Translational Advances in Lipid Nanoparticle-Mediated Nucleic Acid Therapeutics for Cancer Immunotherapy: Overcoming Resistance and Engineering Immunity

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Abstract

Recent breakthroughs in lipid nanoparticle (LNP)-mediated nucleic acid delivery have revolutionized cancer immunotherapy, addressing critical limitations of traditional modalities such as immune checkpoint inhibitors and ex vivo adoptive cell therapies. LNPs enable safe, efficient, and programmable delivery of mRNA and other nucleic acids directly to target immune cells or tumor microenvironments, overcoming pharmacokinetic, biological, and manufacturing barriers. Innovations in LNP design including ionizable lipids, targeted surface modifications, and AI-guided formulation have enhanced endosomal escape, tissue specificity, and reduced off-target effects. LNPs now facilitate in vivo engineering of CAR-T cells, personalized neoantigen vaccines, and myeloid cell reprogramming, showing significant efficacy in both hematologic and solid tumors. However, clinical translation faces challenges related to immunogenicity, accelerated blood clearance, cold-chain logistics, and regulatory complexities. Future prospects focus on next-generation RNA formats (circular RNA, self-amplifying RNA), stimuli-responsive and lyophilizable nanocarriers, and multidisciplinary collaboration to unlock potent, scalable, and universally accessible cancer immunotherapies.

Keywords

Lipid nanoparticles (LNPs), Cancer immunotherapy, mRNA vaccines, In vivo CAR-T engineering, Tumor microenvironment, Nanomedicine translation

1. Introduction

The Evolution of Cancer Immunotherapy:

Cancer immunotherapy has shifted from broad cytotoxicity to harnessing host immunity. Immune checkpoint inhibitors (ICIs targeting CTLA-4, PD-1/PD-L1) offer durable responses but are limited by poor efficacy in immunologically "cold" tumors and immune related adverse events (irAEs). Adoptive cellular therapies (ACTs), notably ex vivo CAR-T (e.g., anti-CD19 tisagenlecleucel), achieve curative outcomes in hematologic malignancies but face high costs, long production times, solid-tumor barriers (hostile TME), and toxicities like CRS and ICANS(1). Next-generation ICIs include bispecific checkpoint antibodies (e.g., anti-PD-1/VEGF) and novel targets like LAG-3 (relatlimab approved) and TIGIT. For ACT, allogeneic ("off-the-shelf") CAR-T cells (e.g., from CRISPR-edited healthy donors) have reduced costs and wait times, with early approvals in lymphoma; armored CAR-T cells engineered to secrete cytokines (e.g., IL-18) show improved solid tumor infiltration; and FDA-approved CAR-T for autoimmune diseases (lupus, 2025–2026) marks a major expansion beyond oncology(2).

The Nanomedicine Breakthrough:

Nanomedicine overcomes conventional chemotherapy's poor solubility, rapid clearance, off-target toxicity (myelosuppression, cardiotoxicity), and multidrug resistance by exploiting the EPR effect for spatiotemporal drug release(3–7). The 1995 FDA approval of Doxil® (pegylated liposomal doxorubicin) proved lipid-based nanocarriers could prolong circulation and reduce cardiotoxicity. As of 2026, over 65 nanomedicines are FDA-approved. Next generation LNPs now incorporate ionizable lipids with improved endosomal escape and tissue-specific targeting (e.g., anti-PECAM lung delivery). Beyond Doxil, newer formulations like Onivyde® (liposomal irinotecan, approved 2020–2025 for pancreatic cancer) and Vyxeos®(liposomal cytarabine+daunorubicin(8), 2017 for acute myeloid leukemia) demonstrate clinical synergy. The first ferumoxytol-based immune imaging nanomedicine (NanoLymph, 2025) now visualizes ICI responses. Moreover, Onpattro's LNP platform has directly enabled all current mRNA therapies (including COVID-19 vaccines and ongoing cancer vaccines), with next-gen tissue-selective LNPs(e.g., anti-CD117-targeted) now delivering mRNA to hematopoietic stem cells in vivo, eliminating ex vivo manipulation(8).

Building on Onpattro's LNP architecture, the COVID-19 mRNA-LNP vaccines (Comirnaty®, Spikevax®) validated safety, scalability, and immunogenicity in billions of patients, accelerating their shift from infectious disease prophylaxis to precision oncology. Modern LNPs (ionizable cationic lipids, helper phospholipids, cholesterol, PEGylated lipids) protect mRNA payloads, enable selective tissue tropism, and drive cytosolic translation(9). In oncology, personalized cancer vaccines are now a clinical reality: the Phase 2b KEYNOTE-942 trial of mRNA-4157 (V940) plus pembrolizumab achieved a 44% reduction in recurrence death risk in resected high-risk melanoma,recently, the Phase 3 INTerpath-001 trial (2025)

met its primary endpoint, leading to FDA breakthrough designation. BioNTech's BNT111 (four TAAs) showed potent T-cell expansion and tumor regression in PD-1-refractory melanoma, with Phase 2 data (2025) reporting a 30% objective response rate. Beyond vaccines, targeted LNPs surface-functionalized with anti-CD3 or anti-CD8 antibodies now enable in vivo CAR-T engineering, directly transfecting circulating T cells. Recent updates, In 2025, the first in vivo CAR-T for heart failure (targeting fibroblast activation protein) entered clinical trials, and a CD5-targeted LNP-CAR for T-cell acute lymphoblastic leukemia (T-ALL) showed complete remission in murine models(10). Capstan Therapeutics (2025–2026) reported the first human in vivo CAR-T for solid tumors (mesothelin-targeted LNP). This off-the-shelf approach circumvents ex vivo manufacturing delays, high costs, and toxicities like CRS/ICANS, heralding programmable immunity for both hematologic and solid malignancies(11).

2. Rational Design, Manufacturing, and Optimization of LNPs

Structural Composition The clinical and translational success of lipid nanoparticles (LNPs) is fundamentally driven by their modular and customizable molecular architecture. A state-of-the-art LNP formulation typically comprises four synergistic components ionizable cationic lipids, cholesterol, neutral helper phospholipids, and PEGylated lipids(12).

Ionizable Cationic Lipids:

Widely considered the most critical component for nucleic acid delivery, ionizable lipids (e.g., DLin-MC3-DMA, ALC-0315, SM-102) are engineered with a specific pKa (typically 6.0–7.0) to remain neutrally charged at physiological pH, thereby minimizing systemic toxicity, non-specific protein binding, and rapid clearance by the mononuclear phagocyte system (MPS)(13). Upon endocytosis into the acidic tumor or cellular endosome (pH < 6.5), these lipids undergo rapid protonation. This positive charge facilitates electrostatic interactions with anionic endosomal membrane lipids, promoting a structural transition into an inverted hexagonal (HII) phase that disrupts the endosomal membrane and ensures the cytosolic release of therapeutic payloads(14). Next-generation iterations, such as "aroLNPs", incorporate aromatic rings to enhance tissue tropism (e.g., lymph node targeting) and drastically reduce off-target hepatic accumulation, while biodegradable ester-linkages are being integrated to accelerate lipid clearance and reduce cumulative toxicity(15).

Cholesterol: Cholesterol acts as a critical stabilizing agent by filling the interstitial geometric gaps between phospholipids, modulating membrane fluidity, and conferring structural rigidity. It bolsters the LNP's integrity against shear forces in systemic circulation, prevents premature cargo leakage, and critically facilitates membrane fusion during the endosomal escape process. Moreover, recent structural analyses reveal that optimizing the cholesterol ratio can dictate the formation of polymorphic structures and actively tune the inherent immunostimulatory/adjuvant properties of the LNP(16).

Helper Lipids: Neutral helper phospholipids, such as 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) or 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), dictate the structural backbone and polymorphic phase tendencies of the nanoparticle(17). DSPC possesses a cylindrical geometry that favors stable bilayer formation and rigidity, whereas the

unsaturated acyl chains of DOPE make it highly fusogenic, further synergizing with ionizable lipids to optimize intracellular payload release(18).

PEGylated Lipids: Polyethylene glycol (PEG)-conjugated lipids (e.g., DMG-PEG2000) coat the LNP surface with a hydrophilic "stealth" corona, providing steric stabilization that prevents particle aggregation during storage and limits opsonization by serum proteins *in vivo* (19). This effectively prolongs the systemic circulation half-life. However, repeated administration can elicit anti-PEG antibodies, leading to the Accelerated Blood Clearance (ABC) phenomenon and hypersensitivity(20). To mitigate this, the field is actively investigating alternatives like poly(2-oxazoline) (POx) and zwitterionic phosphorylcholine (PC) headgroups to achieve stealth properties with reduced immunogenicity(21). (See Figure 1)

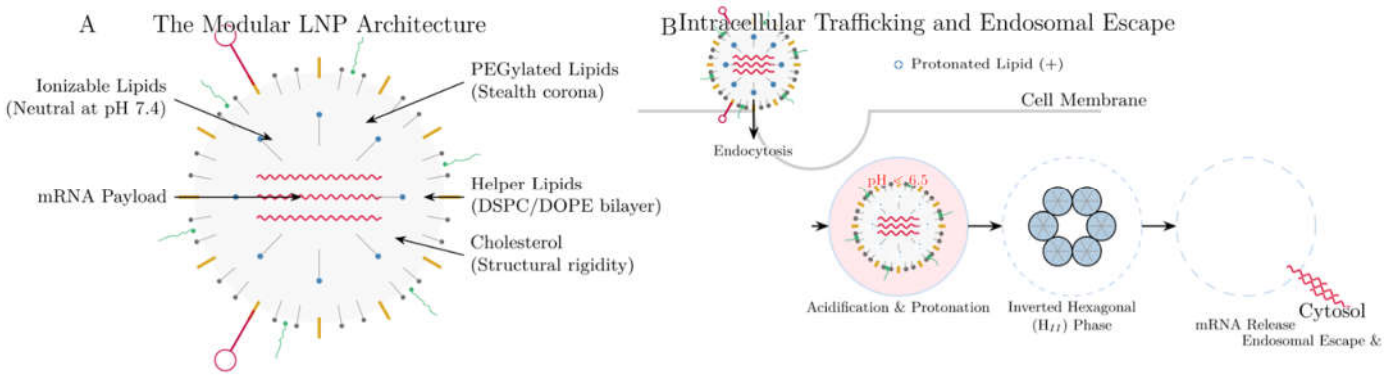

Figure 1: LNP Structural Architecture and the Mechanism of Endosomal Escape (Source : GPAI)

Advanced Manufacturing Techniques The transition of LNPs from bench-scale discovery to commercial mass production necessitates reproducible, scalable, and robust manufacturing protocols. Historically, wet chemistry bottom-up approaches have been preferred(22).

Thin-Film Hydration: A traditional laboratory technique where lipids are dissolved in an organic solvent, rotary evaporated to yield a thin lipid film, and subsequently hydrated with an aqueous buffer(23). While effective for encapsulating diverse payloads, the resulting multilamellar vesicles are highly heterogeneous in size, mandating repetitive, energy-intensive post-processing steps like sonication or membrane extrusion to achieve uniform nanoscale dimensions. This multi-step nature introduces batch-to-batch variability and restricts its viability for continuous industrial scale-up(24).

Nanoprecipitation: This solvent displacement technique relies on the continuous mixing of an organic lipid-ethanol phase with an acidic aqueous nucleic acid phase under magnetic stirring. The sudden shift in polarity triggers spontaneous lipid self-assembly(25). Although simpler, macroscopic bulk mixing is plagued by slow, uncontrolled diffusion rates, resulting in locally inhomogeneous supersaturation. This leads to high polydispersity indices (PDI), inconsistent payload encapsulation, and difficulties in reproducing critical quality attributes (CQAs) across varying scales(26).

Microfluidic-Based Mixing: Microfluidics represents the current gold standard for LNP manufacturing, overcoming the limitations of bulk mixing by confining fluids within micrometer-scale channels(27). Devices employing staggered herringbone micromixers (SHM) or bifurcating channels (e.g., NxGen™) disrupt laminar flow to induce chaotic

advection, achieving rapid and homogenous mixing in under 10 milliseconds. This highly controlled environment allows for the rapid generation of uniform LNPs (typically 50–150 nm) with exceptionally high encapsulation efficiencies and PDI values frequently below 0.1(28). Crucially, microfluidic production demonstrates robust scalability. Through "numbering-up" or the parallelization of microfluidic arrays (e.g., Parallelized Microfluidic Devices or PMDs), throughput can be escalated from microliters per minute in discovery phases to hundreds of milliliters per minute for GMP-compliant industrial manufacturing without altering the fundamental physics of particle formation(29). Innovations such as impingement jet mixing (IJM) and the use of perfluorodecalin (PFD) anti-fouling lubricant layers further prevent channel clogging, enabling continuous, reproducible, and commercial-scale output suitable for global supply(30).

Artificial Intelligence in Formulation The vast combinatorial space of lipid chemistries and processing parameters renders traditional trial-and-error formulation highly inefficient. Machine Learning (ML) and Artificial Intelligence (AI) are now fundamentally revolutionizing the inverse design, optimization, and predictive modeling of LNP therapeutics(31).

Predicting Lipid Properties and Inverse Design: Advanced AI frameworks integrate combinatorial chemistry with deep learning to execute the rapid, *in silico* screening of vast virtual lipid libraries(32). Platforms such as AGILE and deep learning-based generative models evaluate thousands of ionizable lipid structures, predicting their pKa, fusogenicity, and mRNA transfection efficiency to identify elite candidates that outperform legacy lipids(33). Furthermore, transformer-based models (e.g., TransLNP) utilize sequential molecular features and 3D spatial representations to execute inverse design, automating the discovery of synergistic lipid combinations tailored for precise cellular contexts and organ-specific tropism(34).

Optimizing Microfluidic Manufacturing: Beyond chemical discovery, ML algorithms including XGBoost and Bayesian optimization models are increasingly deployed to master the complex parameter landscape of microfluidic LNP manufacturing. By training on empirical datasets, these algorithms accurately predict the optimal lipid molar ratios, aqueous-to-organic flow rate ratios, and mixing speeds required to consistently yield ideal particle sizes, maximize encapsulation efficiency, and ensure batch-to-batch reproducibility during scale-up. By mapping the interplay between formulation inputs and Critical Quality Attributes (CQAs), AI-driven approaches drastically shorten development timelines, reduce costs, and pave the way for customized, highly potent LNP vectors in precision oncology(35).

3. LNP-Mediated mRNA Cancer Vaccines: Mechanisms and Clinical Landscape

Optimization of the mRNA Payload The therapeutic efficacy of mRNA-based cancer vaccines hinges upon the meticulous molecular engineering of the mRNA construct to maximize cytosolic stability and translational efficiency while controlling innate immunogenicity(36). To mimic endogenous eukaryotic mRNA, synthetic transcripts must be equipped with a 5' cap structure (e.g., 7-methylguanosine linked via a triphosphate bridge), which is essential for binding the eukaryotic translation initiation factor 4E (eIF4E) and protecting the transcript from 5' exonucleases. Simultaneously, the 3' poly(A) tail synergizes with the 5' cap through poly(A) binding proteins to regulate mRNA metabolism, preventing premature degradation and driving robust ribosomal translation(37). Furthermore, the strategic modulation of the 5' and 3' untranslated regions (UTRs) plays a crucial role in post-

transcriptional regulation, dictating subcellular localization, message stability, and the ultimate yield of the encoded antigen(38).

A paramount breakthrough in mRNA therapeutics is the incorporation of modified nucleosides, such as pseudouridine or N1-methylpseudouridine. Unmodified exogenous mRNA is typically recognized as a viral pathogen-associated molecular pattern (PAMP) by endosomal innate immune sensors, such as Toll-like receptors (TLR3, TLR7, and TLR8), which can trigger robust type I interferon responses and halt cellular translation(39). Substituting uridine with pseudouridine evades these sensors, thereby drastically reducing innate immune activation, blunting systemic reactogenicity, and significantly increasing the translational capacity and biological stability of the mRNA payload(40).

Targeting Antigen-Presenting Cells (APCs) To generate potent anti-tumor immunity, LNP-formulated vaccines must efficiently deliver mRNA encoding tumor-associated antigens (TAAs) or patient-specific neoantigens directly to professional APCs, most notably dendritic cells (DCs)(29). Upon systemic or localized administration, LNPs are engulfed by DCs via receptor-mediated endocytosis. The ionizable lipid component of the LNP then undergoes protonation in the acidic endosome, causing membrane destabilization and ensuring the successful cytosolic escape of the mRNA(41).

Once translated by the host cell's ribosomes, endogenous antigens are degraded by the proteasome into short peptide fragments. These fragments are transported into the endoplasmic reticulum and loaded onto Major Histocompatibility Complex (MHC) class I molecules, which are subsequently presented on the DC surface to prime robust CD8+ cytotoxic T lymphocyte (CTL) responses(42). In parallel, secreted antigens or antigens processed via lysosomal compartments are presented via MHC class II molecules, thereby activating CD4+ helper T cells. The concurrent activation of both CD8+ and CD4+ T cell compartments is critical, as CD4+ T cells secrete cytokines that sustain CTL effector functions and promote long-term immunological memory(43). To further boost DC selectivity, precision-engineered LNPs are being developed with surface-conjugated targeting ligands, such as mannose (to engage the CD206 receptor) or CLEC9A-specific nanobodies, which selectively target type 1 conventional dendritic cells (cDC1s) to enhance antigen cross-presentation and maximize the resulting T cell responses(44).

Clinical Trial Efficacy The translational success of LNP-mediated mRNA vaccines has recently been validated in landmark clinical trials, establishing a new pillar in precision oncology. A highly anticipated milestone is the Phase 2b KEYNOTE-942 trial, which evaluated mRNA-4157 (V940) a personalized neoantigen vaccine encoding up to 34 patient-specific mutations administered in combination with the PD-1 inhibitor pembrolizumab(45). In patients with completely resected, high-risk melanoma, this combinatorial approach achieved a profound 44% reduction in the risk of disease recurrence or

death compared to pembrolizumab monotherapy. This success provides definitive proof-of-concept that personalized mRNA-LNPs can successfully synergize with immune checkpoint blockade to overcome the immunosuppressive tumor microenvironment(46).

In parallel, "off-the-shelf" fixed-antigen vaccines have also demonstrated significant clinical potential. BNT111, an intravenously administered mRNA-lipoplex vaccine, encodes four non-mutated, shared TAAs commonly expressed in melanoma (NY-ESO-1, MAGE-A3, tyrosinase, and TPTE)(47). In a Phase 2 trial involving patients with advanced melanoma that

was refractory to prior anti-PD-(L)1 therapies, the combination of BNT111 and the PD-1 inhibitor cemiplimab yielded compelling clinical activity. The regimen achieved an 18.1% overall response rate (ORR) and a 55.3% disease control rate, demonstrating that mRNA vaccines can successfully reverse resistance to immune checkpoint inhibitors and re-engage host anti-tumor immunity in heavily pretreated patient populations(48).

4. Pioneering *In Vivo* CAR-T and Immune Cell Engineering

Overcoming *Ex Vivo* Limitations

Traditional *ex vivo* chimeric antigen receptor (CAR) T-cell therapy involves complex, multi-step Good Manufacturing Practice (GMP) protocols that require patient leukapheresis, viral transduction, and extended *ex vivo* cell expansion. This multi-week manufacturing process is highly expensive and introduces critical delays that can be detrimental for patients with rapidly progressing diseases. Furthermore, the clinical application of *ex vivo* CAR-T therapy is frequently marred by severe, potentially life-threatening toxicities, notably Cytokine Release Syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which are often exacerbated by the requisite lymphodepleting chemotherapy conditioning. In stark contrast, in vivo reprogramming generates CAR-T cells directly within the patient via the systemic administration of nanovectors. This transformative approach bypasses costly *ex vivo* manipulation, eliminates the need for toxic lymphodepletion, and offers a highly scalable, rapid, and economical "off-the-shelf" therapeutic modality capable of broadening patient access globally(49).

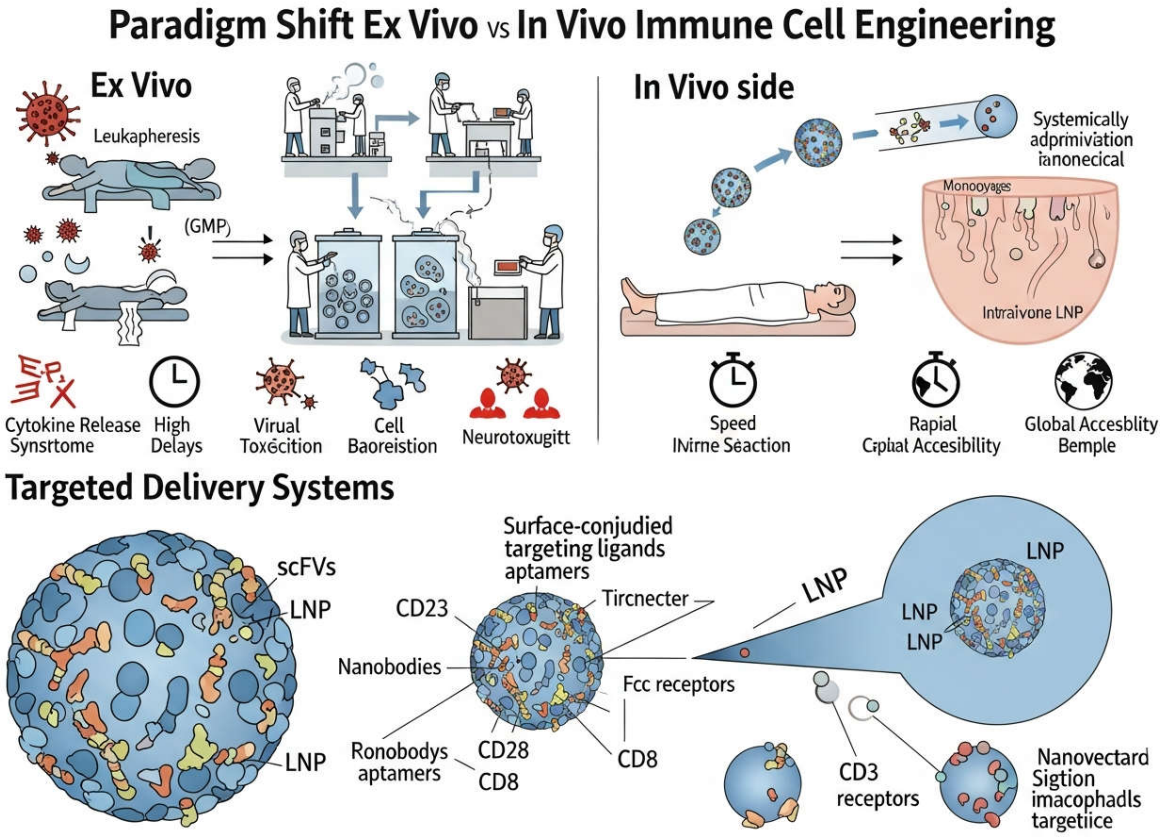

Figure 2: Paradigm Shift Ex Vivo Vs In Vivo Immune Cell Engineering

Targeted Delivery Systems

To achieve precision *in vivo* transfection, LNPs are intricately bioengineered with surface-conjugated targeting ligands, such as single-chain variable fragments (scFvs), nanobodies, or aptamers, which direct the nanoparticle to specific immune cell receptors(50,51). Targeting the CD3 receptor is a prominent strategy that not only directs the LNP to T cells but simultaneously provides a necessary activation signal. For instance, APC-mimetic LNPs decorated with both anti-CD3 and anti-CD28 antibodies can dramatically streamline the engineering process by concurrently activating resting T cells and delivering the CAR transgene in a single step. Conversely, CD8-targeted LNPs represent a highly established approach designed to selectively transfect the cytotoxic T cell compartment without inducing non-specific, widespread T cell activation(52).

Beyond lymphoid cells, advanced lipid-based platforms are successfully reprogramming the myeloid compartment to generate *in vivo* CAR-macrophages (CAR-M) and CAR-monocytes. These myeloid-tropic LNPs can utilize specific surface ligands (e.g., Fcα receptors) or rely on optimized lipid compositions and the endogenous protein corona to drive preferential phagocytic uptake. Reprogramming tumor-associated macrophages (TAMs) into a pro-inflammatory CAR-M state offers a unique advantage in remodeling the immunosuppressive solid tumor microenvironment and stimulating adaptive immunity(53).

Transient vs. Genomic Integration

A critical design consideration in *in vivo* CAR therapies is the duration of transgene expression and its associated safety profile. Transient expression utilizing LNP-mediated mRNA or circular RNA (circRNA) operates via a "hit-and-run" mechanism, wherein the payload is translated in the cytosol without nuclear entry or genomic integration. This transient profile offers a superior safety margin by enabling precise dose titration, providing a controlled therapeutic window, and completely eliminating the risks of insertional mutagenesis or prolonged immune-related toxicities. However, achieving optimal, sustained anti-tumor efficacy in transient systems typically necessitates repeated dosing schedules(54).

Conversely, for malignancies requiring lifelong CAR persistence, next-generation LNP platforms are being engineered to mediate permanent genomic integration. Systems utilizing the Sleeping Beauty transposase co-deliver mRNA-encoded transposase alongside DNA-encoded CARs to achieve stable integration into the T cell genome, providing durable tumor control from a single administration. To mitigate the inherent genotoxic risks associated with random insertion, cutting-edge technologies employ CRISPR/Cas9 or Large Serine Recombinases (LSRs) delivered via targeted LNPs. These highly advanced platforms facilitate precise, site-specific CAR integration into defined safe-harbor loci within the T cell genome, virtually eliminating insertional mutagenesis while ensuring potent, durable anti-tumor immunity(55).

5. Overcoming the Tumor Microenvironment (TME) and Drug Resistance Biological Barriers

The tumor microenvironment (TME) represents a highly dynamic, hostile, and heterogeneous ecosystem that actively impedes the delivery and efficacy of oncological therapeutics. A primary obstacle to pharmacological intervention is the dense, rigid extracellular matrix (ECM**)** composed heavily of cross-linked collagen, fibronectin, and hyaluronan which is

actively deposited by cancer-associated fibroblasts (CAFs). This pronounced desmoplasia creates a formidable physical barrier and significantly elevates interstitial fluid pressure, which profoundly restricts the intratumoral diffusion and uniform distribution of systemically administered nanoparticles. Furthermore, the rapid and uncontrolled proliferation of malignant cells outstrips the local vascular supply, generating regions of severe hypoxia and a highly acidic extracellular interstitium. Hypoxia not only drives aberrant, leaky angiogenesis but also fosters an immunosuppressive niche that protects the tumor from cytotoxic immune cells(56).

Embedded within this protective TME are Cancer Stem Cells (CSCs), a highly resilient, self-renewing subpopulation of malignant cells. CSCs share multiple features with normal stem cells, including relative cellular quiescence, hyperactive DNA damage repair mechanisms, and the overexpression of drug efflux pumps (e.g., ABC transporters). Because conventional chemotherapies and radiotherapies primarily target rapidly dividing cells, they frequently spare the quiescent CSC niche. Consequently, CSCs are widely recognized as the root cause of intrinsic and acquired multidrug resistance, tumor metastasis, and clinical relapse, making their selective eradication a critical imperative for curative cancer therapy(57).

Reprogramming Immunosuppression To overcome the immunologically "cold" nature of many solid tumors, advanced LNP platforms are being deployed not just to bypass the TME, but to actively reprogram its immunosuppressive cellular networks. Solid tumors are frequently infiltrated by Tumor-Associated Macrophages (TAMs) that have been polarized by the TME into a pro-tumorigenic, immunosuppressive M2-like state. These M2 TAMs actively suppress cytotoxic T-cell responses and promote tumor angiogenesis and metastasis. LNP-mediated therapies are now being engineered to specifically transfect or deliver small-molecule modulators to the myeloid compartment, successfully reprogramming TAMs from a suppressive M2 phenotype into a pro-inflammatory, tumoricidal M1 phenotype. The generation of *in vivo* CAR-macrophages (CAR-M) using mRNA-LNPs exemplifies this strategy, bridging innate and adaptive immunity by driving direct phagocytosis while enhancing antigen presentation.(58)

Another pivotal immunosuppressive axis within the TME is mediated by indoleamine 2,3-dioxygenase-1 (IDO1). Upregulated in numerous solid tumors, IDO1 catalyzes the degradation of the essential amino acid tryptophan into immunosuppressive kynurenine metabolites. This metabolic shift directly induces T-cell anergy, triggers T-cell apoptosis, and drives the expansion of regulatory T cells (Tregs). LNPs have been designed to co-deliver IDO1 inhibitors (such as indoximod or NLG919) alongside immunogenic cell death (ICD)-inducing chemotherapeutics. By silencing the IDO1 pathway while simultaneously generating tumor neoantigens via ICD, these LNPs act synergistically with immune checkpoint inhibitors (ICIs) targeting CTLA-4 or the PD-1/PD-L1 axes. This combinatorial nanomedicine approach effectively reverses T-cell exhaustion, increases the ratio of tumor-infiltrating CD8+ cytotoxic T cells to Tregs, and resensitizes refractory tumors to immune checkpoint blockade(59).

Stimuli-Responsive LNPs To achieve precise, spatiotemporal payload release exclusively within the tumor site, next-generation "smart" LNPs are intricately engineered with stimuli-responsive architectures that react to specific internal or external triggers.(60)

Internal Stimuli-Responsive LNPs: These systems exploit the unique biochemical signatures of the TME, most notably its acidity and altered redox potential. pH-responsive

LNPs frequently incorporate titratable ionizable lipids or fusogenic helper lipids like DOPE. As these LNPs transition from the neutral pH of the bloodstream (pH 7.4) into the mildly acidic TME (pH 6.5–6.8) or the acidic endosomes of cancer cells, the lipids undergo rapid protonation. This triggers a structural phase transition into an inverted hexagonal configuration, which destabilizes the LNP membrane and ensures a rapid, localized burst release of the therapeutic cargo. Redox-responsive LNPs, on the other hand, utilize bio-cleavable disulfide linkages. Because intracellular glutathione (GSH) concentrations in cancer cells are exponentially higher than in the extracellular matrix or systemic circulation, the disulfide bonds are exclusively reduced upon cellular internalization, dismantling the LNP and releasing the drug payload directly into the cytosol(61).

External Stimuli-Responsive LNPs:

To provide an additional layer of clinician-controlled precision, LNPs can be designed to respond to localized exogenous triggers. Thermosensitive liposomes (TSLs) are formulated with lipids that possess a specific phase transition temperature (Tm), typically within the mild hyperthermia range (41–42°C). When combined with localized external heating of the tumor, the TSL membrane transitions from a solid gel to a highly permeable liquid-crystalline phase, triggering massive and instantaneous drug release. Furthermore, hyperthermia actively dilates endothelial gap junctions, enhancing the enhanced permeability and retention (EPR) effect and allowing deep tumor penetration. Similarly, light-responsive LNPs incorporate photosensitizers (e.g., indocyanine green or porphyrin-lipid conjugates) that absorb near-infrared (NIR) light. Upon targeted laser irradiation, these nanocarriers generate localized hyperthermia (PTT) or reactive oxygen species (PDT), physically disrupting the TME stroma, permeabilizing the vasculature, and driving potent anti-tumor immune priming(62).

6. Clinical Translation: Pharmacological Bottlenecks and Regulatory Frameworks

Toxicity and Immunogenicity

Despite the clinical validation of lipid nanoparticles (LNPs) during the COVID-19 pandemic, their application in chronic or repeated-dosing oncological regimens is hindered by complex toxicity and immunogenicity profiles. The ionizable lipid component, while crucial for endosomal escape, possesses an intrinsic adjuvant effect that can trigger significant inflammatory responses, including complement activation, pro-inflammatory cytokine release, and potential dose-limiting hepatotoxicity(63). Furthermore, conventional cationic lipids, such as DOTAP, have a propensity to bind nonspecifically to serum proteins and the extracellular matrix, precipitating aggregation and premature cargo leakage. A far more pervasive clinical barrier is the immunogenicity of the PEGylated lipid corona, which is originally incorporated to provide steric stabilization and prolong systemic circulation. Extensive clinical monitoring reveals that 30–40% of patients develop anti-PEG IgM and IgG antibodies following initial exposure. Upon repeated administration a standard requirement for cancer vaccines and systemic immunotherapies these antibodies rapidly opsonize the nanocarriers, precipitating the Accelerated Blood Clearance (ABC) phenomenon. The ABC phenomenon results in the rapid sequestration of LNPs by the mononuclear phagocyte system (MPS), drastically curtailing the pharmacokinetic half-life and blunting therapeutic efficacy. To circumvent these immunological liabilities, the field is aggressively pivoting toward structurally optimized, biodegradable ionizable lipids with rapid hepatic clearance, as well as PEG alternatives like poly(2-oxazoline) (POx) or zwitterionic phosphorylcholine headgroups that maintain stealth properties while evading anti-polymer antibody induction(64).

Stability and Cold-Chain Logistics

A critical vulnerability of current LNP-mediated mRNA therapeutics is their inherent thermodynamic and chemical instability, which dictates a stringent reliance on ultracold-chain logistics (typically between −80 °C and −20 °C). This absolute cold-chain dependence restricts global distribution, increases clinical costs, and complicates localized deployment in resource-limited settings. To achieve true "off-the-shelf" commercial viability, advancing long-term, room-temperature storage solutions via lyophilization (freeze-drying**)** is an absolute necessity(65). However, the lyophilization process subjects LNPs to severe biophysical trauma; the dual stresses of ice crystallization and vacuum dehydration can cause structural collapse, lipid phase separation, particle aggregation, and catastrophic mRNA leakage. To mitigate structural deformation, formulations must incorporate cryoprotectants and lyoprotectants, predominantly disaccharides such as 5% (w/v) sucrose or trehalose. These excipients function by replacing the water of hydration around the lipid headgroups during the drying phase, maintaining the hydrogen-bonded membrane architecture in an amorphous glassy state. When optimized, lyophilized LNPs demonstrate robust post-reconstitution integrity, preserving critical nanoparticle dimensions, encapsulation efficiency, and *in vivo* transfection potency, thereby bridging the gap to decentralized, temperature-independent manufacturing(65,66).

Regulatory Compliance

The transition of complex LNP nanotherapeutics from the bench to commercial oncology demands strict adherence to rigorous regulatory frameworks mandated by agencies such as the FDA and EMA. Unlike conventional small-molecule drugs, the multicomponent, self-assembling nature of LNPs necessitates extensive Chemistry, Manufacturing, and Controls (CMC) packages. Regulatory bodies mandate the rigorous definition and continuous monitoring of Critical Quality Attributes (CQAs), including particle size, a polydispersity index (PDI) strictly ≤ 0.30 payload encapsulation efficiency, lipid impurity profiles, and overall morphological consistency. To meet these stringent criteria, scalable and highly reproducible Good Manufacturing Practice (GMP) protocols must be employed. Continuous microfluidic mixing platforms have become indispensable for GMP compliance, as they eliminate the batch to batch inconsistencies inherent to traditional bulk mixing. Furthermore, the advent of personalized mRNA neoantigen vaccines has disrupted traditional regulatory paradigms. Because these "N-of-1" therapies combine standardized LNP manufacturing with entirely patient-specific antigen sequences, they bypass conventional "one-size-fits-all" lot release models. Consequently, the FDA’s Center for Biologics Evaluation and Research (CBER) and the EMA require novel, adaptive frameworks focusing on comprehensive dose-ranging, robust validation of the predictive bioinformatics algorithms, real-time biomarker tracking, and meticulous risk benefit assessments for complex, repeated-dosing immunotherapeutic regimens(67).

7. Conclusion and Future Perspectives

Synthesis of Findings

The advent of lipid nanoparticle (LNP) technology has catalyzed a profound paradigm shift in modern medicine, acting as the critical translational bridge between fundamental molecular

biology and clinical oncology. Historically, the clinical utility of nucleic acid therapeutics was severely bottlenecked by their inherent physicochemical vulnerabilities, including rapid enzymatic degradation by ubiquitous nucleases, unfavorable electrostatic properties preventing cellular uptake, and endosomal entrapment. By engineering a sophisticated, multi-component core-shell architecture integrating pH-responsive ionizable lipids, structural phospholipids, cholesterol, and stealth polymers LNPs have successfully overcome these formidable biological barriers. In the context of cancer immunotherapy, this platform has evolved from a simple protective carrier into an active, programmable immunomodulator. Today, LNPs uniquely facilitate the rapid translation of genomic data into precision therapeutics, enabling the *in vivo* generation of chimeric antigen receptor (CAR) T-cells, the targeted delivery of patient-specific neoantigen vaccines, and the spatiotemporal remodeling of the highly immunosuppressive tumor microenvironment (TME).

The Road Ahead: Advancing RNA Architectures

While conventional linear mRNA-LNP platforms have achieved landmark clinical validations, their inherently transient expression kinetics mandate repeated dosing regimens, which can exacerbate cumulative toxicities, induce immune exhaustion, and increase patient burden. To achieve durable anti-tumor immunity and expand the therapeutic window, the field must aggressively pivot toward advanced, next generation RNA formats, most notably circular RNA (circRNA) and self-amplifying RNA (saRNA).

Circular RNA (circRNA):

By utilizing a covalently closed-loop structure that lacks free 5' and 3' termini, circRNA is rendered highly resistant to exonuclease mediated degradation. When formulated within LNPs, this structural stability translates to exponentially prolonged intracellular half-lives, yielding superior and sustained antigen or CAR expression for weeks rather than days, all without the risks of genomic integration.

Self-Amplifying RNA (saRNA):

By incorporating viral replication machinery (e.g., alphavirus RNA-dependent RNA polymerase), saRNA constructs actively amplify the therapeutic transcript within the host cell cytosol. This self-replication mechanism significantly amplifies translational yield, exerting a profound dose sparing effect that requires 10- to 100-fold lower payload concentrations compared to conventional linear mRNA. Consequently, saRNA-LNPs mitigate the systemic reactogenicity and manufacturing costs associated with high lipid-to-RNA ratios while driving potent, long lasting T-cell responses.

A Call for Multidisciplinary Collaboration

The future of LNP-mediated cancer immunotherapy is intrinsically tied to our ability to orchestrate complex, multi-scale biological interventions. Overcoming the remaining clinical bottlenecks such as the physical barriers of desmoplastic solid tumors, the accelerated blood clearance (ABC) phenomenon, and the necessity for ultra-cold chain logistics demands unprecedented, multidisciplinary collaboration. The integration of artificial intelligence and machine learning is required to accelerate neoantigen discovery and automate the inverse design of optimized, organ-tropic lipid chemistries. Concurrently, materials scientists, immunologists, and clinical oncologists must unite to engineer stimuli-responsive and

lyophilizable nanocarriers that bypass cold-chain dependencies. Through this synergistic convergence of scientific disciplines, the ultimate vision of precision oncology can be realized: the development of potent, "off-the-shelf", and universally accessible cancer immunotherapies that provide curative outcomes for patients worldwide.

Conclusion

LNP-mediated nucleic acid therapeutics represent a transformative leap in cancer immunotherapy, bridging the gap between fundamental molecular advances and clinical application. Through modular design, targeted delivery, and programmable immunomodulation, LNPs have enabled new generations of mRNA vaccines and in vivo immune cell engineering approaches that overcome many of the historical barriers associated with conventional cancer treatments. These advances have not only enhanced therapeutic precision and efficacy but also broadened accessibility by streamlining manufacturing and reducing costs.

Despite significant progress, important challenges remain, including overcoming the immunosuppressive tumor microenvironment, mitigating immunogenicity and toxicity, and addressing global distribution and regulatory hurdles. The future of LNP-mediated cancer immunotherapy will rely on multidisciplinary innovation—integrating artificial intelligence, advanced biomaterials, and clinical expertise—to develop next-generation RNA formats and stimuli-responsive nanocarriers. These efforts hold the promise of achieving universally accessible, off-the-shelf cancer immunotherapies that deliver durable and curative outcomes for patients worldwide.

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Conflict of Interest

The authors declare no competing financial interests or personal relationships that could influence the work reported in this manuscript.

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