Model-Informed Drug Development Applications and Opportunities in mRNA-LNP Therapeutics

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The utilization of lipid nanoparticles (LNP) for encapsulating mRNA has revolutionized the field of therapeutics, enabling the rapid development of COVID-19 vaccines and cancer vaccines. However, the clinical development of mRNA-LNP therapeutics faces numerous challenges due to their complex mechanisms of action and limited clinical experience. To overcome these hurdles, Model-Informed Drug Development (MIDD) emerges as a valuable tool that can be applied to mRNA-LNP therapeutics, facilitating the evaluation of their safety and efficacy through the integration of data from all stages into appropriate modeling and simulation techniques. In this review, we provide an overview of current MIDD applications in mRNA-LNP therapeutics clinical development using *in vivo* data. A variety of modeling methods are reviewed, including quantitative system pharmacology (QSP), physiologically based pharmacokinetics (PBPK), mechanistic pharmacokinetics/pharmacodynamics (PK/PD), population PK/PD, and model-based meta-analysis (MBMA). Additionally, we compare the differences between mRNA-based therapeutics, small interfering RNA, and adeno-associated virus-based gene therapies in terms of their clinical pharmacology, and discuss the potential for mutual sharing of MIDD knowledge between these therapeutics. Furthermore, we highlight the promising future opportunities for applying MIDD approaches in the development of mRNA-LNP drugs. By emphasizing the importance of applying MIDD knowledge throughout mRNA-LNP therapeutics development, this review aims to encourage stakeholders to recognize the value of MIDD and its potential to enhance the safety and efficacy evaluation of mRNA-LNP therapeutics.

The COVID-19 pandemic highlighted the effectiveness and speed of mRNA-Lipid Nanoparticles (LNP) vaccine platforms developed by Pfizer/BioNTech and Moderna in addressing emerging crises.[1] Currently, three mRNA-LNP drugs have been approved by the Food and Drug Administration (FDA), all of which are prophylactic vaccines (**Table 1**). The advantages of mRNA-LNP therapeutics include safety, versatility, flexibility, fast manufacturing speed, and cost-effectiveness.[2] These attributes have broadened the potential applications of mRNA-LNP beyond infectious diseases, encompassing cancer vaccines, protein replacement therapy, antibody encoding, cellular reprogramming, and gene editing.[1]

Over the past three decades, significant progress has been made in nucleoside-based modification techniques and efficient carrier platforms like LNP systems, making mRNA-based therapeutics a reality. The structure of mRNA-LNP is depicted in **Figure 1**. The synthetic mRNA part is composed of five key elements, including 5′ Cap, 5′ and 3′ untranslated regions (UTRs), open reading frame (ORF), and Poly(A) sequence.[1] Various types of mRNA, such as self-amplifying mRNA, trans-amplifying mRNA, and circular mRNA, may have slightly different structures.[3] Efforts have been dedicated to enhancing mRNA stability and reducing immunogenicity through chemical modification, product purification, and sequence optimization.[2,4] The LNP formulations consist of helper lipids, cholesterol, a polyethylene glycol (PEG)-lipid, and an ionizable lipid. The ionizable lipid is crucial for the delivery of mRNA into cells and enhances the immune response for vaccines while maintaining low toxicity profiles. The PEG-lipid helps control particle size and prolong mRNA-LNP stability. Several stable and effective LNP technologies have been developed to protect mRNA from degradation and facilitate efficient delivery into cells and organs.[4]

Despite the remarkable progress in mRNA-LNP technology, challenges remain in clinical development. *In vivo* delivery obstacles, such as nuclease degradation, lack of stability, endosomal trapping, and immunotoxicity responses, hinder the efficacy of mRNA-LNP therapeutics.[5] Additionally, understanding dose-safety, dose-efficacy, and pharmacokinetics/pharmacodynamics (PK/PD) profiles in specific populations, like pediatrics or individuals with liver impairment, requires further investigation.[6] Model-informed drug development (MIDD) methods, which integrate data generated from all stages of development with quantitative approaches and modeling and simulation, can address these challenges.[7] Successful application of MIDD methods in

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mRNA-LNP development can enhance clinical trial efficiency, optimize dose selection, increase the likelihood of regulatory success, and accelerate the development process. This paper summarizes the current applications of MIDD in mRNA-LNP therapeutics and discusses the opportunities of using quantitative approaches and modeling and simulation methods to facilitate their future development.

CHALLENGES IN mRNA-LNP CLINICAL DEVELOPMENT

Complicated delivery mechanisms

One of the main challenges of mRNA-LNP development comes from the delivery of mRNA to the site of action. The mRNA-LNP utilizes an LNP delivery system to transport mRNA molecules into cells. Once inside the cells, the mRNA is translated into proteins that can have various therapeutic effects. This approach can be used to produce therapeutic proteins, such as antibodies or enzymes, to treat diseases. It can also be employed to deliver vaccine antigens to promote protective immune responses or to edit specific genes for targeted gene therapy (**Figure 2**). The clinical outcome of mRNA-LNP therapeutics depends on the complex interplay between pharmacological factors (biodistribution, cellular uptake, mRNA translation) and target modulations or immune responses that drive clinical efficacy and safety.[6] The development of MIDD and quantitative modeling platforms addresses these complexities by facilitating an improved understanding of PK/PD relationships, dose optimization, and efficacy and safety predictions for mRNA-LNP therapeutics (**Figure 2**).

Distinctive PK/PD characteristics

Another challenge in the clinical development of mRNA-based therapeutics is the lack of a clear understanding of the PK/PD relationship.[6] Unlike traditional small molecules or antibodies, where pharmacokinetics (PK) drives pharmacodynamics (PD) or efficacy, mRNA-LNP therapeutics exhibit significant delays between the delivery of RNA and the onset of pharmacologic responses. Such delays occur due to the multi-step process of LNP uptake and endosomal trafficking, mRNA release, and translation. Furthermore, LNPs can be recycled within the cell endosome and traffic back into circulation, often resulting in a second peak in the plasma PK profile for mRNA-LNPs.[8,9] Similar recycling has also been observed with small interfering RNA (siRNA)-LNPs.[10] These unique PK/PD characteristics were summarized in **Figure 2**. The pharmacological interpretation of LNP recycling may vary depending on the investigation methods. Studies using quantitation of the oligonucleotides as a means of tracking PK of the LNP detected oligonucleotides outside cells, which may indeed result from cellular leakage or turnover rather than re-packaged, functional delivery systems. Since recycled or released oligonucleotides are unlikely to retain effective delivery capabilities, their impact on the therapeutic outcome is minimal

Table 1 A summary of FDA-approved mRNA-LNP therapeutics until 2024

Drug/Trade name	Date of approval or authorization	Routes of administration	Indication and usage	Reference
mRNA-1345/mRESVIA	May 31, 2024	Intramuscular	To protect adults aged 60 years and older from lower respiratory tract disease caused by respiratory syncytial virus (RSV)	93
mRNA-1273/SPIKEVAX	December 18, 2020 (first emergency use authorization)	Intramuscular	For the prevention of COVID-19 disease in individuals 18 years of age or older	94
BNT162b2/COMIRNATY	August 23, 2021 (first emergency use authorization)	Intramuscular	For the prevention of COVID-19 disease in individuals 16 years of age and older. (The authorization of younger age groups came later)	95

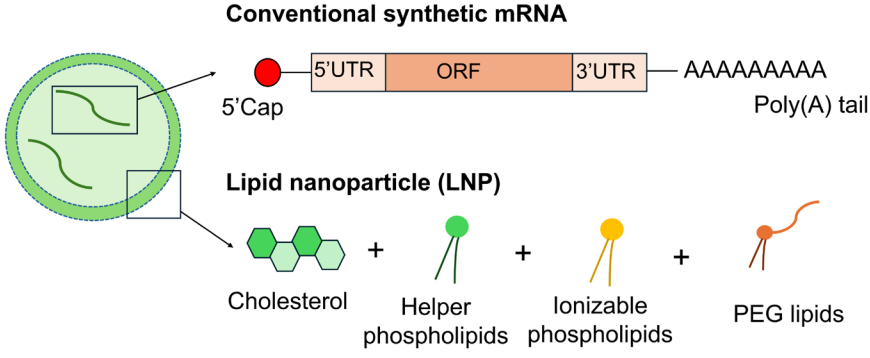

Figure 1 mRNA-LNP components. The conventional synthetic mRNA in the mRNA-LNP therapeutics has five key elements: 5′-Cap structure, 5′UTR, ORF, 3′UTR, and Poly(A) sequence tail. The LNP delivery system for mRNA generally is comprised of cholesterol, helper phospholipids, ionizable phospholipids, and a PEG-lipid. ORF, open reading frame; PEG, polyethylene glycol; UTR, untranslated region.

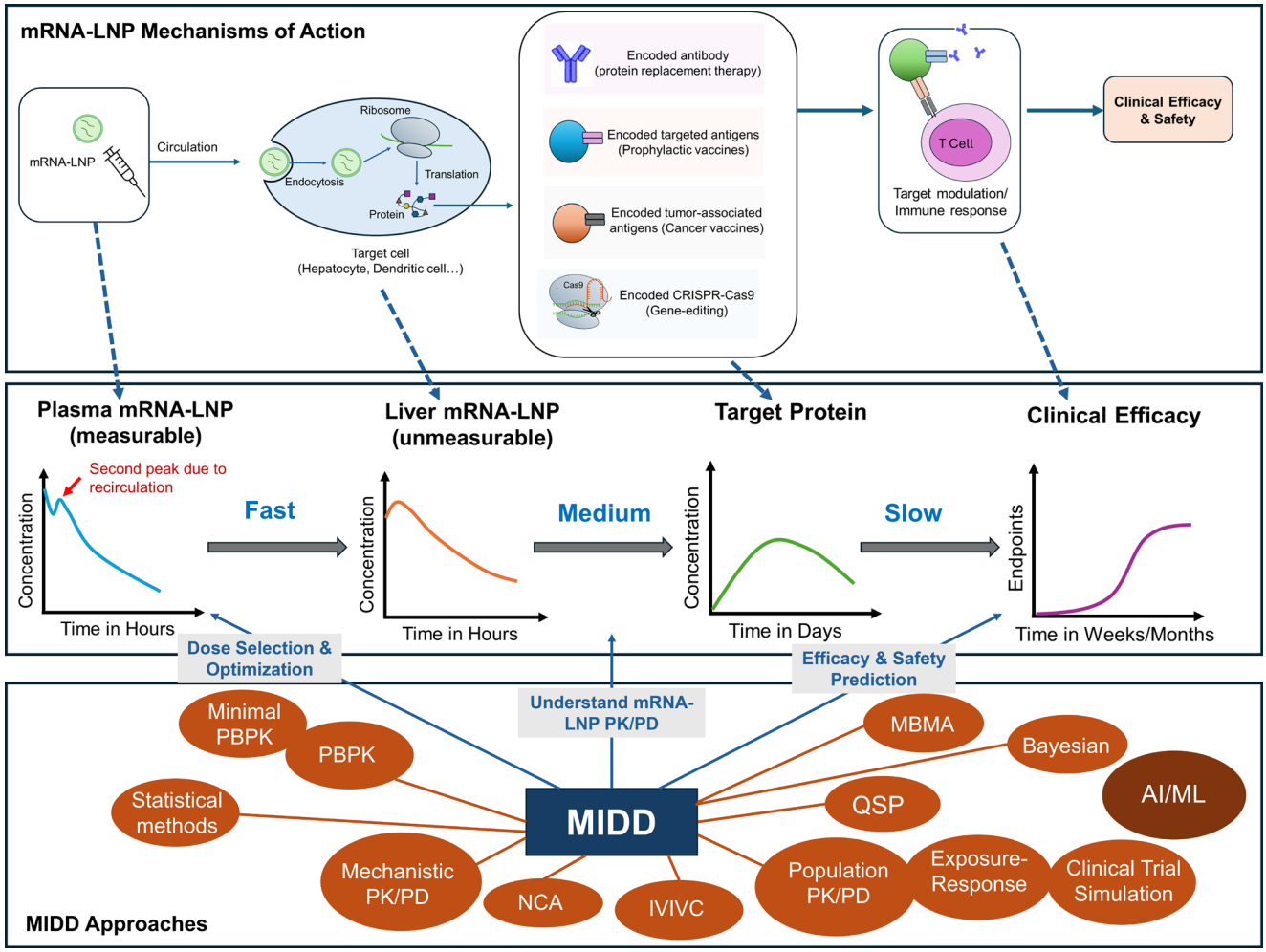

Figure 2 MIDD approaches could be applied to understand PK/PD, optimize dose, and predict efficacy and safety for mRNA-LNP therapeutics. AI/ML, artificial intelligence/machine learning; Cas9, CRISPR-associated protein 9; CRISPR, clustered regularly interspaced short palindromic repeats; IVIVC, *in vitro - in vivo* correlations; LNP, lipid nanoparticles; MBMA, model-based meta-analysis; MIDD, model-informed drug development; NCA, non-compartmental analysis; PBPK, physiologically based pharmacokinetics; PK/PD, pharmacokinetics/pharmacodynamics; QSP, quantitative system pharmacology.

or negligible.[11,12] Quantitative modeling techniques, such as stochastic models and ordinary differential equations, have been applied to describe the uptake, intracellular trafficking, and ultimate delivery of mRNA using cell data *in vitro*.[13–15] However, because of the limited *in vivo* and clinical data, the factors that determine the PK/PD relationships in humans remain largely unexplored.

Limited distribution beyond the liver

Despite the therapeutic potential in various diseases, the clinical translation of mRNA-LNP is hindered by the limited distribution to organs beyond the liver. The predominant distribution of mRNA-LNP in the liver, particularly after systemic administration, is attributed to the unique physiological characteristics of the liver.[16] First, the proteins in the plasma adsorb onto the LNPs, forming a protein corona that facilitates uptake by liver-resident macrophages.[17] Second, the liver's fenestrated endothelium allows LNP retention, further enhancing hepatic accumulation.[16] In contrast, non-hepatic organs such as the lungs, brain, and muscles possess low vascular permeability, significantly restricting LNP extravasation and limiting their clinical application to hepatic diseases and vaccines. Overcoming these barriers requires strategic modifications, such as reducing particle size, LNPs engineering,or using targeting ligands to enhance tissue-specific delivery of mRNA-LNPs.[17]

Lack of effective bioanalytical approaches

Despite progress in the clinical development of mRNA-LNP therapeutics, bioanalytical methods for studying mRNA-LNP *in vivo* biodistribution remain underdeveloped and loosely defined by current regulations.[18] Assay validation efforts have primarily focused on preclinical biodistribution studies to characterize the presence, persistence, and clearance of mRNA-LNP in target tissues.[18] Quantitative whole-body autoradiography (QWBA) is considered the industry standard for preclinical biodistribution, enabling assessment of mRNA products, their carrier components, and degradation products using radioactive isotope technology.[19] Mass spectrometry (MS)-based assays, such as liquid chromatography mass spectrometry (LC–MS)/MS, are used to quantify synthetic

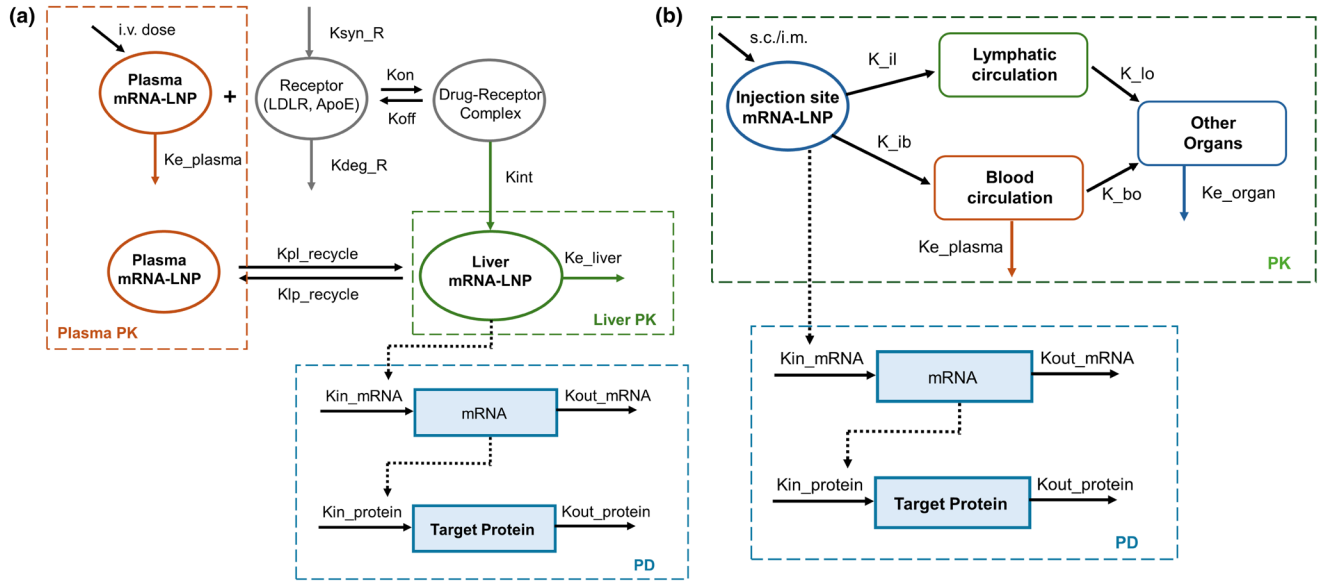

Figure 3 Semi-mechanistic PK/PD model for mRNA-LNP therapeutics via i.v. (**a**) or s.c./i.m. administration route (**b**). ApoE, Apolipoprotein E; i.m., intramuscular; i.v., intravenous; K_bo, rate constant of drug distribution from blood to other organs; K_ib, rate constant of drug distribution from injection site to blood circulation; K_il, rate constant of drug distribution from injection site to lymphatic circulation; K_lo, rate constant of drug distribution from lymphatic system to other organs; K_pt, mRNA generation rate constant; Kdeg_R, receptor degradation rate constant; Ke_liver, liver elimination rate constant; Ke_organ, elimination rate constant in other organs; Ke_plasma, plasma elimination rate constant; Kin_mRNA, target mRNA release rate constant; Kin_protein, target protein synthesis rate constant; Kint, internalization rate constant of drug-receptor complex; Koff, the dissociation rate constant for drug-receptor complex; Kon, the association rate constant of drug to receptor; Kout_mRNA, degradation rate constant of target mRNA; Kout_protein, degradation rate constant of target protein; Kpl_recycle and Klp_recycle, LNP recirculation rate constant; Ksyn_R, receptor synthesis rate constant; LDLR, low-density lipoprotein receptor; s.c., subcutaneous.

lipid components in plasma or tissue samples, providing insights into the PK of mRNA-LNP.[20,21] Hybridization techniques like fluorescence *in situ* hybridization (FISH) are the gold standard for single-molecular RNA visualization and could be used to detect mRNA biodistribution.[22,23] Other methods, including reverse transcription quantitative polymerase chain reaction (RT-qPCR), enzyme-linked immunosorbent assay (ELISA), fluorescence imaging, and bioluminescence, have also been explored for mRNA-LNP bioanalytical studies.[24] However, validated bioanalytical assays for evaluating mRNA-LNP PK/PD in humans remain limited. This gap presents significant challenges in generating robust data to support the development of MIDD platforms and enhance their predictive utility.

MIDD APPLICATIONS IN mRNA-LNP THERAPEUTICS DEVELOPMENT

As detailed in subsequent sections, most MIDD approaches for mRNA-LNP therapeutics fall into two categories: (a) semi-mechanistic PK/PD approaches and (b) more detailed QSP approaches. Depending on the key development question being addressed, these approaches are associated with their own advantages and disadvantages. For each of these approaches, we schematically summarize the key features that have been explored in the MIDD literature for mRNA-LNP therapeutics. **Figure 3** illustrates semi-mechanistic PK/PD models for mRNA-LNP, while **Figure 4** presents the QSP model schematics. Considering the distinct biodistribution patterns associated with different administration routes, intravenous and subcutaneous/intramuscular delivery are depicted separately. For intravenous administration, the liver is the primary organ of distribution, with the potential recirculation of LNPs from the liver to the systemic circulation highlighted in the figures.[9,25–27] In contrast, subcutaneous or intramuscular injections result in mRNA-LNP being largely retained at the injection site, where they initiate immune responses via antigen-presenting cells (APC).[28,29] These visual summaries serve as a foundational guide, with subsequent sections providing detailed examples and discussions from the literature on MIDD applications in mRNA-LNP development.

MIDD approaches to understand the PK/PD of mRNA-LNP therapeutics

LNP was originally developed as delivery vehicles for siRNA, and their success paved the way for the subsequent development of mRNA-based therapeutics. MIDD approaches have been widely established for siRNA-LNP therapeutics, including FDA-approved patisiran,[30,31] facilitating current understanding of biodistribution, clearance, and dose–response relationships.[32] Given the shared PK and delivery mechanisms of siRNA-LNP and mRNA-LNP systems, insights into LNP PK and biodistribution can be derived from established MIDD approaches for siRNA-LNP therapies. Leveraging insights from siRNA-LNP therapies is particularly important when considering the limited clinical data for mRNA-LNP therapies.[33,34] A survey of published clinical PK data of LNP-RNA therapies indicates multiple phases of plasma

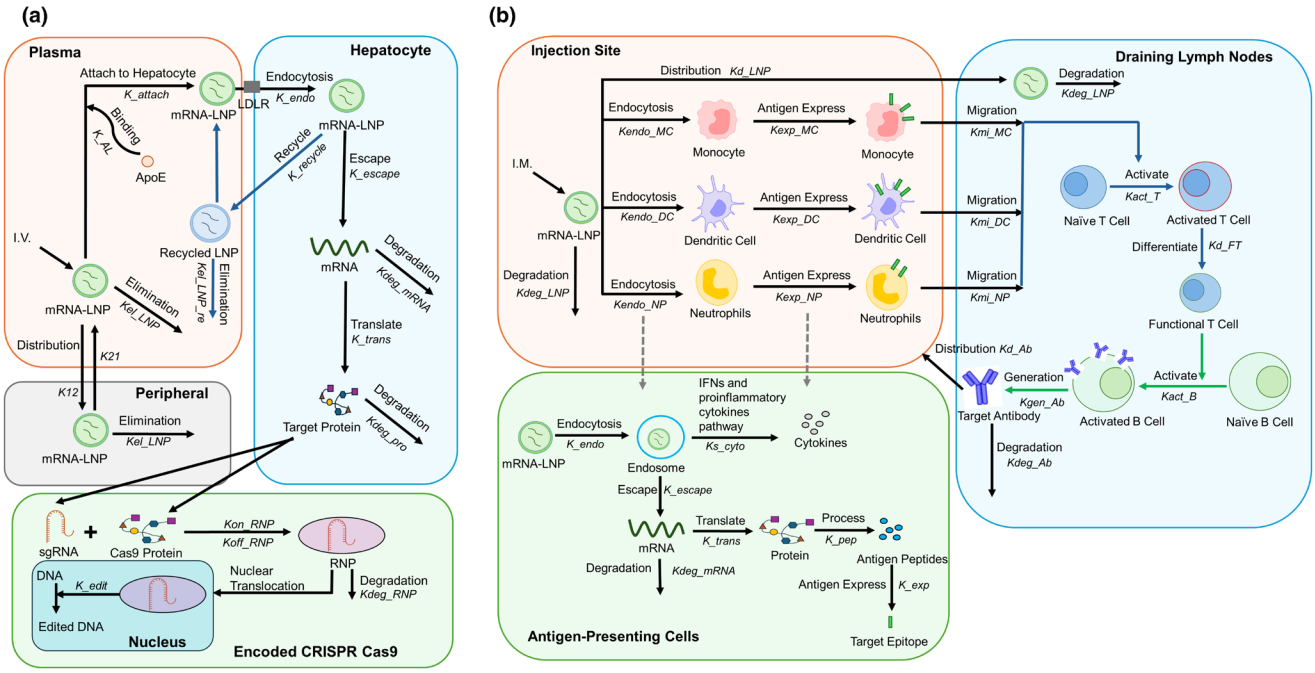

Figure 4 Schematic plots of quantitative systems pharmacology model for mRNA-LNP therapeutics. (**a**) Quantitative system pharmacology model of mRNA-LNP encode CRISPR Cas9 via i.v. administration. (**b**) Quantitative system pharmacology model of mRNA-LNP vaccine via i.m. administration. ApoE, Apolipoprotein E; Cas9, CRISPR-associated protein 9; i.m., intramuscular; i.v., intravenous; IFN, interferon; LDLR, low-density lipoprotein receptor; RNP, Ribonucleoprotein; sgRNA, single-guide RNA.

clearance.[35–37] This has been characterized as a rapid clearance from the plasma and corresponding uptake in the liver, followed by a redistribution from the liver back to the plasma and a subsequent slower clearance through the liver. Three-compartment population PK models have been utilized to capture this phenomenon.[9,30] Mechanisms for LNP processing in mRNA-LNPs are similar. Upon administration of mRNA-LNPs, the rapid dissociation of PEG-lipid from LNP allows apolipoprotein E (ApoE) binding to the LNP, which interacts with cell surface receptors and triggers receptor-mediated endocytosis. Physiologically based pharmacokinetic (PBPK) modeling has shown that organs expressing varying levels of LNP-related receptors have different rates and fractions of adsorption.[38] The low-density lipoprotein receptor (LDLR), a key receptor facilitating receptor-mediated endocytosis of LNPs, is primarily expressed in the liver compared to other organs. This helps explain why the liver is the organ where mRNA-LNPs are predominantly distributed after intravenous administration.[39] Other receptors may compensate for LDLR in its absence but are beyond the scope of this review. Upon internalization, LNPs can undergo endosomal recycling, lysosomal degradation, or endosomal escape, with only the latter process resulting in mRNA release into the cytosol for translation into proteins. These processes are subject to intricate regulatory control with varying timescales and can be influenced by the specific physicochemical properties of LNP formulations. Thus, concentration-time profiles of LNPs measured in blood, while routinely accessible, are a poor surrogate for pharmacologically relevant intracellular mRNA concentrations from e.g. the liver, which are infeasible to collect in practice. As a result, the application of standard phenomenological exposure-response methods for LNP-mRNAs might not apply. This has motivated the development of more mechanistic modeling approaches to further study the efficiency of LNP endosomal escape. For example, recently published PBPK models have been able to describe the impact of biophysical properties of different ionizable lipids and nanoparticle coatings on cellular uptake and endosomal escape rates.[38] Furthermore, large amounts of mRNA still exist in the LNPs that went through endosomal recycling and will redistribute to circulation. A coupled Quantitative Systems Pharmacology (QSP) -PBPK model has been developed to describe such mRNA-LNP kinetics and the protein expression dynamics in protein replacement therapy, highlighting that LNP recycling and mRNA redistribution to circulation can result in a second peak in the mRNA PK profile.[25] This model represents liver uptake by assuming that mRNA-LNPs are taken up from the vascular space into the liver's Kupffer cell compartment, and a hepatocyte sub-compartment also takes up mRNA-LNPs from the interstitial space. The model also assumes that mRNA-LNP distribution to non-hepatic organs occurs through the vascular space, driven by tissue-specific volumetric flow rates. This model accurately captured the experimental data; however, it does not account for ApoE adsorption and LDLR-mediated endocytosis in the liver uptake.

Target proteins, once translated, need to redistribute in the system to enable target modulation or stimulate immune responses for clinical efficacy. The PBPK modeling approach was applied to estimate substantial differences in target protein expression across organs, which are likely related to the differences in endothelial surface area.[40] This relationship supports the potential of PBPK

models for scaling mRNA-LNP protein expression from mice to higher species.

Unlike small molecules, LNPs are approximately 100 megadaltons in size. Consequently, they cannot passively diffuse across the cell membrane and rely on endocytosis for cellular uptake.[41] This brings additional challenges for LNP delivery, including PEG-lipid shedding, which can influence circulation time and biodistribution[42,43]; opsonization, where serum proteins mark LNPs for immune clearance[44]; and the effects of cationic lipids, which play a role in endosomal escape but may also induce toxicity.[45] One of the objectives of studying the PK/PD of mRNA-LNPs is to identify the factors that influence their delivery, and thus to overcome these barriers and enhance the clinical effectiveness of mRNA-LNPs. The QSP-PBPK model simulations revealed that the interplay of the rate of LNP degradation and endosomal escape plays a critical role in LNP delivery and target protein expression.[40] Additionally, a QSP model was developed to illustrate how mRNA vaccines induce immune responses through intramuscular administration for protection against viral infection. The model also suggested that optimizing the LNP delivery system can improve cellular uptake and overall efficacy.[46] This model incorporated dendritic cells, monocytes, and neutrophils as APCs and described their migration, as well as the stimulation of T cell and B cell kinetics to produce targeted antibodies. Sensitivity analyses of the QSP model indicated that APC recruitment and mRNA-LNP uptake by APCs are the most critical factors determining drug efficacy and should be prioritized in drug optimization.

MIDD approaches in optimizing dosing for mRNA-LNP therapeutics

MIDD approaches have been widely used to establish dose–response relationships for therapeutics such as small molecules or monoclonal antibodies, using preclinical and clinical data.[7] These approaches enable the selection of an optimal drug dose and dosing regimen that balance efficacy and toxicity. However, for mRNA-LNP therapeutics, which target a broad range of biological pathways and can elicit immune responses, determining clear dose–response relationships for optimizing drug dose and dosing regimens is challenging. Therefore, it is crucial to employ quantitative dose-optimization strategies from a clinical pharmacology perspective to enhance the development of mRNA-LNP therapeutics.

MIDD platforms have been proposed as useful tools to optimize dose and dosing regimen selection for vaccines, including mRNA-LNP vaccines.[28,47] A key challenge in assessing dose–response relationships for mRNA-LNP vaccines is that, in addition to temporal discordance between mRNA-LNP administration and antigenic protein translation, vaccines induce a cellular and humoral immune cascade that is sensitive to the properties of the translated antigen and LNP formulation. The ionizable lipid component used in LNP formulations for mRNA vaccines also has intrinsic adjuvant activity and can promote both innate and adaptive immune responses.[48,49] In human-derived dendritic cells (DC) and monocytes, empty LNP particles were found to promote DC maturation and induce cytokine production, with a lesser immune response in cells derived from older (>65 years old) individuals.[50] Excessive immune stimulation can also hinder mRNA translation, thus necessitating the selection of a dose that balances adequately protective antigenic protein generation with adjuvant-induced immune stimulation.[51] Moreover, given that vaccine-induced immune responses can ultimately impact the biodistribution of both antigenic protein and mRNA-LNP, dose–response relationships for multi-dose vaccine regimens can be complex. Recent mechanistic modeling approaches to address these challenges have involved adapting prior QSP models of monoclonal antibodies (mAb) immunogenicity[52] and coupling them to QSP models of mRNA-LNP-based protein translation. These models incorporated vaccine doses (implicitly accounting for antigen and adjuvant exposure) and immune response dynamics to identify vaccine dosing schedules that maximize immunogenicity while mitigating unwanted reactogenicity. For example, a QSP model by Giorgi *et al*. predicted a bell-shaped dose response curve for the primary series COVID-19 mRNA vaccine booster dose with a predicted optimal dosing interval of 7–8 weeks.[28] Such models have also been used to predict optimal vaccination schedules for different populations, such as healthy individuals and immunocompromised cancer patients, to minimize vulnerability to breakthrough infections while maintaining vaccine efficacy.[53]

MIDD approaches can be beneficial in guiding the first-in-human (FIH) dose selection for mRNA-LNP therapeutics during early stages of development. Using model-informed scaling factors that account for physiological differences across species outperforms empirical scaling factors in FIH dose selection.[7] In the context of mRNA-LNP protein replacement therapies, MIDD approaches have been employed to optimize FIH doses by developing translational PK/PD models based on preclinical data.[9,26] These models represent the target protein expression rate as a linear function of plasma mRNA concentration with a delay in effect. Allometric scaling[54] was applied to mRNA PK model parameters to extrapolate across species, and target protein clearance could also be allometrically scaled based on protein characteristics. These models are fit-for-purpose and effective for translating preclinical data into clinical predictions. However, they do not account for detailed processes like LNP uptake by circulating monocytes and tissue-resident macrophages. Such complexities are more effectively addressed using a QSP modeling approach. A QSP model was developed by Apgar *et al*. to calibrate preclinical data of modified mRNA-LNP therapy.[8] This model considers the varying production rates and clearances of target proteins between animals and humans, enabling the selection of appropriate FIH doses. In the context of mRNA-LNP gene therapy, a QSP model was developed to describe LNP delivery of mRNA for CRISPR-associated protein 9 (Cas9) and a guide RNA.[27] The model illustrated the intracellular processes involved in translating the Cas9 mRNA to protein, performing gene editing, and downstream formation of protein from the edited gene. The model was calibrated using FIH PK/PD data and subsequently used to support dose selection for a dose expansion cohort. A recently published QSP model incorporated additional LNP delivery processes, including LNP binding to opsonins in the liver vasculature, LDLR-mediated endocytosis in the liver, and mRNA and sgRNA disposition via exocytosis and clathrin-mediated endocytosis.[55] This model effectively captured

the biodistribution and dose-exposure of mRNA-LNP encoded with the CRISPR-Cas9 modality.

The LNP delivery has proven effective for Chimeric Antigen Receptor (CAR)-T cell manufacturing, offering advantages over traditional viral, electroporation, or lipofection methods by reducing immunogenicity, toxicity, and improving safety.[56,57] The mRNA-LNP-engineered CAR-T cells are transient, as mRNA-based therapies do not alter the host cell's genetic information.[58] Consequently, repeated dosing is necessary, and understanding the dose-CAR protein expression relationship is crucial for optimizing CAR-T cell engineering. A translational PK-PD model was developed using a target-mediated drug disposition (TMDD) approach to characterize LNP binding and internalization in CD8+ T cells, along with an Emax model to describe mRNA translation into CAR proteins.[59] As experimental and clinical data continue to accumulate, additional MIDD approaches are being developed to deepen the understanding of this process.

MIDD approaches in predicting the efficacy of mRNA-LNP therapeutics

Population-based MIDD methods, such as population pharmacokinetics (PopPK) or population PK/PD (PopPK/PD) modeling, facilitate clinical drug development by integrating data from diverse populations. These methods enhance drug efficacy prediction by considering interindividual variability and covariates, leading to safer and more effective treatments.[7] Population models have been developed to predict the long-term durability of the immune response to mRNA COVID vaccines.[60,61] These models fitted clinical data on vaccine-induced neutralizing antibodies and cytokine levels under different doses of mRNA vaccines using the non-linear mixed effects (NLME) method and evaluated covariate effects on model-predicted immune responses. The model predictions emphasized the importance of a recommended third booster dose to maintain efficacy levels.[60,61] Moreover, population-based modeling methods can predict the mRNA vaccine efficacy in diverse populations, including both healthy individuals and those with immunosuppression, and can optimize dosing strategies accordingly.[61] A recent publication developed a semi-mechanistic immunostimulatory/immunodynamic (IS/ID) model for the COVID-19 mRNA vaccine to guide pediatric dose selection.[62] The model successfully captured pooled neutralizing antibody titer data from multiple phase II/III clinical studies and described B cell activation and antibody production.[62] However, it was unable to account for more complex immune responses, such as T cell activation or antigen-presenting cell migration, due to limited clinical data on the biodistribution and PK of mRNA vaccines. This gap highlights an important area for future research.

Understanding of biomarker dynamics can then be linked to predictive models of clinical outcomes. This can be particularly valuable for the development of vaccines with established immunological correlates of protection (CoP), that is, immunological biomarkers that are predictive of vaccine efficacy. For cases where sufficient evidence for putative immunological CoP exists, model-based approaches have been developed with the aim of using emerging individual-level immunogenicity data from early clinical trials to inform key development milestones, such as go/no-go criteria for advancing vaccine candidates.[63,64] Recently developed CoP-based methods have been shown to provide more precise efficacy estimates compared to approaches that rely only on incidence rates from clinical trial readouts.[64] Such approaches can be used to inform confidence in the clinical rationale for novel mRNA vaccines, especially when benchmarking against data from licensed traditional vaccine comparators and thus significantly de-risk pivotal clinical trial decisions.

Relatedly, model-based meta-analysis (MBMA) has been a useful MIDD tool that integrates data from multiple studies to develop a quantitative model that can be used to predict the efficacy and safety of a treatment across different populations and study designs.[65] The MBMA model established by Kandala *et al.* integrated published rhesus macaque and human data to quantify the relationship between immunogenicity (serum neutralized titers) and vaccine protection for COVID-19 mRNA vaccines.[66] The model was used to assess covariates and predict the clinical efficacy of new vaccine candidates against different variants of COVID-19.

MIDD approaches have also been employed to evaluate potential factors that may affect efficacy in mRNA-LNP therapeutics. For example, LNP uptake is influenced by PEG-lipid dissociation and concurrent protein adsorption.[4] Previous research has indicated that the presence of circulating antibodies specifically binding to PEG, known as anti-PEG antibodies, can compromise the integrity of LNP-mRNA formulations, leading to premature mRNA release and triggering the release of complement activation products.[67] PBPK and minimal-PBPK models have been developed to assess the impact of anti-PEG antibodies on the PK/PD of PEGylated therapeutics.[68,69] These models have suggested that high titers of pre-existing anti-PEG antibodies are likely to impact the PK profiles of PEGylated drugs. However, this hypothesis still requires further validation in humans, as studies by Kent *et al.* and Guerrini *et al.* did not find significant correlations between the PEG-specific antibody and the clearance of the mRNA COVID vaccine in PK.[70,71] While clinical evidence suggests anti-PEG antibodies do not impact vaccine PK, the absence of pharmacometrics models presents a research opportunity. Ongoing studies on anti-PEG antibodies in other mRNA-LNP therapeutics may provide further insights.

Despite the use of a different delivery method, population approaches used in the context of adeno-associated virus-based gene therapy may also be relevant for mRNA-LNP therapeutics.[72] Example analyses of long-term factor IX (FIX) concentrations resulting from hemophilia B gene therapy include linear mixed effects models, providing predictions for the durability of response over time.[73] The MIDD models successfully accounted for both within- and between-participant variability in factor IX activity levels and predicted that over 80% of future participants would exhibit durable factor IX activity without the need for the administration of prophylactic FIX replacement products 25.5 years post-infusion. Another example population analysis was able to account for these factors, using a longitudinal model combining a two-compartment model describing the translation of mRNA to FIX and a three-compartment model describing the pharmacodynamics of FIX.[74] Accounting for the full FIX profile

resulting from gene therapy, this model was then used to predict the required dose and frequency of recombinant FIX to achieve similar efficacy.

UNLOCKING THE POTENTIAL OF MIDD IN THE FUTURE DEVELOPMENT OF mRNA-LNP THERAPEUTICS

Unraveling interindividual variability in mRNA-LNP cancer vaccines using MIDD

Clinical trials are currently underway to develop cancer vaccines utilizing mRNA-LNP technology.[2] However, a significant challenge in these treatments is the wide variability in patient responses to mRNA-LNP cancer vaccines. This variability in outcomes may arise from diverse factors, including mRNA delivery, translational efficiency, immune responses, and tumor heterogeneity. To comprehend the sources of interindividual variability in mRNA-LNP cancer vaccine outcomes, MIDD approaches can be employed. Previous studies employing population modeling tools have explored variability in treatment responses among colorectal cancer patients at both the organ and individual levels under targeted therapies.[75,76] These efforts provide a valuable foundation for applying MIDD methodologies to comprehend the sources of interindividual variability in mRNA-LNP cancer vaccine outcomes. In novel therapeutic areas with limited data availability, MBMA serves as a valuable MIDD approach to integrate sparse information and improve the understanding of these emerging treatments.[65] For example, an MBMA study has been conducted for CAR-T cell therapy to evaluate the relationship between cellular kinetics and patient responses across various tumor types.[77] Similar studies could be undertaken for mRNA cancer vaccines to support the design of personalized dosing strategies and regimens. By leveraging MIDD approaches, we can gain valuable insights into the interindividual variability in mRNA-LNP cancer vaccine responses, enabling personalized vaccines for patients.

Reducing the risks of mRNA-LNP therapeutics toxicities using MIDD

As more mRNA-LNP therapeutics enter clinical development, early identification of unacceptable toxicity will become increasingly important. Like biologics, mRNA-based therapeutics designed to produce target proteins may lead to toxicities.[78] Additionally, mRNA drugs delivered via lipid nanoparticles (LNPs), which include buffer and small-molecule lipid components, also have the potential to induce adverse effects.[79] Common clinical safety concerns for mRNA-LNP therapeutics include liver and spleen toxicities due to the pronounced hepatic and splenic biodistribution of LNP-mRNA, adverse immunological responses such as reactogenicity observed with mRNA vaccines, and inflammasome activation with subsequent cytokine release triggered by LNP components.[79–81] Quantitative systems toxicology (QST) models are increasingly being used to predict toxicity risks in drug development for novel modalities.[82] For example, a quantitative model was developed to characterize cytokine dynamics following bispecific antibody administration in solid tumors, enabling the prediction of cytokine release syndrome risk.[83] Similarly, we can anticipate the development of QST models tailored for mRNA-LNP therapeutics to support toxicity and safety prediction. Furthermore, post-marketing surveillance data from clinically approved mRNA vaccines provide an opportunity to apply MIDD approaches for safety monitoring. Previously, pharmacovigilance studies employing disproportionality analysis have been used to investigate tumor lysis syndrome associated with monoclonal antibodies in multiple myeloma patients.[84] Similar MIDD approaches could be adapted for mRNA-LNP pharmacovigilance studies to enhance safety monitoring and risk mitigation.

The liver plays a crucial role in the delivery, translation, and induction of immune responses in mRNA-LNP therapeutics. The majority of LNPs bind apolipoprotein E (ApoE) and adsorb in the liver through low-density lipoprotein receptor (LDLR) after systemic delivery.[4] Understanding the delivery, translation, and immune responses of mRNA-LNP therapeutics in individuals with liver impairment is essential for ensuring their efficacy and safety. Population PK/PD modeling and simulation approaches can incorporate data from various populations receiving mRNA-LNP drugs and help interpret the variability of PK/PD in subjects with liver impairment. Such approaches are widely exemplified in anti-infective drug development.[85–87] Additionally, mechanistic PK/PD and PBPK models can be developed to account for transporter deficiency and hepatocyte differences in populations with liver impairment. For example, PBPK models have been used to simulate changes in drug transporters to study the impact of liver cirrhosis on pharmacokinetics.[88] These models can predict the altered efficacy and potential adverse effects in populations with liver dysfunction, thus facilitating the optimization of mRNA-LNP doses for special populations.

Applying PBPK models to enhance organ-specific delivery of mRNA-LNP therapeutics

One of the key challenges in the development of mRNA-LNP therapeutics lies in achieving efficient delivery to organs beyond the liver. More recently, extensive efforts are being made to develop LNP formulations and delivery strategies with greater selectivity for organs other than the liver, such as the lung and spleen, through non-LDLR-mediated internalization mechanisms.[89] PBPK models hold great potential in integrating the physiological characteristics of different organs with the physicochemical properties of the drug to understand the biodistribution of drugs in various organs.[90] However, the full potential of PBPK models in the context of mRNA-LNP therapeutics has yet to be fully explored. One promising future direction is to develop nanoparticle property-disposition relationships using PBPK models to optimize the *in vivo* biodistributions of LNPs.[91] For example, Li *et al*. developed a permeability-limited PBPK model to analyze the disposition of five PEGylated polyacid nanoparticles with varying PEG content. This model demonstrated the potential to predict the biodistribution of nanoparticles with different chemical compositions.[92] By incorporating organ-specific factors such as tissue composition, blood flow, and cellular interactions into these PBPK models, we can gain insights into the factors influencing the distribution and uptake of mRNA-LNP therapeutics in different organs. This research will provide strategies to improve

the targeted delivery of LNPs to specific organs, ultimately enhancing the efficacy and therapeutic potential of mRNA-LNP therapeutics.

CONCLUSIONS

The mRNA-LNP therapeutics are a relatively new class of drugs that have shown great promise in various diseases. With many mRNA-LNP therapeutics clinical trials ongoing, the application of MIDD approaches presents significant opportunities in understanding mRNA-LNP PK/PD, optimizing dose selection and dosing regimens, as well as offering valuable insights into the efficacy and safety. Furthermore, MIDD can facilitate the integration of sparse data and aid in the interpretation of limited information; therefore, it can de-risk the development of mRNA-LNP therapeutics in special populations. Overall, MIDD applications in mRNA-LNP therapeutics hold immense promise for improving patient outcomes and accelerating the development of novel and effective therapeutic interventions.

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CONFLICT OF INTEREST

J.Z. is a former employee of Pfizer and may own stock in Pfizer. All other authors are current employees of Pfizer and may own stock in Pfizer.

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