturally tailored public-health campaigns responsive to local epistemic norms. Achieving sustained public trust in mRNA technologies demands coordinated action among policymakers, healthcare professionals, behavioral scientists, and communication specialists, rather than reliance on single-channel messaging approaches.[152]

Intellectual property and open science in global health

The intellectual-property (IP) landscape governing mRNA vaccines is characterized by dense and overlapping patent portfolios, often requiring multiple licensing

agreements that can pose significant barriers to entry for LMIC manufacturers. Active litigation over core platform patents further complicates technology diffusion and may complicate technology diffusion and coordination across innovation ecosystems.[153,154] In response, the WHO-supported Medicines Patent Pool (MPP) mRNA Technology Transfer Programmed launched in 2021 with South Africa as its central hub aims to establish regional manufacturing capacity and reduce dependency on multinational suppliers. While the programmed represents a meaningful advance toward equitable access, its reliance on voluntary participation, limited affordability provisions, and market-oriented licensing structures may limit the degree of operational autonomy available to recipient countries.[155,156] Nonetheless, the initiative provides a critical proof-of-concept for WHO-led, platform-based technology transfer models, particularly when coupled with workforce training, regulatory strengthening, and sustained financing. The tension between IP protection and equitable diffusion remains unresolved, contributing to ongoing discussions regarding open-science frameworks and patent-pooling mechanisms, patent pooling, and conditional licensing arrangements tailored to pandemic preparedness and global health equity.[156] Regulatory frameworks, public trust, and intellectual-property governance represent important determinants of the broader societal impact of mRNA vaccine technologies.While emergency pathways demonstrated the feasibility of rapid deployment, their long-term success depends on preserving scientific credibility, countering misinformation through evidence-based communication, and enabling equitable technology diffusion. Addressing these dimensions will be essential for embedding mRNA platforms as durable components of global health infrastructure.

Challenges and limitations

Despite substantial advances, RNA vaccine technologies continue to face multidimensional and interdependent challenges that span molecular design, delivery systems, manufacturing scalability, clinical translation, and global deployment. These limitations do not negate the transformative potential of RNA platforms but instead delineate the boundaries within which realistic and sustainable innovation must occur. At the molecular level, the intrinsic instability of RNA remains an important technical constraint. Unmodified RNA transcripts are highly susceptible to extracellular RNases and spontaneous hydrolysis, necessitating chemical modifications such as N1-methyl-pseudouridine. While such modifications enhance translational efficiency and attenuate innate immune sensing, they introduce additional complexity into in vitro transcription workflows and introduce additional considerations related to manufacturing complexity and regulatory characterization, regulatory characterization, and intellectual-property constraints. saRNA platforms offer potential dose-sparing advantages; however, their larger molecular size increases vulnerability to shear stress, compromises formulation robustness, and complicates delivery optimization, thereby posing additional constraints on near-term scalability.

Manufacturing pipelines introduce further bottlenecks. In vitro transcription frequently generates dsRNA byproducts that activate pattern-recognition receptors and attenuate antigen expression. Although enzymatic strategies, including engineered and psychrophilic RNA polymerases, together with cellulose-based purification, have been reported to reduce dsRNA contamination, the scalability, reproducibility, and cost-effectiveness of these approaches in continuous-flow industrial settings remain under active evaluation. Cold-chain dependence represents an additional structural limitation. Most clinically approved LNP-mRNA formulations still require frozen or refrigerated storage, constraining distribution in resource-limited settings. While lyophilization and emerging stabilizing excipients have extended stability under refrigerated conditions, robust room-temperature formulations supported by regulatory-grade long-term potency data remain uncommon. Delivery technologies particularly LNPs pose both technical and biological challenges. Formulation parameters must strike a narrow balance between efficient endosomal escape and avoidance of excessive inflammatory signaling mediated by interactions between lipid components and innate immune receptors such as TLRs or NLR-associated pathways. In addition, off-target biodistribution to the liver and spleen may contribute to dose-limiting toxicities in some contexts, complicating repeated dosing strategies. Achieving reproducible nanoparticle characteristics at commercial scale further demands stringent control over particle size, morphology, and encapsulation efficiency, with microfluidic manufacturing platforms showing promise but not yet universally implemented across production pipelines. Clinical and translational challenges remain substantial. Vaccine immunogenicity varies across age groups, comorbidity profiles, and pathogen classes, limiting uniform efficacy. For highly mutable viruses such as HIV-1 and influenza, the induction of broadly neutralizing antibodies through conserved epitope targeting remains unresolved, despite advances in antigen design. In oncology, personalized neoantigen vaccines consistently demonstrate safety and immunogenicity, yet tumor heterogeneity, immune-escape mechanisms, and suboptimal epitope-prediction algorithms characterized by high false-positive rates remain important challenges for achieving durable clinical responses. At the global level, inequities in manufacturing capacity, regulatory infrastructure, and technology access remain major barriers to equitable deployment. Complex intellectual-property landscapes, coupled with the limited reach and mixed effectiveness of existing technology-transfer mechanisms, continue to restrict autonomous production in low- and middle-income countries. Finally, persistent public-perception challenges—including vaccine hesitancy driven by misinformation and distrust of novel biotechnologies—can undermine adoption even

in the presence of favorable safety and efficacy profiles. Collectively, these limitations underscore the need for coordinated advances in RNA molecular engineering, scalable and reproducible manufacturing, transparent and adaptive regulatory frameworks, and culturally informed public-engagement strategies. Addressing these challenges in parallel, rather than in isolation, will be essential for realizing the broader public-health potential of RNA vaccine platforms.

Future directions and disruptive innovations

Recent advances in RNA vaccine science suggest that the field is entering a phase characterized not only by incremental optimization but also by platform-level innovations that may influence durability, scalability, and adaptability, scalability, and adaptability. Emerging directions—including circular RNA platforms, artificial-intelligence-guided antigen and construct design, and smart delivery systems—aim to address persistent limitations related to RNA stability, antigen persistence, and immunological memory. Collectively, these developments signal a transition toward more stable, modular, and adaptable RNA vaccine technologies. These directions are discussed here as emerging research trajectories rather than clinically validated solutions.

mRNA 2.0 and circular RNA vaccines

Circular RNA (circRNA) vaccines have emerged as a next-generation RNA modality designed to overcome several intrinsic limitations of linear mRNA platforms. Preclinical studies evaluating circRNA constructs encoding trimeric receptor-binding domains (RBDs) of the SARS-CoV-2 spike protein have demonstrated neutralizing antibody responses and antigen-specific T-cell immunity and antigen-specific T-cell immunity in murine and non-human primate models and were associated with protection against viral challenge in preclinical models. Compared with base-modified linear mRNA, circRNA vaccines exhibit prolonged antigen expression and a tendency toward Th1-biased immune polarization, which in some studies has been associated with antiviral and antitumor immune responses.[157] The covalently closed loop structure of circRNAs confers intrinsic resistance to exonuclease-mediated degradation, enhancing molecular stability and sustaining protein translation.[158] In addition, some studies suggest that circRNAs may exhibit reduced recognition by certain innate immune sensors, including RIG-I and certain Toll-like receptors, thereby reducing excessive innate activation while preserving effective adaptive immune priming.[159] Beyond infectious disease models, preclinical cancer vaccine studies have reported durable, antigen-specific $CD8^+$ T-cell responses persisting during extended observation periods in murine studies, highlighting potential applications in therapeutic oncology.[160] Despite these promising attributes, current evidence for circRNA vaccines remains confined to preclinical systems, and key challenges—including scalable manufacturing, delivery optimization, and regulatory characterization—must be resolved before clinical translation. Human safety and efficacy data are still lacking, underscoring the need for cautious interpretation of early results.

AI-driven antigen prediction and RNA design

Artificial intelligence is increasingly shaping RNA vaccine development by integrating machine-learning models with immunoinformatics and synthetic-biology pipelines. Advanced architectures such as GraphEPN—combining graph neural networks, vector-quantized variational autoencoders, and graph transformers—capture both structural and sequence-level features to achieve competitive performance in B-cell epitope prediction tasks.[161] For T-cell epitope discovery, transformer-based and hybrid CNN–transformer models, including MITNet, MLPT, and TransHLA, have improved peptide–MHC–TCR interaction modeling, demonstrating improved classification performance in several benchmark datasets.[162–164]

Within RNA vaccine design, AI-driven workflows enable simultaneous optimization of antigen sequence engineering, codon harmonization, untranslated-region design, and RNA secondary-structure prediction, with the potential to improve predicted translational efficiency and construct stability.[165,166] Machine-learning approaches are also being applied to rationally engineer lipid-nanoparticle formulations, optimizing pharmacokinetic and pharmacodynamic behavior of RNA vaccines.[13]

Together, these tools support a computationally guided development workflow in which antigen selection, RNA engineering, and delivery optimization are iteratively refined prior to experimental validation, accelerating candidate prioritization while reducing empirical trial-and-error.

The algorithmic workflow of AI-powered RNA vaccine design is illustrated in Fig. 4.

Smart delivery and self-boosting vaccine systems

Innovations in delivery technologies are enabling the development of self-boosting and sustained-release RNA vaccine formulations designed to prolong antigen exposure and enhance immunological memory. Hydrogel-based slow-release systems, including polymer–nanoparticle (PNP) composites, create tunable immunological microenvironments that recruit antigen-presenting cells and extend antigen availability, and have been associated in preclinical studies with broader and more durable humoral responses in preclinical studies compared with conventional bolus administration. In SARS-CoV-2 models, sustained delivery of receptor-binding-domain subunit vaccines via PNP hydrogels conferred improved protection against variants of concern, whereas conventional bolus administration produced weaker neutralizing responses in some experimental models.[167–169] Advances in time-release polymer carriers further expand this concept. Poly(β-amino amide) (PBAA) systems

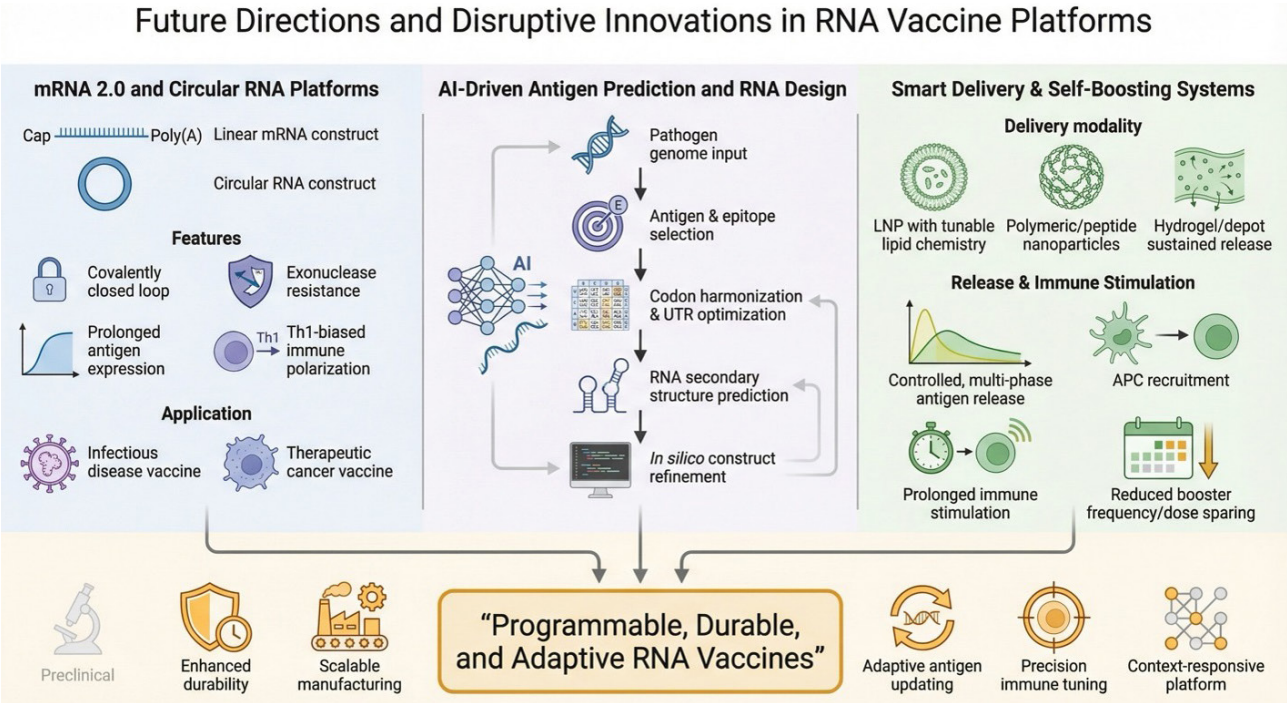


Fig. 4. Future directions and disruptive innovations in RNA vaccine platforms. Next-generation RNA vaccine technologies are evolving toward programmable, durable, and adaptive systems through convergent advances in RNA engineering, artificial intelligence, and smart delivery modalities. mRNA 2.0 and circular RNA (circRNA) platforms enable prolonged antigen expression, exonuclease resistance, and immune polarization control via covalently closed RNA constructs, supporting applications in infectious diseases and therapeutic cancer vaccination. AI-driven antigen prediction and RNA design integrate pathogen genomic inputs with computational epitope selection, codon harmonization, untranslated region (UTR) optimization, and RNA secondary structure modeling, allowing in silico refinement of vaccine constructs prior to experimental validation. Smart delivery and self-boosting systems, including LNPs with tunable lipid chemistry, polymeric or peptide-based nanoparticles, and hydrogel-based depot formulations, enable controlled multi-phase antigen release, enhanced antigen-presenting cell recruitment, prolonged immune stimulation, and reduced booster frequency through dose-sparing strategies. Collectively, these innovations support scalable manufacturing, enhanced durability, adaptive antigen updating, and precision immune tuning, defining a new paradigm of context-responsive RNA vaccine platforms.

incorporating redox-cleavable disulfide linkages enable controlled release kinetics and have demonstrated efficient in vitro gene editing activity while maintaining stability under short-term refrigerated conditions.[170] Poly(L-lactic acid) (PLA) matrices and poly(lactide-co-glycolide) (PLGA) microspheres offer biphasic or triphasic release profiles, substantially improving RNA and oligonucleotide stability over extended durations.[171,172] Additional biomaterials including chitosan, cyclodextrins, dextran, polyglutamic acid, hyaluronic acid, and gelatin are under active investigation for delivery of small non-coding RNA therapeutics.[173] Collectively, these smart-delivery platforms point toward next-generation RNA vaccines with the potentially enable lower dosing requirements, reduced booster frequency, and improved durability of immune responses, although clinical translation and regulatory validation remain ongoing challenges.

Conclusion

Over the past decade, RNA vaccines have transitioned from an experimental concept to a clinically validated immunization platform with demonstrable real-world relevance. This evolution has been driven not by a single breakthrough, but by the convergence of advances in molecular biology, synthetic chemistry, delivery nanotechnologies, computational modeling, and clinical development strategies. The COVID-19 pandemic functioned as both a large-scale validation and an accelerator, compressing development timelines, catalyzing regulatory innovation, and revealing both the strengths and structural vulnerabilities of global vaccine ecosystems. The clinical deployment of nucleoside-modified mRNA vaccines formulated in lipid nanoparticles established RNA vaccination as a credible and versatile modality within contemporary immunotherapy. At a mechanistic level, these platforms integrate multiple technological advances: precise in vitro transcription enabling rapid antigen encoding; rational antigen sequence engineering, untranslated region optimization, and nucleoside modification to enhance stability and translational efficiency; and delivery systems most prominently lipid nanoparticles that protect RNA cargo while facilitating cellular uptake and immune activation. Together, these elements enable coordinated innate and adaptive immune responses across diverse pathogen targets. Beyond prophylactic vaccination, the extension of RNA technologies into oncology, particularly through personalized neoantigen-based strategies, has expanded their potential role in individualized cancer immunotherapy. Exploratory applications in immune tolerance induction and enzyme replacement further illustrate the breadth of potential therapeutic use cases. In parallel, advances in manufacturing science, including microfluidic nanoparticle production and

formulation stabilization approaches, are progressively addressing historical challenges related to scalability, reproducibility, and distribution. Despite these advances, equitable global access remains an important scientific, logistical, and ethical challenge. Initiatives supporting regional manufacturing capacity, technology transfer, and workforce development represent important steps toward more geographically distributed RNA vaccine production. Lessons from the pandemic underscore that rapid technological progress, if not coupled with equitable deployment frameworks, risks perpetuating structural disparities in global health preparedness. Significant challenges nevertheless persist. Mechanistic questions at the nano–bio interface, heterogeneity in immune responses across populations, and complex intellectual property landscapes continue to shape translational trajectories. Public trust also remains fragile; vaccine hesitancy—often amplified by misinformation and skepticism toward emerging biotechnologies—can undermine uptake even when safety and efficacy profiles are favorable. Regulatory systems are therefore tasked with balancing flexibility and rigor, ensuring that accelerated development pathways reinforce, rather than compromise, long-term confidence in biomedical innovation. Looking ahead, next-generation RNA formats, including circular RNA, self-amplifying constructs, and computationally guided immunogen design, are being explored as avenues to enhance durability, breadth, and programmability of immune responses. Advances in smart delivery and controlled-release systems may further reduce dosing requirements and improve deployment in resource-limited settings. These directions should be viewed as active areas of investigation rather than clinically established solutions. In summary, RNA vaccines stand at the intersection of multidisciplinary science, industrial innovation, and global health policy. Their significance extends beyond pandemic response, offering a flexible framework for addressing endemic infections, emerging pathogens, and selected chronic diseases. Realizing this potential will depend not only on continued scientific progress, but also on sustained collaboration among molecular scientists, engineers, clinicians, regulators, public health institutions, and communities worldwide, helping ensure that technological advances translate into sustainable and equitable health benefits.

Authors' Contribution

Conceptualization: Qamar Abuhassan, Ahmed Aldulaimi.
Data curation: Ahmed Aldulaimi, O. S. Waleed.
Formal analysis: Subbulakshmi Ganesan, V. Kavitha.
Funding acquisition: Qamar Abuhassan.
Investigation: O. S. Waleed, Laxmidhar Maharana.
Methodology: Subbulakshmi Ganesan, V. Kavitha.
Project administration: Qamar Abuhassan.
Resources: Renu Sharma, Mutabar Latipova.
Supervision: Qamar Abuhassan.
Validation: Laxmidhar Maharana, Nazira Madatova.
Writing–original draft: Ahmed Aldulaimi, O. S. Waleed.
Writing–review & editing: Qamar Abuhassan, Subbulakshmi Ganesan, V. Kavitha, Laxmidhar Maharana, Renu Sharma, Mutabar Latipova, Nazira Madatova, Doniyor Jumanazarov, Aseel Smerat.

Competing Interests

None.

Data Availability Statement

Not applicable.

Ethical Approval

Not applicable.

Funding

No funding.

References

1. Tani H. Recent advances and prospects in RNA drug development. *Int J Mol Sci* **2024**; 25: 12284. doi:10.3390/ijms252212284
2. Szabó GT, Mahiny AJ, Vlatkovic I. COVID-19 mRNA vaccines: platforms and current developments. *Mol Ther* **2022**; 30: 1850-68. doi:10.1016/j.ymthe.2022.02.016
3. Perenkov AD, Sergeeva AD, Vedunova MV, Krysko DV. In vitro transcribed RNA-based platform vaccines: past, present, and future. *Vaccines (Basel)* **2023**; 11: 1600. doi:10.3390/vaccines11101600
4. Granados-Riveron JT, Aquino-Jarquin G. Engineering of the current nucleoside-modified mRNA-LNP vaccines against SARS-CoV-2. *Biomed Pharmacother* **2021**; 142: 111953. doi:10.1016/j.biopha.2021.111953
5. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. *Nat Rev Drug Discov* **2018**; 17: 261-79. doi:10.1038/nrd.2017.243
6. Zhou DW, Wang K, Zhang YA, Ma K, Yang XC, Li ZY, et al. mRNA therapeutics for disease therapy: principles, delivery, and clinical translation. *J Mater Chem B* **2023**; 11: 3484-510. doi:10.1039/d2tb02782h
7. Pollard C, De Koker S, Saelens X, Vanham G, Grooten J. Challenges and advances towards the rational design of mRNA vaccines. *Trends Mol Med* **2013**; 19: 705-13. doi:10.1016/j.molmed.2013.09.002
8. Yılmaz E. [New hopes in vaccine technology: mRNA vaccines]. *Mikrobiyol Bul* **2021**; 55: 265-84. doi:10.5578/mb.20219912
9. Parvin N, Mandal TK, Joo SW. The impact of COVID-19 on RNA therapeutics: a surge in lipid nanoparticles and alternative delivery systems. *Pharmaceutics* **2024**; 16: 1366. doi:10.3390/pharmaceutics16111366
10. Leong KY, Tham SK, Poh CL. Revolutionizing immunization: a comprehensive review of mRNA vaccine technology and applications. *Virol J* **2025**; 22: 71. doi:10.1186/s12985-025-02645-6
11. McDonald I, Murray SM, Reynolds CJ, Altmann DM, Boyton RJ. Comparative systematic review and meta-analysis of reactogenicity, immunogenicity and efficacy of vaccines against SARS-CoV-2. *NPJ Vaccines* **2021**; 6: 74. doi:10.1038/s41541-021-00336-1
12. Lu Y, Qian C, Huang Y, Ren T, Xie W, Xia N, et al. Advancing mRNA vaccines: a comprehensive review of design, delivery, and efficacy in infectious diseases. *Int J Biol Macromol* **2025**; 319: 145501. doi:10.1016/j.ijbiomac.2025.145501
13. Imani S, Li X, Chen K, Maghsoudloo M, Jabbarzadeh Kaboli P, Hashemi M, et al. Computational biology and artificial intelligence in mRNA vaccine design for cancer immunotherapy. *Front Cell Infect Microbiol* **2024**; 14: 1501010. doi:10.3389/fcimb.2024.1501010
14. Swierczewska M, Lee S, Chen X. Moving theranostics trom bench to bedside in an interdisciplinary research team. *Ther Deliv* **2011**; 2: 165-70. doi:10.4155/tde.10.106
15. Mahmoudi M. Debugging nano-bio interfaces: systematic strategies to accelerate clinical translation of nanotechnologies. *Trends Biotechnol* **2018**; 36: 755-69. doi:10.1016/j.tibtech.2018.02.014
16. Woodcock J, Woosley R. The FDA critical path initiative and its influence on new drug development. *Annu Rev Med* **2008**; 59: 1-12. doi:10.1146/annurev.med.59.090506.155819
17. Goldman M. The innovative medicines initiative: a European response to the innovation challenge. *Clin Pharmacol Ther* **2012**; 91: 418-25. doi:10.1038/clpt.2011.321

18. Vaudano E. The innovative medicines initiative: a public private partnership model to foster drug discovery. *Comput Struct Biotechnol J* **2013**; 6: e201303017. doi:10.5936/csbj.201303017
19. Hinkson IV, Madej B, Stahlberg EA. Accelerating therapeutics for opportunities in medicine: a paradigm shift in drug discovery. *Front Pharmacol* **2020**; 11: 770. doi:10.3389/fphar.2020.00770
20. Casmil IC, Jin J, Won EJ, Huang C, Liao S, Cha-Molstad H, et al. The advent of clinical self-amplifying RNA vaccines. *Mol Ther* **2025**; 33: 2565-82. doi:10.1016/j.ymthe.2025.03.060
21. Liu Y, Li Y, Hu Q. Advances in saRNA vaccine research against emerging/re-emerging viruses. *Vaccines (Basel)* **2023**; 11: 1142. doi:10.3390/vaccines11071142
22. Geall AJ, Kis Z, Ulmer JB. Vaccines on demand, part II: future reality. *Expert Opin Drug Discov* **2023**; 18: 119-27. doi:10.1080/17460441.2022.2147501
23. Quintana V, Caillava J, Byk LA, Mondotte JA, Battini L, Tarte P, et al. Improvement in the potency of a N1-methylpseudouridine-modified self-amplifying RNA through mutations in the RNA-dependent RNA polymerase. *J Biol Chem* **2025**; 301: 110487. doi:10.1016/j.jbc.2025.110487
24. Sun Z, Liu Y, Zhang H, Ge T, Pan Y, Liu Y, et al. Next-generation saRNA platforms: systematic screening and engineering enhances superior protein expression and organ-specific targeting for RNA therapeutics. *bioRxiv* [Preprint]. March 30, **2025**. Available from: https://www.biorxiv.org/content/10.1101/2025.03.30.644708v1.
25. Gong Y, Yong D, Liu G, Xu J, Ding J, Jia W. A novel self-amplifying mRNA with decreased cytotoxicity and enhanced protein expression by macrodomain mutations. *Adv Sci (Weinh)* **2024**; 11: e2402936. doi:10.1002/advs.202402936
26. Blakney AK, Zhu Y, McKay PF, Bouton CR, Yeow J, Tang J, et al. Big is beautiful: enhanced saRNA delivery and immunogenicity by a higher molecular weight, bioreducible, cationic polymer. *ACS Nano* **2020**; 14: 5711-27. doi:10.1021/acsnano.0c00326
27. Shakya AK, Chowdhury MY, Tao W, Gill HS. Mucosal vaccine delivery: current state and a pediatric perspective. *J Control Release* **2016**; 240: 394-413. doi:10.1016/j.jconrel.2016.02.014
28. Vajdy M, Srivastava I, Polo J, Donnelly J, O'Hagan D, Singh M. Mucosal adjuvants and delivery systems for protein-, DNA- and RNA-based vaccines. *Immunol Cell Biol* **2004**; 82: 617-27. doi:10.1111/j.1440-1711.2004.01288.x
29. Demissie EA, Park SY, Moon JH, Lee DY. Comparative analysis of codon optimization tools: advancing toward a multi-criteria framework for synthetic gene design. *J Microbiol Biotechnol* **2025**; 35: e2411066. doi:10.4014/jmb.2411.11066
30. Goulet DR, Yan Y, Agrawal P, Waight AB, Mak AN, Zhu Y. Codon optimization using a recurrent neural network. *J Comput Biol* **2023**; 30: 70-81. doi:10.1089/cmb.2021.0458
31. Zhang H, Zhang L, Lin A, Xu C, Li Z, Liu K, et al. Algorithm for optimized mRNA design improves stability and immunogenicity. *Nature* **2023**; 621: 396-403. doi:10.1038/s41586-023-06127-z
32. Gong H, Wen J, Luo R, Feng Y, Guo J, Fu H, et al. Integrated mRNA sequence optimization using deep learning. *Brief Bioinform* **2023**; 24: bbad001. doi:10.1093/bib/bbad001
33. Ward M, Richardson M, Metkar M. mRNA folding algorithms for structure and codon optimization. *Brief Bioinform* **2025**; 26: bbaf386. doi:10.1093/bib/bbaf386
34. Angov E, Hillier CJ, Kincaid RL, Lyon JA. Heterologous protein expression is enhanced by harmonizing the codon usage frequencies of the target gene with those of the expression host. *PLoS One* **2008**; 3: e2189. doi:10.1371/journal.pone.0002189
35. Yadava A, Ockenhouse CF. Effect of codon optimization on expression levels of a functionally folded malaria vaccine candidate in prokaryotic and eukaryotic expression systems. *Infect Immun* **2003**; 71: 4961-9. doi:10.1128/iai.71.9.4961-4969.2003
36. Yu CH, Dang Y, Zhou Z, Wu C, Zhao F, Sachs MS, et al. Codon usage influences the local rate of translation elongation to regulate co-translational protein folding. *Mol Cell* **2015**; 59: 744-54. doi:10.1016/j.molcel.2015.07.018
37. Nakahigashi K, Takai Y, Shiwa Y, Wada M, Honma M, Yoshikawa H, et al. Effect of codon adaptation on codon-level and gene-level translation efficiency in vivo. *BMC Genomics* **2014**; 15: 1115. doi:10.1186/1471-2164-15-1115
38. Hernandez-Alias X, Benisty H, Schaefer MH, Serrano L. Translational efficiency across healthy and tumor tissues is proliferation-related. *Mol Syst Biol* **2020**; 16: MSB199275. doi:10.15252/msb.20199275
39. Krisko A, Copic T, Gabaldón T, Lehner B, Supek F. Inferring gene function from evolutionary change in signatures of translation efficiency. *Genome Biol* **2014**; 15: R44. doi:10.1186/gb-2014-15-3-r44
40. Halder B, Malakar AK, Chakraborty S. Nucleotide composition determines the role of translational efficiency in human genes. *Bioinformation* **2017**; 13: 46-53. doi:10.6026/97320630013046
41. Svitkin YV, Cheng YM, Chakraborty T, Presnyak V, John M, Sonenberg N. N1-methyl-pseudouridine in mRNA enhances translation through eIF2α-dependent and independent mechanisms by increasing ribosome density. *Nucleic Acids Res* **2017**; 45: 6023-36. doi:10.1093/nar/gkx135
42. Bernard MC, Bazin E, Petiot N, Lemdani K, Commandeur S, Verdelet C, et al. The impact of nucleoside base modification in mRNA vaccine is influenced by the chemistry of its lipid nanoparticle delivery system. *Mol Ther Nucleic Acids* **2023**; 32: 794-806. doi:10.1016/j.omtn.2023.05.004
43. Lewis CJ, Xie LH, Bhandarkar SM, Jin D, Abdallah K, Draycott AS, et al. Quantitative profiling of human translation initiation reveals elements that potently regulate endogenous and therapeutically modified mRNAs. *Mol Cell* **2025**; 85: 445-59.e5. doi:10.1016/j.molcel.2024.11.030
44. Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* **2005**; 23: 165-75. doi:10.1016/j.immuni.2005.06.008
45. Durbin AF, Wang C, Marcotrigiano J, Gehrke L. RNAs containing modified nucleotides fail to trigger RIG-I conformational changes for innate immune signaling. *mBio* **2016**; 7: e00833-16. doi:10.1128/mBio.00833-16
46. Nelson J, Sorensen EW, Mintri S, Rabideau AE, Zheng W, Besin G, et al. Impact of mRNA chemistry and manufacturing process on innate immune activation. *Sci Adv* **2020**; 6: eaaz6893. doi:10.1126/sciadv.aaz6893
47. Hellmuth I, Freund I, Schlöder J, Seidu-Larry S, Thüring K, Slama K, et al. Bioconjugation of small molecules to RNA impedes its recognition by toll-like receptor 7. *Front Immunol* **2017**; 8: 312. doi:10.3389/fimmu.2017.00312
48. Tatematsu M, Funami K, Seya T, Matsumoto M. Extracellular RNA sensing by pattern recognition receptors. *J Innate Immun* **2018**; 10: 398-406. doi:10.1159/000494034
49. Chen N, Xia P, Li S, Zhang T, Wang TT, Zhu J. RNA sensors of the innate immune system and their detection of pathogens. *IUBMB Life* **2017**; 69: 297-304. doi:10.1002/iub.1625
50. Burkert S, Schumann RR. RNA sensing of *Mycobacterium tuberculosis* and its impact on TB vaccination strategies. *Vaccines (Basel)* **2020**; 8: 67. doi:10.3390/vaccines8010067
51. Georg P, Sander LE. Innate sensors that regulate vaccine responses. *Curr Opin Immunol* **2019**; 59: 31-41. doi:10.1016/j.coi.2019.02.006
52. Zelkoski AE, Lu Z, Sukumar G, Dalgard C, Said H, Alameh MG, et al. Ionizable lipid nanoparticles of mRNA vaccines elicit NF-κB and IRF responses through toll-like receptor 4. *NPJ Vaccines* **2025**; 10: 73. doi:10.1038/s41541-025-01124-x
53. Chaudhary N, Kasiewicz LN, Newby AN, Arral ML, Yerneni SS, Melamed JR, et al. Amine headgroups in ionizable lipids drive immune responses to lipid nanoparticles by binding to the receptors TLR4 and CD1d. *Nat Biomed Eng* **2024**; 8: 1483-98. doi:10.1038/s41551-024-01256-w
54. Swaminathan G, Lin SA, Patel M, DiFelice K, Smith J, Gindy M, et al. Activation of the TLR2-MyD88 pathway is required for in-vivo efficacy of lipid nanoparticle-based vaccine formulation. *J Immunol* **2017**; 198: 79.2-.2. doi:10.4049/jimmunol.198.Supp.79.2
55. Catenacci L, Rossi R, Sechi F, Buonocore D, Sorrenti M, Perteghella S, et al. Effect of lipid nanoparticle physico-chemical properties and composition on their interaction with the immune system. *Pharmaceutics* **2024**; 16: 1521. doi:10.3390/pharmaceutics16121521
56. Laidlaw BJ, Ellebedy AH. The germinal centre B cell response

to SARS-CoV-2. *Nat Rev Immunol* **2022**; 22: 7-18. doi:10.1038/s41577-021-00657-1
57. Kim W, Zhou JQ, Horvath SC, Schmitz AJ, Sturtz AJ, Lei T, et al. Germinal centre-driven maturation of B cell response to mRNA vaccination. *Nature* **2022**; 604: 141-5. doi:10.1038/s41586-022-04527-1
58. Matz HC, Yu TG, Zhou JQ, Peyton L, Madsen A, Han F, et al. mRNA-based influenza vaccine expands breadth of B cell response in humans. *bioRxiv* [Preprint]. October 13, **2024**. Available from: https://www.biorxiv.org/content/10.1101/2024.10.10.617255v1.
59. Jiang W, Maldeney AR, Yuan X, Richer MJ, Renshaw SE, Luo W. Ipsilateral immunization after a prior SARS-CoV-2 mRNA vaccination elicits superior B cell responses compared to contralateral immunization. *Cell Rep* **2024**; 43: 113665. doi:10.1016/j.celrep.2023.113665
60. Johansen P, Storni T, Rettig L, Qiu Z, Der-Sarkissian A, Smith KA, et al. Antigen kinetics determines immune reactivity. *Proc Natl Acad Sci U S A* **2008**; 105: 5189-94. doi:10.1073/pnas.0706296105
61. Cosma GL, Lobby JL, Fay EJ, Siciliano NA, Langlois RA, Eisenlohr LC. Kinetically distinct processing pathways diversify the CD8 + T cell response to a single viral epitope. *Proc Natl Acad Sci U S A* **2020**; 117: 19399-407. doi:10.1073/pnas.2004372117
62. Bachmann MF, Beerli RR, Agnellini P, Wolint P, Schwarz K, Oxenius A. Long-lived memory CD8 + T cells are programmed by prolonged antigen exposure and low levels of cellular activation. *Eur J Immunol* **2006**; 36: 842-54. doi:10.1002/eji.200535730
63. Zaiss DM, Boog CJ, van Eden W, Sijts AJ. Considerations in the design of vaccines that induce CD8 T cell mediated immunity. *Vaccine* **2010**; 28: 7716-22. doi:10.1016/j.vaccine.2010.08.101
64. Debnath M, Forster J 3rd, Ramesh A, Kulkarni A. Protein corona formation on lipid nanoparticles negatively affects the NLRP3 inflammasome activation. *Bioconjug Chem* **2023**; 34: 1766-79. doi:10.1021/acs.bioconjchem.3c00329
65. Anand PK. From fat to fire: the lipid-inflammasome connection. *Immunol Rev* **2025**; 329: e13403. doi:10.1111/imr.13403
66. Pizzuto M, Pelegrin P, Ruysschaert JM. Lipid-protein interactions regulating the canonical and the non-canonical NLRP3 inflammasome. *Prog Lipid Res* **2022**; 87: 101182. doi:10.1016/j.plipres.2022.101182
67. Anand PK. Lipids, inflammasomes, metabolism, and disease. *Immunol Rev* **2020**; 297: 108-22. doi:10.1111/imr.12891
68. Vadovics M, Zhao W, Daley EF, Lam K, Daly O, Rashid K, et al. Tailoring the adjuvanticity of lipid nanoparticles by PEG lipid ratio and phospholipid modifications. *Nat Nanotechnol* **2025**; 20: 1312-22. doi:10.1038/s41565-025-01958-5
69. Han X, Alameh MG, Butowska K, Knox JJ, Lundgreen K, Ghattas M, et al. Adjuvant lipidoid-substituted lipid nanoparticles augment the immunogenicity of SARS-CoV-2 mRNA vaccines. *Nat Nanotechnol* **2023**; 18: 1105-14. doi:10.1038/s41565-023-01404-4
70. Jangra S, Lamoot A, Singh G, Laghlali G, Chen Y, Ye T, et al. Lipid nanoparticle composition for adjuvant formulation modulates disease after influenza virus infection in quadrivalent influenza vaccine vaccinated mice. *Front Immunol* **2024**; 15: 1370564. doi:10.3389/fimmu.2024.1370564
71. Ren Y, Lin L, Abdallah M, Zhu X, Liu H, Fabb SA, et al. Impact of ionizable lipid type on the pharmacokinetics and biodistribution of mRNA-lipid nanoparticles after intravenous and subcutaneous injection. *J Control Release* **2025**; 384: 113945. doi:10.1016/j.jconrel.2025.113945
72. Barbieri BD, Peeler DJ, Samnuan K, Day S, Hu K, Sallah HJ, et al. The role of helper lipids in optimising nanoparticle formulations of self-amplifying RNA. *J Control Release* **2024**; 374: 280-92. doi:10.1016/j.jconrel.2024.08.016
73. Dehghani-Ghahnaviyeh S, Smith M, Xia Y, Dousis A, Grossfield A, Sur S. Ionizable amino lipids distribution and effects on DSPC/cholesterol membranes: implications for lipid nanoparticle structure. *J Phys Chem B* **2023**; 127: 6928-39. doi:10.1021/acs.jpcb.3c01296
74. Somu Naidu G, Rampado R, Sharma P, Ezra A, Kundoor GR, Breier D, et al. Ionizable lipids with optimized linkers enable lung-specific, lipid nanoparticle-mediated mRNA delivery for treatment of metastatic lung tumors. *ACS Nano* **2025**; 19: 6571-87. doi:10.1021/acsnano.4c18636
75. Shepherd SJ, Warzecha CC, Yadavali S, El-Mayta R, Alameh MG, Wang L, et al. Scalable mRNA and siRNA lipid nanoparticle production using a parallelized microfluidic device. *Nano Lett* **2021**; 21: 5671-80. doi:10.1021/acs.nanolett.1c01353
76. Shepherd SJ, Han X, Mukalel AJ, El-Mayta R, Thatte AS, Wu J, et al. Throughput-scalable manufacturing of SARS-CoV-2 mRNA lipid nanoparticle vaccines. *Proc Natl Acad Sci U S A* **2023**; 120: e2303567120. doi:10.1073/pnas.2303567120
77. Wu W, Oliveira LT, Jain A, Karpov Y, Olsen K, Wu Y, et al. Process development of tangential flow filtration and sterile filtration for manufacturing of mRNA-lipid nanoparticles: a study on membrane performance and filtration modeling. *Int J Pharm* **2025**; 675: 125520. doi:10.1016/j.ijpharm.2025.125520
78. Hourdel L, Lebaz N, Peral F, Ripoll M, Briançon S, Bensaid F, et al. Overview on LNP-mRNA encapsulation unit operation: mixing technologies, scalability, and influence of formulation & process parameters on physico-chemical characteristics. *Int J Pharm* **2025**; 672: 125297. doi:10.1016/j.ijpharm.2025.125297
79. Murphy DE, de Jong OG, Evers MJ, Nurazizah M, Schiffelers RM, Vader P. Natural or synthetic RNA delivery: a stoichiometric comparison of extracellular vesicles and synthetic nanoparticles. *Nano Lett* **2021**; 21: 1888-95. doi:10.1021/acs.nanolett.1c00094
80. Duechler M. Vehicles for small interfering RNA transfection: exosomes versus synthetic nanocarriers. *DNA RNA Nanotechnol* **2013**; 1: 16-26. doi:10.2478/rnan-2013-0002
81. Nordmeier S, Ke W, Afonin KA, Portnoy V. Exosome mediated delivery of functional nucleic acid nanoparticles (NANPs). *Nanomedicine* **2020**; 30: 102285. doi:10.1016/j.nano.2020.102285
82. Jiang L, Vader P, Schiffelers RM. Extracellular vesicles for nucleic acid delivery: progress and prospects for safe RNA-based gene therapy. *Gene Ther* **2017**; 24: 157-66. doi:10.1038/gt.2017.8
83. Schmidt MR, McGinnes LW, Kenward SA, Willems KN, Woodland RT, Morrison TG. Long-term and memory immune responses in mice against Newcastle disease virus-like particles containing respiratory syncytial virus glycoprotein ectodomains. *J Virol* **2012**; 86: 11654-62. doi:10.1128/jvi.01510-12
84. Quan FS, Huang C, Compans RW, Kang SM. Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. *J Virol* **2007**; 81: 3514-24. doi:10.1128/jvi.02052-06
85. Dai W, Xing M, Sun L, Lv L, Wang X, Wang Y, et al. Lipid nanoparticles as adjuvant of norovirus VLP vaccine augment cellular and humoral immune responses in a TLR9- and type I IFN-dependent pathway. *J Virol* **2024**; 98: e0169924. doi:10.1128/jvi.01699-24
86. Beltran-Garcia J, Han S, Perez BS, Gunn J, Kwon E, Kaufman D. Development of novel lipid nanoparticles and virus-like particles for in vivo engineering of immune cells for targeted cancer therapy. *Blood* **2023**; 142: 3632. doi:10.1182/blood-2023-184312
87. Liao H, Liao J, Zeng L, Cao X, Fan H, Chen J. Strategies for organ-targeted mRNA delivery by lipid nanoparticles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* **2024**; 16: e2004. doi:10.1002/wnan.2004
88. Thomas M, Kularatne SA, Qi L, Kleindl P, Leamon CP, Hansen MJ, et al. Ligand-targeted delivery of small interfering RNAs to malignant cells and tissues. *Ann N Y Acad Sci* **2009**; 1175: 32-9. doi:10.1111/j.1749-6632.2009.04977.x
89. Dietmair B, Humphries J, Mercer TR, Thurecht KJ, Howard CB, Cheetham SW. Targeted mRNA delivery with bispecific antibodies that tether LNPs to cell surface markers. *Mol Ther Nucleic Acids* **2025**; 36: 102520. doi:10.1016/j.omtn.2025.102520
90. Bessa J, Schmitz N, Hinton HJ, Schwarz K, Jegerlehner A, Bachmann MF. Efficient induction of mucosal and systemic immune responses by virus-like particles administered intranasally: implications for vaccine design. *Eur J Immunol* **2008**; 38: 114-26. doi:10.1002/eji.200636959
91. Yahyaei S, Abdoli A, Jamali A, Teimoori A, Arefian E, Eftekhari Z, et al. Targeting respiratory viruses: the efficacy of intranasal mRNA vaccination in generating protective mucosal and systemic immunity against influenza A (H1N1). *Influenza Other Respir Viruses* **2025**; 19: e70093. doi:10.1111/irv.70093

92. Jearanaiwitayakul T, Seesen M, Chawengkirttikul R, Limthongkul J, Apichirapokey S, Sapsutthipas S, et al. Intranasal administration of RBD nanoparticles confers induction of mucosal and systemic immunity against SARS-CoV-2. *Vaccines (Basel)* **2021**; 9:768. doi:10.3390/vaccines9070768
93. Anthi AK, Kolderup A, Vaage EB, Bern M, Benjakul S, Tjärnhage E, et al. An intranasal subunit vaccine induces protective systemic and mucosal antibody immunity against respiratory viruses in mouse models. *Nat Commun* **2025**; 16: 3999. doi:10.1038/s41467-025-59353-6
94. Fatima M, Park PG, Hong KJ. Clinical advancements in mRNA vaccines against viral infections. *Clin Immunol* **2025**; 271: 110424. doi:10.1016/j.clim.2024.110424
95. Karlsson Hedestam GB, Fouchier RA, Phogat S, Burton DR, Sodroski J, Wyatt RT. The challenges of eliciting neutralizing antibodies to HIV-1 and to influenza virus. *Nat Rev Microbiol* **2008**; 6: 143-55. doi:10.1038/nrmicro1819
96. Pauthner MG, Hangartner L. Broadly neutralizing antibodies to highly antigenically variable viruses as templates for vaccine design. *Curr Top Microbiol Immunol* **2020**; 428: 31-87. doi:10.1007/82_2020_221
97. Ahmed S, Herschhorn A. mRNA-based HIV-1 vaccines. *Clin Microbiol Rev* **2024**; 37: e0004124. doi:10.1128/cmr.00041-24
98. Sparrow E, Hasso-Agopsowicz M, Kaslow DC, Singh K, Rao R, Chibi M, et al. Leveraging mRNA platform technology to accelerate development of vaccines for some emerging and neglected tropical diseases through local vaccine production. *Front Trop Dis* **2022**; 3: 844039. doi:10.3389/fitd.2022.844039
99. Ganley M, Holz LE, Minnell JJ, de Menezes MN, Burn OK, Poa KC, et al. mRNA vaccine against malaria tailored for liver-resident memory T cells. *Nat Immunol* **2023**; 24: 1487-98. doi:10.1038/s41590-023-01562-6
100. Matarazzo L, Bettencourt PJG. mRNA vaccines: a new opportunity for malaria, tuberculosis and HIV. *Front Immunol* **2023**; 14: 1172691. doi:10.3389/fimmu.2023.1172691
101. Kodysh J, Finnigan JP, Rubinsteyn A. Abstract B079: evaluation of tools for predicting mutated tumor antigens from exome and RNA sequencing. *Cancer Immunol Res* **2019**; 7: B079. doi:10.1158/2326-6074.CRICIMTEATIAACR18-B079
102. De Mattos-Arruda L, Vazquez M, Finotello F, Lepore R, Porta E, Hundal J, et al. Neoantigen prediction and computational perspectives towards clinical benefit: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol* **2020**; 31: 978-90. doi:10.1016/j.annonc.2020.05.008
103. Tang Y, Wang Y, Wang J, Li M, Peng L, Wei G, et al. TruNeo: an integrated pipeline improves personalized true tumor neoantigen identification. *BMC Bioinformatics* **2020**; 21: 532. doi:10.1186/s12859-020-03869-9
104. Huber F, Arnaud M, Stevenson BJ, Michaux J, Benedetti F, Thevenet J, et al. A comprehensive proteogenomic pipeline for neoantigen discovery to advance personalized cancer immunotherapy. *Nat Biotechnol* **2025**; 43: 1360-72. doi:10.1038/s41587-024-02420-y
105. Pourseif MM, Baradaran Hosseini SA, Khoshraftar SH, Omidi Y. The role of bioinformatics algorithms in modern biopharmaceutical design: progress, challenges, and future perspectives. *Bioimpacts* **2025**; 15: 33072. doi:10.34172/bi.33072
106. Gomez-Randulfe I, Lavender H, Symeonides S, Blackhall F. Impact of systemic anticancer therapy timing on cancer vaccine immunogenicity: a review. *Ther Adv Med Oncol* **2025**; 17: 17588359251316988. doi:10.1177/17588359251316988
107. Collins JM, Redman JM, Gulley JL. Combining vaccines and immune checkpoint inhibitors to prime, expand, and facilitate effective tumor immunotherapy. *Expert Rev Vaccines* **2018**; 17: 697-705. doi:10.1080/14760584.2018.1506332
108. Becker W, Olkhanud PB, Seishima N, Moreno PA, Goldfarbmuren KC, Maeng HM, et al. Second-generation checkpoint inhibitors and Treg depletion synergize with a mouse cancer vaccine in accordance with tumor microenvironment characterization. *J Immunother Cancer* **2024**; 12. doi:10.1136/jitc-2024-008970
109. Pourseif MM, Masoudi-Sobhanzadeh Y, Azari E, Parvizpour S, Barar J, Ansari R, et al. Self-amplifying mRNA vaccines: mode of action, design, development and optimization. *Drug Discov Today* **2022**; 27: 103341. doi:10.1016/j.drudis.2022.103341
110. Mofidi Chelan E, Parhizkar A, Nemati M, Kiani J, Almassian B, Ghasemi Moghaddam H, et al. Targeted cytosolic delivery of mRNA immunotherapeutics: from vaccine delivery to protein replacement. *Biomed Pharmacother* **2026**; 198: 119181. doi:10.1016/j.biopha.2026.119181
111. Omidi Y, Pourseif MM, Ansari RA, Barar J. Design and development of mRNA and self-amplifying mRNA vaccine nanoformulations. *Nanomedicine (Lond)* **2024**; 19: 2699-725. doi :10.1080/17435889.2024.2419815
112. Krienke C, Kolb L, Diken E, Streuber M, Kirchhoff S, Bukur T, et al. A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis. *Science* **2021**; 371: 145-53. doi:10.1126/science.aay3638
113. Furlan R. A tolerizing mRNA vaccine against autoimmunity? *Mol Ther* **2021**; 29: 896-7. doi:10.1016/j.ymthe.2021.02.003
114. Song Y, Li J, Wu Y. Evolving understanding of autoimmune mechanisms and new therapeutic strategies of autoimmune disorders. *Signal Transduct Target Ther* **2024**; 9: 263. doi:10.1038/s41392-024-01952-8
115. DeRosa F, Smith L, Shen Y, Huang Y, Pan J, Xie H, et al. Improved efficacy in a Fabry disease model using a systemic mRNA liver depot system as compared to enzyme replacement therapy. *Mol Ther* **2019**; 27: 878-89. doi:10.1016/j.ymthe.2019.03.001
116. Zhu X, Yin L, Theisen M, Zhuo J, Siddiqui S, Levy B, et al. Systemic mRNA therapy for the treatment of Fabry disease: preclinical studies in wild-type mice, Fabry mouse model, and wild-type non-human primates. *Am J Hum Genet* **2019**; 104: 625-37. doi:10.1016/j.ajhg.2019.02.003
117. Feng S, Rcheulishvili N, Jiang X, Zhu P, Pan X, Wei M, et al. A review on Gaucher disease: therapeutic potential of β-glucocerebrosidase-targeted mRNA/saRNA approach. *Int J Biol Sci* **2024**; 20: 2111-29. doi:10.7150/ijbs.87741
118. Chandler RJ. Messenger RNA therapy as an option for treating metabolic disorders. *Proc Natl Acad Sci U S A* **2019**; 116: 20804-6. doi:10.1073/pnas.1914673116
119. Nagaraj S, Stankiewicz-Drogon A, Darzynkiewicz E, Grzela R. RNA sensor response in HeLa cells for transfected mRNAs prepared in vitro by SP6 and HiT7 RNA polymerases: a comparative study. *Front Bioeng Biotechnol* **2022**; 10: 1017934. doi:10.3389/fbioe.2022.1017934
120. Xia H, Yu B, Jiang Y, Cheng R, Lu X, Wu H, et al. Psychrophilic phage VSW-3 RNA polymerase reduces both terminal and full-length dsRNA byproducts in in vitro transcription. *RNA Biol* **2022**; 19: 1130-42. doi:10.1080/15476286.2022.2139113
121. Baiersdörfer M, Boros G, Muramatsu H, Mahiny A, Vlatkovic I, Sahin U, et al. A facile method for the removal of dsRNA contaminant from in vitro-transcribed mRNA. *Mol Ther Nucleic Acids* **2019**; 15: 26-35. doi:10.1016/j.omtn.2019.02.018
122. Siew YY, Zhang W. Removing immunogenic double-stranded RNA impurities post in vitro transcription synthesis for mRNA therapeutics production: a review of chromatography strategies. *J Chromatogr A* **2025**; 1740: 465576. doi:10.1016/j.chroma.2024.465576
123. Bezelya A, Küçüktürkmen B, Bozkır A. Microfluidic devices for precision nanoparticle production. *Micro* **2023**; 3: 822-66. doi:10.3390/micro3040058
124. Valencia PM, Farokhzad OC, Karnik R, Langer R. Microfluidic technologies for accelerating the clinical translation of nanoparticles. In: *Nano-Enabled Medical Applications*. Jenny Stanford Publishing; **2020**. p. 93-112. doi:10.1201/9780429399039-3
125. Kastner E, Kaur R, Lowry D, Moghaddam B, Wilkinson A, Perrie Y. High-throughput manufacturing of size-tuned liposomes by a new microfluidics method using enhanced statistical tools for characterization. *Int J Pharm* **2014**; 477: 361-8. doi:10.1016/j.ijpharm.2014.10.030
126. Yonet-Tanyeri N, Parker RS, Falo LD Jr, Little SR. Investigation of the impact of manufacturing methods on protein-based long-acting injectable formulations: a comparative assessment for microfluidics vs. conventional methods. *Pharmaceutics* **2024**; 16: 1264. doi:10.3390/pharmaceutics16101264
127. Gerhardt A, Voigt E, Archer M, Reed S, Larson E, Van

Hoeven N, et al. A thermostable, flexible RNA vaccine delivery platform for pandemic response. *bioRxiv* [Preprint]. February 02, **2021**. Available from: https://www.biorxiv.org/content/10.1101/2021.02.01.429283v1.
128. Suzuki Y, Hyodo K, Tanaka Y, Ishihara H. siRNA-lipid nanoparticles with long-term storage stability facilitate potent gene-silencing in vivo. *J Control Release* **2015**; 220: 44-50. doi:10.1016/j.jconrel.2015.10.024
129. Ruppl A, Kiesewetter D, Köll-Weber M, Lemazurier T, Süss R, Allmendinger A. Formulation screening of lyophilized mRNA-lipid nanoparticles. *Int J Pharm* **2025**; 671: 125272. doi:10.1016/j.ijpharm.2025.125272
130. Ohgi S, Coulon L, Muller R, Muller-Cohn J, Clement O. Stabilizing RNA at room temperature in RNAstable®. *Biotechniques* **2010**; 48: 470-. doi:10.2144/000113453
131. Chua A, Tran TT, Pu S, Park JW, Hadinoto K. Lyophilization of curcumin-albumin nanoplex with sucrose as cryoprotectant: aqueous reconstitution, dissolution, kinetic solubility, and physicochemical stability. *Int J Mol Sci* **2022**; 23: 11731. doi:10.3390/ijms231911731
132. Allison SD, Anchordoquy TJ. Mechanisms of protection of cationic lipid-DNA complexes during lyophilization. *J Pharm Sci* **2000**; 89: 682-91. doi:10.1002/(sici)1520-6017(200005)89:5<682::Aid-jps14>3.0.Co;2-#
133. Herrmann A, Abbina S, Bathula NV, Malek Mohammadi Nouri P, Chafeeva I, Constantinescu I, et al. An ultrahydrating polymer that protects protein therapeutics and RNA-lipid nanoparticles against freezing, heat and lyophilization stress. *Adv Funct Mater* **2024**; 34: 2406878. doi:10.1002/adfm.202406878
134. Anchordoquy TJ, Armstrong TK, Molina M. Low molecular weight dextrans stabilize nonviral vectors during lyophilization at low osmolalities: concentrating suspensions by rehydration to reduced volumes. *J Pharm Sci* **2005**; 94: 1226-36. doi:10.1002/jps.20353
135. Paremoer L, Pollock A. "A passion to change the landscape and drive a renaissance": The mRNA Hub at Afrigen as decolonial aspiration. *Front Public Health* **2022**; 10: 1065993. doi:10.3389/fpubh.2022.1065993
136. Doua J, Ndembi N, Auerbach J, Kaseya J, Zumla A. Advancing local manufacturing capacities for vaccines within Africa - opportunities, priorities and challenges. *Vaccine* **2025**; 50: 126829. doi:10.1016/j.vaccine.2025.126829
137. Saied AA. Building Africa's first mRNA vaccine facility. *Lancet* **2023**; 402: 287-8. doi:10.1016/s0140-6736(23)01119-4
138. Wilson KR, Kohler JC, Ovtcharenko N. The make or buy debate: considering the limitations of domestic production in Tanzania. *Global Health* **2012**; 8: 20. doi:10.1186/1744-8603-8-20
139. Hendriks J. Technology transfer in human vaccinology: a retrospective review on public sector contributions in a privatizing science field. *Vaccine* **2012**; 30: 6230-40. doi:10.1016/j.vaccine.2012.07.087
140. Salicrup LA, Fedorková L. Challenges and opportunities for enhancing biotechnology and technology transfer in developing countries. *Biotechnol Adv* **2006**; 24: 69-79. doi:10.1016/j.biotechadv.2005.06.004
141. Kristensen D, Chen D. Strategies to advance vaccine technologies for resource-poor settings. *Vaccine* **2013**; 31: B157-62. doi:10.1016/j.vaccine.2012.10.113
142. Tran A, Witek TJ Jr. The emergency use authorization of pharmaceuticals: history and utility during the COVID-19 pandemic. *Pharmaceut Med* **2021**; 35: 203-13. doi:10.1007/s40290-021-00397-6
143. Ostad Ali Dehaghi R, Khadem Broojerdi A, Magdy A, Valentin M, Dahlan J, Malik O, et al. Strengthening vaccine regulation: Insights from COVID-19 vaccines, best practices, and lessons for future public health emergencies. *Vaccines (Basel)* **2025**; 13: 638. doi:10.3390/vaccines13060638
144. Thomson K, Nachlis H. Emergency use authorizations during the COVID-19 pandemic: lessons from hydroxychloroquine for vaccine authorization and approval. *JAMA* **2020**; 324: 1282-3. doi:10.1001/jama.2020.16253
145. Iqbal SM, Rosen AM, Edwards D, Bolio A, Larson HJ, Servin M, et al. Opportunities and challenges to implementing mRNA-based vaccines and medicines: lessons from COVID-19. *Front Public Health* **2024**; 12: 1429265. doi:10.3389/fpubh.2024.1429265
146. Gergen J, Petsch B. mRNA-based vaccines and mode of action. *Curr Top Microbiol Immunol* **2022**; 440: 1-30. doi:10.1007/82_2020_230
147. Le T, Sun C, Chang J, Zhang G, Yin X. mRNA vaccine development for emerging animal and zoonotic diseases. *Viruses* **2022**; 14: 401. doi:10.3390/v14020401
148. Bettini E, Locci M. SARS-CoV-2 mRNA vaccines: immunological mechanism and beyond. *Vaccines (Basel)* **2021**; 9: 147. doi:10.3390/vaccines9020147
149. Whitehead HS, French CE, Caldwell DM, Letley L, Mounier-Jack S. A systematic review of communication interventions for countering vaccine misinformation. *Vaccine* **2023**; 41: 1018-34. doi:10.1016/j.vaccine.2022.12.059
150. Vivion M, Anassour Laouan Sidi E, Betsch C, Dionne M, Dubé E, Driedger SM, et al. Prebunking messaging to inoculate against COVID-19 vaccine misinformation: an effective strategy for public health. *J Commun Healthc* **2022**; 15: 232-42. doi:10.1080/17538068.2022.2044606
151. Schmid P, Betsch C. Benefits and pitfalls of debunking interventions to counter mRNA vaccination misinformation during the COVID-19 pandemic. *Sci Commun* **2022**; 44: 531-58. doi:10.1177/10755470221129608
152. Kisa S, Kisa A. A comprehensive analysis of COVID-19 misinformation, public health impacts, and communication strategies: scoping review. *J Med Internet Res* **2024**; 26: e56931. doi:10.2196/56931
153. Adekola TA. When mRNA technology meets patent law: innovation, barriers and public health. *J Intellect Prop Law Pract* **2023**; 18: 867-77. doi:10.1093/jiplp/jpad086
154. Burrows R, Lambrix E. mRNA vaccines: a growing and complex IP landscape. *Vaccine Insights* **2022**; 1: 191-9. doi:10.18609/vac.2022.029
155. Herder M, Benavides X. 'Our project, your problem?' A case study of the WHO's mRNA technology transfer programme in South Africa. *PLOS Glob Public Health* **2024**; 4: e0003173. doi:10.1371/journal.pgph.0003173
156. Gsell PS, Giersing B, Gottlieb S, Wilder-Smith A, Wu L, Friede M. Key considerations for the development of novel mRNA candidate vaccines in LMICs: a WHO/MPP mRNA Technology Transfer Programme meeting report. *Vaccine* **2023**; 41: 7307-12. doi:10.1016/j.vaccine.2023.10.027
157. Qu L, Yi Z, Shen Y, Lin L, Chen F, Xu Y, et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. *Cell* **2022**; 185: 1728-44.e16. doi:10.1016/j.cell.2022.03.044
158. Xie J, Ye F, Deng X, Tang Y, Liang JY, Huang X, et al. Circular RNA: a promising new star of vaccine. *J Transl Int Med* **2023**; 11: 372-81. doi:10.2478/jtim-2023-0122
159. Wesselhoeft RA, Kowalski PS, Parker-Hale FC, Huang Y, Bisaria N, Anderson DG. RNA circularization diminishes immunogenicity and can extend translation duration in vivo. *Mol Cell* **2019**; 74: 508-20.e4. doi:10.1016/j.molcel.2019.02.015
160. Liu X. Small circular RNA for cancer immunotherapy. *Cancer Rese* **2025**; 85: 847. doi:10.1158/1538-7445.AM2025-847
161. Wang F, Dai X, Shen L, Chang S. GraphEPN: a deep learning framework for B-cell epitope prediction leveraging graph neural networks. *Appl Sci* **2025**; 15: 2159. doi:10.3390/app15042159
162. Darmawan JT, Leu JS, Avian C, Ratnasari NR. MITNet: a fusion transformer and convolutional neural network architecture approach for T-cell epitope prediction. *Brief Bioinform* **2023**; 24: bbad202. doi:10.1093/bib/bbad202
163. Ashwini S, Minu RI, Jeevan Kumar M. Predicting antigenic peptides using a multi-level pooling-based transformer model with enhanced Kolaskar & Tongaonkar's algorithm for feature selection. *Comput Biol Chem* **2026**; 120: 108615. doi:10.1016/j.compbiolchem.2025.108615
164. Lu T, Wang X, Nie W, Huo M, Li S. TransHLA: a hybrid transformer model for HLA-presented epitope detection. *Gigascience* **2025**; 14: giaf008. doi:10.1093/gigascience/giaf008
165. Zhao Y, Wang H. Artificial intelligence-driven circRNA vaccine development: multimodal collaborative optimization and a new

paradigm for biomedical applications. *Brief Bioinform* **2025**; 26: bbaf263. doi:10.1093/bib/bbaf263

166. Kong H. Advances in personalized cancer vaccine development: AI applications from neoantigen discovery to mRNA formulation. *Biochem (Basel)* **2025**; 5: 5. doi:10.3390/biochem5020005
167. Meany EL, Klich JH, Jons CK, Mao T, Chaudhary N, Utz A, et al. Generation of an inflammatory niche in a hydrogel depot through recruitment of key immune cells improves efficacy of mRNA vaccines. *Sci Adv* **2025**; 11: eadr2631. doi:10.1126/sciadv.adr2631
168. Roth GA, Saouaf OM, Smith AA, Gale EC, Hernández MA, Idoyaga J, et al. Prolonged codelivery of hemagglutinin and a TLR7/8 agonist in a supramolecular polymer-nanoparticle hydrogel enhances potency and breadth of influenza vaccination. *ACS Biomater Sci Eng* **2021**; 7: 1889-99. doi:10.1021/acsbiomaterials.0c01496
169. Gale EC, Powell AE, Roth GA, Meany EL, Yan J, Ou BS, et al. Hydrogel-based slow release of a receptor-binding domain subunit vaccine elicits neutralizing antibody responses against SARS-CoV-2. *Adv Mater* **2021**; 33: e2104362. doi:10.1002/adma.202104362
170. Yang X, Xiao J, Zhai Y, Liao L, Qiu H, Chen Y, et al. Biodegradable poly(β-amino amide)s enable efficient RNA delivery and spleen targeting. *bioRxiv* [Preprint]. March 11, **2025**. Available from: https://www.biorxiv.org/content/10.1101/2025.03.03.641302v1.
171. Hudson AJ, Lewis KJ, Rao MV, Akhtar S. Biodegradable polymer matrices for the sustained exogenous delivery of a biologically active c-Myc hammerhead ribozyme. *Int J Pharm* **1996**; 136: 23-9. doi:10.1016/0378-5173(96)04474-2
172. Lewis KJ, Irwin WJ, Akhtar S. Development of a sustained-release biodegradable polymer delivery system for site-specific delivery of oligonucleotides: characterization of P(LA-GA) copolymer microspheres in vitro. *J Drug Target* **1998**; 5: 291-302. doi:10.3109/10611869808995882
173. Mokhtarzadeh A, Alibakhshi A, Hashemi M, Hejazi M, Hosseini V, de la Guardia M, et al. Biodegradable nano-polymers as delivery vehicles for therapeutic small non-coding ribonucleic acids. *J Control Release* **2017**; 245: 116-26. doi:10.1016/j.jconrel.2016.11.017