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EDITORIAL

The RNA delivery dilemma—lipid *versus* polymer nanoparticle platforms

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Abstract

Since the first market authorization of RNA therapies, just eight years ago, the field has witnessed an extraordinary expansion, ranging from hepatic delivery for rare genetic diseases to global-scale vaccination during the COVID-19 pandemic,

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and now to cutting-edge cancer vaccines and gene editing strategies entering late-stage clinical trials. In parallel, the RNA therapeutics landscape has evolved rapidly, progressing from small interfering RNAs to next-generation and combinatorial RNA modalities. None of these breakthroughs would have been possible without the development of sophisticated RNA delivery technologies capable of navigating complex biological environments, enabling precise cellular targeting, and facilitating efficient intracellular trafficking. In this Editorial Note, we take a step back to reflect on key lessons learned throughout the RNA delivery journey. Featuring insights from leading and experienced voices in the field, this manuscript highlights critical milestones, persistent challenges, and the roles of lipid nanoparticles (LNPs) and polymer nanoparticles (PNPs) as RNA delivery platforms. These experts reflect on the features that have positioned LNPs as the current RNA delivery gold standard, while also exploring the untapped potential and distinctive advantages of polymer-based nanosystems. Collectively, these perspectives underscore a striking truth: we are only beginning to unlock the full therapeutic potential of RNA, and nanomedicine will certainly continue to shape the future clinical translation of RNA-based therapies.

Keywords Nanomedicine · Genetics · Clinical translation · Extrahepatic delivery · Manufacturing scalability · Regulatory readiness

Introduction

The field of RNA delivery has witnessed remarkable progress over the past decade, with nanomedicine emerging as a key enabler in bringing these therapies to the clinic. This is because, for RNA-based gene therapies to be effective, therapeutic RNA must reach the intracellular milieu of target cells while avoiding off-target toxicity. However, due to their inherently high molecular weight and anionic nature, RNAs cannot readily traverse the cell membrane and typically require a delivery system [1, 2]. A longstanding dilemma in the field focuses on the advantages and limitations of lipid nanoparticles (LNPs) and polymeric nanoparticles (PNPs) as the most suitable non-viral delivery systems for this purpose.

LNPs are currently the most established RNA delivery platform, largely due to their central role in the development of COVID-19 vaccines and their expanding use in a wide range of diseases. Their impact builds on decades of research, as highlighted in a recent review by Pieter Cullis and Philip Felgner, two pioneers in lipid-based nucleic acid delivery, who summarized the historical contributions of LNPs over the past six decades [3]. Philip Felgner is also widely recognized for his discovery of Lipofectamine, one of the earliest and most influential synthetic lipid formulations for transfection, which paved the way for many of today's delivery technologies [4]. LNPs emerged from foundational work on liposomes, a concept introduced by Bangham et al. in 1965 [5], and subsequent refinements established the four-component architecture of modern LNPs: ionizable amino lipids, helper lipids (*e.g.*, phospholipids and zwitterionic lipids), sterols, and polyethylene glycol (PEG) lipids. A major advancement came with the addition of ionizable lipids, which transformed their delivery potential, leading to potency increases of up to 1,000-fold compared with earlier lipid systems [6]. Ionizable lipids are key to the function of LNPs in delivering RNA into cells: while uncharged in neutral environments, their positively chargeable head determines the LNP pKa, enabling both nucleic acid encapsulation and interaction with anionic endosomal membranes [7]. Together with Pieter Cullis, whose work laid the foundation for clinically approved LNP systems, Robert Langer and Daniel Anderson are widely regarded as leaders at the forefront of lipid-based drug delivery [8–11]. With decades of pioneering work behind them, they have recently advanced the field further by applying machine learning to accelerate the discovery of new ionizable lipids [12].

In addition to LNPs, less attention has been paid to different types of polymers that can be used to formulate PNPs for RNA delivery [13–16]. Like ionizable lipids, many polymers used in PNP formulations carry a positive charge, allowing them to interact with and compact negatively charged RNA into stable particles. These polymers often feature a mix of primary, secondary and tertiary amine groups, which can enhance cellular uptake and promote release from endosomes. However, their overall positive surface charge tends to attract negatively charged serum proteins, which can compromise physiological stability. Among polymer-based nanosystems, polyethyleneimine (PEI) has long been the most widely studied, though its high cytotoxicity has prompted the search for safer alternatives [17–20]. Thanks to their chemical versatility, polymers offer a promising delivery platform beyond the liver, with the potential to be tailored for a wide range of therapeutic needs. Ongoing efforts to design new polymer structures that meet the specific physicochemical demands of RNA delivery are likely to expand the toolkit available for future gene therapies.

As RNA therapeutics evolve toward increasingly complex applications, such as the co-delivery of large and small RNAs or even combinations of RNA and DNA, there is growing interest in pushing the limits of current

nanoparticle (NP) platforms. Efforts are focused on fine-tuning the ratios and chemistries of lipid components or adjusting polymer backbones or side chains to create formulations that can better handle this diversity. At the same time, not every therapeutic need fits comfortably within the LNP framework. Certain applications demand features like nuclear transport, tunable targeting, prolonged release features or improved stability, and these are areas where lipid systems still face limitations. Polymers, with their vast chemical flexibility and unique ability to condense RNA via multivalent interactions, remain compelling candidates to fill these gaps. Though they are earlier in their clinical development, PNPs may offer the potential for controlled release formulations, enhanced stability and broader targeting capabilities. However, realizing this promise requires continued advances in materials science and mechanistic understanding, delivery strategies, safety, and standardization.

These evolving challenges and opportunities will be explored in depth through the expert perspectives featured in this article. This article includes perspectives from Professors Michael Mitchell, Dan Peer, Yvonne Perrie, Daniel Siegwart, and María José Alonso, world-leading experts in the field of RNA delivery.

Michael Mitchell is Associate Professor in the Department of Bioengineering at the University of Pennsylvania as well as the Leader of the Lipid Nanoparticle Delivery Systems Group and the Director of the Lipid Nanoparticle Synthesis Core, both located at the Penn Institute for RNA Innovation. At the interface of biomaterials science, drug delivery and cellular and molecular bioengineering, the Mitchell lab focuses on the synthesis of novel biomaterials and NPs for the delivery of nucleic acids (siRNA, miRNA, mRNA, CRISPR-Cas9) for cancer therapy; engineering of immune cells for immunotherapy and vaccines; investigating the influence of biomaterial chemical structure on in vivo transport to target cells and tissues; and novel drug delivery technologies for tissue engineering and regenerative medicine.

Dan Peer is Professor of Nanomedicine and Immunology at Tel Aviv University and the director of the Laboratory of Precision NanoMedicine at the same University. He is also the Founder and Managing Director of the SPARK Tel Aviv Center for Translational Medicine and has been elected member of the Israel Young Academy, US National Academy of Engineering and Fellow of the US National Academy of Inventors and the Controlled Release Society (CRS). The Peer lab works at the interface of materials science, chemistry, molecular biology, and immunology, to discover and validate novel therapeutic targets at the molecular level, and to develop specific genetic medicines for therapeutics and disease management. His lab pioneered work in developing cell-type specific delivery strategies of novel RNA and DNA molecular medicines, and novel genome editing strategies. In addition, the lab has generated a very large library of structurally unique lipids, some of which have been tested clinically as carriers for different types of RNAs as novel vaccines and therapeutics.

Yvonne Perrie is Professor and the Chair in Drug Delivery within Strathclyde Institute for Pharmacy and Biomedical Sciences at the University of Strathclyde. She is also a Fellow of the Society of Biology, a Fellow of the Royal Society of Chemistry, the Royal Pharmaceutical Society and an Eminent Fellow of the Academy of Pharmaceutical Sciences. Moreover, she has been president of CRS and a Member of the Order of the British Empire for services to pharmaceutical innovation and regulation. The Perrie Lab focuses on the design, formulation, and manufacture of nanomedicines, developing practical solutions to address current healthcare challenges.

Daniel J. Siegwart is Professor in the Department of Biomedical Engineering, Department of Biochemistry, and the Simmons Comprehensive Cancer Center at the University of Texas Southwestern Medical Center. He holds the W. Ray Wallace Distinguished Chair in Molecular Oncology Research and serves as the Director of the Program in Genetic Drug Engineering and Director of the Drug Delivery Program in Biomedical Engineering. The Siegwart lab uses a materials chemistry approach to enable targeted NP delivery of genomic medicines. Notably, his lab has been at the forefront in the design of synthetic carriers for gene editing and has applied these technologies for correction of genetic diseases and treatment of cancer.

María José Alonso is Full Professor at the University of Santiago de Compostela and a fellow of the American Institute for Medical and Biological Engineering and of the CRS. She was also president of the CRS (2018–2020) and a member of three Academies in Spain, the US National Academy of Medicine, the Royal Academy of Medicine of Belgium, and the Academy of Pharmacy and Biochemistry of Argentina. María José Alonso's lab has pioneered the design and development of novel nanostructures based on biopolymers intended to the targeted delivery of drugs, notably biological drugs. More specifically, in the field of vaccination, her lab has collaborated in the development of needle-free vaccination strategies for several vaccines, including a series of mRNA nasal vaccines.

The RNA delivery milestone journey

The exceptional efficacy of LNPs in RNA delivery builds upon a decades-long foundation in liposome research, representing a major refinement of early phospholipid vesicle systems that were originally explored for polynucleotide

transport. Yet the field's true turning point arrived in 2018 when the U.S. Food and Drug Administration (FDA) approved Onpattro, developed by Alnylam Pharmaceuticals, as the first ever siRNA therapy to treat hereditary transthyretin-mediated (hATTR) amyloidosis, setting LNPs as safe and effective nanocarriers to deliver RNA therapies to the liver [21, 22]. All interviewees unanimously identified Onpattro as the first major milestone in RNA therapeutics, which not only proved that RNA delivery following intravenous administration was possible in humans, but also catalyzed a wave of development in versatile LNP systems capable of safely delivering other types of nucleic acid therapeutics. Siegwart emphasized that Onpattro represented a watershed moment, not just for its therapeutic impact but also for the mechanistic insights it provided into NP biodistribution, endosomal interactions, and intracellular trafficking that shaped development of subsequent delivery strategies.

Alonso added that the next transformative moment followed in 2019 with the approval of Givlaari (Alnylam Pharmaceuticals), the first N-acetylgalactosamine (GalNAc)-siRNA conjugate to reach the clinic, specifically for the treatment of acute hepatic porphyria [23]. By exploiting the asialoglycoprotein receptor, which is found almost exclusively on hepatocytes, Givlaari marked a distinct shift toward polymer-based RNA delivery, although still focused on the liver. The main differences between Onpattro's LNPs and GalNAc conjugates are GalNAc's precise liver targeting, less invasive subcutaneous administration, streamlined manufacturing and improved safety profile [24]. Following Givlaari, three additional GalNAc–siRNA therapies were approved, namely Oxlumo (Alnylam Pharmaceuticals, 2020) for primary hyperoxaluria type 1, Leqvio (Novartis, 2021) for primary hypercholesterolemia or mixed dyslipidemia, and Amvuttra (Alnylam Pharmaceuticals, 2022) for hATTR amyloidosis. While numerous other polymer platforms have shown promise in research, GalNAc conjugates remain the only polymeric RNA platform to achieve regulatory approval to date.

The field accelerated dramatically during the COVID-19 pandemic, with the global deployment of mRNA vaccines from Moderna (Spikevax) and BioNTech/Pfizer (Comirnaty) in 2020–2021 [25, 26]. This event, widely cited by all interviewees as another major milestone, expanded RNA delivery from rare diseases to population-scale immunization. Mitchell noted that while siRNA had already reached the clinic for specialized indications, the pandemic brought RNA nanomedicine to the forefront of biomedical innovation, shifting much of the field's research energy towards LNPs and mRNA. The impact of this milestone continues to unfold, with the field now exploring new respiratory vaccines beyond the pandemic context. Perrie pointed to the FDA approval of Moderna's respiratory syncytial virus (RSV) vaccine, mRESVIA (2024), making it their second mRNA vaccine, using the exact same LNP formulation as for the COVID-19 mRNA vaccine. This formulation is also used in Moderna's next-generation COVID-19 vaccine, mNEXSPIKE, approved in 2025, and differing from Spikevax by delivering a one-fifth dose with a refined spike protein target, along with improved refrigerator stability for easier distribution [27].

However, the critical momentum in RNA delivery has prompted the entrance into clinical trials of RNA therapies beyond infectious diseases. New mRNA cancer vaccines are being developed, including V940 (Merck/Moderna) for melanoma in the phase III INTerpath-001 trial (NCT05933577) [28, 29], and for non-small cell lung cancer in the phase III INTerpath-002 and INTerpath-009 trials (NCT06077760 and NCT06623422, respectively) [30, 31]. Genome editors combining mRNA with a guide RNA (gRNA) are also entering trials, such as NTLA-2001 (Intellia Therapeutics, CRISPR-Cas9) for transthyretin amyloidosis with cardiomyopathy in the phase III MAGNITUDE trial (NCT06128629) [32, 33] and for transthyretin amyloidosis with polyneuropathy in the phase III MAGNITUDE-2 trial (NCT06672237) [34]. Verve Therapeutics advanced the first clinical in vivo base-editing program with VERVE-101 (phase I, Heart-1 trial, NCT05398029), which provided early proof-of-concept but was subsequently discontinued due to safety issues. The company has now moved on to the next-generation VERVE-102 (developed with Lilly, phase I, Heart-2 trial, NCT06164730), which uses actively targeted GalNAc-conjugated LNPs to deliver adenine base-editing components for heterozygous familial hypercholesterolemia or premature coronary artery disease. Circular RNA delivery is also being explored in clinical trials. Peer highlighted this shift in payload as both scientifically and clinically significant, pointing to recent clinical dosing in March 2025 by RiboX Therapeutics, his affiliated company, as the first in-human administration of a circular RNA LNP therapeutic (RXRG001), specifically developed for radiation-induced xerostomia-1 (NCT06714253, phase I/IIa) [35]. This development not only introduces a new class of RNA with enhanced stability and translational durability but also illustrates the expanding versatility of LNP delivery platforms.

The most recent milestone highlights a key advancement in actively targeted LNP-mediated mRNA delivery for in vivo engineering of CAR T cells [36–39], dosed in May 2025 in Australia by Capstan Therapeutics (NCT06917742, phase I). This in vivo CAR T approach integrates an anti-CD8 antibody fragment on the LNP surface for specific T cell targeting (CPTX2309), signaling a new era where mRNA can enable cellular reprogramming directly inside the patient without the need for ex vivo manipulation. This marks the beginning of a new class of targeted non-viral cell therapies, expected to rapidly diversify in the coming years.

Figure 1 summarizes the major milestones in the lipid *versus* polymer RNA delivery journey.

The interviewees see the past decade of RNA delivery progress as a landmark era in the evolution of biomedical science. Looking back on their yearlong careers, they consider this era the most impactful they have experienced, highlighting the significant shift it has brought to how clinical scenarios are addressed through nucleic acid-based approaches.

What makes lipid nanoparticles the go-to RNA carriers?

All interviewees agreed that the dominance of LNPs over PNPs for RNA delivery is not the result of a single breakthrough, but rather the outcome of decades of accumulated research, favorable physical and regulatory properties, rapid manufacturing scalability and broad community engagement. While alternative types of NPs still hold potential, including PNPs, LNPs have clearly moved ahead in clinical translation, particularly following their success in delivering mRNA during the COVID-19 pandemic [40, 41].

Mitchell discussed how the modular nature of LNPs contributes to their adaptability [40, 42]. When switching between different RNA cargos, the molar ratios of the LNP components can be adjusted rather than redesigning the entire NP. This flexibility simplifies the development pipeline and reduces the need for new material synthesis. In contrast, with polymers, such changes frequently require the creation of entirely new polymer structures, which introduces additional complexities in synthesis, optimization and characterization. In Mitchell's opinion, LNP modularity was one of the key reasons the field was able to quickly transition from the formulation of Onpattro to the formulations used for COVID-19 vaccines. Moreover, challenges in terms of entrapment efficiency and chemical variability were pointed out by Peer as obstacles for the translation of PNPs. Each polymer may require a different formulation strategy, and the field lacks the robust analytical frameworks and regulatory confidence that lipids currently enjoy. While Peer acknowledged that the potential of polymers remains significant, especially in non-hepatic or specialized delivery applications, he believes that polymers are inherently more complex and require more time to mature as a clinical platform. A balanced view was provided by Perrie, noting that her team has compared LNPs and PNPs for RNA delivery and found that both systems offer distinct advantages and disadvantages depending on the context [43, 44]. Perrie emphasized that both systems hold considerable potential, particularly as a broader range of materials and formulations are explored and optimized through further research.

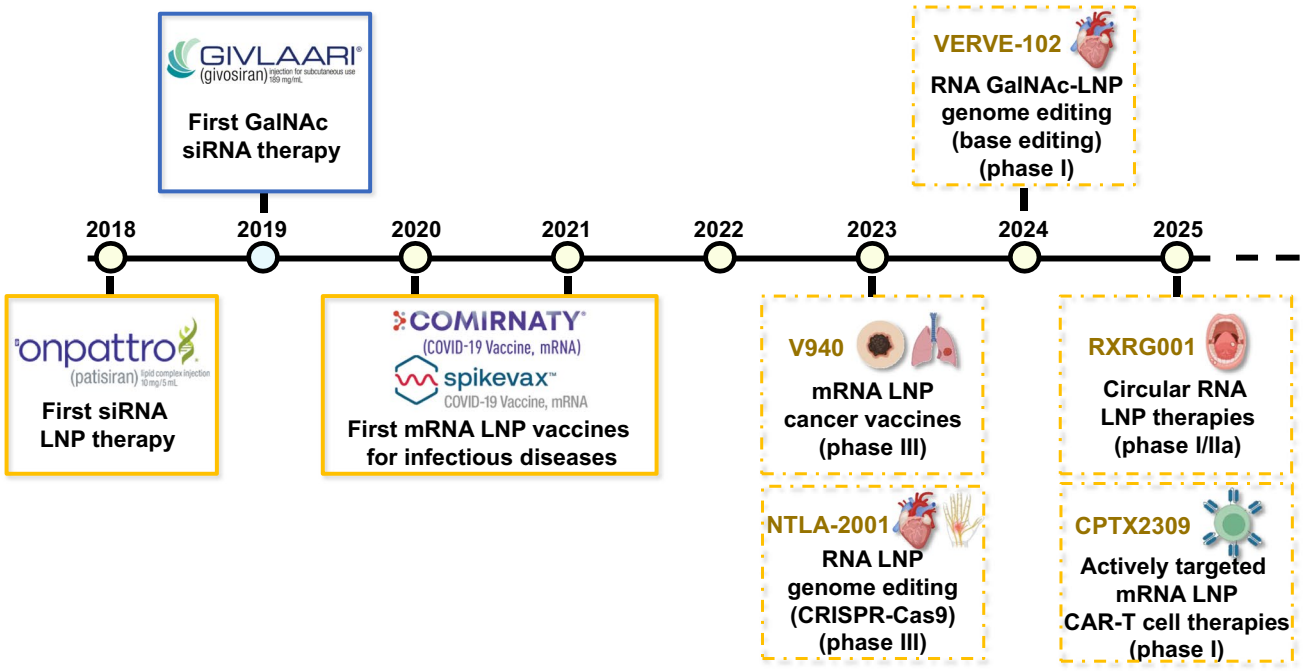

Fig. 1 Major milestones according to interviewees in the lipid (yellow boxes) *versus* polymer (blue box) RNA delivery journey: from approved siRNA and mRNA therapies to clinical trials of cancer vaccines, genome editing, circular RNA, and actively targeted approaches. Boxes with solid outlines represent systems that have received regulatory approval, whereas boxes with dashed outlines represent systems that have entered clinical trials

In terms of scalability, Siegwart recalled that in the late 2010 s, there was significant skepticism from venture capitalists and industry leaders about whether LNPs could ever be produced at scale, which was seen as a manufacturing hurdle. However, the global rollout of COVID-19 mRNA vaccines disproved these doubts—over six billion doses of LNP-based vaccines were produced and shipped to more than 180 countries. Siegwart attributed this breakthrough to the relative simplicity of the production process, namely the ethanol dilution method and the use of pumps and T-mixers, which enable continuous flow manufacturing [40]. The "very clean" and "relatively easy" production process, as Alonso noted based on discussions at Bill & Melinda Gates Foundation meetings, has led some African and Asian countries to establish their own RNA vaccine manufacturing facilities, reducing reliance on foreign supply and helping control costs. Delving deeper into this idea, Perrie emphasized a key lesson from the pandemic: the critical importance of local manufacturing in preventing supply chain strain. Industrial scalability has now become routine, and the fundamental challenge of large-scale production has clearly been overcome, while such large-scale processes are not yet optimized and validated for PNPs.

Another important factor contributing to the rise of LNPs is the sheer scale of global participation in their development. Alonso observed that nowadays "millions of people" are working with LNPs, and this popularity is partly due to the LNP protocols. The widespread use of LNPs feeds back into their success, as more researchers adopt, test and refine these systems, creating a virtuous cycle of optimization and application.

Peer emphasized that regulatory agencies are already familiar with lipid systems. This familiarity translates into a regulatory advantage, since these agencies know what safety, efficacy, and chemistry, manufacturing and controls (CMC) data to expect, and standard analytical tools already exist for assessing LNP quality and stability. These standardizations are not inherently easier for lipids, but they benefit from many more years of accumulated experience and optimization, which is not yet the case for polymers. Alonso added that, many polymers, while promising, have not yet reached clinical trials, and concerns about their toxicity remain unresolved. This places polymers at a disadvantage, at least in the short term, when compared to the clinically validated profile of LNPs.

The key characteristics that make LNPs the go-to RNA carriers over PNPs are summarized in Fig. 2.

Mind the polymer landscape: LNPs are not one-size-fits-all

Despite the dominance of LNPs in RNA delivery and the momentum they have gained in clinical translation, with over 200 clinical trials currently underway, all interviewees believe this does not mark the end for polymer-based delivery systems. Instead, polymers may find their niche in applications where LNPs are less suitable, thus offering unique advantages. The future of nucleic acid delivery will likely not be dictated by a single platform but by a diversified toolkit that includes both LNPs and PNPs, and even other material types, depending on the therapeutic goal and delivery context. The challenge is not that PNPs are inferior to LNPs, but rather that they probably require more time, optimization and strategic alignment with the right therapeutic indications.

One of the major limitations of LNPs is their inability to provide prolonged release, as Peer underscored. LNPs are inherently burst-release systems: once inside the cell, they rapidly discharge their payloads, which is suitable for some applications like vaccines or short-term gene silencing but might be suboptimal for long-term therapies. Moreover, while both LNPs and PNPs must pass through the endosomal compartment, the degradation kinetics of polymers can be precisely tuned to enable stimulus-responsive RNA release, regulate endosomal escape, and ultimately control when and how the RNA becomes available in the cytosol [45].

Toxicity remains one of the most pressing concerns for LNP-based systems. Ionizable lipids, the functional core of most LNPs, are not inert, as Peer pointed out**.** They can be immunogenic, inflammatory and potentially toxic, particularly to the liver and kidneys. Peer's team has shown that LNPs can trigger innate and adaptive immune responses, for example, through the amine headgroups of ionizable lipids, which interact with toll-like receptors such as TLR4, triggering inflammatory signaling cascades [46–51]. Mitchell added that LNPs commonly elevate systemic levels of inflammatory cytokines, namely IL-6 and TNF-α [52–55]. Thus, avenues are open in the field of polymer chemistry to design new NP matrices with fewer reactive groups, incorporate biodegradable motifs, and finely tune the balance between efficacy and toxicity, ultimately offering a more favorable safety profile. In Alonso's view, the key to reducing toxicity lies in minimizing the amount of cationic material in the formulation, whether it is a lipid or a polymer. To reduce potential LNP toxicity and enable safe delivery of therapeutic RNAs to diseased tissues, Siegwart's team has engineered extensive dendrimer-like lipid libraries, leveraging the systematic integration of ester-based degradable motifs with chemically diverse cores, peripheries and generations [56–59]. In an alternative strategy, Mitchell's team has utilized chemical evolution to progressively optimize the structure of ionizable lipids through combinatorial chemistry, improving their biocompatibility by increasing their biodegradability [60]. Peer has addressed biocompatibility through the design of ionizable lipids with different biodegradable linkers [61]. More on the polymer side, Alonso

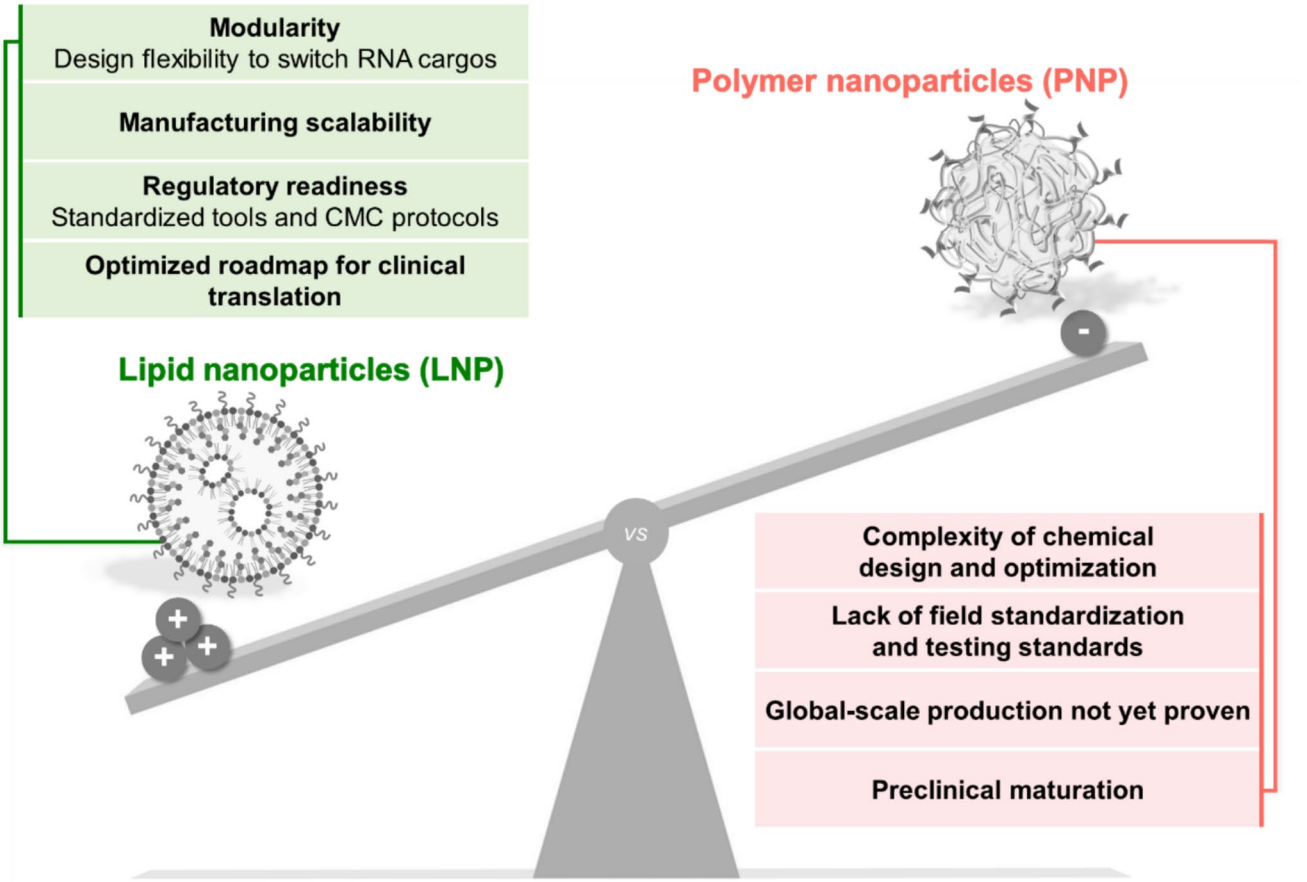

Fig. 2 Key characteristics that make LNPs the go-to RNA carriers over PNPs: modularity, scalability, regulatory familiarity and optimized roadmaps for clinical translation

has proposed polymer enveloping systems as biocompatible and non-immunogenic nanocarriers, and even as coating layers for lipid-based NPs, such as by using hyaluronic acid, polyglutamic acid, polyarginine and chitosan [62–64]. Peer collaborated in the development of PNPs based on PEI, chitosan or dextran-diaminobutane paired with a macrophage-targeted anionic polysaccharide for siRNA delivery [65].

LNPs typically contain a PEG lipid to stabilize the NP and control circulation time. However, the interviewees flagged that repeated exposure to PEGylated compounds can lead to the formation of anti-PEG antibodies, as reported since the early 2000 s [66–68]. These antibodies may reduce efficacy over time and complicate redosing, especially in lifelong therapies [69]. Although the interviewees relativized the regulatory risk posed by anti-PEG antibodies, pointing to PEG's long history of use such as in cosmetics, they acknowledged that their existence is well documented [70]. Siegwart referred to recent studies showing that patients receiving repeated Moderna or BioNTech/Pfizer LNP doses developed measurable levels of anti-PEG antibodies [71, 72], though the immune system appeared to tolerize after some time [73]. As emphasized by both Siegwart and Mitchell, this issue opens the door for polymer chemists to substitute PEG with alternative hydrophilic polymers, namely poly(oxazoline), zwitterionic polymers or other PEG mimetics. For instance, Peer has exploited the use of polysarcosine [74], while Siegwart has recently proposed a new class of brush-shaped polymer lipids that reduce anti-PEG antibody binding [75]. The area of redesigning the stealth component of RNA NPs represents a major opportunity to enhance tolerability and performance.

The inherent immunogenicity of LNPs, while acceptable and even advantageous for single-dose vaccine settings, is far less tolerable for chronic therapies. To manage inflammation, patients in the APOLLO trial were immunosuppressed with dexamethasone prior to administration of Onpattro [76]. Mitchell explained that, for conditions like cancer and protein replacement therapies, requiring monthly or more frequent administrations, this level of inflammation is unsustainable and immunosuppression could be counterproductive. Therefore, it is crucial to develop delivery vehicles that are less inflammatory and more redosable. Ideally, delivery platforms should be "immune silent", triggering minimal systemic response even with repeated dosing.

LNPs typically release their cargo into the cytoplasm, which is well suited for mRNA therapies and cytosolic gene editing. However, this mechanism may be less effective for strategies that require nuclear delivery of more complex payloads, such as in genome insertion therapies, as noted by Siegwart. Although Alonso cautiously noted that we are not yet far enough to identify a clear therapeutic scenario in which PNPs might outperform LNPs as RNA delivery systems, polymers, by contrast, could potentially be engineered to enhance nuclear uptake, coordinate the delivery of multiple components into distinct intracellular compartments or even improve transfection efficiency per unit mass of payload.

PNPs generally offer greater design flexibility compared to LNPs, particularly in minimizing the number of components required, as both Perrie and Mitchell noted. Developing polymer-based systems that can function as single-component NPs [77] could simplify large-scale manufacturing and reduce costs for certain applications. For example, polyion complex (PIC) micelles are self-assembling amphiphilic polymeric nanostructures that have been explored for siRNA delivery [78, 79]. They are formed by the electrostatic interaction between a negatively charged siRNA and a positively charged polymer segment, which is linked to a neutral, hydrophilic polymer like PEG [80]. This creates a core–shell structure with the RNA-polymer complex in the core and a PEG shell on the outside.

Perrie added that polymers also have advantages in storage and stability. Many polymeric systems can be formulated as dry powders with longer shelf lives, unlike LNPs, which typically require cold-chain logistics. Lyophilization can be accomplished by the addition of lyoprotectants, which has been exploited by Moderna for its phase III mRNA LNP vaccine against cytomegalovirus (NCT05085366) [42, 81]. Perrie emphasized that, although lyophilization of LNPs is possible and has been studied, it is costly and has yet to be adopted at commercial scale.

Figure 3 summarizes the major opportunities for the future applicability of PNPs in RNA delivery.

Current considerations and challenges for translation

Translating NP systems from the lab to the clinic requires addressing a range of interrelated scientific, technical and regulatory challenges.

Targetability beyond the liver

While current LNP formulations primarily accumulate in the liver after IV administration, a range of new strategies is being developed to redirect NPs toward extrahepatic targets. As noted by Perrie, expanding LNP applications beyond the liver will depend heavily on our ability to guide these particles to specific tissues or cell types. Among NP targeting mechanisms, active and endogenous targeting have been the focus of numerous studies as the most promising approaches [82].

Active targeting involves the surface modification of NPs with ligands that strategically bind to specific receptors on target cells [83]. However, controversial meta-analysis of 57 data sets revealed a delivery efficiency to the intended tissue of only 0.9% [84]. Mitchell's team recently proposed modifying the surface of mRNA LNPs with moieties such as folate to improve tumor retention [85], peptides to provide brain targeting [86], and antibody or antibody fragments to target pan-T cell markers [87]. Peer work has focused on mRNA LNP surface modification with antibodies against receptor tyrosine kinases, CD38 and PD-L1 for tumor targeting [88–92], as well as against specific α4β7 integrin conformations [93] and Ly6C [94] for leukocyte targeting. Alonso's team has exploited the surface functionalization of LNPs with the Lyp1 truncated peptide for tumor active targeting [95]. In a recent study supported by Biogen, brain-targeted mRNA LNPs were developed based on the modification of ionizable lipids with a variety of small molecules known for their ability to penetrate the blood–brain barrier (BBB) [96]. Perrie recalled that, despite over 50 years of research into active targeting using NPs, no actively targeted NP drug has yet been approved for clinical use. One reason is the complexity introduced by the protein corona, which is a layer of plasma proteins that forms around NPs upon entry into the bloodstream, one of the main focuses of Alonso's research [15, 97–102]. The protein corona remains a major barrier to reliable active targeting, and addressing this challenge requires a detailed understanding of its composition and formation dynamics – tools and protocols for isolation and characterization of the protein corona have been developed [103–107]. Importantly, substancial inter-species differences in plasma protein composition mean that coronas formed in rodent or other animal models may differ significantly from those formed in human blood. For this reason, characterizing the human-specific protein corona is essential for accurately predicting NP behavior in patients and improving translational relevance. Despite the translational gap, Peer expects advances in active targeting strategies in the near future, especially given the recent launch of clinical trials by Capstan Therapeutics using their CD8-targeted LNP technology for mRNA delivery to enable *in vivo* engineering of CAR T cells [36–39, 108]. According to Peer, it may be beneficial to use antibodies or antibody fragments as targeting ligands due to their well-established safety profiles and proven conjugation chemistries, which can help facilitate translational applications.

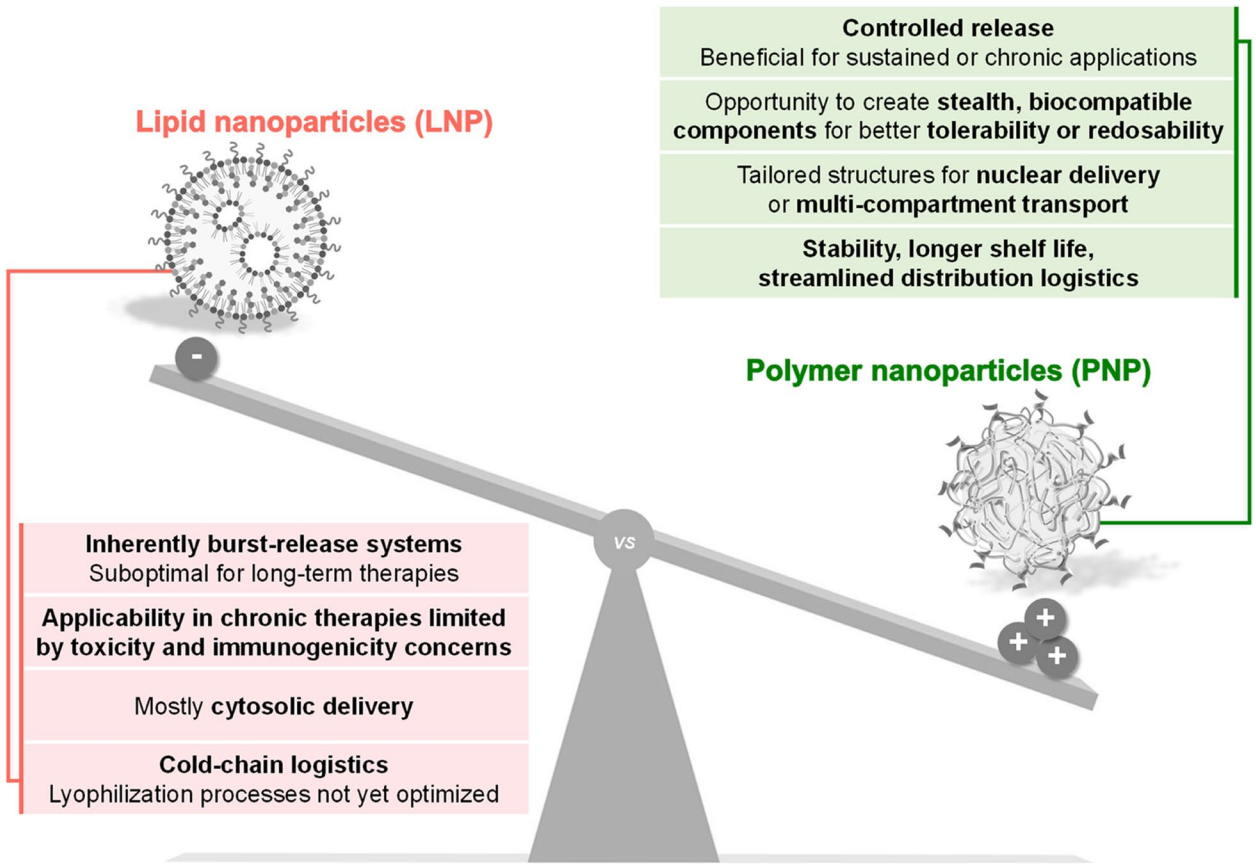

Fig. 3 Major opportunities for the applicability of PNPs over LNPs in RNA delivery: controlled release, design of better tolerated or redosable structural components, design of nuclear or multi-compartment transport strategies and long-term stability

Endogenous targeting leverages the natural association between NPs and plasma proteins in the bloodstream. Once formed, the protein corona can serve as an "endogenous identity", guiding NPs to specific cells via receptor-mediated mechanisms. Siegwart's team has contributed extensively to this area. In the polymer field, they started by screening hundreds of polyester variants and identified polyplex formulations capable of selectively delivering siRNA to tumor cells [109, 110]. Due to the unpredictability of polymer-protein corona interactions and the challenge of identifying broadly effective polyplexes outside of high-throughput screening, the team shifted focus to classical LNPs. In the lipid field, the development of Selective Organ Targeting (SORT) RNA LNPs has been particularly impactful [58, 59, 111–122]. Siegwart's team introduced a fifth lipid to traditional LNP formulations, which altered both pKa and protein corona characteristics. By carefully titrating this fifth component in SORT LNPs, often a quaternary ammonium lipid, they could shift mRNA expression from the liver to the spleen, lungs, kidneys or bone marrow in a dose-dependent manner. Peer has modulated RNA LNP endogenous targeting by proposing comprehensive libraries of proprietary ionizable lipid head–tail linker segments for targetability beyond the liver [123–125]. These segments present capability for leukocyte-specific [92, 126], lung-specific [61] and bone marrow-specific [89] delivery, but the team has also exploited LNP phospholipid content for higher accumulation in the colon [127]. In Mitchell's work, the team has altered the RNA LNP composition and consequent protein corona [128–135], such as by specifically modifying the lipid backbone with siloxane [136], dendron-like structures [137], piperazine backbone and bisphosphate moieties [138], amidine [54], oxidized motifs [139], bile acids [140] and anisamide groups [141], to provide lung [54, 128, 130, 136], spleen [54, 136, 137], bone [138], immune cell [129, 139, 140] and liver cell niche [141] tropism. Over the past years, Mitchell became particularly interested in studying the biological fate of LNP technologies in pregnant mice, since pregnant women are often excluded from clinical trials. His team found that spleen-tropic LNPs designed for extrahepatic delivery can also deliver to the placenta in pregnant mice, likely due to changes in blood flow distribution and protein corona composition [142]. Although these LNPs are not able to reach the placenta-protected fetus, they are still able to efficiently

transfect placental cells, which they leveraged to treat preeclampsia with vascular endothelial growth factor mRNA [142–146].

In Siegwart's observations, endogenous targeting tends to result in high organ-level enrichment but often lacks cell-type specificity, whereas active targeting might enable higher cell specificity, even when only a small fraction of the dose reaches the target organ. Perrie believes that a key future direction may involve combining active and endogenous targeting strategies. Given that the formation of a protein corona is essentially unavoidable, a more pragmatic approach may involve understanding and exploiting the biocorona rather than trying to eliminate it. Identifying proteins that preferentially bind to NP surfaces could help direct delivery more precisely to target tissues [147].

Mechanistic understanding

Both Alonso and Siegwart stressed the importance of gaining deeper mechanistic insight into how delivery systems function at both the extracellular and intracellular levels. In polymeric systems, knowledge is still limited regarding how to encapsulate and release a wide variety of payloads, especially those with challenging solubility or charge properties.

Siegwart added that, for nucleic acid therapies, greater knowledge of intracellular trafficking pathways is vital, especially given that current data in this area remains limited and often contradictory. Endosomal escape continues to be a major bottleneck for NP-based RNA delivery, resulting in suboptimal transfection efficiency [148]. Studies have indicated that only about 1–4% of RNA delivered via LNPs successfully escapes from late endosomes into the cytosol [149, 150]. This highlights the pressing need to design new NP materials, such as ionizable lipids or polymers, that can more effectively facilitate endosomal escape. In this context, Siegwart's team reported new lipids with biodegradable linkers with potential for accelerating the entrapped RNA payload release [151], as well as zwitterionic phospholipidation of cationic polymers to enable RNA delivery to spleen and lymph nodes with increased endosomal escape ability [20]. Mitchell, in turn, developed a new class of branched ionizable lipids that improve endosomal escape, increasing hepatic RNA and ribonucleoprotein complex delivery and gene editing efficiency, as well as T cell transfection [152]. While the cytoplasm is the primary site of action for siRNA and mRNA, other nucleic acid modalities, like plasmid DNA or donor templates for gene correction, require nuclear localization. Siegwart noted that a major translational challenge is to understand how to design delivery systems that can bypass endosomal sequestration and reach the nucleus efficiently. Progress in this area could unlock the full potential of gene insertion and genome editing therapies, dramatically improving therapeutic outcomes.

Solutions for hard-to-reach tissues

As Alonso noted, several biological barriers remain difficult to overcome for achieving RNA delivery to tissues beyond the liver, spleen, or lungs following IV administration. Alternatively, Siegwart foresees promise in local delivery for the treatment of hard-to-treat-tissues. These routes may allow localized expression of therapeutic agents, while minimizing systemic exposure.

Mitchell highlights the heart and brain as particularly challenging tissue targets, emphasizing that the BBB poses a major hurdle specifically for neurological delivery. For the brain, Alonso sees potential in alternative administration routes such as intranasal, intraparenchymal, intraventricular and intrathecal, that could help circumvent the need for BBB crossing [153]. Alonso's team is actively investigating the intranasal route by developing different ionizable lipid nanoemulsions and lipid-polymer nanocapsules with capacity to provide a robust antigen-specific T cell response [154], penetrate deep into the brain and reach the hippocampus [62]. In what concerns intraparenchymal administration, Alonso has proposed ionizable lipid nanocarriers (nanoemulsions and nanocapsules) with exceptional diffusivity and selective transfection of neurons [155]. Mitchell echoed this enthusiasm for local central nervous system delivery, describing the preclinical work of his team using LNPs injected into cerebral ventricles with capacity to provide mRNA transfection in neuron populations [156]. Notably, Alnylam Pharmaceuticals' ongoing phase II clinical trials for an intrathecal siRNA lipid-based therapy targeting Alzheimer's-related tauopathies have shown that nucleic acids can reach the brain via cerebrospinal fluid after regional injection (NCT05231785, NCT06393712) [157]. These findings underscore the promise of bypassing the BBB via ventricular or spinal routes to reach otherwise inaccessible neural tissues. In addition to regional delivery routes, transient and non-invasive BBB-disruption strategies offer an alternative means to enable brain delivery following systemic administration. In particular, microbubble-enhanced focused ultrasound has emerged as a powerful approach to locally and reversibly open the BBB, enabling the delivery of both LNPs and PNPs carrying mRNA to the brain after intravenous injection. This technique has demonstrated spatially controlled transfection of neuronal and glial populations while minimizing off-target exposure, highlighting its potential as a complementary strategy for overcoming BBB limitations without direct central nervous system injection [158–160]. Despite these encouraging advances, the journey towards effective RNA delivery to the brain remains far from complete. The critical challenge lies in identifying and developing brain-homing RNA nanocarriers capable not only of reaching their targets but also of exerting precise therapeutic effects within the intricate neural environment. Thus, a

long and rigorous path remains to translate these innovative approaches into broadly effective clinical solutions.

Mitchell's team is also exploring intratumoral delivery as a route of administration to reach the cancer site [161]. This approach is conceived not only as a local delivery, but also as a strategy to generate an on-site immune response than can subsequently target tumors systemically. In other words, the goal of intratumoral injection is not to eliminate every single cell directly, but rather to induce tumor cell lysis, allowing the released antigens to be taken up by antigen-presenting cells. This, in turn, acts similar to a therapeutic vaccine in that it stimulates an immune response capable of eradicating cancer cells throughout the body.

Ultimately, the route of administration for an RNA delivery system dictates the formulation design. For example, in the case of LNPs, as reported by Mitchell, the choice of ionizable lipids should be tailored to the specific biological microenvironment that they are expected to encounter.

Improved toxicity profiles, duration of response and redosability

As RNA delivery systems move from local to systemic administration, safety and redosability become central concerns. Alonso highlighted the need to better understand toxicity profiles, including dose-dependent effects that could be overlooked without robust mechanistic studies. As NP platforms expand into non-cancer indications such as autoimmune diseases or neurological disorders, safety becomes paramount. Peer drew parallels with vaccine development, emphasizing that for applications in otherwise healthy individuals, tolerability must be exceptionally high. For example, current clinical trials using CAR T approaches in autoimmune diseases [162] demand stringent safety benchmarks, as even minor toxicity could compromise development. Ongoing investigations are aimed at identifying lipids that are less inflammatory or at co-delivering LNPs with agents that can reduce inflammation. Mitchell sees this as one of the next iterations of LNPs, namely formulations that are both highly active and well-tolerated, thus enabling improvements not only in vaccines but across a wide range of therapeutic applications. Another key limitation of current LNP technologies is the transient endosomal disruption they induce to release nucleic acids, which is a process that can trigger inflammation [163]. Siegwart identified this as a priority for next-generation development.

Peer highlighted that, with the rise of targeting strategies, particularly for those involving ligand-functionalized NPs, additional layers of safety studies are required. Chemical conjugation steps must be thoroughly assessed to ensure no residual cross-linkers or unintended reactivity remains, which could trigger harmful biological effects. The regulatory familiarity with antibody-based therapeutics, including formats as antibody–drug conjugates, provides a useful model, but careful validation is still necessary. Additionally, some ligands may unintentionally activate receptor-mediated signaling, which must be avoided in sensitive indications.

Another relevant aspect that directly impacts the relevance of toxicity profile and the need for redosability is the duration of therapeutic effects, as outlined by Alonso. Siegwart considers genome editing especially promising, pointing to preclinical work where lung-targeting LNPs successfully corrected a disease-causing mutation with effects that lasted the lifetime of the treated animals [120]. Siegwart views such permanent or near-permanent outcomes, achievable through one or two treatments, as a fundamental shift in what is possible with RNA delivery. More broadly, Alonso stresses that durability depends not only on the carrier system but also on the chemical structure of the RNA itself. Modified RNAs can enhance both stability and duration of effect, which is especially important in applications like immunotherapy or cell therapy, where sustained gene expression or silencing is often required. Peer points to late-stage trials by companies like Intellia Therapeutics and Verve Therapeutics, where LNPs are being used for single-dose systemic delivery of gene editors to the liver (NCT06128629/NCT06672237 and NCT06164730, respectively) that result in durable editing. In these examples, editing tools are used to treat genetic diseases like hATTR amyloidosis or to permanently reduce LDL cholesterol by disrupting genes such as PCSK9. These developments, in Peer's view, mark a transition from conceptual to clinical reality in RNA genome editing.

Solutions for alternative RNA modalities and standarization

Perrie's team has exploited the use of self-amplifying RNA (saRNA), which enables high protein expression at much lower doses than conventional mRNA [43, 164, 165]. This makes saRNA an attractive platform for vaccines and other applications requiring sustained protein expression kinetics over extended timeframes compared to standard mRNA. Alonso remains optimistic, noting that although the same formulation technology cannot be readily extrapolated from one RNA to another, current evidence suggests that for RNA delivery experts, transitioning between different RNA types is relatively straightforward. However, Perrie noted that the longer and more fragile structure of saRNA poses formulation challenges, as it is more difficult and expensive to work with. Overcoming these barriers could lead to broader adoption of saRNA as a next-generation nucleic acid platform. Moreover, new RNA modalities like saRNA require specially designed nanocarrier systems to overcome challenges related to stability, efficient encapsulation and large-scale manufacturing [165]. Perrie has extensively contributed to

the manufacturing field by investigating the most favorable operating and formulation parameters to successfully develop RNA nanoformulations, including phase or mixing ratios and production speeds [166–168], buffer molarity [169], relatively-low-cost microfluidic mixers that do not compromise the efficiency and integrity of the resulting nanocarriers [170, 171], predictability boundaries for critical quality attributes [172], and orthogonal analytical pipelines to physicochemically characterize nanocarrier properties in early formulation stages [173, 174].

Apart from formulation optimization to open new avenues for alternative RNA modalities, the nanomedicine field is in urgent need of more consistent standards across formulation, characterization and preclinical evaluation, as strongly emphasized by Peer. Unlike the well-established frameworks for biologics such as monoclonal antibodies, NP systems still suffer from variability that compromises reproducibility and regulatory approval. Standardization efforts must extend across material types, whether lipid- or polymer-based, and cover not only physical characterization but also biological performance. Perrie also underscored that such harmonization to ensure quality, safety, and efficacy should ideally be pursued through worldwide collaboration. Past work, including major multi-author publications [175–178], has already laid the foundation for this initiative, but more universal implementation is needed.

Sustainable development of RNA delivery systems

Improving accessibility and global equity is another key goal. Alonso points to the need for formulations intended for mucosal administration, notably, nasal administration suitable for needle-free delivery, being this an important consideration in low-resource settings. Perrie expands on this, advocating for decentralized and modular manufacturing models that can reduce cost and enable local vaccine production. Perrie envisions a future in which personalized RNA-based cancer therapies could be produced on demand, close to the point of care, thereby reducing logistical and environmental burdens.

The RNA delivery dilemma on the horizon: divide or unite?

With over 13 billion doses of mRNA COVID-19 vaccines administered globally, RNA delivery systems have proven their capacity to drive large-scale clinical impact. What began as targeted therapies for rare genetic conditions has expanded to redefine the therapeutic landscape across multiple disease areas.

As Siegwart pointed out, it took nearly two decades for the first siRNA-based medicine to receive approval, as it always takes time to identify the most appropriate indications to develop a different class of medicine—be it RNA, antibody, protein or small molecule—and address foundational challenges, including entry into specific cell types, potential immunogenicity or inflammation induced by the therapy.

Yet, the field remains in its early chapters. Therapeutic scenarios where RNA delivery can make a difference are likely to thrive. In this regard, both Mitchell and Alonso anticipate broader applications beyond COVID-19, with RNA vaccines being developed for infectious diseases like influenza and HIV and expanding into oncology. Mitchell sees particular promise in personalized cancer vaccines, which would use patient-specific tumor neoantigens to elicit targeted immune responses [179]. Immune-modulating therapies also represent an emerging frontier. Alonso highlighted the potential of RNA-based therapies for autoimmune diseases, while Mitchell's group is exploring in vivo reprogramming of T cells using LNPs, a strategy that could bypass the complexity of ex vivo CAR T cell manufacturing [152]. Peer emphasized the untapped potential of the non-coding genome**.** While most current gene therapies focus on the small fraction of the genome that codes for proteins, regulatory elements within non-coding regions play critical roles in gene expression and cellular behavior. As biological understanding of these regions grows, new therapeutic strategies may emerge.

To meet these therapeutic demands and with a wide range of therapeutic cargos, each posing distinct challenges, the delivery technologies must evolve in step. There is broad agreement that both LNPs and PNPs have essential roles to play, and hybrid strategies that harness the strengths of each are likely to gain momentum. Alonso, in particular, views this period as a pivotal moment in the maturation of NP technologies, with both lipids and polymers poised for complementary success. Alonso's team has actively researched lipid-polymer hybrid nanocapsules for RNA delivery, offering a highly promising platform that combines the strengths of lipid and polymer nanocarriers and points toward the next generation of versatile, efficient and tunable RNA therapeutics [63, 99, 102, 154].

Polymers are not out of the picture but, for now, LNPs have carved a clear path forward. LNPs have come to dominate the field of RNA delivery due to a combination of favorable biophysical properties, relative simplicity in production and optimization, a deep historical foundation and regulatory readiness. From a structural and functional perspective, interviewees highlighted that it is not only the composition of the NP that matters but also how the components are organized and interact within the system. LNPs are designed to condense and protect RNA, and they must navigate several physiological barriers to ensure effective delivery. This extensive foundation has given LNPs a head start,

supported by decades of work and an established scientific community familiar with lipid-based delivery technologies.

While LNPs have taken the lead, especially for RNA vaccines and liver-targeted therapies, there may be contexts in which polymeric systems perform better or fill specific gaps. Toxicity, inflammation, redosing barriers and lack of sustained release are all pressing issues that polymers may be better equipped to solve. Polymers offer unparalleled chemical diversity, opportunities for sustained or targeted delivery, and customizable toxicity profiles. Although only siRNA-polymer GalNAc conjugates have reached the market so far, one could argue that PNPs today are where LNPs were in 2018, i.e., on the cusp of broader clinical translation.

As the RNA delivery field continues to evolve, it is increasingly likely that we will see not a substitution of LNPs, but a complementary landscape where PNPs fill critical gaps and enable unlocking the full potential of next-generation RNA therapies. With eight years of clinical track record behind them, RNA delivery systems still have a long way to go, and the future is likely hybrid, collaborative, and just beginning to unfold.

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Declarations

Ethics approval Not applicable.

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Consent to publish All authors read and approved the final manuscript.

Competing interests M.J.M., D.J.S. and M.J.A. are named on patents describing the use of nanoparticles and nanoparticle compositions for nucleic acid delivery that are discussed in this perspective article. M.J.M. is a Scientific Advisor to and holds equity in Capstan Therapeutics. D.P. receives licensing fees (to patents on which he was an inventor) from, invested in, consults (or on scientific advisory boards or boards of directors) for, lectured (and received a fee), or conducts sponsored research at Tel Aviv University for the following entities: ART Biosciences, BioNtech SE, Earli Inc., Kernal Biologics, LAND Therapeutics, Merck, Newphase Ltd., NeoVac Ltd., RiboX Therapeutics, Roche, SirTLabs Corporation, Teva Pharmaceuticals Inc. D.J.S. discloses financial interests in ReCode Therapeutics, Signify Bio, Jumble Therapeutics, and Pegasus Bio. M.J.A conducts sponsored research in this field for Eli Lilly, and is also the founder and shareholder of LiberaBio. All other authors have no competing interests to declare.

References

1. Bhatia SN, Dahlman JE. RNA delivery systems. Proc Natl Acad Sci U S A. 2024;121:e2315789121.
2. Paunovska K, Loughrey D, Dahlman JE. Drug delivery systems for RNA therapeutics. Nat Rev Genet. 2022;23:265–80.
3. Cullis PR, Felgner PL. The 60-year evolution of lipid nanoparticles for nucleic acid delivery. Nat Rev Drug Discov. 2024;23:709–22.
4. Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wenz M, et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc Natl Acad Sci U S A. 1987;84:7413–7.
5. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol. 1965;13:238-IN227.
6. Kulkarni JA, Witzigmann D, Thomson SB, Chen S, Leavitt BR, Cullis PR, et al. The current landscape of nucleic acid therapeutics. Nat Nanotechnol. 2021;16:630–43.
7. Wang W, Chen K, Jiang T, Wu Y, Wu Z, Ying H, et al. Artificial intelligence-driven rational design of ionizable lipids for mRNA delivery. Nat Commun. 2024;15:10804.
8. Jiang AY, Witten J, Raji IO, Eweje F, MacIsaac C, Meng S, et al. Combinatorial development of nebulized mRNA delivery formulations for the lungs. Nat Nanotechnol. 2024;19:364–75.
9. Li B, Jiang AY, Raji I, Atyeo C, Raimondo TM, Gordon AGR, et al. Enhancing the immunogenicity of lipid-nanoparticle mRNA vaccines by adjuvanting the ionizable lipid and the mRNA. Nat Biomed Eng. 2025;9:167–84.
10. Witten J, Raji I, Manan RS, Beyer E, Bartlett S, Tang Y, et al. Artificial intelligence-guided design of lipid nanoparticles for pulmonary gene therapy. Nat Biotechnol. 2024. https://doi.org/10.1038/s41587-024-02490-y.
11. Li B, Manan RS, Liang S-Q, Gordon A, Jiang A, Varley A, et al. Combinatorial design of nanoparticles for pulmonary mRNA delivery and genome editing. Nat Biotechnol. 2023;41:1410–5.
12. Li BW, Raji IO, Gordon AGR, Sun LZ, Raimondo TM, Oladimeji FA, et al. Accelerating ionizable lipid discovery for mRNA delivery using machine learning and combinatorial chemistry. Nat Mater. 2024;23:13.
13. Chen S, Huang X, Xue Y, Álvarez-Benedicto E, Shi Y, Chen W, et al. Nanotechnology-based mrna vaccines. Nat Rev Methods Primers. 2023;3:63.
14. Linderman SW, DeRidder L, Sanjurjo L, Foote MB, Alonso MJ, Kirtane AR, et al. Enhancing immunotherapy with tumour-responsive nanomaterials. Nat Rev Clin Oncol. 2025;22:262–82.
15. López-Estévez AM, Lapuhs P, Pineiro-Alonso L, Alonso MJ. Personalized cancer nanomedicine: overcoming biological barriers for intracellular delivery of biopharmaceuticals. Adv Mater. 2024;36:e2309355.
16. Jain M, Yu X, Schneck JP, Green JJ. Nanoparticle targeting strategies for lipid and polymer-based gene delivery to immune cells in vivo. Small Sci. 2024;4:2400248.
17. Dahlman JE, Barnes C, Khan OF, Thiriot A, Jhunjunwala S, Shaw TE, et al. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. Nat Nanotechnol. 2014;9:648–55.
18. Guimarães PPG, Figueroa-Espada CG, Riley RS, Gong N, Xue L, Sewastianik T, et al. In vivo bone marrow microenvironment siRNA delivery using lipid–polymer nanoparticles for multiple myeloma therapy. Proc Natl Acad Sci USA. 2023;120:e2215711120.
19. Figueroa-Espada CG, Guimarães PPG, Riley RS, Xue L, Wang K, Mitchell MJ. SiRNA lipid-polymer nanoparticles targeting

E-selectin and cyclophilin A in bone marrow for combination multiple myeloma therapy. Cell Mol Bioeng. 2023;16:383–92.
20. Liu S, Wang X, Yu X, Cheng Q, Johnson LT, Chatterjee S, et al. Zwitterionic phospholipidation of cationic polymers facilitates systemic mRNA delivery to Spleen and lymph nodes. J Am Chem Soc. 2021;143:21321–30.
21. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang C-C, Ueda M, Kristen AV, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med. 2018;379:11–21.
22. Akinc A, Maier MA, Manoharan M, Fitzgerald K, Jayaraman M, Barros S, et al. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nat Nanotechnol. 2019;14:1084–7.
23. Sardh E, Harper P, Balwani M, Stein P, Rees D, Bissell DM, et al. Phase 1 trial of an RNA interference therapy for acute intermittent porphyria. N Engl J Med. 2019;380:549–58.
24. Debacker AJ, Voutila J, Catley M, Blakey D, Habib N. Delivery of oligonucleotides to the liver with GalNAc: from research to registered therapeutic drug. Mol Ther. 2020;28:1759–71.
25. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020;383:2603–15.
26. Baden LR, Sahly HME, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384:403–16.
27. Stewart-Jones GBE, Elbashir SM, Wu K, Lee D, Renzi I, Ying B, Koch M, Sein CE, Choi A, Whitener B, Garcia-Dominguez D, Henry C, Woods A, Ma L, Montes Berrueta D, Avena LE, Quinones J, Falcone S, Hsiao CJ, Scheaffer SM, Thackray LB, White P, Diamond MS, Edwards DK, Carfi A. Development of SARS-CoV-2 mRNA vaccines encoding spike N-terminal and receptor binding domains, bioRxiv. 2022.
28. Weber JS, Carlino MS, Khattak A, Meniawy T, Ansstas G, Taylor MH, et al. Individualised neoantigen therapy mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab monotherapy in resected melanoma (KEYNOTE-942): a randomised, phase 2b study. Lancet. 2024;403:632–44.
29. Weber JS, Luke JJ, Carlino MS, Khattak MA, Meehan RS, Brown M, et al. INTerpath-001: pembrolizumab with V940 (mRNA-4157) versus pembrolizumab with placebo for adjuvant treatment of high-risk stage II-IV melanoma. J Clin Oncol. 2024;42:TPS9616-TPS9616.
30. Lee JM, Spicer J, Nair S, Khattak A, Brown M, Meehan RS, et al. The phase 3 INTerpath-002 study design: Individualized neoantigen therapy (INT) V940 (mRNA-4157) plus pembrolizumab vs placebo plus pembrolizumab for resected early-stage non–small-cell lung cancer (NSCLC). J Clin Oncol. 2024;42:TPS8116-TPS8116.
31. Cascone T, Peled N, Zer A, Chism D, Martincic D, Ojalvo LS, et al. Abstract CT251: phase 3 INTerpath-009 study: individualized neoantigen therapy V940 (mRNA-4157) plus pembrolizumab for resected stage II-IIIB (N2) NSCLC with incomplete pathological response to neoadjuvant immunochemotherapy. Cancer Res. 2025;85:CT251-CT251.
32. Maurer MS, Fontana M, Claggett B, Hernandez A, Januzzi JL, Lam C, et al. Design and rationale for MAGNITUDE, a phase 3, randomised, placebo-controlled trial of NTLA-2001, a CRISPR-based gene editing therapy, in patients with transthyretin amyloidosis with cardiomyopathy. Eur J Heart Fail. 2024;26:494–494.
33. Fontana M, Solomon SD, Kachadourian J, Walsh L, Rocha R, Lebwohl D, et al. CRISPR-Cas9 gene editing with Nexiguran Ziclumeran for ATTR cardiomyopathy. N Engl J Med. 2024;391:2231–41.
34. Gillmore JD, Gane E, Taubel J, Kao J, Fontana M, Maitland ML, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med. 2021;385:493–502.
35. A Phase I/IIa, dose escalation study evaluating the safety, tolerability, and preliminary efficacy of intraductal administration of RXRG001 to Parotid Gland(s) in adults with radiation-induced xerostomia and hyposalivation. 2024.
36. Rurik JG, Tombácz I, Yadegari A, Méndez Fernández PO, Shewale SV, Li L, et al. CAR T cells produced in vivo to treat cardiac injury. Science. 2022;375:91–6.
37. Adams G, Soldevila F, Matsuda D, Zhang Y, Bao Y, Ross B, et al. 1202 In vivo engineering of CAR T cells using a novel targeted LNP-mRNA technology. J ImmunoTherapy Cancer. 2023;11:A1326–A1326.
38. Tilsed CM, Sadiq BA, Papp TE, Areesawangkit P, Kimura K, Noguera-Ortega E, et al. IL7 increases targeted lipid nanoparticle-mediated mRNA expression in T cells *in vitro* and *in vivo* by enhancing T cell protein translation. Proc Natl Acad Sci U S A. 2024;121:e2319856121.
39. Tilsed CM, Sadiq BA, Papp TE, Areesawangkit P, Noguera-Ortega E, Kimura K, et al. 312 IL7 increases targeted lipid nanoparticle-mediated mRNA protein expression in T cells in vitro and in siitu by enhancing T cell translation. J ImmunoTherapy of Cancer. 2023;11:A355–A355.
40. Huang X, Kon E, Han X, Zhang X, Kong N, Mitchell MJ, et al. Nanotechnology-based strategies against SARS-CoV-2 variants. Nat Nanotechnol. 2022;17:1027–37.
41. Elia U, Ramishetti S, Rosenfeld R, Dammes N, Bar-Haim E, Naidu GS, et al. Design of SARS-CoV-2 hFc-conjugated receptor-binding domain mRNA vaccine delivered via lipid nanoparticles. ACS Nano. 2021;15:9627–37.
42. Kon E, Elia U, Peer D. Principles for designing an optimal mRNA lipid nanoparticle vaccine. Curr Opin Biotechnol. 2022;73:329–36.
43. Anderluzzi G, Lou G, Woods S, Schmidt ST, Gallorini S, Brazzoli M, et al. The role of nanoparticle format and route of administration on self-amplifying mRNA vaccine potency. J Control Release. 2022;342:388–99.
44. Anderluzzi G, Lou G, Gallorini S, Brazzoli M, Johnson R, O'Hagan DT, Baudner BC, Perrie Y. Investigating the impact of delivery system design on the efficacy of self-amplifying RNA vaccines, vaccines (Basel). 2020;8.
45. Cheung TH, Shoichet MS. The interplay of endosomal escape and RNA release from polymeric nanoparticles. Langmuir. 2025;41:7174–90.
46. Landesman-Milo D, Peer D. Altering the immune response with lipid-based nanoparticles. J Control Release. 2012;161:600–8.
47. Kon E, Ad-El N, Hazan-Halevy I, Stotsky-Oterin L, Peer D. Targeting cancer with mRNA–lipid nanoparticles: key considerations and future prospects. Nat Rev Clin Oncol. 2023;20:739–54.
48. Kedmi R, Ben-Arie N, Peer D. The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. Biomaterials. 2010;31:6867–75.
49. Peer D. Immunotoxicity derived from manipulating leukocytes with lipid-based nanoparticles. Adv Drug Deliv Rev. 2012;64:1738–48.
50. Sharma P, Breier D, Peer D. Immunogenic amines on lipid nanoparticles. Nat Biomed Eng. 2024;8:1332–3.
51. Sharma P, Hoorn D, Aitha A, Breier D, Peer D. The immunostimulatory nature of mRNA lipid nanoparticles. Adv Drug Deliv Rev. 2024;205:115175.
52. Parhiz H, Brenner JS, Patel PN, Papp TE, Shahnawaz H, Li Q, et al. Added to pre-existing inflammation, mRNA-lipid nanoparticles induce inflammation exacerbation (IE). J Control Release. 2022;344:50–61.
53. Atsavapranee E, Haley RM, Billingsley MM, Chan A, Ruan B, Figueroa-Espada CG, et al. Ionizable lipid nanoparticles for RAS protease delivery to inhibit cancer cell proliferation. J Control Release. 2024;370:614–25.

54. Han X, Alameh M-G, Gong N, Xue L, Ghattas M, Bojja G, et al. Fast and facile synthesis of amidine-incorporated degradable lipids for versatile mRNA delivery in vivo. Nat Chem. 2024;16:1687–97.
55. Riley RS, Kashyap MV, Billingsley MM, White B, Alameh MG, Bose SK, et al. Ionizable lipid nanoparticles for in utero mRNA delivery. Sci Adv. 2021. https://doi.org/10.1126/sciadv.aba1028.
56. Zhou K, Nguyen LH, Miller JB, Yan Y, Kos P, Xiong H, et al. Modular degradable dendrimers enable small RNAs to extend survival in an aggressive liver cancer model. Proc Natl Acad Sci USA. 2016;113:520–5.
57. Farbiak L, Cheng Q, Wei T, Álvarez-Benedicto E, Johnson LT, Lee S, et al. All-in-one dendrimer-based lipid nanoparticles enable precise HDR-mediated gene editing *in vivo*. Adv Mater. 2021;33:e2006619.
58. Lian X, Chatterjee S, Sun Y, Dilliard SA, Moore S, Xiao Y, et al. Bone-marrow-homing lipid nanoparticles for genome editing in diseased and malignant haematopoietic stem cells. Nat Nanotechnol. 2024;19:1409–17.
59. Lee SM, Cheng Q, Yu X, Liu S, Johnson LT, Siegwart DJ. A systematic study of unsaturation in lipid nanoparticles leads to improved mRNA transfection in vivo. Angew Chem Int Ed Engl. 2021;60:5848–53.
60. Han X, Alameh MG, Xu Y, Palanki R, El-Mayta R, Dwivedi G, et al. Optimization of the activity and biodegradability of ionizable lipids for mRNA delivery via directed chemical evolution. Nat Biomed Eng. 2024;8:1412–24.
61. Somu Naidu G, Rampado R, Sharma P, Ezra A, Kundoor GR, Breier D, et al. Ionizable lipids with optimized linkers enable lung-specific, lipid nanoparticle-mediated mRNA delivery for treatment of metastatic lung tumors. ACS Nano. 2025;19:6571–87.
62. Samaridou E, Walgrave H, Salta E, Álvarez DM, Castro-López V, Loza M, et al. Nose-to-brain delivery of enveloped RNA - cell permeating peptide nanocomplexes for the treatment of neurodegenerative diseases. Biomaterials. 2020;230:119657.
63. Ledo AM, Sasso MS, Bronte V, Marigo I, Boyd BJ, Garcia-Fuentes M, et al. Co-delivery of RNAi and chemokine by polyarginine nanocapsules enables the modulation of myeloid-derived suppressor cells. J Control Release. 2019;295:60–73.
64. Raviña M, Cubillo E, Olmeda D, Novoa-Carballal R, Fernandez-Megia E, Riguera R, et al. Hyaluronic acid/chitosan-g-poly(ethylene glycol) nanoparticles for gene therapy: an application for pDNA and siRNA delivery. Pharm Res. 2010;27:2544–55.
65. Gao H, Li S, Lan Z, Pan D, Naidu GS, Peer D, et al. Comparative optimization of polysaccharide-based nanoformulations for cardiac RNAi therapy. Nat Commun. 2024;15:5398.
66. Ishida T, Masuda K, Ichikawa T, Ichihara M, Irimura K, Kiwada H. Accelerated clearance of a second injection of PEGylated liposomes in mice. Int J Pharm. 2003;255:167–74.
67. Ishida T, Ichihara M, Wang X, Yamamoto K, Kimura J, Majima E, et al. Injection of PEGylated liposomes in rats elicits PEG-specific IgM, which is responsible for rapid elimination of a second dose of PEGylated liposomes. J Control Release. 2006;112:15–25.
68. Semple SC, Harasym TO, Clow KA, Ansell SM, Klimuk SK, Hope MJ. Immunogenicity and rapid blood clearance of liposomes containing polyethylene glycol-lipid conjugates and nucleic acid. J Pharmacol Exp Ther. 2005;312:1020–6.
69. Chen Y, Su Y-C, Roffler SR. Polyethylene glycol immunogenicity in nanomedicine. Nat Rev Bioeng. 2025. https://doi.org/10.1038/s44222-025-00321-6.
70. López-Estevez AM, Gref R, Alonso MJ. A journey through the history of PEGylated drug delivery nanocarriers. Drug Deliv Transl Res. 2024;14:2026–31.
71. Ju Y, Lee WS, Pilkington EH, Kelly HG, Li S, Selva KJ, et al. Anti-PEG antibodies boosted in humans by SARS-CoV-2 lipid nanoparticle mRNA vaccine. ACS Nano. 2022;16:11769–80.
72. Ju Y, Carreño JM, Simon V, Dawson K, Krammer F, Kent SJ. Impact of anti-PEG antibodies induced by SARS-CoV-2 mRNA vaccines. Nat Rev Immunol. 2023;23:135–6.
73. Koeberl D, Schulze A, Sondheimer N, Lipshutz GS, Geberhiwot T, Li L, et al. Interim analyses of a first-in-human phase 1/2 mRNA trial for propionic acidaemia. Nature. 2024;628:872–7.
74. Nogueira SS, Schlegel A, Maxeiner K, Weber B, Barz M, Schroer MA, et al. Polysarcosine-functionalized lipid nanoparticles for therapeutic mRNA delivery. ACS Appl Nano Mater. 2020;3:10634–45.
75. Xiao Y, Lian X, Sun Y, Sung YC, Vaidya A, Chen Z, Gupta A, Chatterjee S, Zheng L, Guerrero E, Wang X. High-density brush-shaped polymer lipids reduce anti-PEG antibody binding for repeated administration of mRNA therapeutics. Nat Mater. 2025;1-2.
76. Adams D, Suhr OB, Dyck PJ, Litchy WJ, Leahy RG, Chen J, et al. Trial design and rationale for APOLLO, a phase 3, placebo-controlled study of patisiran in patients with hereditary ATTR amyloidosis with polyneuropathy. BMC Neurol. 2017;17:181.
77. Jogdeo CM, Siddhanta K, Das A, Ding L, Panja S, Kumari N, et al. Beyond lipids: exploring advances in polymeric gene delivery in the lipid nanoparticles era. Adv Mater. 2024;36:2404608.
78. Zhao J, Liu T, Li Y, Yang X, Wang X, Fu Y, et al. Hepatocellular carcinoma epi-immunotherapy with polyion complex micelles co-delivering HDAC8 inhibitor and PD-L1 siRNA. Chem Eng J. 2025;503:158138.
79. Schneider P, Zhang H, Simic L, Dai Z, Schrörs B, Akilli-Öztürk Ö, et al. Multicompartment polyion complex micelles based on triblock Polypept(o)ides mediate efficient *siRNA* delivery to cancer-associated fibroblasts for antistromal therapy of hepatocellular carcinoma. Adv Mater. 2024;36:2404784.
80. Cabral H, Miyata K, Osada K, Kataoka K. Block copolymer micelles in nanomedicine applications. Chem Rev. 2018;118:6844–92.
81. Akingbola A, Adegbesan A, Adewole O, Adegoke K, Benson AE, Jombo PA, et al. The mRNA-1647 vaccine: a promising step toward the prevention of cytomegalovirus infection (CMV). Hum Vaccin Immunother. 2025;21:2450045.
82. Dilliard SA, Siegwart DJ. Passive, active and endogenous organ-targeted lipid and polymer nanoparticles for delivery of genetic drugs. Nat Rev Mater. 2023;8:282–300.
83. Tang S, Han EL, Mitchell MJ. Peptide-functionalized nanoparticles for brain-targeted therapeutics. Drug Deliv Transl Res. 2025;1-20.
84. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, et al. Analysis of nanoparticle delivery to tumours. Nat Rev Mater. 2016;1:16014.
85. Jester M, Haley RM, Billingsley MM, Figueroa-Espada C, Joseph RA, Han X, et al. Ionizable lipid nanoparticles with functionalized PEG-lipids increase retention in the tumor microenvironment. Mol Ther Methods Clin Dev. 2025. https://doi.org/10.1016/j.omtm.2025.101457.
86. Han EL, Tang S, Kim D, Murray AM, Swingle KL, Hamilton AG, et al. Peptide-functionalized lipid nanoparticles for targeted systemic mRNA delivery to the brain. Nano Lett. 2025;25:800–10.
87. Billingsley MM, Gong N, Mukalel AJ, Thatte AS, El-Mayta R, Patel SK, et al. In vivo mRNA CAR T cell engineering via targeted ionizable lipid nanoparticles with extrahepatic tropism. Small. 2024;20:e2304378.
88. Masarwy R, Breier D, Stotsky-Oterin L, Ad-El N, Qassem S, Naidu GS, et al. Targeted CRISPR/Cas9 lipid nanoparticles

elicits therapeutic genome editing in head and neck cancer. Adv Sci. 2025;12:2411032.
89. Tarab-Ravski D, Hazan-Halevy I, Goldsmith M, Stotsky-Oterin L, Breier D, Naidu GS, et al. Delivery of therapeutic RNA to the bone marrow in multiple myeloma using CD38-targeted lipid nanoparticles. Adv Sci. 2023;10:2301377.
90. Yong S-B, Ramishetti S, Goldsmith M, Diesendruck Y, Hazan-Halevy I, Chatterjee S, et al. Dual-targeted lipid nanotherapeutic boost for chemo-immunotherapy of cancer. Adv Mater. 2022;34:2106350.
91. Kampel L, Goldsmith M, Ramishetti S, Veiga N, Rosenblum D, Gutkin A, et al. Therapeutic inhibitory RNA in head and neck cancer via functional targeted lipid nanoparticles. J Control Release. 2021;337:378–89.
92. Rosenblum D, Gutkin A, Kedmi R, Ramishetti S, Veiga N, Jacobi AM, et al. CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Sci Adv. 2020;6:eabc9450.
93. Dammes N, Goldsmith M, Ramishetti S, Dearling JLJ, Veiga N, Packard AB, et al. Conformation-sensitive targeting of lipid nanoparticles for RNA therapeutics. Nat Nanotechnol. 2021;16:1030–8.
94. Veiga N, Goldsmith M, Diesendruck Y, Ramishetti S, Rosenblum D, Elinav E, et al. Leukocyte-specific siRNA delivery revealing IRF8 as a potential anti-inflammatory target. J Control Release. 2019;313:33–41.
95. Anthiya S, Öztürk SC, Yanik H, Tavukcuoglu E, Şahin A, Datta D, et al. Targeted siRNA lipid nanoparticles for the treatment of KRAS-mutant tumors. J Control Release. 2023;357:67–83.
96. Wang C, Xue Y, Markovic T, Li H, Wang S, Zhong Y, et al. Blood–brain-barrier-crossing lipid nanoparticles for mRNA delivery to the central nervous system. Nat Mater. 2025. https://doi.org/10.1038/s41563-024-02114-5.
97. Berrecoso G, Crecente-Campo J, Alonso MJ. Unveiling the pitfalls of the protein corona of polymeric drug nanocarriers. Drug Deliv Transl Res. 2020;10:730–50.
98. Pineiro-Alonso L, Rubio-Prego I, Lobyntseva A, González-Freire E, Langer R, Alonso MJ. Nanomedicine for targeting brain neurodegeneration: critical barriers and circadian rhythm considerations. Adv Drug Deliv Rev. 2025;222:115606.
99. Berrecoso G, Bravo SB, Arriaga I, Abrescia N, Crecente-Campo J, Alonso MJ. Controlling the protein corona of polymeric nanocapsules: effect of polymer shell on protein adsorption. Drug Deliv Transl Res. 2024;14:918–33.
100. Quattrini F, Berrecoso G, Crecente-Campo J, Alonso MJ. Asymmetric flow field-flow fractionation as a multifunctional technique for the characterization of polymeric nanocarriers. Drug Deliv Transl Res. 2021;11:373–95.
101. Dacoba TG, Anthiya S, Berrecoso G, Fernández-Mariño I, Fernández-Varela C, Crecente-Campo J, et al. Nano-oncologicals: a tortoise trail reaching new avenues. Adv Funct Mater. 2021;31:2009860.
102. Berrecoso G, Crecente-Campo J, Alonso MJ. Quantification of the actual composition of polymeric nanocapsules: a quality control analysis. Drug Deliv Transl Res. 2022;12:2865–74.
103. Abdulrahman R, Punnabhum P, Capomaccio R, Treacher K, Perrie Y, Rattray Z. Frit-inlet asymmetric flow field-flow fractionation for the analysis of lipid nanoparticle-protein interactions. J Chromatogr A. 2025;1743:465663.
104. Daramy K, Punnabhum P, Hussain M, Minelli C, Pei Y, Rattray NJW, et al. Nanoparticle isolation from biological media for protein corona analysis: the impact of incubation and recovery protocols on nanoparticle properties. J Pharm Sci. 2024;113:2826–36.
105. Abdulrahman R, Ibrahem H, Mahmood A, Treacher K, Vasey C, Capomaccio R, et al. Assessing lipid nanoparticle protein corona formation and cytocompatibility. Br J Pharm. 2023;8:S1–2.
106. Daramy K, Punnabhum P, Perrie Y, Rattray Z. The development of an AF4 characterization pipeline for polymeric nanoparticle-protein interactions. Br J Pharmacol. 2023;8:S1-2.
107. Daramy K, Walker J, Pei Y, Minelli C, Perrie Y, Rattray Z. Investigating the impact of shear flow on nanoparticle-protein interactions. Br J Pharmacol. 2022;7:S1-2.
108. Hunter TL, Bao Y, Zhang Y, Matsuda D, Riener R, Wang A, et al. In vivo CAR T cell generation to treat cancer and autoimmune disease. Science. 2025;388:1311–7.
109. Yan Y, Liu L, Xiong H, Miller JB, Zhou K, Kos P, et al. Functional polyesters enable selective siRNA delivery to lung cancer over matched normal cells. Proc Natl Acad Sci U S A. 2016;113:E5702-5710.
110. Yan Y, Xiong H, Zhang X, Cheng Q, Siegwart DJ. Systemic mRNA delivery to the lungs by functional polyester-based carriers. Biomacromol. 2017;18:4307–15.
111. Wang X, Liu S, Sun Y, Yu X, Lee SM, Cheng Q, et al. Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. Nat Protoc. 2023;18:265–91.
112. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing. Nat Nanotechnol. 2020;15:313–20.
113. Dilliard SA, Sun Y, Brown MO, Sung Y-C, Chatterjee S, Farbiak L, et al. The interplay of quaternary ammonium lipid structure and protein corona on lung-specific mRNA delivery by selective organ targeting (SORT) nanoparticles. J Control Release. 2023;361:361–72.
114. Álvarez-Benedicto E, Tian Z, Chatterjee S, Orlando D, Kim M, Guerrero ED, et al. Spleen SORT LNP generated *in situ* CAR T cells extend survival in a mouse model of lymphoreplete B cell lymphoma. Angew Chem Int Ed Engl. 2023;62:e202310395.
115. Vaidya A, Moore S, Chatterjee S, Guerrero E, Kim M, Farbiak L, et al. Expanding RNAi to kidneys, lungs, and spleen via selective ORgan targeting (SORT) siRNA lipid nanoparticles. Adv Mater. 2024;36:e2313791.
116. Kim M, Song ES, Chen JC, Chatterjee S, Sun Y, Lee SM, Wu S, Patel P, Tian Z, Kantor A, Wustman BA. Dual SORT LNPs for multi-organ base editing. Nat Biotechnol. 2025;1-9.
117. Dilliard SA, Cheng Q, Siegwart DJ. On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. Proc Natl Acad Sci U S A. 2021. https://doi.org/10.1073/pnas.2109256118.
118. Wei T, Cheng Q, Farbiak L, Anderson DG, Langer R, Siegwart DJ. Delivery of tissue-targeted scalpels: opportunities and challenges for in vivo CRISPR/Cas-based genome editing. ACS Nano. 2020;14:9243–62.
119. Álvarez-Benedicto E, Farbiak L, Márquez Ramírez M, Wang X, Johnson LT, Mian O, et al. Optimization of phospholipid chemistry for improved lipid nanoparticle (LNP) delivery of messenger RNA (mRNA). Biomater Sci. 2022;10:549–59.
120. Sun Y, Chatterjee S, Lian X, Traylor Z, Sattiraju SR, Xiao Y, et al. In vivo editing of lung stem cells for durable gene correction in mice. Science. 2024;384:1196–202.
121. Lin L, Su K, Zhang X, Shi L, Yan X, Fu Q, et al. A versatile strategy to transform cationic polymers for efficient and organ-selective mRNA delivery. Angew Chem Int Ed. 2025;64:e202500306.
122. Liu S, Cheng Q, Wei T, Yu X, Johnson LT, Farbiak L, et al. Membrane-destabilizing ionizable phospholipids for organ-selective mRNA delivery and CRISPR–Cas gene editing. Nat Mater. 2021;20:701–10.
123. Naidu GS, Yong S-B, Ramishetti S, Rampado R, Sharma P, Ezra A, et al. A combinatorial library of lipid nanoparticles for cell type-specific mrna delivery. Adv Sci. 2023;10:2301929.

124. Chatterjee S, Naidu GS, Hazan-Halevy I, Grobe H, Ezra A, Sharma P, et al. Therapeutic gene silencing of *CKAP5* leads to lethality in genetically unstable cancer cells. Sci Adv. 2023;9:eade4800.
125. Kon E, Levy Y, Elia U, Cohen H, Hazan-Halevy I, Aftalion M, et al. A single-dose F1-based mRNA-LNP vaccine provides protection against the lethal plague bacterium. Sci Adv. 2023;9:eadg1036.
126. Ramishetti S, Hazan-Halevy I, Palakuri R, Chatterjee S, Naidu Gonna S, Dammes N, et al. A combinatorial library of lipid nanoparticles for RNA delivery to leukocytes. Adv Mater. 2020;32:1906128.
127. Rampado R, Naidu GS, Karpov O, Goldsmith M, Sharma P, Ezra A, et al. Lipid nanoparticles with fine-tuned composition show enhanced colon targeting as a platform for mRNA therapeutics. Adv Sci. 2025;12:2408744.
128. Haley RM, Padilla MS, El-Mayta RD, Joseph RA, Weber JA, Figueroa-Espada CG, et al. Lipid nanoparticles for in vivo lung delivery of CRISPR-Cas9 ribonucleoproteins allow gene editing of clinical targets. ACS Nano. 2025;19:13790–804.
129. Hamilton AG, Swingle KL, Thatte AS, Mukalel AJ, Safford HC, Billingsley MM, et al. High-throughput in vivo screening identifies differential influences on mRNA lipid nanoparticle immune cell delivery by administration route. ACS Nano. 2024;18:16151–65.
130. Xue L, Hamilton AG, Zhao G, Xiao Z, El-Mayta R, Han X, et al. High-throughput barcoding of nanoparticles identifies cationic, degradable lipid-like materials for mRNA delivery to the lungs in female preclinical models. Nat Commun. 2024;15:1884.
131. Han X, Xu J, Xu Y, Alameh M-G, Xue L, Gong N, et al. In situ combinatorial synthesis of degradable branched lipidoids for systemic delivery of mRNA therapeutics and gene editors. Nat Commun. 2024;15:1762.
132. Hamilton AG, Swingle KL, Joseph RA, Mai D, Gong N, Billingsley MM, et al. Ionizable lipid nanoparticles with integrated immune checkpoint inhibition for mRNA CAR T cell engineering. Adv Healthc Mater. 2023;12:e2301515.
133. Thatte AS, Hamilton AG, Nachod BE, Mukalel AJ, Billingsley MM, Palanki R, et al. mRNA lipid nanoparticles for ex vivo engineering of immunosuppressive T cells for autoimmunity therapies. Nano Lett. 2023;23:10179–88.
134. Han X, Alameh M-G, Butowska K, Knox JJ, Lundgreen K, Ghattas M, et al. Adjuvant lipidoid-substituted lipid nanoparticles augment the immunogenicity of SARS-CoV-2 mRNA vaccines. Nat Nanotechnol. 2023;18:1105–14.
135. Han X, Zhang H, Butowska K, Swingle KL, Alameh M-G, Weissman D, et al. An ionizable lipid toolbox for RNA delivery. Nat Commun. 2021;12:7233.
136. Xue L, Zhao G, Gong N, Han X, Shepherd SJ, Xiong X, et al. Combinatorial design of siloxane-incorporated lipid nanoparticles augments intracellular processing for tissue-specific mrna therapeutic delivery. Nat Nanotechnol. 2025;20:132–43.
137. Xue L, Xiong X, Zhao G, Molina-Arocho W, Palanki R, Xiao Z, et al. Multiarm-assisted design of dendron-like degradable ionizable lipids facilitates systemic mRNA delivery to the spleen. J Am Chem Soc. 2025;147:1542–52.
138. Yoon I-C, Xue L, Chen Q, Liu J, Xu J, Siddiqui Z, et al. Piperazine-derived bisphosphonate-based ionizable lipid nanoparticles enhance mRNA delivery to the bone microenvironment. Angewandte Chemie International Edition. 2025;64:e202415389.
139. Mukalel AJ, Hamilton AG, Billingsley MM, Li J, Thatte AS, Han X, et al. Oxidized mrna lipid nanoparticles for in situ chimeric antigen receptor monocyte engineering. Adv Funct Mater. 2024. https://doi.org/10.1002/adfm.202312038.
140. Patel SK, Billingsley MM, Mukalel AJ, Thatte AS, Hamilton AG, Gong N, et al. Bile acid-containing lipid nanoparticles enhance extrahepatic mRNA delivery. Theranostics. 2024;14:1–16.
141. Han X, Gong N, Xue L, Billingsley MM, El-Mayta R, Shepherd SJ, et al. Ligand-tethered lipid nanoparticles for targeted RNA delivery to treat liver fibrosis. Nat Commun. 2023;14:75.
142. Swingle KL, Hamilton AG, Safford HC, Geisler HC, Thatte AS, Palanki R, et al. Placenta-tropic VEGF mRNA lipid nanoparticles ameliorate murine pre-eclampsia. Nature. 2025;637:412–21.
143. Safford HC, Shuler CF, Geisler HC, Thatte AS, Swingle KL, Han EL, et al. Probing the role of lipid nanoparticle elasticity on mRNA delivery to the placenta. Nano Lett. 2025;25:4800–8.
144. Safford HC, Swingle KL, Geisler HC, Hamilton AG, Thatte AS, Ghalsasi AA, et al. Orthogonal design of experiments for engineering of lipid nanoparticles for mRNA delivery to the placenta. Small. 2024;20:e2303568.
145. Geisler HC, Safford HC, Mitchell MJ. Rational design of nanomedicine for placental disorders: birthing a new era in women's reproductive health. Small. 2024;20:2300852.
146. Swingle KL, Safford HC, Geisler HC, Hamilton AG, Thatte AS, Billingsley MM, et al. Ionizable lipid nanoparticles for in vivo mRNA delivery to the placenta during pregnancy. J Am Chem Soc. 2023;145:4691–706.
147. Veiga N, Diesendruck Y, Peer D. Targeted nanomedicine: lessons learned and future directions. J Control Release. 2023;355:446–57.
148. Chatterjee S, Kon E, Sharma P, Peer D. Endosomal escape: a bottleneck for LNP-mediated therapeutics. Proc Natl Acad Sci USA. 2024;121:e2307800120.
149. Gilleron J, Querbes W, Zeigerer A, Borodovsky A, Marsico G, Schubert U, et al. Image-based analysis of lipid nanoparticle–mediated siRNA delivery, intracellular trafficking and endosomal escape. Nat Biotechnol. 2013;31:638–46.
150. Wittrup A, Ai A, Liu X, Hamar P, Trifonova R, Charisse K, et al. Visualizing lipid-formulated siRNA release from endosomes and target gene knockdown. Nat Biotechnol. 2015;33:870–6.
151. Chen Z, Tian Y, Yang J, Wu F, Liu S, Cao W, et al. Modular design of biodegradable ionizable lipids for improved mRNA delivery and precise cancer metastasis delineation in vivo. J Am Chem Soc. 2023;145:24302–14.
152. Padilla MS, Mrksich K, Wang Y, Haley RM, Li JJ, Han EL, et al. Branched endosomal disruptor (BEND) lipids mediate delivery of mRNA and CRISPR-Cas9 ribonucleoprotein complex for hepatic gene editing and T cell engineering. Nat Commun. 2025;16:996.
153. Borrajo ML, Alonso MJ. Using nanotechnology to deliver biomolecules from nose to brain — peptides, proteins, monoclonal antibodies and RNA. Drug Deliv Transl Res. 2022;12:862–80.
154. Borrajo ML, Lou G, Anthiya S, Lapuhs P, Álvarez DM, Tobío A, et al. Nanoemulsions and nanocapsules as carriers for the development of intranasal mRNA vaccines. Drug Deliv Transl Res. 2024;14:2046–61.
155. Borrajo ML, Quijano A, Lapuhs P, Rodriguez-Perez AI, Anthiya S, Labandeira-Garcia JL, et al. Ionizable nanoemulsions for RNA delivery into the central nervous system – importance of diffusivity. J Control Release. 2024;372:295–303.
156. Palanki R, Bose SK, Dave A, White BM, Berkowitz C, Luks V, et al. Ionizable lipid nanoparticles for therapeutic base editing of congenital brain disease. ACS Nano. 2023;17:13594–610.
157. Brown KM, Nair JK, Janas MM, Anglero-Rodriguez YI, Dang LTH, Peng H, et al. Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. Nat Biotechnol. 2022;40:1500–8.
158. Shumer-Elbaz M, Ad-El N, Chulanova Y, Brier D, Goldsmith M, Bismuth M, et al. Low-frequency ultrasound-mediated blood-brain barrier opening enables non-invasive lipid nanoparticle RNA delivery to glioblastoma. J Control Release. 2025;385:114018.

159. Kwak G, Grewal A, Slika H, Mess G, Li H, Kwatra M, et al. Brain nucleic acid delivery and genome editing via focused ultrasound-mediated blood-brain barrier opening and long-circulating nanoparticles. ACS Nano. 2024;18:24139–53.
160. Ogawa K, Kato N, Yoshida M, Hiu T, Matsuo T, Mizukami S, et al. Focused ultrasound/microbubbles-assisted BBB opening enhances LNP-mediated mRNA delivery to brain. J Control Release. 2022;348:34–41.
161. Chou MM, Henrich IC, Billingsley MM, Jain K, Mondal S, Quick LN, Young R, Nguyen N, Oliveira AM, Blobel GA, Mitchell MJ. Ubiquitin-specific protease 6 (USP6) mRNA lipid nanoparticles ignite anti-tumor immunity and suppress tumorigenesis in Ewing sarcoma, Mol. Cancer Ther. 2025.
162. English EP et al., Engineering CAR-T therapies for autoimmune disease and beyond. Sci. Transl. Med. 16, eado2084 (2024). https://doi.org/10.1126/scitranslmed.ado2084
163. Omo-Lamai S, Wang Y, Patel MN, Milosavljevic A, Zuschlag D, Poddar S, et al. Limiting endosomal damage sensing reduces inflammation triggered by lipid nanoparticle endosomal escape. Nat Nanotechnol. 2025;20:1285–97.
164. Lou G, Anderluzzi G, Schmidt ST, Woods S, Gallorini S, Brazzoli M, et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: the impact of cationic lipid selection. J Control Release. 2020;325:370–9.
165. Webb C, Ip S, Bathula NV, Popova P, Soriano SKV, Ly HH, et al. Current status and future perspectives on mRNA drug manufacturing. Mol Pharm. 2022;19:1047–58.
166. Roces CB, Lou G, Jain N, Abraham S, Thomas A, Halbert GW, et al. Manufacturing considerations for the development of lipid nanoparticles using microfluidics. Pharmaceutics. 2020;12:1095.
167. Hussain M, Binici B, O'Connor L, Perrie Y. Production of mrna lipid nanoparticles using advanced crossflow micromixing. J Pharm Pharmacol. 2024;76:1572–83.
168. McMillan C, Druschitz A, Rumbelow S, Borah A, Binici B, Rattray Z, et al. Tailoring lipid nanoparticle dimensions through manufacturing processes. RSC Pharm. 2024;1:841–53.
169. Binici B, Borah A, Watts JA, McLoughlin D, Perrie Y. The influence of citrate buffer molarity on mRNA-LNPs: exploring factors beyond general critical quality attributes. Int J Pharm. 2025;668:124942.
170. Forrester J, Davidson CG, Blair M, Donlon L, McLoughlin DM, Obiora CR, et al. Low-cost microfluidic mixers: are they up to the task? Pharmaceutics. 2025;17:566.
171. Ali-Jerman H, Zainab A-Q, Ashish M, Yvonne P, Tr J, Margaret M, et al. Enhancing transfection efficacy in glioma cells: a comparison of microfluidic versus manual polypropylenimine dendriplex formation. Int J Nanomed. 2024;19:12189–203.
172. Binici B, Rattray Z, Zinger A, Perrie Y. Exploring the impact of commonly used ionizable and pegylated lipids on mRNA-LNPs: a combined in vitro and preclinical perspective. J Control Release. 2025;377:162–73.
173. Davidson CG, Abdulrahman R, Punnabhum P, Cairns M, Rattray NJW, Capomaccio R, et al. The use of orthogonal analytical approaches to profile lipid nanoparticle physicochemical attributes. Nano Futures. 2024;8:035001.
174. Davidson CG, Abdulrahman R, Cairns M, Perrie Y, Rattray Z. Higher or lower?: the resolution of analytical pipelines for the evaluation of lipid nanoparticle critical quality attributes. Br J Pharm. 2023;8:S1-2.
175. Howard KA, Peer D. Providing the full picture: a mandate for standardizing nanoparticle-based drug delivery. Nanomedicine. 2013;8:1031–3.
176. Leong HS, Butler KS, Brinker CJ, Azzawi M, Conlan S, Dufés C, et al. On the issue of transparency and reproducibility in nanomedicine. Nat Nanotechnol. 2019;14:629–35.
177. Joyce P, Allen CJ, Alonso MJ, Ashford M, Bradbury MS, Germain M, et al. A translational framework to DELIVER nanomedicines to the clinic. Nat Nanotechnol. 2024;19:1597–611.
178. Anchordoquy T, Artzi N, Balyasnikova IV, Barenholz Y, La-Beck NM, Brenner JS, et al. Mechanisms and barriers in nanomedicine: progress in the field and future directions. ACS Nano. 2024;18:13983–99.
179. Gong N, Alameh M-G, El-Mayta R, Xue L, Weissman D, Mitchell MJ. Enhancing in situ cancer vaccines using delivery technologies. Nat Rev Drug Discov. 2024;23:607–25.