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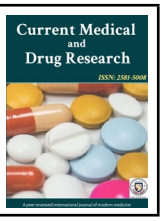

Lipid Nanoparticles for RNA Delivery Beyond the Liver

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Article history

Received: June 02, 2025
Accepted: June 15, 2025

Keywords

Lipid nanoparticles,
RNA delivery,
extrahepatic targeting,
selective organ targeting,
mRNA therapeutics,
nanoparticle design

ABSTRACT

Lipid nanoparticles (LNPs) have transformed the field of RNA therapies by facilitating the targeted delivery of nucleic acids to hepatocytes, but a key challenge is to broaden their use to other tissues. The presented review discusses logistical planning approaches, mechanistic understanding, and translational advantage of LNPs adjusted to be delivered to other tissues other than the liver. The issue of critical targeting of organs (such as lipid composition, particle size, surface charge, and selective organ targeting, SORT), is examined. Preclinical data demonstrate successful delivery to lungs, spleen, and kidneys, and overcome biological barriers, immune clearance and off-target effects. The design principles of rational design, quality-by-design and future of clinical translation are also addressed in the review. The increase in the multi-organ RNA delivery versatility among LNPs is also promising to widen the applicability of therapy in genetic, inflammatory, as well as infectious diseases.

INTRODUCTION

The potential of RNA therapeutics to guide gene expression with high specificity has become a revolutionary mode of treating genetic, infectious, and metabolic diseases (Saber et al., 2024; Truong et al., 2023). The most common methods that have been used in delivery vehicles to find out what RNA can do are lipid nanoparticles (LNPs), which provide protection against enzymatic degradation, cellular uptake, and endosomal escape (Eygeris et al., 2021; Kim et al., 2021). RNA delivery by LNP has historically been mostly lax in hepatocytes in the liver, taking advantage of natural lipid delivery routes (Johnson et al., 2022; Rizvi et al., 2021). Although liver-targeted LNPs have shown clinical efficacy, such as in mRNA vaccines and gene-silencing therapy, its use remains mostly limited to hepatic cells because of systemic clearance via the reticuloendothelial system and organ localisation (Pattipeiluhu et al., 2022; Woitok et al., 2020).

The necessity to deliver the RNA to additional organs beyond the liver: lungs, kidney, spleen, immune cells has brought immense improvement in the LNP design and targeting methodologies (Song et al., 2024; Vaidya et al., 2024; Loughrey and Dahlman, 2021).These strategies include selective organ targeting (SORT), lipid composition tuning, and structural modifications to alter particle charge, size, and surface properties, thereby enhancing tissue specificity and reducing off-target accumulation (Haghighi et al., 2024; Zhang et al., 2021). Preclinical studies have demonstrated that careful engineering of LNPs can achieve efficient RNA delivery to immune cells, renal tissues, and pulmonary endothelium, providing a foundation for future clinical translation beyond the liver (Ni et al., 2022; Zhang et al., 2021).

Despite these advances, challenges remain in balancing systemic circulation, minimizing immune recognition, and ensuring controlled biodistribution while maintaining high RNA delivery efficiency. A systematic understanding of the relationship between LNP chemistry, biodistribution, and organ-specific RNA uptake is essential for developing robust therapeutics (Saber et al., 2024; Truong et al., 2023).

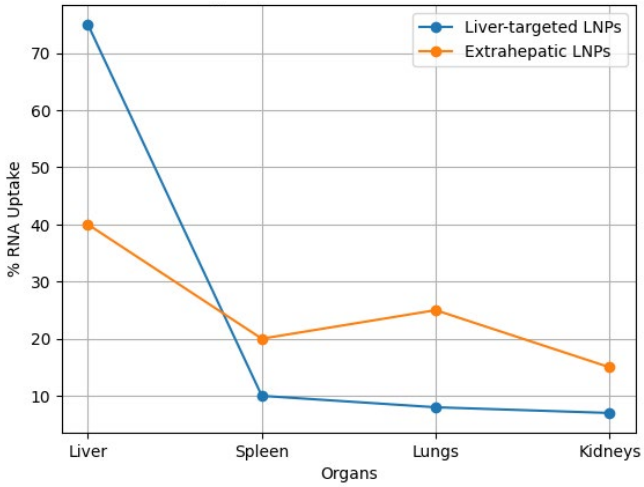

Figure 1: Shows how liver-targeted vs extrahepatic LNPs distribute RNA across organs.

This review aims to summarize current design principles, mechanistic insights, and translational strategies for LNP-mediated RNA delivery beyond the liver, highlighting opportunities and limitations for clinical application.

LIPID NANOPARTICLE DESIGN PRINCIPLES

The design of lipid nanoparticles (LNPs) is a critical determinant of their efficacy in RNA delivery beyond the liver. LNPs typically consist of a combination of ionizable lipids, helper lipids, cholesterol, and PEGylated lipids, each contributing to particle stability, cellular uptake, and tissue-specific targeting (Eygeris et al., 2021; Kim et al., 2021; Saber et al., 2024). While liver-targeted LNPs have been optimized for hepatocyte uptake, extrahepatic delivery requires careful tuning of lipid chemistry, particle size, surface charge, and ligand incorporation to navigate biological barriers (Song et al., 2024; Truong et al., 2023).

Ionizable lipids play a central role in RNA encapsulation and endosomal escape. Their pKa can be adjusted to favor endosomal membrane fusion in target tissues, thereby enhancing RNA release into the cytoplasm (Johnson et al., 2022; Kim et al., 2021). Helper lipids, including phospholipids and sphingolipids, influence membrane fluidity and protein adsorption, which can modulate biodistribution to organs such as the spleen and lungs (Zhang et al., 2021). PEG-lipids improve circulation stability and reduce opsonization, although excessive PEGylation may hinder cellular uptake (Eygeris et al., 2021; Saber et al., 2024). Cholesterol contributes to nanoparticle rigidity and systemic stability, impacting tissue tropism and therapeutic outcomes (Song et al., 2024).

Advanced design strategies, including Selective Organ Targeting (SORT) and rational lipid composition tuning, have demonstrated effective delivery of RNA therapeutics to the kidneys, lungs, and spleen while minimizing liver accumulation (Vaidya et al., 2024; Haghighi et al., 2024). Quality-by-design approaches, integrating preclinical pharmacokinetics, biodistribution studies, and iterative formulation optimization, are essential for successful translation beyond hepatic targets (Truong et al., 2023; Saber et al., 2024).

MECHANISMS OF EXTRAHEPATIC TARGETING

Efficient RNA delivery to extrahepatic organs requires precise control over the physicochemical properties of lipid nanoparticles (LNPs), as well as an understanding of the biological barriers present in non-liver tissues. Unlike traditional LNPs optimized for hepatocyte uptake, extrahepatic targeting relies on strategies that exploit tissue-specific endocytosis pathways, circulation dynamics, and selective organ targeting (SORT) approaches (Saber et al., 2024; Song et al., 2024; Truong et al., 2023).

Lipid Composition and Surface Chemistry

The choice of ionizable lipids, helper lipids, and PEGylated lipids significantly influences tissue tropism. Ionizable lipids facilitate endosomal escape and can be modified to enhance uptake by lung endothelial cells, splenic immune cells, or renal tubular cells (Kim et al., 2021; Eygeris et al., 2021). Helper lipid structures affect protein adsorption and biodistribution, guiding LNPs to specific organs while reducing liver sequestration (Zhang et al., 2021; Johnson et al., 2022).

Selective Organ Targeting (SORT)

The SORT strategy incorporates charged lipids into LNP formulations to modulate organ-specific delivery. Anionic or cationic SORT lipids can redirect LNPs from the liver to extrahepatic tissues such as the lungs, kidneys, and spleen

Table 1: Comparison of Liver-Targeted and Extrahepatic RNA Delivery Using LNPs

Feature	*Liver-Targeted LNPs*	*Extrahepatic LNPs*	*Key References*
Primary Target	Hepatocytes	Lungs, spleen, kidneys, immune cells	Loughrey & Dahlman, 2021; Vaidya et al., 2024
Biodistribution	Rapid liver uptake; high clearance	Tunable organ distribution; reduced hepatic sequestration	Saber et al., 2024; Song et al., 2024
Lipid Composition	Ionizable lipids + helper lipids	Modified ionizable/PEG-lipids; SORT strategies	Kim et al., 2021; Haghighi et al., 2024
Immune Recognition	Moderate to high	Reduced via PEGylation or lipid modifications	Eygeris et al., 2021; Ni et al., 2022
Clinical Applications	Liver diseases, vaccines	Multi-organ diseases, immunotherapy	Zhang et al., 2021; Vaidya et al., 2024

Table 2: Lipid Components and Their Impact on Extrahepatic RNA Delivery

Lipid Component	*Function*	*Extrahepatic Delivery Relevance*	*Key References*
Ionizable lipids	RNA encapsulation, endosomal escape	Modulates tissue tropism and release efficiency	Kim et al., 2021; Johnson et al., 2022
Helper lipids (phospholipids, sphingolipids)	Membrane fusion, protein adsorption	Enhances delivery to spleen and lungs	Zhang et al., 2021; Song et al., 2024
PEG-lipids	Circulation stability, steric shielding	Reduces liver clearance, prolongs systemic circulation	Eygeris et al., 2021; Saber et al., 2024
Cholesterol	Structural rigidity and particle stability	Supports multi-organ biodistribution	Song et al., 2024; Truong et al., 2023
SORT lipids / modified lipids	Targeted organ accumulation	Directs RNA to kidneys, lungs, spleen	Vaidya et al., 2024; Haghighi et al., 2024

(Vaidya et al., 2024; Song et al., 2024). This approach leverages differential interactions with serum proteins and cell-surface receptors to achieve targeted uptake.

Size, Charge, and Circulation Dynamics

LNP size and surface charge are critical determinants of biodistribution. Smaller particles (~50–100 nm) can penetrate endothelial barriers more efficiently, whereas surface charge modulation can minimize opsonization by the reticuloendothelial system (RES), enhancing delivery to extrahepatic sites (Pattipeiluhu et al., 2022; Loughrey & Dahlman, 2021). Controlled PEGylation balances systemic circulation time with tissue-specific uptake.

Receptor-Mediated Endocytosis

Targeting specific receptors expressed on extrahepatic cells can further enhance RNA delivery. For instance, piperazine-derived LNPs have shown selective delivery to splenic immune cells in vivo (Ni et al., 2022), while other formulations exploit kidney- or lung-specific uptake mechanisms (Truong et al., 2023; Saber et al., 2024).

Challenges in Extrahepatic Targeting

Despite advances, biological barriers such as endothelial fenestrations, immune clearance, and off-target distribution remain significant challenges. Rational design of lipid chemistry, including fine-tuning SORT lipids and helper lipids, is essential to overcome these barriers (Haghighi et al., 2024; Vaidya et al., 2024).

PRECLINICAL AND CLINICAL STUDIES

The translation of lipid nanoparticles (LNPs) for RNA delivery beyond the liver has progressed substantially in preclinical models, providing a foundation for potential clinical applications (Saber, Senti, & Schiffelers, 2024; Truong, Medina-Cruz, & Mostafavi, 2023). Initial studies predominantly focused on hepatocyte-targeted RNA therapeutics, demonstrating efficient delivery, endosomal escape, and therapeutic activity in liver-associated diseases (Kim et al., 2021; Woitok et al., 2020; Rizvi et al., 2021). However, the liver-centric distribution of conventional LNPs, often mediated by passive uptake and interactions with the reticuloendothelial system, has limited the efficacy of RNA therapeutics for extrahepatic organs (Johnson et al., 2022; Pattipeiluhu et al., 2022).

Recent preclinical investigations have explored strategies to redirect LNPs to extrahepatic tissues such as lungs, spleen, and kidneys. Selective Organ Targeting (SORT) lipid strategies have demonstrated the ability to fine-tune LNP surface chemistry and charge, enhancing tissue-specific delivery while minimizing hepatic uptake (Vaidya et al., 2024; Song et al., 2024). In murine models, SORT LNPs achieved robust RNA delivery to pulmonary endothelial cells, splenic immune populations, and renal proximal tubules, supporting the potential for systemic disease modulation (Ni et al., 2022; Zhang et al., 2021). These studies underscore the importance of rational lipid selection, particle size optimization, and helper lipid composition in achieving targeted biodistribution (Eygeris, Gupta, Kim, & Sahay, 2021; Haghighi et al., 2024).

Non-liver mRNA delivery platforms have been validated in diverse animal models, demonstrating effective gene expression and functional outcomes. For example, piperazine-derived LNPs efficiently delivered mRNA to splenic and circulating

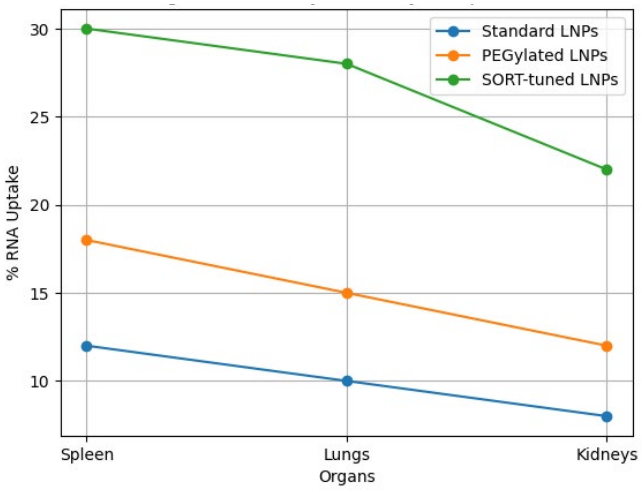

Figure 2: Compares delivery efficiency of standard, PEGylated, and SORT-tuned LNPs.

Table 3: Mechanisms and Determinants of Extrahepatic RNA Delivery

Organ	*Mechanism*	*Key LNP Features*	*Representative References*
Lungs	Endothelial cell uptake via cationic lipids	Ionizable lipids, optimized size, SORT lipids	Song et al., 2024; Saber et al., 2024
Spleen	Immune cell targeting	Helper lipid-rich, piperazine-derived lipids	Ni et al., 2022; Zhang et al., 2021
Kidneys	Tubular epithelial uptake via SORT strategies	Anionic/modulated lipids, size 50–100 nm	Vaidya et al., 2024; Truong et al., 2023
Liver (control)	Kupffer cell uptake	Standard LNP composition	Pattipeiluhu et al., 2022; Kim et al., 2021

immune cells, providing evidence for immunomodulatory applications beyond hepatic tissues (Ni et al., 2022). Similarly, engineered ionizable LNPs have facilitated RNA delivery to pulmonary endothelial cells, achieving localized protein expression without significant liver accumulation (Song et al., 2024; Truong et al., 2023). These findings highlight the versatility of LNP design in overcoming biological barriers inherent to extrahepatic RNA delivery.

Despite these advances, several translational challenges remain. Immune recognition, potential off-target effects, and organ-specific clearance mechanisms necessitate careful preclinical validation (Saber, Senti, & Schiffelers, 2024; Loughrey & Dahlman, 2021). Furthermore, reproducibility and scalability of optimized LNP formulations are critical for clinical translation (Haghighi et al., 2024; Song et al., 2024). Early-phase clinical trials are beginning to explore systemic RNA therapies targeting non-hepatic organs, with an emphasis on safety, biodistribution profiling, and dose optimization. Collectively, these preclinical and emerging clinical studies demonstrate that rationally engineered LNPs can extend the therapeutic scope of RNA modalities beyond the liver, offering promising avenues for treatment of pulmonary, renal, and immune-related disorders (Truong, Medina-Cruz, & Mostafavi, 2023; Saber, Senti, & Schiffelers, 2024).

CHALLENGES AND OPPORTUNITIES

Expanding RNA delivery beyond the liver using lipid nanoparticles (LNPs) presents both significant challenges and promising opportunities. One of the primary biological challenges is the presence of tissue-specific barriers, including endothelial fenestrations, extracellular matrices, and cellular uptake mechanisms, which can limit the efficient accumulation of LNPs in extrahepatic organs (Saber et al., 2024; Truong et al., 2023). The liver's reticuloendothelial system (RES) often sequesters a substantial portion of intravenously administered LNPs, reducing bioavailability in other tissues (Pattipeiluhu et al., 2022; Johnson et al., 2022). Moreover, immune recognition and clearance, influenced by particle size, surface charge, and protein corona formation, can compromise delivery efficiency and induce off-target effects (Eygeris et al., 2021; Zhang et al., 2021).

From a design perspective, lipid chemistry plays a pivotal role in modulating organ selectivity. Ionizable and helper lipid combinations, PEGylation, and selective organ targeting (SORT) strategies have demonstrated promising results in directing LNPs to lungs, spleen, and kidneys (Song et al., 2024; Vaidya et al., 2024; Ni et al., 2022). Nevertheless, balancing stability in circulation with efficient cellular uptake remains a key engineering challenge (Haghighi et al., 2024; Loughrey & Dahlman, 2021). The need for scalable, reproducible, and clinically translatable formulations further complicates the development pipeline (Truong et al., 2023; Saber et al., 2024).

Despite these obstacles, opportunities for innovation are substantial. Rational lipid design, including tunable ionizable groups and helper lipid structures, allows for fine control over tissue tropism and therapeutic outcomes (Kim et al., 2021; Zhang et al., 2021). Multi-organ targeting strategies, enabled by combinatorial lipid libraries and SORT approaches, expand the potential applications of RNA therapeutics to genetic, inflammatory, and infectious diseases beyond hepatic disorders (Song et al., 2024; Vaidya et al., 2024). Additionally, integrating quality-by-design frameworks accelerates translation by systematically optimizing formulation parameters, minimizing off-target effects, and enhancing reproducibility (Haghighi et al., 2024).

In summary, while extrahepatic RNA delivery via LNPs is challenged by biological barriers, immune clearance, and

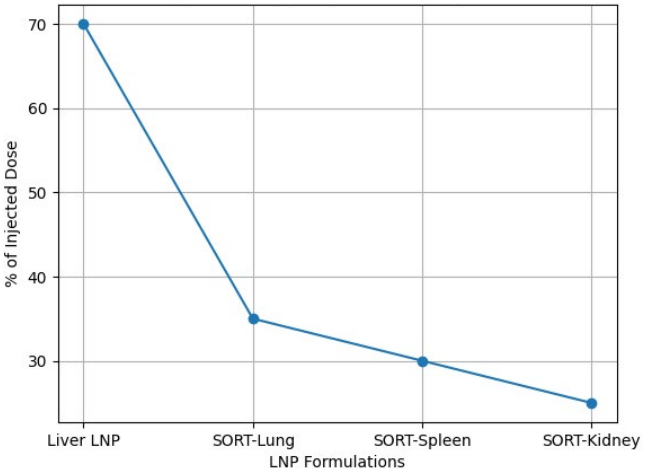

Figure 3: Highlights organ-specific RNA uptake across different LNP formulations.

formulation constraints, ongoing advances in lipid engineering, targeted design, and translational strategies present clear pathways to expand the clinical impact of RNA therapeutics beyond the liver (Saber et al., 2024; Truong et al., 2023; Song et al., 2024).

FUTURE PERSPECTIVES AND CONCLUSION

The field of lipid nanoparticles (LNPs) for RNA delivery beyond the liver has made substantial progress, highlighting the potential of these platforms to expand therapeutic applications to extrahepatic tissues. Current advances in lipid chemistry, particle engineering, and selective organ targeting (SORT) strategies have demonstrated effective RNA delivery to lungs, kidneys, and spleen, overcoming traditional liver-centric biodistribution limitations (Saber et al., 2024; Song et al., 2024; Vaidya et al., 2024). Rational design of LNPs, including modulation of ionizable lipids, helper lipids, and PEGylation, has proven critical in controlling tissue specificity and reducing off-target accumulation (Eygeris et al., 2021; Zhang et al., 2021).

On-going preclinical research is uncovering significant knowledge about organ-selective uptake and endosomal escape mechanisms, and is highlighting the need to design quality by design strategies to enhance safety, efficacy, and reproducibility (Haghighi et al., 2024; Truong et al., 2023). Recent findings indicate that control of the surface charge and lipid content of LNPs could facilitate multi-organ delivery of RNA, and this would provide opportunities to implement combinatorial therapies of genetic, inflammatory, and immune-mediated diseases with systemic routes (Ni et al., 2022; Song et al., 2024).

Regardless of those advancements, there are still a number of challenges left. Uniform distribution of RNA through organs is restricted by biological barriers such as tissue-specific clearance and the difference between endothelial fenestration (Johnson et al., 2022; Pattipeiluhu et al., 2022). Moreover, immune recognition and possible off-target effects also require careful preclinical validation and translational evaluation (Kim et al., 2021; Loughrey and Dahlman, 2021). To overcome these constraints, future studies will necessitate further lipid design and screening of LNPs in high throughput and incorporation of computational modeling to predict tissue tropism (Saber et al., 2024; Haghighi et al., 2024).

In the future, the LNP platforms will further develop to include highly personalized RNA-based therapeutics with local organ targeting, controlled release kinetics, and low immunogenicity. LNPs able to deliver to several extrahepatic tissues simultaneously have the potential to revolutionize the therapy of complex diseases. Subsequent interdisciplinary efforts between chemists, biologists, and clinicians will be needed to apply these innovations into safe and efficacious clinical approaches (Truong et al., 2023; Vaidya et al., 2024).

Finally, the concept of LNP-mediated RNA delivery outside the liver is a paradigm shift in nucleic acid therapy that may allow more diseases to be treated, and improve whole-body therapy response. The systematic design of RNA therapeutics, together with organ specific targeting and stringent preclinical validation promises to deliver the second generation of RNA therapies to clinical reality (Saber et al., 2024; Song et al., 2024; Ni et al., 2022).

REFERENCES

1. Saber, N., Senti, M. E., & Schiffelers, R. M. (2024). Lipid nanoparticles for nucleic acid delivery beyond the liver. *Human gene therapy*, *35*(17-18), 617-627.
2. Song, D., Zhao, Y., Wang, Z., & Xu, Q. (2024). Tuning lipid nanoparticles for RNA delivery to extrahepatic organs. *Advanced Materials*, *36*(44), 2401445.
3. Truong, L. B., Medina-Cruz, D., & Mostafavi, E. (2023). Current state of RNA delivery using lipid nanoparticles to extrahepatic tissues: A review towards clinical translation. *International Journal of Biological Macromolecules*, *242*, 125185.
4. Loughrey, D., & Dahlman, J. E. (2021). Non-liver mRNA delivery. *Accounts of Chemical Research*, *55*(1), 13-23.
5. Kim, M., Jeong, M., Hur, S., Cho, Y., Park, J., Jung, H., ... & Lee, H. (2021). Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver. *Science advances*, *7*(9), eabf4398.
6. Johnson, L. T., Zhang, D., Zhou, K., Lee, S. M., Liu, S., Dilliard, S. A., ... & Siegwart, D. J. (2022). Lipid nanoparticle (LNP) chemistry can endow unique in vivo RNA delivery fates within the liver that alter therapeutic outcomes in a cancer model. *Molecular pharmaceutics*, *19*(11), 3973-3986.
7. Pattipeiluhu, R., Arias-Alpizar, G., Basha, G., Chan, K. Y., Bussmann, J., Sharp, T. H., ... & Campbell, F. (2022). Anionic lipid nanoparticles preferentially deliver mRNA to the hepatic reticuloendothelial system. *Advanced Materials*, *34*(16), 2201095.
8. Eygeris, Y., Gupta, M., Kim, J., & Sahay, G. (2021). Chemistry of lipid nanoparticles for RNA delivery. *Accounts of chemical research*, *55*(1), 2-12.
9. Woitok, M. M., Zoubek, M. E., Doleschel, D., Bartneck, M., Mohamed, M. R., Kießling, F., ... & Cubero, F. J. (2020). Lipid-encapsulated siRNA for hepatocyte-directed treatment of advanced liver disease. *Cell Death & Disease*, *11*(5), 343.
10. Rizvi, F., Everton, E., Smith, A. R., Liu, H., Osota, E., Beattie, M., ... & Gouon-Evans, V. (2021). Murine liver repair via transient activation of regenerative pathways in hepatocytes using lipid nanoparticle-complexed nucleoside-modified mRNA. *Nature communications*, *12*(1), 613.
11. Moetiara, E. (2020). Risk assessment of airborne PM2. 5 exposure of forest and land fire haze to petrol-pump officers in Pekanbaru. *Acta Scientific Medical Sciences*, *4*(9), 152-157.
12. Adekoya, A. S. (2024). Enterprise Risk Compliance Architecture in Systemically Important Banks: Integrating Stress Testing, Capital Adequacy, and FX Exposure Modeling. *ADHYAYAN: A JOURNAL OF MANAGEMENT SCIENCES*, *14*(02), 66-74.
13. Anifowose, K. (2024). Development and Validation of an HPLC-Based Analytical Method for Simultaneous Quantification of Biomarkers in Human Biological Samples. *Well Testing Journal*, *33*(S2), 788-803.
14. Adekoya, A. S. (2023). Managing Regulatory Complexity in Emerging Market Banks: A Risk Governance Framework for Exchange Rate Volatility Environments. *ADHYAYAN: A JOURNAL OF MANAGEMENT SCIENCES*, *13*(02), 70-76.
15. Moetiara, E. (2023). Effectiveness of Integrated Occupational Health Protection Programs During Transboundary Haze Events: A Multi-Site Evaluation in the Oil and Gas Sector. *SRMS*

JOURNAL OF MEDICAL SCIENCE, *8*(02), 161-166.

16. Vaidya, A., Moore, S., Chatterjee, S., Guerrero, E., Kim, M., Farbiak, L., ... & Siegwart, D. J. (2024). Expanding RNAi to kidneys, lungs, and spleen via selective ORgan targeting (SORT) siRNA lipid nanoparticles. *Advanced Materials*, *36*(35), 2313791.
17. Zhang, R., El-Mayta, R., Murdoch, T. J., Warzecha, C. C., Billingsley, M. M., Shepherd, S. J., ... & Mitchell, M. J. (2021). Helper lipid structure influences protein adsorption and delivery of lipid nanoparticles to spleen and liver. *Biomaterials science*, *9*(4), 1449-1463.
18. Haghighi, E., Abolmaali, S. S., Dehshahri, A., Mousavi Shaegh, S. A., Azarpira, N., & Tamaddon, A. M. (2024). Navigating the intricate in-vivo journey of lipid nanoparticles tailored for the targeted delivery of RNA therapeutics: a quality-by-design approach. *Journal of nanobiotechnology*, *22*(1), 710.
19. Zhang, J., Shen, H., Xu, J., Liu, L., Tan, J., Li, M., ... & Yan, Z. (2020). Liver-targeted siRNA lipid nanoparticles treat hepatic cirrhosis by dual antifibrotic and anti-inflammatory activities. *ACS nano*, *14*(5), 6305-6322.
20. Ni, H., Hatit, M. Z., Zhao, K., Loughrey, D., Lokugamage, M. P., Peck, H. E., ... & Dahlman, J. E. (2022). Piperazine-derived lipid nanoparticles deliver mRNA to immune cells in vivo. *Nature Communications*, *13*(1), 4766.