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LETTERS

Lipid based self-assembled nanostructures for therapeutic delivery applications

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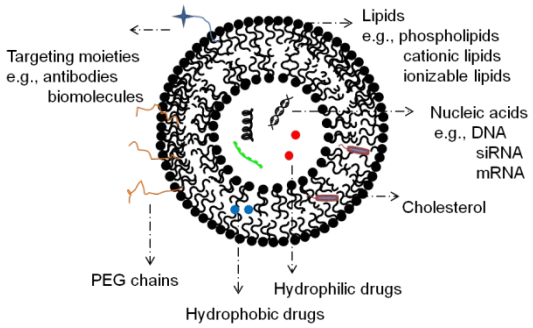
Review

ABSTRACT The evolution of lipid nanoparticles (LNPs) has been remarkably interesting and in beneficent directions for food and health industries working towards human well being. Since the discovery of the first-generation lipid based self-assembled nanostructures, i.e., liposomes in the 1960s, it has witnessed significant advances in their development and distinctive potential in different application domains. Based on the composition and structure, these lipid-based structures have varied from liposome to lipid nanoparticles, e.g., solid lipid nanoparticles (SLNs) & nanostructured lipid carriers (NLCs) to overcome certain limitation pertaining to their use in different fields. The outstanding application of LNPs as therapeutic delivery systems has made them key players to treat different human disorders including the fatal cancers. Their life-saving global contribution has recently been witnessed in the form of mRNA vaccines against deadly COVID-19. They have also significantly served purpose in other domains such as biomedical imaging, cosmetics, nutrition, and agriculture. Their prominent role is in the area of anticancer therapy as delivery vectors for nucleic acids and drugs. Some issues with respect to the cellular delivery of drugs and genes, such as circulation time and stability have been somewhat resolved, but the unmet goal of site-specific substantial delivery remains the main focus in LNPs development research. Despite the promise shown by LNPs in animal studies and the fact that technological advances in LNPs research have made the approval possible of a few formulations, therapeutic outcomes in human are not satisfactory. The LNPs technology has managed to survive due to possible tailoring of their properties by virtue of the possibility of altering the composition and modifying the surface. Therefore, enormous scientific endeavours are on the rise to transform lipid structures, composition along with tinkering with surface of LNPs. The alternative methods to guide LNPs coupled with advances in small molecule nucleic acid therapeutics and drug development technology to make the entry possible to specific cells may be effective in cancer therapy. The development is very promising; however enduring efforts are required till the goal is reached.



Keywords: Nanoliposomes, Lipid nanoparticles, Gene delivery, Drug delivery, Clinical formulations

INTRODUCTION

The amphiphilic molecules tend to self-assemble in aqueous medium wherein the polar heads are exposed to water and hydrophobic tails create a hydrophobic interior. The origin of this notable phenomenon lies in the experiments with phospholipids performed by Bangham in the 60's.[1,2] This reorganisation of phospholipids in aqueous medium led to the discovery of liposomes which consisted of microscopic closed bilayers assemblies resembling to cell membranes with inner aqueous compartments. Interestingly these structures were capable of accommodating hydrophilic, hydrophobic and amphiphilic molecules which presented the doorway to their huge potential applications.[2] Thereafter, the liposomes research saw tremendous upsurge in its development and substantial number of studies were performed by employing different amphiphilic structures, altering their compositions and surface pertaining to different applications. The lipid based nanoparticles composed of only solid lipids were also developed and termed as solid lipid nanoparticles (SLNs) which possess highly ordered crystalline structure. A lipid nanoparticles of next generation that is, nanostructured lipid carriers (NLCs) was also developed by mixing liquid and solid lipids to deal with limitations associated with SLNs.[3] The solid lipids are mainly saturated fatty acids which make them solid at room temperature, however the liquid lipids are mainly those from unsaturated fatty acids which makes them liquid at room temperature.[3] The main areas wherein the properties of these lipid based nanostructures were explored includes drug/gene delivery technology, cosmetics, and food industry.[2] The food industry uses lipid nanoparticles into foods and beverages especially for the encapsulation of hydrophobic biomolecules which improves their stability and increases absorption by the gastrointestinal tract.[3] The therapeutic delivery

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of nucleic acids and drugs using liposomes and different lipid based nanoparticles against different health conditions remains a major field of scientific research till date. The LNPs as drug carriers have shown their potential to deliver hydrophobic drugs against different cancer types. Initially the LNPs based delivery approach suffered the major problem of their recognition by the immune system in the body which was subsequently overcome by their PEGylation which led to their prolonged blood circulation times and the development of clinically applicable drug containing lipid formulations.[4,5] On the other hand, cationic lipid based liposomes were pioneered by Felgner and coworkers to deliver the negatively charged nucleic acids across negatively charged mammalian cell membranes.[1,6] This study revolutionized the field and it was further expanded to delivery of other nucleic acid types like microRNA (miRNA) and small interfering RNA (siRNA).[1,6] The gene therapy field (RNAi and CRISPR/Cas-9) has been highly benefitted with liposome technology and has won approval from FDA for a lipid-siRNA combination against polyneuropathy. Very importantly, the recent development of lipid based mRNA vaccines against COVID-19 is an example which demonstrates the tremendous potential of LNPs based therapeutic delivery.[7,8] In order to reach the therapeutic concentrations of either therapeutic drugs or nucleic acids within the cells, the lipid NPs have been also modified to respond to either some external stimuli (e.g., temperature and light) or specific biological stimuli (e.g., pH and redox potential).[9,10] The nanoparticles release the cargoes in substantial amount in response to such stimuli and thereby increasing the intracellular concentration effective enough to have therapeutic action. This strategy has been quite successful in treating drug resistant cancers where cells acquire resistance against repeated free drug treatments. The non-specific distribution of nanoparticles leads to severe side effects and therefore to achieve the target specific therapeutic affects, the LNPs have also been decorated with targeting ligands such as monoclonal antibodies, aptamers, peptides and certain biomolecules such as folate and biotin.[10] The antibody-coated immunoliposomes (e.g., epidermal growth factor receptor; EGFR) appear to be very promising to selectively target the cancer cells and have been part of clinical trials.[11] The advances in LNPs research has also developed multifunctional NPs equipped with the ability to facilitate optical imaging along with delivery action.[10] In addition, the advances in production technology for LNPs such as microfluidics have the potential to facilitate their commercial and clinical applications.[12] The following text discusses in brief the progress shown by lipid nanoparticles in therapeutic delivery applications.

Application of lipid nanoparticles (LNPs) in medicinal field: The lipid based nanocarriers ranging from liposomes to next generation lipid-based nanoparticles viz. NLCs and SLNs, have been widely appreciated for their use in biomedical applications.[13] The ability of these nanostructures to hold variety of drug molecule and nucleic acids (DNA, mRNA and siRNA) (Figure 1) has led to their potential application against different diseases including the deadliest cancers and life-threatening viral infections such as COVID-19 to deliver therapeutic outcomes.[13,14]

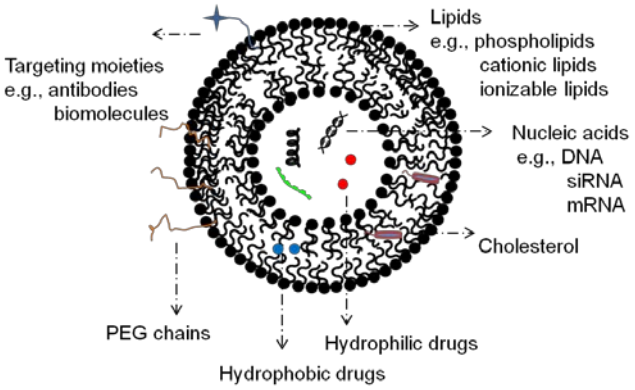

Figure 1. The schematic depiction of the liposome as delivery vehicle for therapeutic drugs and nucleic acids.

LNPs in cancer treatment: The chemotherapy using cytotoxic small molecules is one of the most generally prescribed methods to treat cancer. However, the major restriction in eliciting their full potential is the hydrophobic nature of many potential anticancer drug molecules and lack of specificity which leads to extensive damage to healthy tissues. The LNPs are widely utilized in cancer treatment because of their capacity to encapsulate hydrophobic drugs and thereby increasing their bioavailability. The surface functionalization of such drug carriers has the potential to reduce the possibility of off-target release of drug molecules and thus decreasing the toxicity.[13] Several lipid based anticancer formulations have been approved for use in patients. The Doxil, was the first marketed liposome formulation for cancer treatment such as ovarian cancer and breast cancer etc.[15,16] This formulation contains anticancer drug, doxorubicin inside aqueous core of liposomes which was derived from the PEGylated phospholipids.[16] It takes the advantage of enhanced permeability and retention (EPR) phenomenon to enter the cancer tissue because of being nanoscale in diameter. The EPR effect allows substantial accumulation of liposomes in tumors than in normal tissues by virtue of leaky vasculature and inefficient lymphatic drainage.[4,5] However in normal tissues such entries are relatively restricted owing to tight junctions of endothelial cells. This doxorubicin formulation provides longer blood retention time, increase tumor uptake, and decreased chances of cardiotoxicity originating from treatment of patients with free doxorubicin.[17] Vyxeos is another approved liposome based formulation which encapsulates the combination of daunorubicin with cytarabine (1:5 molar ratio) for treating the acute myeloid leukemia. This formulation is also based upon liposomes derived from composition of phospholipids and cholesterol. Such peculiar combinations of two or more chemotherapeutics provide synergistic effect with superior pharmacokinetic profile when compared to free drugs.[18,19] Similarly other formulations based on different lipid compositions and drugs have been approved and are undergoing clinical trials for the treatment of different cancer types.

Apart from the chemotherapeutic delivery, lipid formulations have also garnered success in delivering therapeutic nucleic acid

molecules which by themselves cannot cross the barrier of negatively charged cell membranes and are prone to nuclease mediated degradation.[20] This type of non-viral gene delivery approaches proved to be superior to overcome different physiological responses associated with gene delivery methodologies.[21] The discovery of RNAi was revolutionary happening which made intracellular gene silencing possible with the use of small interfering RNA (siRNA) molecules and short hairpin RNA (shRNA).[7] The significant potential was harnessed using LNPs technology as first prescription medicine against polyneuropathy caused by hereditary ATTR amyloidosis.[7,22] The success has a long history of development of suitable ionizable cationic lipids as well as PEGylated lipids. The deep understanding of variation in lipid structures and resulting properties facilitating a desirable intracellular effect paved the way to their successful clinical application.[22] Thereafter LNPs were studied extensively in preclinical models to deliver siRNA therapeutics to selectively kill cancer cells. The LNPs derived from arginine conjugated cholesterol and dioleoyl phosphatidylethanolamine (DOPE) for effective loading of siRNA and silencing the target gene (kinesin spindle protein) in several cancer cells that inhibited the tumor growth in prostate cancer model in *in-vivo*.[23] However, clinical development of LNP based RNAi anticancer therapeutics still remains challenging owing to induction of immune response.[7,24] The selection of appropriate marker and alternative administration route has been shown to exploit RNAi therapeutics to produce clinically viable formulation. One such example is the development of DFP-10825, a composition containing cationic liposomes with shRNA targeting thymidylate synthase to treat peritoneally disseminated cancers by i.p. injection.[24] A few other formulations in the category underwent clinical trial but fell short of desired performance. The LNP technology is serving to advance the delivery potentials of these carriers for mRNA delivery. The mRNA vaccines have been developed using lipid nanoparticles and are currently in clinical trials.[25] The melanoma FixVac (BNT111) is a recent example of cationic liposomal RNA vaccine against tumour-associated antigens undergoing clinical trial.[26] This study is focused on the immune responses induced by the formulation. The anti-PD1 antibodies in combination with melanoma FixVac has been reported to enhance antitumor effects in patients. Similarly, a novel mRNA-LNPs therapeutic formulation, mRNA-2416 has also been developed for intra-tumoral injections in solid tumor patients to induce pro-inflammatory activity. The effect of mRNA-2416 has also been evaluated in combination with the anti-PD-L1 inhibitor durvalumab to look for synergistic effects.[27] The intravenous administration method for various mRNA-LNPs suffers their sequestration in the liver.[28] Various efforts therefore have been made to modify their surface strategically to guide them to bind with affinity ligands for targeted delivery. The ionizable lipid based nanoparticles were chemically conjugated with a monovalent αPV1 antibody against plasmalemma vesicle-associated protein (caveolae-associated protein) for targeted delivery to lungs.[28] The lipid nanoparticles have also been engineered for selective entry of RNA into hepatocytes and sinusoidal endothelial cells associated with liver related diseases following ApoE-mediated- and mannose ligand mediated cellular uptake.[29] Very recently a synthetic lipid based formulation was fabricated to treat pulmonary lymphangioleiomyomatosis following mRNA delivery to restore tumor suppressor, Tsc2 which led to notable therapeutic effect.[30] The targeting ability of these nanoparticles is found to be interesting in terms that the presence of amide linkage in the lipid structure of the LNPs allows adsorption of lung targeting plasma proteins and thereby lung selective delivery. However similar set of LNPs with ester bond in lipidoid tails led to specific mRNA delivery to liver. Therefore a keen grasp on structure-activity relationships has the potential to develop LNPs with ability to target specific organs. The in vivo activity shown by amino lipids to deliver therapeutic nucleic acids can serve as guide to optimize new such lipids for desirable delivery and safety.[31]

Application of LNPs for the treatment of fungal infection: The LNP based drug formulations have also found application to treat fungal infection by delivering antibiotics, e.g., amphotericin B at the target site. This broad-spectrum antibiotic is used to treat fungal infection because of its good affinity towards ergosterol in cell membrane of fungus; however, it is associated with several limitations for the effective treatment of fungal infections.[32] These limitations include low water solubility, poor membrane permeability and other health related side effects etc. Due to the amphipathic nature of the antibiotic, different types of aggregation occurs in aqueous solution which directly influences the solubility and toxicity associated with this molecule and in turn correlates with the efficacy of the treatment.[33] These LNPs help in controlling the aggregation state amphotericin B in aqueous medium and thereby improve the therapeutic effect. There are some clinically approved LNP-amphotericin B formulations (e.g., Abelcet® and AmBisome®) which are able to treat serious fungal infections with reduced toxicity side effects.[32] The abundance of lipase enzyme in the inflammatory cells of host and fungal cells promotes the release of less toxic monomeric form of amphotericin B which is also facilitated by its higher affinity towards the ergosterol thereby increasing the therapeutic index.[32,34] Various other approaches were also undertaken to improve the safety of this drug formulation based on the change in liposome composition and preparation. One such formulation was Fungisome™, developed in India showed improved stability, better biodistribution and therapeutic effect at lower concentration of drug which reduced side effect significantly.[32] The treatment of cutaneous infections through systemic medication has limitations of poor efficacy and severe side effects.[35] The lipid nanoparticles, both SLNs and NLCs have gained tremendous attention in developing antifungal formulations for topical applications.[35] The research on LNPs based topical delivery formulations of different and potent antifungal agents has shown advantageous therapeutic effects following adequate skin permeation. The clinical assessment of SLNs based topical gel containing antifungal agent Fluconazole, revealed superior outcomes over commercially available antifungal creams in the treatment of Pityriasis Versicolor.[36] Therefore, the use of LNPs in developing antifungal formulations

assure to deal with limitations associated with many potent antifungal drugs and give rise to commercially acceptable medication.

Application of LNPs for the treatment of viral infection: The lipid nanoparticles have a recognized history of their application in vaccine development against viral infections. The mRNA vaccine development has numerous advantages over conventional technologies.[25] For the fact that liposomes have demonstrated effective protection capability and substantial delivery of nucleic acid molecules, the use and effect of first mRNA vaccine was described using liposomes against influenza virus infection following mice immunization.[25,37] This led to the understanding and subsequent progress in global research and the development of other mRNA-LNPs vaccines against deadly viral infections. The mRNA-1440 and mRNA-1325 are examples of LNPs based vaccines undergoing clinical trial for their effect against Influenza H10N8 and Zika virus infections respectively.[37,38] The lipid nanoparticles have recently seen their moment in the development of vaccines against COVID-19. These vaccines contained LNPs to deliver therapeutic mRNA to human cells to combat COVID-19 pandemic. The mRNAs are negatively charged and cannot cross the cell membranes and also very prone to degradation in the body. Therefore, an efficient delivery of mRNA is a key challenge to look for its therapeutic effect in vaccines. In the two conditionally approved mRNA vaccines developed by the pharmaceutical companies, Moderna and Pfizer/BioNTech, the viral mRNA was encapsulated within the LNPs to provide stability and prevent its degradation by RNAse.[39] Both phospholipid based formulations were comprised of an ionizable lipid which acquires positive charge at low pH of endosomes and help releasing the LNPs with its contents into the cytoplasm of the cells. The incorporation of PEGylated lipid improved the retention time in the systemic circulation while avoiding the immune attack of host system. The size of these LNPs were ~ (80-100) nm with a capacity to carry 100 mRNAs/LNPs.[13,31] In the cytosol mRNA gets translated to protein (Spike protein in case of COVID-19) which activates immune system to produce antibodies against it.[39,40] It was the time to appreciate liposome-based delivery technology and mRNA therapeutics as well, which saved lives of millions across the globe. There is still lot more to do and the design and development of new ionizable lipids with appropriate pKa values and surface optimization has the potential to advance vaccine based therapeutics.[37]

LNPs in other biomedical application: Apart from the ability of LNPs to deliver drugs and genes, these nanoparticles have achieved significant importance in other biomedical applications such as medical imaging, cosmetics, nutraceutical products etc. The encapsulation of fluorescence probe (quantum dot) with anticancer drugs is found to be suitable for theranostics purpose.[41,42] To increase the efficacy of traditional imaging approaches such as computed tomography (CT) and magnetic resonance imaging (MRI), LNPs can be radio-labelled for the detection of spreading of cancer.[43] LNPs got importance in cosmetic filed to increase the stability of bioactive and their better penetration inside the skin. Different products already came in commercial grades where different components e.g., collagen, hyaluronic acid etc. were encapsulated in the liposomal formulations.[44] The utility of LNPs is very versatile which includes agriculture, nanoreactors, and applicability as a system to explore different biological mechanisms.

OUTLOOK

The improvements delivering the desirable clinical impact using LNPs based nanomedicines seem to be very much realistic, though it would require smart thinking and rational reasoning based on the progress achieved so far in different aspects relating to LNPs and certainly enduring efforts before we realize the goal. The lack of general consensus on the properties of liposomes that may yield therapeutic formulations is grounded upon the distinct performance of LNPs against different targets in different applications. However, it is evident that the therapeutic effect is significantly influenced by the type of lipid components, their part in the composition and resulting surface of the lipid nanoparticles. The comprehensive investigation of lipid structures that have managed to yield therapeutic benefits can be further considered to be modified by considering all the regions (head group, linker and tail) of the structure because it is the structure of lipids which imparts different properties to liposomes or lipid nanoparticles and determines their pharmacokinetic behaviour and intracellular fate. In this realm, the prominence of ionizable cationic lipids has been well recognized because of their excellent potential to impact cellular fate of lipid nanoparticles, which otherwise was very difficult with cationic and neutral lipids owing to their toxicity, immune activation and less efficacy. These ionizable lipids remain nearly neutral at physiological pH, but acquire positive charge at low pH of endosomes and thereby interact with their membranes and facilitate release therefrom. Therefore, development of such lipids in combination with PEGylated molecules offers an opportunity to fabricate relatively more viable and efficacious lipid formulations. Similarly, the surface of the LNPs is very crucial as it regulates their circulation after administration in the body and interaction with cellular membranes. It is noteworthy to mention that the PEGylation of liposomes significantly altered the surface properties and assisted them escape from reticuloendothelial system (RES) and stay longer in body which eventually laid the foundation for the discovery of DOXIL™. The PEGylation reduces the interaction of particles with different plasma proteins and does not allow the formation of surrounding protein corona. Interestingly, not all the protein corona leads to adverse event of RES recognition and subsequent clearance, instead function in targeting the tumor cells. The type of plasma protein binding to liposomes has been correlated with type of lipid structure and the composition used in the formulation. This provides a practical and effective approach to closely follow the physiological environment and allows the development of targeted liposomes. Therefore, considering the potential of lipid structure chemistry itself in deciding their circulation property and intracellular fate and/or targeting ability, further modulations based on current understanding can be very useful. The another notable achievement in exploiting the surface of lipid

nanoparticles is decorating them with monoclonal antibodies (Mabs) which has led to the development of highly promising delivery tool, i.e., immunoliposomes (ILs). These immunoliposomes target different tumor types by virtue of the ability of Mabs to tightly bind to their receptors on cells. The ILs, however, could not escape the fate of their recognition by immune system during circulation. Nonetheless, based on ongoing research and recent history of managing to succeed in clinical trials, engineering suitable antibodies and optimizing their part in suitable liposome composition has the potential to deliver desirable outcomes. Similarly, the use of lipid nanoparticles in antitumor immunotherapy or vaccination, has delivered many formulations which are under clinical trial phase. The liposome composition and properties influence the outcome in immunotherapeutic delivery and therefore has the potential to bring a paradigm shift in cancer treatment. The LNPs have manifested as remarkable and meaningful support to RNAi therapeutics which has reflected towards the clinical acceptance of Onpattro to treat Amyloidosis. The lipid nanoparticles have made the successful entry to clinic to deliver siRNA & mRNA. The therapeutic outcomes are by and large influenced by design of lipid nanoparticles addressing physiological barriers. The selection of appropriate markers and alternative methods to treat tumors can also enhance the therapeutic outcomes. Therefore, it is perceived that different properties arising from various lipid compositions can be amalgamated with different potential modalities to provide optimized therapeutic delivery systems.

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CONFLICT OF INTEREST

Authors declare no conflict of interest (financial, academic or otherwise) for publication of this work.

REFERENCES AND NOTES

1. K. K. Ewert, P. Scodeller, L. Simón-Gracia, V. M. Steffes, E. A. Wonder, T. Teesalu, C. R. Safinya. Cationic Liposomes as Vectors for Nucleic Acid and Hydrophobic Drug Therapeutics. *Pharmaceutics* **2021**, 13, 1365.
2. A. Laouini, C. Jaafar-Maalej, I. Limayem-Blouza, S. Sfar, C. Charcosset, H. Fessi. Preparation, characterization and applications of liposomes: state of the art. *J. Colloid Sci. Biotechnol.* **2012**, 1, 147-168.
3. V.D. Santos, A.P.B. Ribeiro, M.H.A. Santana. Solid lipid nanoparticles as carriers for lipophilic compounds for applications in foods. *Food Res Int* **2019**, 122, 610-626.
4. K. He, M. Tang. Safety of novel liposomal drugs for cancer treatment: advances and prospects. *Chem. Biol. Interact.* **2018**, 295, 13-19.
5. H. Harashima, T. Ishida, Editorial: Advanced Liposome Research. *Adv. Drug Deliv. Rev.* **2020**, 154-155, 1.
6. F. Ponti, M. Campolungo, C. Melchiori, N. Bono, G. Candiani. Cationic lipids for gene delivery: many players, one goal. *Chem. Phys. Lipids*, **2021**, 235, 105032.
7. J.A. Kulkarni, D. Witzigmann, S. Chen, P.R. Cullis, R. Van Der Meel. Lipid nanoparticle technology for clinical translation of siRNA therapeutics. *Acc. Chem. Res.* **2019**, 52, 2435-2444.
8. X. Hou, T. Zaks, R. Langer, Y. Dong. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Mater.* **2021**, 6, 1078-1094.
9. Y. Lee, D.H. Thompson. Stimuli-responsive liposomes for drug delivery. *Wiley Interdisciplinary Rev. Nanomed. Nanobiotechnol*. **2017**, 9, e1450.
10. J. Majumder, T. Minko. Multifunctional and stimuli-responsive nanocarriers for targeted therapeutic delivery. *Exp. Opin. Drug Deliv.* **2021**, 18, 205-227.
11. D. Wang, Y. Sun, Y. Liu, F. Meng, R. J. Lee. Clinical translation of immunoliposomes for cancer therapy: Recent perspectives. *Exp. Opin. Drug Deliv.* **2018**, 15, 893-903.
12. S. Shah, V. Dhawan, R. Holm, M. S. Nagarsenker, Y. Perrie. Liposomes: Advancements and innovation in the manufacturing process. *Adv. Drug Deliv. Rev.* **2020**, 154, 102-122.
13. R. Tenchov, R. Bird, A. E. Curtze, Q. Zhou. Lipid Nanoparticles-From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. *ACS nano* **2021**, 15, 16982-17015.
14. World Health Organization. Background document on the mRNA-1273 vaccine (Moderna) against COVID-19: background document to the WHO Interim recommendations for use of the mRNA-1273 vaccine (Moderna), 3 February 2021 (No. WHO/2019 nCoV/vaccines/SAGE_recommendation/mRNA-1273/background/2021.1). World Health Organization 2021.
15. M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas, R. Langer. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discov.* **2021**, 20, 101-124.
16. R.N.V.S. Mamidi, S. Weng, S. Stellar et.al. Pharmacokinetics, efficacy and toxicity of different pegylated liposomal doxorubicin formulations in preclinical models: is a conventional bioequivalence approach sufficient to ensure therapeutic equivalence of pegylated liposomal doxorubicin products? *Cancer Chemother. Pharmacol.* **2010**, 66, 1173-1184.
17. Y. Barenholz. Doxil®—the first FDA-approved nano-drug: lessons learned. *J. Control. Release* **2012**, 160, 117-134.
18. A.C. Anselmo, S. Mitragotri. Nanoparticles in the clinic: an update. *Bioeng. & Transl. Med.* **2019**, 4, e10143.
19. E.J. Feldman, J.E. Lancet, J.E. Kolitz et.al. First-in-man study of CPX-351: a liposomal carrier containing cytarabine and daunorubicin in a fixed 5: 1 molar ratio for the treatment of relapsed and refractory acute myeloid leukemia. *J. Clin. Oncol*., **2011**, 29, 979-985.
20. B. Maiti, S. Bhattacharya. Liposomal nanoparticles based on steroids and isoprenoids for nonviral gene delivery. *Wiley Interdisciplinary Rev. Nanomed. Nanobiotechnol*. **2022**, 14, e1759.
21. A. Bajaj, S.K. Mishra, P. Kondaiah, S. Bhattacharya. Effect of the headgroup variation on the gene transfer properties of cholesterol based cationic lipids possessing ether linkage. *Biochim. Biophys. Acta* **2008**, 1778, 1222-1236.
22. S. Yonezawa, H. Koide, T. Asai. Recent advances in siRNA delivery mediated by lipid-based nanoparticles. *Adv. Drug Deliv. Rev.* **2020**, 154, 64-78.
23. J. Lee, P.E. Saw, V. Gujrati, Y. Lee, H. Kim, S. Kang, M. Choi, J. I. Kim, S. Jon. Mono-arginine cholesterol-based small lipid nanoparticles as a systemic siRNA delivery platform for effective cancer therapy. *Theranostics* **2016**, 6, 192-203.
24. H. Ando, T. Ishida. An RNAi therapeutic, DFP-10825, for intraperitoneal and intrapleural malignant cancers. *Adv. Drug Deliv. Rev.* **2020**, 154, 27-36.
25. X. Hou, T. Zaks, R. Langer, Y. Dong. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Mater.* **2021**, 6, 1078-1094.
26. U. Sahin et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* **2020**, 585, 107-112.
27. A. Jimeno et al, A phase 1/2, open-label, multicenter, dose escalation and efficacy study of mRNA-2416, a lipid nanoparticle encapsulated mRNA encoding human OX40L, for intratumoral injection alone or in combination with durvalumab for patients with advanced malignancies [abstract]. In: Proceedings of the Annual Meeting of the American Association for Cancer Research 2020; 2020 Apr 27-28 and Jun 22-24. Philadelphia (PA): AACR; Cancer Res **2020**;80(16 Suppl):Abstract nr CT032.

28. Q. Li, C. Chan, N. Peterson, et. al. Engineering Caveolae-targeted lipid nanoparticles to deliver mRNA to the lungs. *ACS Chem. Biol*. **2020**, 15, 830-836.
29.M. Kim, M. Jeong, S. Hur, Y. Cho, J. Park, H. Jung, Y. Seo, H.A. Woo, K.T. Nam, K. Lee, H. Lee. Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver. *Sci. Adv*. **2021**, 7, eabf4398.
30. M. Qiu, Y. Tang, J. Chen, R. Muriph, Z. Ye, C. Huang, J. Evans, E. P. Henske, Q. Xu. Lung-selective mRNA delivery of synthetic lipid nanoparticles for the treatment of pulmonary lymphangioleiomyomatosis. *Proc. Natl. Acad. Sci.* **2022**, 119, e2116271119.
31. S. Sabnis et al. A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. *Mol. Ther.* **2018**, 26, 1509-1519.
32. C. Faustino, L. Pinheiro. Lipid systems for the delivery of amphotericin B in antifungal therapy. *Pharmaceutics* **2020**, 12, 29.
33.J. Starzyk, M. Gruszecki, K. Tutaj, R. Luchowski, R. Szlazak, P. Wasko, W. Grudzinski, J. Czub, W.I. Gruszecki. Self-association of amphotericin B: spontaneous formation of molecular structures responsible for the toxic side effects of the antibiotic. *J. Phys. Chem. B* **2014**, 118, 13821-13832.
34. A. Adedoyin et al. Pharmacokinetic profile of ABELCET (amphotericin B lipid complex injection): combined experience from phase I and phase II studies. *Antimicrob. Agents Chemother.* **1997**, 41, 2201-2208.
35. S. Nami, A. Aghebati-Maleki, L. Aghebati-Maleki. Current applications and prospects of nanoparticles for antifungal drug delivery. *EXCLI j.* **2021**, 20, 562-584.
36. S. El-Housiny, M. A. Shams Eldeen, Y. A. El-Attar, H. A. Salem, D. Attia, E. R. Bendas, M. A. El-Nabarawi. Fluconazole-loaded solid lipid nanoparticles topical gel for treatment of pityriasis versicolor: formulation and clinical study. *Drug deliv.* **2018**, 25, 78-90.
37. E. H. Pilkington et al. From influenza to COVID-19: Lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. *Acta Biomater*. **2021**, 131, 16-40.
38. I. Knezevic, M. A. Liu, K. Peden, T. Zhou, H. N. Kang. Development of mRNA vaccines: scientific and regulatory issues. *Vaccines* **2021**, 9, 81.
39. Y. Li, R. Tenchov, J. Smoot, C. Liu, S. Watkins, Q. Zhou. A comprehensive review of the global efforts on COVID-19 vaccine development. *ACS Cent. Sci.* **2021**, 7, 512-533.
40. B.S. Chhikara, R. Kumar, Poonam, P. Bazard, R.S. Varma. Viral infection mitigations using advanced nanomaterials and tools: lessons from SARS-CoV-2 for future prospective interventions. *J. Mater. Nanosci.* **2021**, 8 (2), 64–82.
41. W. T. Al-Jamal, K. Kostarelos. Liposomes: from a clinically established drug delivery system to a nanoparticle platform for theranostic nanomedicine. *Acc. Chem. Res.* **2011**, 44, 1094-1104.
42. B.S. Chhikara, B. Rathi, K. Parang. Critical evaluation of pharmaceutical rational design of Nano-Delivery systems for Doxorubicin in Cancer therapy. *J. Mater. Nanosci.* **2019**, 6 (2), 47–66.
43. N. Lamichhane, T. S. Udayakumar, W. D. D'Souza, C. B. Simone II, S. R. Raghavan, J. Polf, J. Mahmood. Liposomes: clinical applications and potential for image-guided drug delivery. *Molecules* **2018**, 23, 288-305.
44. X. Wu, R. H. Guy. Applications of nanoparticles in topical drug delivery and in cosmetics. *J. Drug Deliv. Sci. Technol.* **2009**, 19, 371-384.

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