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Review

mRNA-Lipid Nanoparticle Vaccines for Infectious Diseases: Mechanisms of Action and Translational Progress

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Abstract

Six years after the first emergency use authorizations, mRNA-lipid nanoparticle (mRNA-LNP) vaccines have gone from a platform without a licensed product to one that supplies a large share of the world's COVID-19 and respiratory syncytial virus (RSV) vaccine doses and has completed phase 3 trials for seasonal influenza. Their performance depends on mechanisms that are being resolved with unusual speed at the level of individual cell types, receptors and molecular interactions, and several textbook assumptions have had to be revised in the process. This review follows the vaccine from the formulation vial to the germinal centre. We first describe how the RNA is engineered for efficient translation and for partial escape from cytosolic and endosomal RNA sensors, and why N1-methylpseudouridine is neither immunologically silent nor translationally neutral. We then examine how the ionizable lipid, the helper phospholipid, cholesterol and the PEG-lipid determine particle behaviour, cell uptake and endosomal release, and how the lipid and RNA components act as separable adjuvant signals. Evidence from mouse models and from human lymph node sampling is compared, with attention to the substantial species differences in innate cytokine control that limit extrapolation from mice to people. The clinical section summarizes efficacy and immunogenicity data for COVID-19, RSV and influenza vaccines, and the preclinical and early clinical status of candidates against cytomegalovirus, mpox, HIV, malaria, Nipah virus and Clostridioides difficile. Safety data are discussed in absolute terms rather than as signals alone. We close with the questions that currently block rational improvement: the molecular identity of the lipid sensor, the difficulty of separating immunogenicity from reactogenicity, the absence of a quantitative description of endosomal escape, and the design of antigens for pathogens that do not tolerate a single fixed immunogen.

Keywords: mRNA vaccine; lipid nanoparticle; ionizable lipid; germinal centre; innate immunity; self-amplifying RNA; vaccine safety

1. Introduction

The idea that a messenger RNA could serve as a vaccine is older than the technology that made it work. For most of the 1990s and 2000s, exogenous RNA was both unstable and inflammatory, and the two problems were assumed to be inseparable. The demonstration that incorporation of naturally occurring modified nucleosides, in particular pseudouridine, suppressed recognition by Toll-like receptors while preserving translation changed that assumption, and it did so by turning an obstacle into a design parameter [1]. Two further developments were needed before the platform could be

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tested at scale: a delivery vehicle that would protect RNA from nucleases and carry it into the cytosol, and a manufacturing process that could produce hundreds of millions of doses within a year. Lipid nanoparticles supplied the first, and in vitro transcription combined with microfluidic formulation supplied the second [2–4].

The clinical results were unambiguous. In the two registration trials, two mRNA vaccines given as two-dose regimens reduced symptomatic infection by more than 90% in adults, with efficacy against severe disease close to complete during the pre-variant phase of the pandemic [5–7]. Between 2021 and 2026, billions of doses were administered, and the platform was extended to RSV, to influenza, to cytomegalovirus and to a growing list of pathogens that had resisted conventional vaccine approaches. The speed of this translation was not matched by an equally fast understanding of mechanism. For several years the working model, drawn largely from mouse studies and from the gene-silencing literature, treated the lipid nanoparticle as an inert carrier and the modified RNA as an immunologically silent cargo, with immunogenicity attributed to the encoded antigen and to unspecified adjuvant effects of the formulation. Work published since 2021 has shown that both halves of that model are wrong in interesting ways: the RNA is sensed, the lipid is sensed, and the two signals do different things [8–10].

Why does the mechanism matter beyond curiosity? Three practical reasons. First, the platform's main liabilities are immunological. Reactogenicity, the rare cardiac events that came to attention during mass vaccination, and the loss of efficacy against successive variants are all consequences of how the vaccine interacts with the immune system, not of the antigen alone. Second, dose and formulation are still chosen empirically; the approved COVID-19 vaccines use 30 μg and 100 μg of mRNA respectively, a threefold difference that was never derived from a mechanistic comparison. Third, the pathogens that remain without effective vaccines, including HIV, tuberculosis, malaria and several haemorrhagic fever viruses, require immune responses that the current formulation does not reliably produce, in particular mucosal immunity and broadly neutralizing antibodies.

This review is organized around the sequence of events that follows intramuscular injection. Section 2 deals with the RNA and its design constraints, section 3 with the lipid particle and its intracellular itinerary, section 4 with innate sensing of both components, and section 5 with the adaptive response that results, up to the germinal centre and the bone marrow. Section 6 compares the replicating and circular alternatives to conventional mRNA, section 7 summarizes clinical results in infectious disease, and sections 8 and 9 address safety, stability and manufacturing. Section 10 sets out the questions we regard as unresolved. Where conclusions rest on human data we say so; where they rest on mice we say that too, because the gap between the two is larger for this platform than for most vaccines.

2. The RNA Component: Design Constraints and Their Consequences

2.1. Anatomy of a Vaccine mRNA

A vaccine mRNA is a linear single-stranded molecule of roughly 2,000 to 4,500 nucleotides with five functional elements: a 5′ cap, a 5′ untranslated region, an open reading frame, a 3′ untranslated region and a poly(A) tail. Each has been optimized empirically, and in several cases the optimization has produced effects that were not anticipated.

The cap is the primary determinant of both translation and stability. Vaccines use a cap 1 structure, either added co-transcriptionally by a capping enzyme or incorporated during synthesis as a CleanCap analogue, because cap 0 RNA is recognized by the innate sensor RIG-I and by cellular restriction factors. Capping efficiency below about 90% leaves uncapped transcripts that are immunogenic and poorly translated, which is one reason why in vitro transcription products are usually purified by high-performance liquid chromatography or cellulose-based methods to remove double-stranded RNA by-products and abortive transcripts [2].

The untranslated regions contribute to translational efficiency through secondary structure and through the binding of RNA-binding proteins. Systematic combinatorial replacement of 5′ and 3′

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UTRs has shown that the effect of a given UTR depends on the open reading frame and on the cell type, and that no single UTR is optimal across contexts [11]. Codon usage is a further variable: synonymous codons differ in their cognate tRNA pools and in the stability of local secondary structure, and machine-learning models trained on large experimental datasets can now generate sequences with improved translational capacity relative to hand-optimized designs [12]. The poly(A) tail, typically 100 to 150 adenosines, sets the rate at which the transcript is deadenylated and degraded, and its length is a compromise between translation and persistence.

One structural feature deserves emphasis because it is often presented as settled. The encoded antigen determines the antibody response far more than any UTR or codon choice: a poorly stabilized glycoprotein will produce a poor response regardless of how well it is expressed, which is why antigen engineering, discussed in section 10, has consistently yielded larger gains than sequence optimization.

2.2. N1-Methylpseudouridine: Three Claims, Only Some of Which Hold

Nearly all licensed mRNA vaccines replace uridine entirely with N1-methylpseudouridine (m1Ψ). Three claims are commonly made for this substitution: that it abolishes innate immune activation, that it increases protein output, and that it has no effect on the fidelity of translation. The first two are broadly supported; the third is not.

Early work with pseudouridine and m1Ψ established reduced activation of TLR7 and TLR8, reduced phosphorylation of PKR and of the OAS-RNase L pathway, and higher protein expression per microgram of RNA in cultured cells and in mice. The mechanism of the reduced TLR activation has only recently been resolved. Pseudouridine-containing RNA is a poor substrate for the endolysosomal exonucleases RNase T2, PLD3 and PLD4, so fewer TLR-agonistic degradation products are generated; in addition, TLR8 does not accept pseudouridine in its first binding pocket and TLR7 does not accept pseudouridine-containing RNA in its second [13]. The same study found that m1Ψ, unlike pseudouridine, escapes nuclease processing but can directly activate TLR8, which means that the modification reduces but does not eliminate endosomal sensing. This is consistent with clinical observations of transient local and systemic inflammatory symptoms after vaccination and with the residual adjuvant activity that appears to be required for good antibody responses.

Translation is affected in ways that are still being characterized. m1Ψ changes the kinetics with which the ribosome traverses individual codons, and the effect depends on the surrounding sequence; this is one reason why the same modification can increase protein output from one open reading frame and reduce it from another [14]. More troubling, m1Ψ-containing RNA is prone to +1 ribosomal frameshifting at specific sequence motifs, producing chimeric peptides that are displayed on MHC class I and can be recognized by T cells [15]. The magnitude of this phenomenon in humans is unknown, and its practical significance is debated; the original reports that m1Ψ produces faithful protein products remain valid for the intended open reading frame [16], and the frameshifted species are a minority product. But frameshifting is a real effect with two implications: candidate sequences should be screened for slippery motifs, and immunological monitoring after vaccination should not assume that the only antigen expressed is the one encoded.

A related point concerns the double-stranded RNA content of the final product. In vitro transcription generates dsRNA and abortive products that activate PKR, OAS and MDA5; the level of these impurities varies between manufacturers and between batches, and it influences both reactogenicity and immunogenicity in mice. Removal by chromatography reduces but does not abolish them, and the residual amount is not a specification that is publicly reported for licensed products. This is one of the few areas where product heterogeneity between otherwise similar vaccines could plausibly explain differences in reactogenicity, although direct evidence in humans is lacking.

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2.3. What the RNA Does Not Do

Two negative findings are worth recording. First, the RNA component is not the principal driver of the neutrophil-rich inflammation seen at the injection site in mice; that response is reproduced by empty lipid nanoparticles at the same lipid dose [17]. Second, the adaptive response to BNT162b2 in mice does not require signalling through TLR2, TLR3, TLR4, TLR5, TLR7, the inflammasome, necroptosis or pyroptosis; the CD8+ T cell response instead depends on type I interferon acting through MDA5 [18]. The contrast between this result and the TLR4-dependent lipid sensing described below is one of the unresolved contradictions in the field, discussed in section 4.

3. The Lipid Nanoparticle: Composition, Structure and Intracellular Fate

3.1. Four Components, One of Which Does the Work

A vaccine LNP contains an ionizable lipid, a helper phospholipid, cholesterol and a PEG-lipid, combined at a defined molar ratio and formulated with RNA in a microfluidic mixing device (Figure 1A). Table 1 lists the compositions of the vaccines that have reached clinical use.

The ionizable lipid is the functional core. It carries a tertiary amine that is uncharged at physiological pH and protonated at endosomal pH, and it is this titration, with an apparent pKa usually between 6.2 and 6.8, that allows the particle to remain neutral in plasma while becoming membrane-active after internalization [3,19]. The first generation of ionizable lipids used in RNA interference, such as DLin-MC3-DMA, were not designed for repeated dosing and are slowly degraded; the lipids in licensed vaccines, ALC-0315 and SM-102, contain ester linkages that are hydrolysed in tissue, which limits accumulation. Systematic structure-activity work over the past five years has produced a large family of biodegradable, branched and multi-tail lipids, and machine learning on combinatorial libraries has shortened the design cycle from months to weeks [19–21].

The helper phospholipid, usually DSPC, is a bilayer-forming lipid that contributes to particle stability and to the lamellar-to-inverted transition that accompanies endosomal release. Cholesterol fills the spaces between the other lipids and modulates membrane rigidity; its depletion increases permeability and reduces particle stability. The PEG-lipid, 1.6 mol% ALC-0159 in the BioNTech/Pfizer product and 1.5 mol% PEG2000-DMG in the Moderna product, projects a hydrophilic polymer brush from the surface that limits aggregation, controls particle size during mixing, and reduces opsonization. It is also the component most often implicated in immediate hypersensitivity reactions, and its density is a design variable with immunological consequences: reducing the PEG-lipid fraction and changing the phospholipid species alters both biodistribution and the quality of the antibody response, and can shift the response towards cellular or humoral dominance [22].

Table 1. Composition of mRNA-lipid nanoparticle vaccines that have reached clinical use.

Vaccine (manufacturer)	Ionizable lipid	Molar ratio	mRNA per dose	Status
BNT162b2 / Comirnaty (Pfizer-BioNTech)	ALC-0315	46.3 : 9.4 : 42.7 : 1.6 (ALC-0159)	30 µg	Licensed
mRNA-1273 / Spikevax (Moderna)	SM-102	50 : 10 : 38.5 : 1.5 (PEG2000-DMG)	100 µg	Licensed
mRNA-1345 / mRESVIA (Moderna)	SM-102	as Spikevax	50 µg	Licensed, adults 60 years and older
mRNA-1010 (Moderna)	SM-102	as Spikevax	37.5 µg (trivalent)	Phase 3
ARCT-154 / Kostaive (Arcturus)	proprietary, biodegradable	not disclosed	single-digit µg	Licensed in Japan, 2023
BNT166 (BioNTech)	proprietary	not disclosed	not disclosed	Phase 1

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Molar ratios are given as ionizable lipid : DSPC : cholesterol : PEG-lipid, as reported for the formulated particle [23]. The ionizable lipid determines the apparent pKa and the endosomal release behaviour; the PEG-lipid determines particle size and surface shielding; neither the ratio nor the lipid identity is interchangeable between products.

3.2. What the Particle Actually Looks like

The name is misleading. A vaccine LNP is not a liposome with an aqueous core; it is a solid, approximately spherical particle of 80 to 100 nm in which the RNA is condensed with the ionizable lipid into an electron-dense core surrounded by a lipid monolayer, with the PEG-lipid on the exterior. Cryo-electron microscopy shows a heterogeneous population: some particles contain ordered lamellar stacks of RNA-lipid, others an amorphous core, and the distribution depends on the mixing conditions and on the lipid composition [23,24]. This matters because the internal structure is thought to determine how the particle disassembles in the endosome, and therefore how much RNA reaches the cytosol.

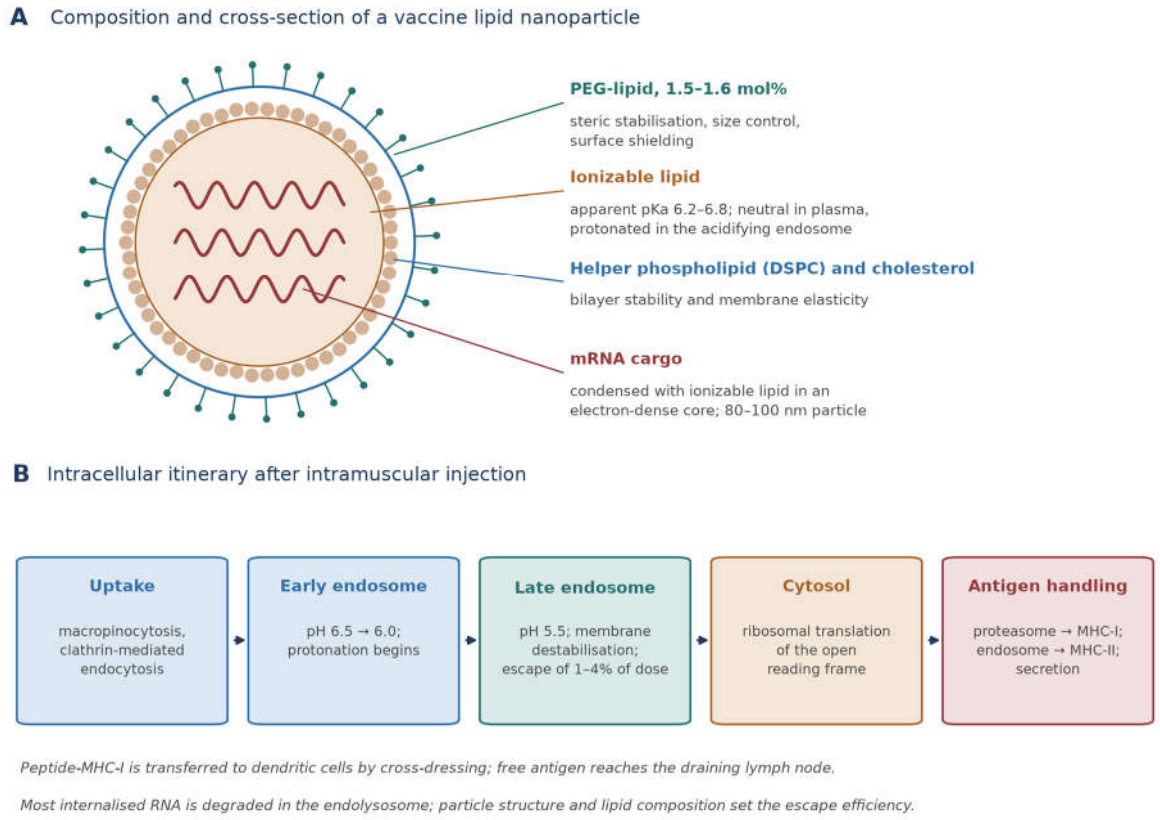

Figure 1. Architecture and intracellular itinerary of an mRNA-lipid nanoparticle vaccine. (A) Cross-section of a vaccine particle. mRNA is condensed with ionizable lipid into an electron-dense core of 80-100 nm, surrounded by a monolayer containing helper phospholipid, cholesterol and PEG-lipid; the molar ratios differ between products (Table 1) [23,24]. (B) After intramuscular injection the particle is internalised, the ionizable lipid is protonated as the endosome acidifies, and a small fraction of the RNA reaches the cytosol for translation; the encoded protein is processed for MHC class I and class II presentation [3,25,26]. Schematic prepared by the authors from the mechanisms described in the cited literature; not to scale.

3.3. Cell Entry and Endosomal Escape

After intramuscular injection, the particle encounters extracellular matrix, serum proteins and interstitial fluid. Systemic administration leads to adsorption of apolipoprotein E and rapid uptake by hepatocytes through the LDL receptor, which is why intravenously delivered LNPs are

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overwhelmingly liver-tropic. Intramuscular delivery avoids most of this: the particle is taken up locally by myocytes, fibroblasts, endothelial cells and resident immune cells, and a fraction reaches the draining lymph node through lymphatics, either as intact particles or carried by migrating dendritic cells [3,27].

Uptake is by endocytosis, predominantly macropinocytosis and clathrin-mediated pathways depending on cell type (Figure 1B). The endosome acidifies over tens of minutes; the ionizable lipid becomes protonated, associates with anionic phospholipids of the endosomal membrane, and destabilizes the bilayer. Exactly how the RNA crosses into the cytosol is not settled. Three models coexist: transient pore formation, flip-flop of the ionizable lipid with phase transition to an inverted hexagonal structure, and release through the membrane of the endosomal recycling compartment [25,26]. Quantitative measurements are difficult because the escaping RNA is a small fraction of the internalized dose, estimates for which range from less than 2% to a few per cent depending on the system and the assay. The absence of a robust, high-throughput method for measuring escape is itself a bottleneck: without it, lipid optimization proceeds by screening rather than by design [26].

3.4. Changing Where the Particle Goes

The default tropism of an LNP is set by its surface chemistry, and two strategies have been used to change it. The first adds an anionic or cationic "SORT" lipid to the formulation, which shifts the apparent pKa and the serum protein corona and redirects the particle to lung, spleen or specific cell types; the mechanism involves desorption of PEG-lipid, binding of distinct serum proteins and receptor-mediated uptake in the target organ [28]. The second alters the ionizable lipid itself. Combinatorial libraries have produced lipids that preferentially transfect muscle rather than liver after intramuscular injection, reducing off-target expression [29], and lipids that recruit albumin to the particle surface, which both reduces liver accumulation and improves the safety profile in mice [30].

Targeting matters for vaccines in a way that it does not for gene therapy. Expression in hepatocytes is not neutral: when mRNA expression was selectively silenced in hepatocytes using microRNA target sites, antigen-specific T cell responses increased, an effect partly mediated by PD-1/PD-L1 signalling. Conversely, silencing expression in professional antigen-presenting cells had no effect on T cell priming, and restricting expression to myocytes produced equal or stronger responses [27]. This result, discussed further in section 5, reframes the design goal: the relevant question is not which cell takes up the particle but which cell presents the antigen, and these are not the same cell.

3.5. Dose, and Why It Is High

The approved COVID-19 vaccines require 30 μg and 100 μg of mRNA per dose, which is two to three orders of magnitude more RNA than the microgram quantities used in some self-amplifying formulations and vastly more than the protein content of a typical subunit vaccine. The excess reflects the inefficiency of the delivery step: if only a few per cent of the internalized RNA reaches the cytosol, and only a fraction of that is translated, then a large input is needed to produce enough antigen to drive a germinal centre response. Reducing the dose without reducing immunogenicity has therefore been the central engineering goal of the last five years, and it is the motivation for the replicating platforms described in section 6.

4. Innate Sensing: Two Signals, Not One

The innate response to an mRNA vaccine is best understood as the sum of two separable contributions, one from the RNA and one from the lipid, which converge on the same dendritic cell (Figure 2).

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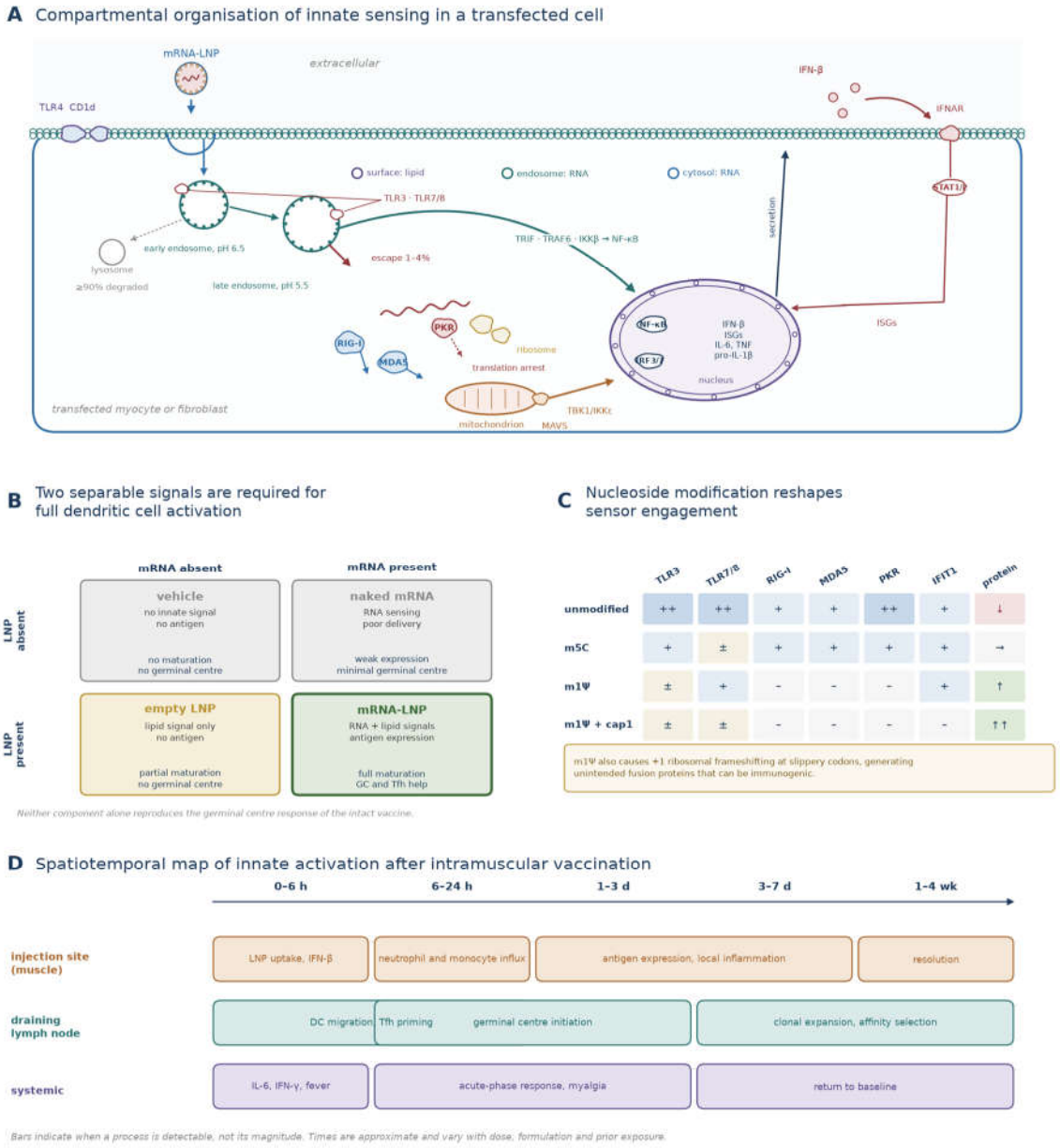

Figure 2. Innate sensing of the RNA and lipid components of mRNA-lipid nanoparticle vaccines. (A) Compartmental organisation in a transfected cell: surface receptors (TLR4, CD1d), endosomal TLR3 and TLR7/8, cytosolic RIG-I, MDA5, PKR and OAS, the MAVS-TBK1-IRF3 axis, NF-κB activation and the type I interferon loop that acts back through IFNAR and STAT1/2. (B) Two separable signals: neither an empty particle nor mRNA alone reproduces the germinal centre response of the intact vaccine. (C) Effect of nucleoside modification on sensor engagement and protein output. (D) Approximate time course at the injection site, in the draining node and systemically. The first pathway drives the cytokine response associated with reactogenicity; the second and third drive dendritic cell maturation and the T follicular helper cell response [9,13,18,31–34]. Schematic prepared by the authors from the mechanisms described in the cited literature; not to scale.

4.1. The RNA Is Sensed, But Not Where It Was Expected

Cytosolic RNA sensors are the cell's defence against viral replication, and they respond to features that distinguish viral from self RNA: length, double-strandedness, the absence of cap 1, and the presence of particular nucleotide modifications. RIG-I detects short double-stranded RNA with a 5′ triphosphate; MDA5 detects long double-stranded RNA and polymerizes into filaments; PKR

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phosphorylates eIF2α and shuts down translation; OAS activates RNase L and degrades RNA. Endosomal TLR3, TLR7 and TLR8 detect double-stranded and single-stranded RNA respectively.

Nucleoside modification blunts these responses, but the residual sensing is not negligible and its anatomical location matters. In mice, the mRNA component induces interferon-β specifically in fibroblasts at the injection site, not in haematopoietic cells, and this interferon-β acts on adjacent cells to induce a population of migratory dendritic cells expressing interferon-stimulated genes; blocking interferon-β signalling at the injection site reduces the cellular immune response to the vaccine [31]. This is a local, fibroblast-driven circuit rather than a systemic cytokine storm, and it explains why the same RNA delivered without a particle produces little immunity: the particle concentrates the RNA in the right cells at the right time.

4.2. The Lipid Is Also Sensed, and the Sensor Is Contested

Empty LNPs injected intramuscularly or intradermally in mice produce a rapid inflammatory response with massive neutrophil infiltration and production of IL-6, IL-1β, TNF and chemokines, at lipid doses equivalent to those used in vaccines [17]. The adjuvant effect of the LNP is not a bystander phenomenon: it is required for the induction of T follicular helper cells, germinal centre B cells, long-lived plasma cells and memory B cells, and it depends on the ionizable lipid and on IL-6 rather than on MyD88- or MAVS-dependent nucleic acid sensing [33]. In humans, RNA vaccines induce IL-1β from blood leukocytes, and the IL-1 pathway amplifies the downstream cytokine response including IL-6 [34].

Which receptor initiates this response is unresolved. One line of evidence implicates the amine headgroup of the ionizable lipid, which binds TLR4 and CD1d and promotes lipid raft formation; in this model, the resulting Th1-biased response includes IgG2c and IgG1 and the inflammatory signal also suppresses anti-PEG IgM, which would otherwise limit repeated dosing [32]. Another line of evidence finds no requirement for TLR2, TLR3, TLR4, TLR5, TLR7 or the inflammasome in the response to BNT162b2 in mice [18]. The two results are not necessarily incompatible, since they used different formulations, doses and readouts, and since TLR4 signalling can be dispensable in vivo while still modulating the response when engaged. But the field does not currently have a single accepted answer, and the 2026 review by Locci and Pardi identifies the identity of the LNP sensor as one of the principal gaps [10].

4.3. Two Signals, Integrated

The most informative recent experiments separate the contributions of the two components rather than ablating one of them. Using dendritic cells exposed to LNPs, mRNA, or both, Castaño and colleagues showed that the mRNA drives type I interferon production, which acts on dendritic cells to enhance maturation and T follicular helper cell differentiation and to favour plasma cell and memory B cell output, while the LNP controls the access of mRNA to dendritic cells within the draining lymph node and shapes the localization of CD25+ dendritic cells [9]. Neither component alone reproduced the germinal centre response of the intact vaccine. Consistent with this, systems-level profiling of BNT162b2 recipients identified a myeloid cluster with an interferon-response transcriptional signature that expanded roughly 100-fold after the second dose, and a monocyte-related signature that correlated with neutralizing antibody titres against the B.1.351 variant [35]. This is a two-signal model in which the RNA provides the maturation signal and the lipid provides the delivery and positioning signal, and it has a practical corollary: formulation changes that alter particle trafficking will change immunogenicity even if the RNA is unchanged, which is exactly what the PEG-lipid and phospholipid substitution experiments show [22].

4.4. Reactogenicity Is the Price of the Same Signals

The inflammatory response that supports immunogenicity is also the cause of the local and systemic symptoms that follow vaccination, and separating the two has proved difficult. The species

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difference is a further complication. Human leukocytes respond to RNA vaccines by producing IL-1β, whereas mouse leukocytes preferentially upregulate the IL-1 receptor antagonist relative to IL-1, which allows mice to tolerate doses more than a thousand-fold higher than those used in humans without cytokine-mediated toxicity [34]. The widely used mouse model therefore underestimates human reactogenicity, and a formulation that is well tolerated in mice may not be in people. This is one reason why the lipid nanoparticle literature contains many reports of "low-reactogenicity" formulations that have not been tested in humans.

5. Adaptive Immunity: From Injection Site to Germinal Centre

The events that link innate activation to durable antibody production are summarized in Figure 3.

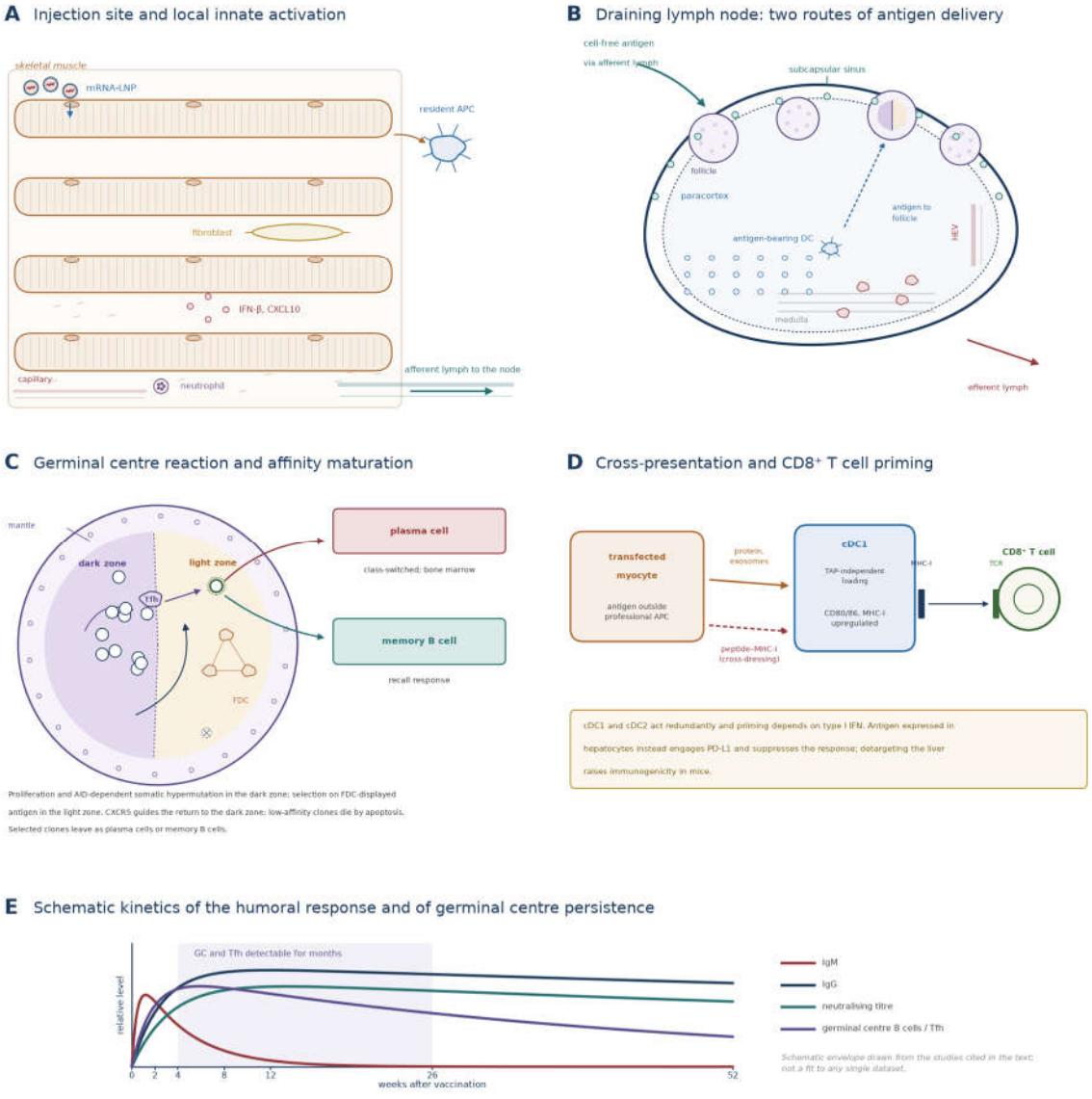

Figure 3. From the injection site to the germinal centre. (A) Transfection of myocytes and fibroblasts at the injection site, local interferon and chemokine production, and uptake by resident antigen-presenting cells. (B) Lymph node architecture: subcapsular sinus, follicles with germinal centres, paracortex and medulla, and the two routes by which antigen arrives. (C) Germinal centre reaction: dark-zone proliferation with AID-dependent somatic hypermutation, light-zone selection on follicular dendritic cells, and output of plasma cells and memory B cells. (D) Cross-presentation of antigen expressed in non-haematopoietic cells and priming of CD8+ T cells

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[9,27,36–39]. (E) Schematic kinetics of the humoral response, drawn from the same studies. Schematic prepared by the authors; not to scale.

5.1. Where the Antigen Comes from

The classical view of mRNA vaccination assumes that dendritic cells take up the particle, translate the mRNA and present the antigen. The evidence now favours a different picture. Selective silencing of mRNA expression in professional antigen-presenting cells, in hepatocytes or in myocytes showed that expression in antigen-presenting cells is dispensable for priming antigen-specific T cells, that expression in myocytes produces equal or stronger responses, and that expression in hepatocytes suppresses the response [27]. The antigen that primes CD8+ T cells is therefore largely acquired from non-haematopoietic cells, either as protein for cross-presentation or as peptide-MHC class I complexes transferred to dendritic cells, a process termed cross-dressing.

How cross-dressing works for mRNA-LNP vaccines has been examined directly. mRNA-LNP vaccines do not require cDC1 cells or the WDFY4-dependent cross-presentation pathway for CD8+ T cell priming; instead they engage cDC1 and cDC2 cells redundantly, and acquisition of peptide-MHC-I complexes from non-haematopoietic cells accounts for a substantial part of the response in a manner dependent on type I interferon [36]. The same study offers a plausible explanation for a puzzling clinical observation, the expansion of T cells specific for antigens that the vaccine does not encode: cross-dressing allows dendritic cells to present peptides derived from any protein made in the surrounding tissue, including antigens from viruses encountered earlier in life.

5.2. T follicular Helper Cells and the Germinal Centre

Antibody responses to mRNA vaccines are unusually durable for a non-replicating vaccine, and the reason is the strength and persistence of the germinal centre reaction. Fine-needle aspiration of draining axillary lymph nodes after BNT162b2 vaccination showed that spike-specific T follicular helper cells persist at nearly constant frequency for at least six months, with an immunodominant HLA-DPB1*04-restricted response to S167-180 in individuals carrying that allele [37]. Lymph node germinal centre B cells remain detectable for months after vaccination [38], and sequencing of 1,540 spike-specific B cell clones from eight individuals showed that somatic hypermutation frequencies in germinal centre B cells increased 3.5-fold within six months, with memory B cells and bone marrow plasma cells accumulating high mutation loads and producing antibodies of increased affinity and neutralization potency [39].

The lipid component is required for this. LNPs alone, mixed with protein antigen, enhance T follicular helper cell and germinal centre responses beyond those obtained with a squalene emulsion adjuvant, and the effect depends on the ionizable lipid and on IL-6 [33]. The RNA component is required as well, through the interferon circuit described above [9]. The two requirements explain why mRNA vaccines induce better germinal centre responses than adjuvanted protein vaccines at comparable antigen doses, and why attempts to reduce reactogenicity by removing the lipid's inflammatory activity tend to reduce antibody durability as well.

5.3. Memory, Boosting and the IgG4 Question

Boosting has effects that are not simply quantitative. Omicron-containing boosters after two or three doses of the ancestral vaccine induce de novo B cell responses rather than only recalling pre-existing memory, which broadens the repertoire but also means that repeated boosting with related antigens continues to engage the germinal centre rather than settling into a stable memory state [40]. Antibody Fc effector functions wane after vaccination and are restored by boosting, with a decay that is slower than that of neutralizing titres for some functions [41].

Repeated bivalent boosting has been associated with an increase in spike-specific IgG4 antibodies and with waning of neutralizing activity [42]. The interpretation of this finding is not settled. IgG4 responses are also seen after repeated exposure to other antigens, including bee venom

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and factor VIII, and their functional consequence for protection against respiratory infection is unclear. What can be said is that the subclass distribution of the antibody response is not fixed, that it shifts with repeated antigen exposure, and that this should be monitored in any programme of frequent boosting.

5.4. T Cell Responses and Their Contribution

mRNA vaccines induce CD4+ and CD8+ T cell responses in most recipients, with CD8+ responses biased towards the spike protein and detectable in blood and in lymph nodes for months. The contribution of these T cells to protection is inferred rather than demonstrated. In humans, the correlation between neutralizing antibody titres and protection is strong and consistent across trials, whereas T cell correlates have been harder to establish because of the difficulty of measuring tissue-resident responses. In mice, T cells can protect in the absence of antibody, particularly against variants that escape neutralization, and mucosal vaccination induces resident memory T cells in the lung that are not generated by intramuscular delivery [43]. Whether the same holds in humans is unknown.

5.5. Correlates of Protection

For COVID-19, the COVE trial provided the clearest quantitative link: vaccine recipients with post-vaccination 50% neutralization titres of 10, 100 and 1,000 had estimated vaccine efficacies of 78%, 91% and 96% respectively [44]. For RSV, day-29 neutralizing antibody titres and prefusion F IgG were inversely associated with RSV lower respiratory tract disease in the mRNA-1345 trial, with a hazard ratio of 0.44 per 10-fold increase in RSV-A neutralizing antibody [45]. These numbers are useful for dose selection and for bridging to new populations, but they are disease-specific: the titre that predicts protection against a systemic infection with a short incubation period is not the titre that predicts protection against a mucosal infection with a long incubation period, and no equivalent threshold exists yet for most of the pathogens in section 7.

6. Replicating and Circular Alternatives

The three formats that differ most from conventional mRNA are compared in Table 2.

6.1. Self-Amplifying RNA

A self-amplifying RNA (saRNA) retains the non-structural genes (nsP1-nsP4) of an alphavirus upstream of the antigen gene, with the antigen expressed from a subgenomic promoter. After delivery to the cytosol, nsP1-4 form a replicase complex that copies the incoming RNA and transcribes the subgenomic mRNA, so that a single molecule produces many antigen transcripts. In principle this lowers the dose by one to two orders of magnitude, and in practice it does: the ARCT-154 vaccine, the first saRNA vaccine to be licensed, is administered at single-digit microgram doses, compared with 30 to 100 μg for conventional mRNA [46,47].

The trade-off is innate immunogenicity. Replication generates double-stranded RNA intermediates that activate PKR, OAS, MDA5 and TLR3, and the response limits both the duration and the magnitude of expression. Partial substitution with modified nucleosides reduces this, and complete substitution with m1Ψ in saRNA suppresses the interferon response and increases potency, at the cost of slower replication and a more complex manufacturing process [48]. Early clinical data reflected the balance: the COVAC1 phase 1 trial of a saRNA vaccine showed dose-dependent immunogenicity with reactogenicity that increased with dose, and modest neutralizing titres at the doses tested [49].

The efficacy data are now available. A pooled analysis of phase 1, 2, 3a and 3b trials of ARCT-154 in Vietnam, conducted largely during the Delta wave, reported 56.6% efficacy against any confirmed COVID-19 and 95.3% against severe disease, with 94.1% seroconversion for neutralizing antibodies four weeks after the second dose [46]. A booster dose of ARCT-154 was non-inferior to

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BNT162b2 for immunogenicity and had a lower incidence of some systemic reactions [50], and an Omicron-specific saRNA booster met its immunogenicity endpoints in a phase 2/3 trial [51]. Regulatory approval in Japan in 2023 established the format as a licensed platform rather than a preclinical curiosity [47].

Two limitations deserve mention. The first is the size of the RNA: an saRNA of roughly 9 to 10 kb is at the upper limit of what current LNPs encapsulate efficiently, and the resulting particles are less stable than those containing conventional mRNA. The second is that the immunology is harder to control. Because the replicon itself is immunostimulatory, formulation changes that would be tolerated with conventional mRNA can produce unacceptable reactogenicity, and the dose-response relationship is steeper.

6.2. Trans-Amplifying RNA

A trans-amplifying design splits the replicon into two molecules, one encoding the replicase and one encoding the antigen with the subgenomic promoter. This reduces the size of each RNA and allows the antigen module to be exchanged without re-engineering the replicase, which is attractive for rapid strain updates. The approach remains preclinical for infectious disease vaccines, and its main uncertainty is whether both molecules reliably reach the same cell.

6.3. Circular RNA

Circular RNA (circRNA) is generated by backsplicing or by chemical ligation, lacks free ends, and is resistant to exonucleases, which gives it a longer intracellular half-life than linear mRNA. It is translated through an internal ribosome entry site or through a modified cap-dependent mechanism, and it does not require a poly(A) tail. In mice and macaques, a circRNA vaccine encoding a trimeric receptor-binding domain produced higher and more durable antigen expression than an m1Ψ mRNA vaccine, elicited a higher proportion of neutralizing antibodies and a Th1-skewed response, and protected against SARS-CoV-2 [52]. CircRNA vaccines also induce potent T cell responses [53], and LNP-formulated circRNA has shown protection against SARS-CoV-2 in preclinical models [54].

The obstacles are manufacturing and purity. In vitro synthesis of circRNA requires either a ribozyme-based splicing strategy or ligation, both of which leave linear species and intronic fragments that must be removed, and the circularization efficiency is sequence-dependent. Immunogenicity of the residual linear RNA and of the splice junctions has not been characterized in humans. No circRNA vaccine has entered phase 1 trials for an infectious disease, and the enthusiasm in the literature should be read with that in mind.

Table 2. Comparison of the mRNA formats used or proposed for infectious disease vaccines.

Format	Replication and architecture	Typical dose	Expression	Innate stimulation	Clinical status
Unmodified mRNA	linear; cap-dependent translation	30-100 μg	hours to days	high (TLR3/7/8, RIG-I, PKR)	not licensed
m1Ψ-modified mRNA	linear; cap-dependent translation	30-100 μg	hours to days	reduced but not absent	licensed
Self-amplifying RNA	alphavirus replicon (nsP1-4) with subgenomic promoter	single-digit μg	days to weeks	high (dsRNA intermediates)	licensed in Japan
Trans-amplifying RNA	replicon split across two molecules	preclinical	days	moderate	preclinical
Circular RNA	covalently closed; IRES- or cap-dependent	preclinical	prolonged	low	preclinical

Dose ranges refer to the antigen-encoding RNA. Self-amplifying and circular formats trade manufacturing complexity for dose sparing or expression durability; only the two conventional formats have licensed products [2,46,48,52].

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7. Clinical Progress in Infectious Disease

Table 3 summarizes the trial results discussed in this section.

7.1. COVID-19

The two registration trials set the benchmark. BNT162b2, given as two 30 μg doses 21 days apart, showed 91.3% efficacy against COVID-19 through six months of follow-up among participants without evidence of prior infection, with 96.7% efficacy against severe disease and a gradual decline over time [7]. mRNA-1273, given as two 100 μg doses 28 days apart, showed 93.2% efficacy at the completion of the blinded phase, 98.2% against severe disease and 63.0% against asymptomatic infection [6]. The earlier primary analyses reported higher point estimates, and the difference between those and the six-month figures is a measure of how quickly the immunological landscape changed as variants emerged.

In children aged 6 months to 5 years, two 25 μg doses of mRNA-1273 produced neutralizing antibody concentrations non-inferior to those in young adults receiving 100 μg, with estimated efficacy of 36.8% in 2-to-5-year-olds and 50.6% in 6-to-23-month-olds during the Omicron period [55]. The lower efficacy in young children is consistent with the lower dose, the shorter interval and the antigenic distance of Omicron from the vaccine strain, and it illustrates the difficulty of extrapolating efficacy across age and variant.

Strain updates have followed the antigenic evolution of the virus. Monovalent XBB.1.5 vaccines, introduced in late 2023, induced neutralizing antibodies that remained stable for at least six months against D614G, BA.5, XBB.1.5 and JN.1, in contrast to the more rapid decline seen after boosters of the original or BA.5-bivalent vaccines [56]. Formulations have since been updated annually, and the 2025-2026 season recommendations rest on observational effectiveness data rather than on new efficacy trials [57]. The consistent finding across these studies is that protection against severe disease is more durable than protection against infection, and that hybrid immunity from infection plus vaccination is more durable than either alone.

7.2. Respiratory Syncytial Virus

The RSV program was the first test of whether the platform could reproduce its COVID-19 performance against a different pathogen. mRNA-1345 encodes a stabilized prefusion F glycoprotein and was tested in adults aged 60 years and older at a single 50 μg dose. In the ConquerRSV trial, efficacy was 83.7% against RSV lower respiratory tract disease with at least two signs or symptoms, 82.4% against disease with at least three signs, and 68.4% against RSV-associated acute respiratory disease, with consistent protection against both RSV A and B subtypes [58]. Local solicited reactions were more frequent than with placebo (58.7% versus 16.2%), systemic reactions were also more frequent (47.7% versus 32.9%), and serious adverse events occurred at 2.8% in both groups.

Two follow-on results are worth noting. Day-29 neutralizing antibody titres were established as correlates of protection in the same trial, which provides a quantitative bridge for future formulation changes [45]. And co-administration with influenza or COVID-19 vaccines did not produce unacceptable interference, which matters for annual campaigns in older adults [59].

7.3. Seasonal Influenza

Influenza has been the hardest test, because the comparator is a licensed vaccine with decades of effectiveness data rather than placebo. A quadrivalent mRNA-1010 vaccine was immunogenic and acceptably tolerated in a phase 1/2 trial [60]. Two phase 3 trials have now reported. In adults aged 18 to 64 years, a quadrivalent modRNA vaccine showed a relative efficacy of 34.5% against laboratory-confirmed influenza-like illness compared with a licensed inactivated vaccine, meeting criteria for both non-inferiority and superiority; the result was driven by A/H3N2 and A/H1N1 strains, with almost no influenza B circulation, and hemagglutination inhibition responses were non-inferior for A strains but not for B strains [61]. In adults aged 50 years and older, a trivalent mRNA-1010

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formulation at 37.5 μg showed a relative vaccine efficacy of 26.6% against RT-PCR-confirmed influenza-like illness compared with a standard-dose comparator, with higher rates of injection-site pain (65.8% versus 29.8%), fatigue (45.1% versus 20.3%) and myalgia (35.4% versus 11.6%) [62].

These are meaningful results: a relative efficacy of 25 to 35% over standard-dose inactivated vaccine is of the same order as the advantage conferred by adjuvanted or high-dose vaccines in older adults. They also set a realistic expectation. The platform does not produce sterilizing immunity against influenza, and the incremental benefit over existing vaccines is modest in absolute terms. The larger opportunity is breadth. A 20-subtype hemagglutinin mRNA vaccine protected mice and ferrets against matched and mismatched strains [63], and a chimeric hemagglutinin mRNA vaccine induced protective immunity and bone marrow plasma cells in rhesus macaques [64]. Whether either approach produces durable breadth in humans is the open question.

7.4. Cytomegalovirus, Mpox, HIV and Other Pathogens

Cytomegalovirus. mRNA-1647 combines mRNA for the gB glycoprotein with mRNA for the pentameric complex, the two principal targets of neutralizing antibody. In a phase 1 comparison, mRNA-1647 elicited broader neutralization and higher antibody-dependent cellular cytotoxicity than the gB/MF59 subunit vaccine, which had shown approximately 50% efficacy against HCMV acquisition in earlier trials [65]. A phase 3 efficacy trial in women of childbearing age is the definitive test, and CMV is arguably the most important current trial of the platform, because it requires protection against a pathogen that establishes latency and has evolved extensive immune evasion.

Mpox. A quadrivalent mRNA vaccine encoding A35, B6, M1 and H3 (BNT166a) induced T cell and neutralizing antibody responses and provided complete protection against vaccinia, clade I and clade IIb mpox in mice, and 100% protection against lethal clade I challenge in cynomolgus macaques [66]. An independent mRNA-LNP vaccine also protected against lethal orthopoxvirus challenge [67]. A phase 1 trial is underway.

HIV. The HIV programme uses mRNA not as a stand-alone vaccine but as a delivery format for germline-targeting immunogens. mRNA-LNP delivery of a germline-targeting trimer and of boosting immunogens generated long-lasting germinal centres, somatic hypermutation and affinity maturation in humanized immunoglobulin knock-in mice [68]. In humans, mRNA encoding membrane-anchored HIV envelope trimers elicited tier 2 neutralizing antibodies in a phase 1 trial, a result that had not previously been achieved with a nucleic acid vaccine [69]. This is early evidence, from a small number of participants, and the titres were low; it is nonetheless the first indication that the platform can engage the B cell lineages that a broadly neutralizing antibody vaccine would need.

Malaria. An mRNA vaccine tailored for liver-resident memory T cells, using a circumsporozoite antigen design intended to favour tissue residency, protected mice against sporozoite challenge [70]. The result is a proof of principle for antigen-design-driven targeting of a non-humoral effector mechanism.

Clostridioides difficile. A multivalent mRNA-LNP vaccine targeting toxins and other virulence factors elicited systemic and mucosal humoral and cellular responses and protected mice against lethal primary and recurrent infection, with improved decolonization when spore and cellular antigens were included [71]. This is the clearest demonstration that the platform can be applied to a bacterial pathogen with a toxin-mediated disease.

Highly pathogenic avian influenza. An mRNA vaccine against clade 2.3.4.4b H5N1 reduced pathogenicity and transmission in ferrets [72], which is the relevant preclinical model for a pandemic response.

Table 3. Selected clinical trials of mRNA vaccines in infectious disease.

Vaccine	Pathogen and antigen	Design and population	Key result	Reference
BNT162b2	SARS-CoV-2, prefusion spike	phase 2/3; 46,429 adults	VE 91.3% through 6 months; 96.7% against severe disease	[7]

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mRNA-1273	SARS-CoV-2, prefusion spike	phase 3; 30,420 adults	VE 93.2%; 98.2% against severe disease; 63.0% against asymptomatic infection	[6]
mRNA-1273, 25 ug	SARS-CoV-2, prefusion spike	phase 2/3; children 6 months to 5 years	nAb non-inferior to young adults; VE 36.8% (2-5 y) and 50.6% (6-23 mo)	[55]
mRNA-1345	RSV, prefusion F	phase 2/3; 35,541 adults 60 years and older	VE 83.7% against RSV-LRTD with 2 or more signs; 68.4% against RSV-ARD	[58]
mRNA-1010, quadrivalent	Influenza A and B, haemagglutinin	phase 3; 18,476 adults 18-64 years	rVE 34.5% versus inactivated vaccine	[61]
mRNA-1010, trivalent	Influenza A and B, haemagglutinin	phase 3; 40,703 adults 50 years and older	rVE 26.6% versus standard-dose vaccine	[62]
ARCT-154	SARS-CoV-2, spike (self-amplifying)	phase 1-3b, pooled; Vietnam	VE 56.6% against any COVID-19; 95.3% against severe disease	[46]
mRNA-1647	Cytomegalovirus, gB and pentamer	phase 1/2; healthy adults	broad neutralization and Fc-mediated effector functions	[65]
BNT166	Monkeypox virus, four antigens	preclinical; phase 1 ongoing	complete protection in mice and macaques	[66]
mRNA-encoded Env trimer	HIV-1, membrane-anchored trimer	phase 1	tier 2 neutralizing antibodies induced	[69]

VE, vaccine efficacy; rVE, relative vaccine efficacy; LRTD, lower respiratory tract disease; ARD, acute respiratory disease; nAb, neutralizing antibody; mo, months; y, years. Efficacy estimates are those reported at the primary analysis and are not directly comparable across trials because of differences in endpoint definitions, circulating variants and follow-up duration.

8. Safety

8.1. Reactogenicity

Solicited reactions after mRNA vaccination are common, dose-dependent and short-lived: injection-site pain in roughly 70 to 85% of recipients, fatigue in 40 to 60%, headache and myalgia in 30 to 50%, with most events mild to moderate and resolving within 72 hours. The rates are consistently higher than those of adjuvanted protein vaccines and of inactivated influenza vaccines, as the head-to-head data in the RSV and influenza trials show [58,61,62]. The mechanism is the same innate activation that provides the adjuvant signal, which is why the two have not been separated (section 4.4).

Serious adverse events in the trials discussed above occurred at rates similar to placebo or comparator: 2.8% in both arms of the RSV trial, and 2.2% versus 1.9% in the influenza trial [58,62]. This is the correct comparator for a vaccine used in healthy populations, and it is the reason why rare events detected only in post-authorization surveillance carry disproportionate weight in the benefit-risk assessment.

8.2. Myocarditis and Pericarditis

Myocarditis after mRNA COVID-19 vaccination is a real association with a strong age and sex gradient. In the US Vaccine Adverse Event Reporting System, 1,626 reports met the case definition among 192 million vaccinated persons, with a median age of 21 years, 82% male, and a median time to onset of two days. Reporting rates within seven days of the second dose were highest in adolescent males aged 12 to 15 years (70.7 per million doses of BNT162b2), 16 to 17 years (105.9 per million) and young men aged 18 to 24 years (52.4 and 56.3 per million for BNT162b2 and mRNA-1273 respectively) [73]. Population-based cohort analysis in England confirmed an excess risk after mRNA vaccination that was higher after infection than after vaccination for most cardiac outcomes [74], and a 2025 review of the evidence placed the rate at 1.3 to 3.1 per 100,000 doses in male adolescents, with lower

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risk associated with longer dosing intervals [57]. Most cases have a mild clinical course with resolution of symptoms and normalization of cardiac markers, although a minority show residual findings on magnetic resonance imaging at follow-up.

8.3. Anaphylaxis and Other Events

Anaphylaxis occurs at approximately 5 cases per million doses: 4.8 per million for BNT162b2 and 5.1 per million for mRNA-1273 in active surveillance [75]. The mechanism is not fully established. PEG-specific IgE is detectable in some cases, but many reactions occur in people without prior PEG exposure and without detectable IgE, and complement activation-related pseudoallergy and direct mast cell activation have been proposed as alternative pathways [76]. The practical consequence is that most people with a history of immediate reaction can be vaccinated under observation, and that polyethylene glycol allergy is not a reliable predictor.

Active surveillance of 11.8 million doses found no signal for ischaemic stroke, acute myocardial infarction or appendicitis when the 1-21 day risk interval was compared with the 22-42 day interval [75]. This design is efficient for common outcomes but insensitive to events that occur with similar frequency in both windows, and it does not replace long-term cohort studies.

8.4. Reducing Toxicity by Design

The toxicity of the platform has two sources: the innate activation required for adjuvanticity, and the biodistribution of the particle. The second is more tractable. Liver accumulation can be reduced by altering the ionizable lipid or by adding albumin-binding lipids [30], and hepatocyte expression can be silenced with microRNA target sites [27]. The first is harder, because the same pathways drive both immunogenicity and reactogenicity, and the strategies under investigation, including lipid headgroup modification, PEG-lipid titration and the use of mRNA-encoded adjuvants, all involve trade-offs that have not been resolved in humans [22,77]. A 2026 review of the mechanistic and public health evidence concludes that the safety profile is acceptable for the indications approved so far but that the reactogenicity-immunogenicity relationship remains the central design constraint [78].

9. Stability, Manufacturing and Access

mRNA is chemically unstable. The phosphodiester backbone is subject to hydrolysis, particularly at elevated pH and temperature, and the 5′ cap and the poly(A) tail are slowly lost; in the lipid particle, hydrolysis of the ionizable lipid ester and particle aggregation add further degradation pathways. Licensed COVID-19 vaccines required storage at −20 °C or −70 °C at launch, which limited deployment in settings without a reliable cold chain [79]. Formulation changes and buffer optimization have since allowed refrigerated storage for up to 30 days, and two strategies promise more. Lyophilization with cryoprotectants preserves potency for months at ambient temperature in preclinical studies [80], and a microneedle patch combined with a thermostable formulation maintained immunogenicity after storage at 25 °C and avoided the need for reconstitution [81]. Neither has yet been adopted for a licensed product at scale.

Manufacturing capacity is the other constraint. Production involves linearization of a DNA template, in vitro transcription, capping, purification, formulation by microfluidic mixing, and fill-finish, each with its own quality attributes and each contributing to cost [82]. The enzymatic step is the most expensive, and the purification step is the most difficult to scale, because the removal of double-stranded RNA and abortive transcripts by chromatography has a limited dynamic range. The investment required to build capacity for a pandemic response is difficult to justify commercially in the absence of a pandemic, which is why the platform's rapid-response claim depends as much on standing capacity as on the biology.

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10. Unresolved Questions and Next-Generation Design

The mechanistic picture that emerges from the work of the past five years is more complicated than the one it replaced, and four questions remain open in a way that limits rational design. Figure 4 groups the design levers that are available for addressing them.

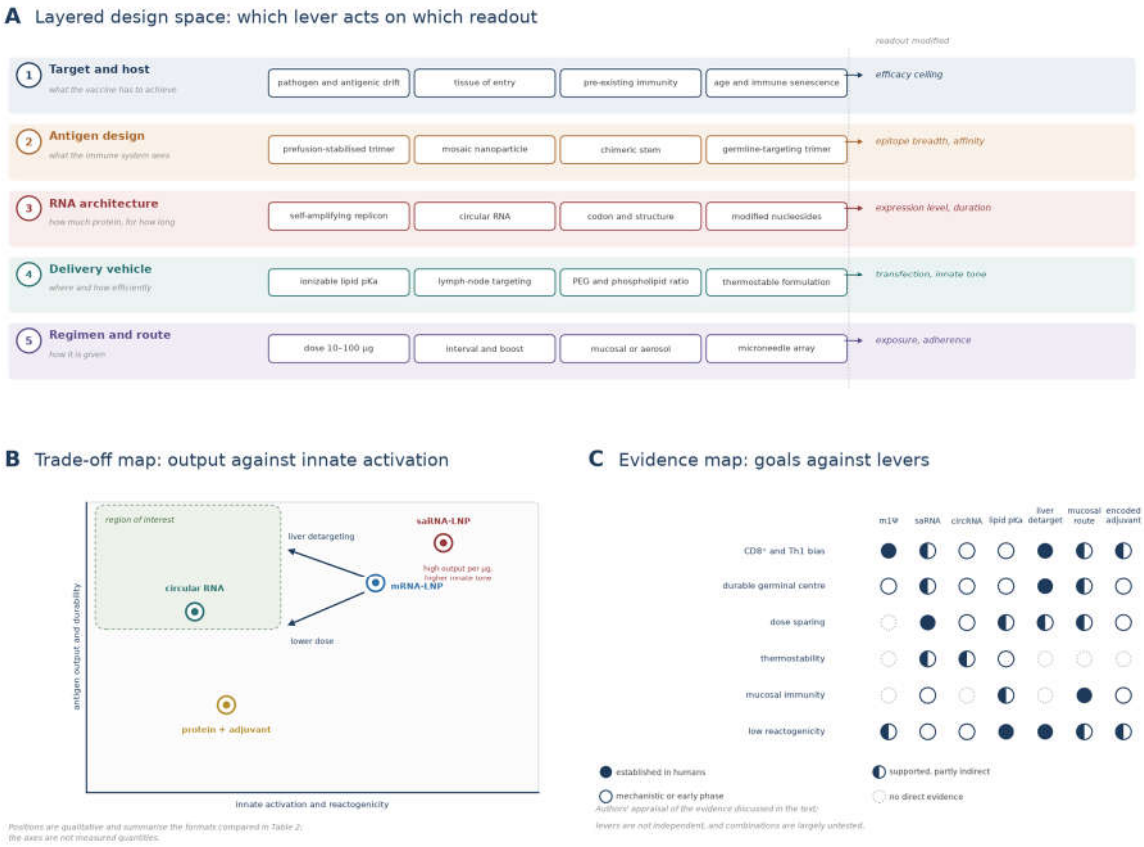

Figure 4. Design space for next-generation mRNA vaccines. (A) Five layers of design, from the target to the regimen, and the readout that each layer modifies. (B) Qualitative trade-off map of the formats compared in Table 2. (C) Evidence map relating design goals to individual levers, coded by the strength of the underlying evidence. Examples are drawn from the studies reviewed in sections 6 to 10 [22,29,46,52,63,64,68,80,81,83]. The levers are complementary rather than alternative, and most combinations have not been tested. Schematic prepared by the authors.

The first is the identity of the lipid sensor. The evidence for TLR4 and CD1d binding by ionizable lipid headgroups is direct and reproducible in vitro [32], but genetic ablation of the corresponding pathways does not abolish the response to the complete vaccine in mice [18]. Possibilities include redundancy between sensors, a contribution from lipid-raft-dependent signalling that does not require a single receptor, and species differences in the expression of the relevant receptors. Until the sensor is identified, the search for less reactogenic lipids will remain empirical.

The second is whether immunogenicity and reactogenicity can be uncoupled at all. The two-signal model implies that the RNA-derived interferon signal and the lipid-derived maturation signal could be tuned independently [9], and the observation that hepatocyte detargeting increases immunogenicity while reducing exposure suggests that some gains are available without an inflammatory cost [27]. But the IL-1 data indicate that the human response is amplified by a pathway that mice regulate differently [34], and a formulation optimized in mice may fail in humans for reasons that have nothing to do with the antigen.

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The third is endosomal escape. The absence of a quantitative assay means that the central step of delivery is measured indirectly, by protein output, which conflates escape with translation and with RNA stability [25,26]. A method that could count escape events per cell would change the design problem from screening to engineering.

The fourth concerns antigen design for variable pathogens. The COVID-19 vaccines work because the spike protein is a single, relatively conserved target within a serotype. Influenza, HIV and dengue do not offer such a target, and the solutions under development, mosaic receptor-binding domain nanoparticles, chimeric hemagglutinins, germline-targeting immunogens and multivalent cocktails, all require the vaccine to induce responses against epitopes that are subdominant in natural infection [63,64,68]. mRNA is well suited to this because multiple antigens can be encoded in one molecule or delivered in one formulation, but the immunological problem, which is immunodominance rather than delivery, is not solved by the platform.

Three further issues are practical rather than mechanistic. Mucosal delivery remains unproven in humans; intranasal mRNA-LNP vaccination protected hamsters against SARS-CoV-2 [83], and repeated mRNA vaccination increases neutralizing antibodies in nasal secretions [84], but the same LNP that is well tolerated intramuscularly caused high mortality when delivered intranasally in mice [17], so the formulation cannot simply be re-routed. Immunosenescence blunts responses in older adults, in whom the fold-increase in antibody titres after vaccination is lower and the decline faster, which is the population that needs RSV and influenza vaccines most [85]. And the platform's dependence on a small number of manufacturers, and on lipid supply chains that were stressed during 2021, has not been resolved; regional manufacturing capacity is a scientific as well as a political problem, because it determines which pathogens get a vaccine and how quickly.

11. Conclusion

The mRNA-LNP platform is now established for COVID-19 and RSV, has completed phase 3 trials in influenza, and is in clinical development for a range of pathogens that have resisted conventional approaches. Its success rests on a combination of properties that were not designed as a system: an RNA that can be engineered for high translation and partial immune evasion, a lipid particle that delivers it to the cytosol and acts as an adjuvant in its own right, and a germinal centre response that is unusually persistent for a non-replicating vaccine. The mechanistic work of the past five years has shown that the RNA and the lipid contribute separable signals, that the antigen is presented largely by cells that do not themselves express it, and that the innate pathways that drive immunity are the same ones that drive reactogenicity.

Progress over the next five years will depend less on new RNA chemistries than on three things: identifying the lipid sensor so that adjuvanticity can be tuned rather than maximized, developing quantitative assays for endosomal escape so that delivery can be engineered rather than screened, and designing antigens that elicit the right responses against pathogens where the dominant epitope is not the protective one. The platform's most important limitation is not that it fails to induce immunity, but that it induces a narrow, strain-specific immunity at the cost of a reactogenicity that is tolerable in a pandemic and less so in an annual campaign. Closing that gap is the central problem of the field.

Ethics of experimentation: not applicable. This review did not involve human participants, human tissue or animal experimentation.

Availability of data and materials: all data discussed are reported in the cited publications. No new datasets were generated.

Generative artificial intelligence: The author declares that no Gen AI was used in the creation of this manuscript.

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conceptualisation; Xiongxiong Lu: writing - review and editing, original draft preparation, methodology and conceptualisation. All authors read and approved the final manuscript.

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Abbreviations

APC, antigen-presenting cell; BMPC, bone marrow plasma cell; circRNA, circular RNA; cDC, conventional dendritic cell; dLN, draining lymph node; dsRNA, double-stranded RNA; GC, germinal centre; IFN, interferon; IL, interleukin; ISG, interferon-stimulated gene; LNP, lipid nanoparticle; m1Ψ, N1-methylpseudouridine; MBC, memory B cell; MDA5, melanoma differentiation-associated protein 5; modRNA, nucleoside-modified mRNA; NHP, non-human primate; nAb, neutralizing antibody; PEG, polyethylene glycol; PKR, protein kinase R; RIG-I, retinoic acid-inducible gene I; saRNA, self-amplifying RNA; SORT, selective organ targeting; Tfh, T follicular helper cell; TLR, Toll-like receptor; UTR, untranslated region; VE, vaccine efficacy.

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