JPRM
Journal of Preventive and Rehabilitative Medicine

2022; 4(1): 14-20
PublishedOnline:14/03/2022
(https://journals.unza.zm/index.php/medicine/about)
DOI: 10.21617/jprm2022.413

ISSN: 2415-038X (Print)

REVIEW ARTICLE Open Access

Lipid Nanoparticle Technology a Key Component of COVID-19 mRNA Vaccines

Petty Miyanda [1*], Surya Gautam [2],

[1]Mulungushi University School of Medicine and Health Sciences, Kabwe 10101 Zambia, [2]CT Institute of Pharmaceutical Sciences, Shahpur Campus, Jalandhar, India

*Corresponding author: miyandapetty@gmail.com

Abstract

To cite: Miyanda P, Suryan Gautam. Lipid Nanoparticle Technology a Key Component of COVID-19 mRNA Vaccines. JPRM 2022, 4(1): 14-20. doi: 10.21617/jprm2022.413

Background: The emergency use authorization (EUA) by the US-FDA for two mRNA-based vaccines BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) has increased the confidence of addressing the COVID-19 pandemic which has killed millions of people worldwide. The aim was to review literature on lipid nanoparticle technology as a key component of COVID-19 mRNA vaccines.
Methods: An introductory literature search was conducted by using keywords including "COVID-19", mRNA vaccines" nanotechnology" and others in the databases; Google Scholar and PubMed.
Results: Using coronavirus disease-2019 vaccine related articles it was evident that lipid nanotechnology has played a significant role in the success of mRNA vaccines. Nanoparticles (NPs) as delivery vehicles render stability to the encased mRNA from ribonucleases, facilitating delivery of high mRNA concentrations into the target cells. The ionisable "lipid nanoparticle" (LNP) facilitates Ribosomal nucleic acid (RNA) complexation and has the capacity to avoid reticuloendothelial clearance and subsequent endocytosis. These unique properties of the lipid nanocarrier technology can be attributed to the overwhelming success of the two-mRNA based COVID-19 vaccines with ~95% efficacy in phase III clinical trials. When compared against the traditional liposomal bilayer carriers, LNPs possess rigid morphology, better stability profile and improved cellular penetration of the cargo.
Conclusion: The efficacy and rate at which BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna) mRNA vaccines were formulated has showcased the potential of nanotechnology in aiding the alleviation of the COVID-19 health crisis and has further demonstrated the undeniable impact of nanomedicine in tackling future health challenges reducing morbidity and mortality worldwide.

Keywords: *COVID-19; mRNA; Lipid nanoparticles; BNT162b;, mRNA-127; Vaccine*

JPRM
Journal of Preventive and Rehabilitative Medicine

Received: *14 October 2021* ***Accepted:*** *11 November 2021* ***Published:*** *15 March 2022*

INTRODUCTION

The development of vaccines against COVID-19 have been met with an unprecedented speed. Particularly, Messenger RNA (mRNA) vaccines — a technology that has conveyed impressive efficacy in clinical trials for other infectious diseases such as Influenza [1]. With BioNTech/Pfizer's BNT162b2 and Moderna's mRNA-1273 vaccines already raising hopes for a near end of the SARS-CoV-2 pandemic [2]. The efficacy of the mRNA vaccines is not without challenges as it relies on the effective delivery of mRNA into the cytoplasm of host cells, where it undergoes transcription and translation to produce antigenic proteins that trigger the generation of neutralizing antibodies [3].

The need for a physical carrier

The physical and chemical characteristics of mRNA molecules intercepts its ability to readily diffuse into cells; the larger magnitude of 105–106 Da in size, the dense negative charge which initiates electrostatic repulsion of the anionic cell membrane, preventing its uptake. Cellular uptake rate of mRNA without a delivery vehicle is less than 1 in 10,000 molecules [4]. With a median half-life of 7 hours [5]. Additionally its protein nature renders it liable to degradation by 5′ exonucleases, 3′ exonucleases and endonucleases [6]. On that account, mRNA vaccines require a delivery vehicle that not only provides stability against the nucleic acid degradation but facilitates the mRNA transfer into cells both in vivo and invitro such as lipid nanoparticle technology [1].

BioNTech/Pfizer's and Moderna's mRNA vaccines both use lipid nanoparticles as mRNA vehicles. The impressive rate at which these vaccines were developed is partly due to the long-standing technology on nucleic acid delivery by lipid nanoparticles that has well investigated, optimized and established by the nanomedicine community [7]. Who have thoroughly studied lipid nanoparticle chemistry, structure, surface morphology, dosage, injection routes, uptake, endosomal escape, cargo release, clearance and most importantly safety [8]. The rising interest in lipid nanoparticle research has been motivated by the advent of numerous potential novel mRNA-based therapies and gene editing technologies for a number of diseases haemophilia B [9] myocardial infarction [10] human immunodeficiency virus (HIV) [11-12] and more than ten types of cancer]13-14] like whose success depends on the availability of a safe and efficient delivery vehicle [15].

Messenger Ribosomal Nucleic Acid (mRNA)

mRNA is a single-stranded RNA molecule that is complementary to one of the DNA strands of a gene .it is neither a permanent genetic blueprint nor a functional end product discovered in 1961. Its transitory nature confers tremendous flexibility and broad therapeutic utility compared to nearly all other classes of known drugs. In vitro-transcribed (IVT) mRNA has several advantages: there is no risk of carcinogenesis [16] as the mRNA does not integrate into the genome and availability of a natural degradation pathway to ensure that its activity is temporary [17].

In the 1970s liposomes were the first carriers used to deliver mRNA into cells by the 1990s, delivery technology had advanced enough to facilitate preclinical studies on mRNA-based cancer immunotherapy and vaccines [4,7]. Ever since, mRNA-based therapeutics have shown considerable promise for both prophylaxis such as antiviral vaccines and the treatment of a wide range of diseases, currently there are ongoing clinical trials for mRNA vaccines for influenza, Zika, HIV and rabies facilitated by potent mRNA delivery to antigen-presenting cells. These advances have been based on an improved pharmacological understanding of IVT mRNA as a drug along with the development of delivery materials that are adequately efficacious in enhancing protein translation in the correct cell type [5,18].

The first step is transfection of the antigen encoding mRNA molecule into the cytosol of an antigen-presenting cell (APC) (1), then mRNA is transcribed and translated into antigenic peptide (step 2). The antigenic peptide is then processed into smaller peptide epitopes (step 3). Which binds to the major histocompatibility complex (MHC) class I (step 4a) or class II (step 4b). Thereafter the MHCs are sequestered to the cell surface, here they present their antigenic epitopes to either CD8+ (cytotoxic) T cells (step 5a) or CD4+ (helper) T cells (step 5b), inducing cellular immunity or an

antigen-specific antibody response, respectively [4]. mRNA vaccines are the most advanced clinical application of mRNA drugs. Vaccines using antigen-encoding mRNA compared to traditional vaccination strategies provide an attractive alternative. Unlike live-attenuated viral vaccines with a risk of reverting to their pathogenic form, mRNA vaccines cannot replicate in vivo [19] besides mRNA vaccines induce both cellular and humoral (antibody-based) immunity [20] (Fig 1). Developing an antigen-specific immunity from an mRNA vaccine requires the transfection of antigen-presenting cells, such as dendritic cells [21]. Typical routes of administration are: intradermal, intramuscular or subcutaneous injection, because dendritic cells are densely populated in the skin tissue [22] and skeletal muscles.

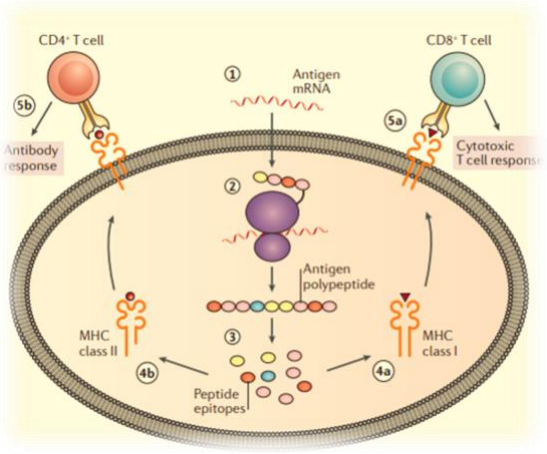

Figure 1: Mechanism of mRNA mediated vaccine

Lipid nanoparticles

Lipid nanoparticles are spherical vesicles that exist on a nanometre scale - below 100 nm in at least one dimension , as earlier alluded to mRNA was initially formulated using liposomes a cationic lipid-based delivery system which showed an enhanced efficacy [23].Liposomal mRNA carriers were associated with toxicity and immunogenicity both in vitro and in vivo studies due to the cationic lipid composition that lead to liver damage , as well as inflammation [24,25] due to the cationic lipid interaction with interferon-γ response in mice. Furthermore, the possible neutralization of positively charged lipids by anionic serum proteins, cause toxicity and reduced efficacy [26]. On this account the urgent need for a better mRNA carrier system extensive optimization research studies came into perspective and lead to the formation of lipid nanoparticles.

Composition

Lipid nanoparticles are composed of ionizable lipids as an alternative lipidic system developed to reduce the toxicity induced by cationic components while retaining their attractive transfection properties. The ionizable lipids possess pH sensitive properties that significantly contribute to the stability and high efficiency. At low pH in acidic buffers these lipids ionize to obtain a positive charge which facilitates RNA complexation and at physiological pH they remain neutral this in tells that post injection there is reduced risk of toxic effects unlike those exhibited by cationic lipids in liposomal carriers [27].

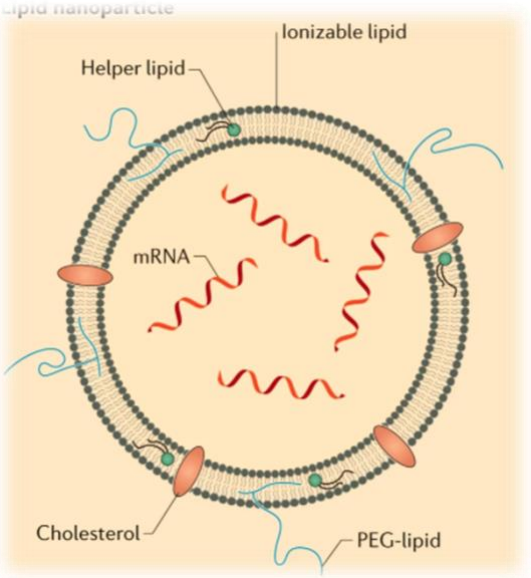

Figure 2: lipid nanoparticle structure

Lipid nanoparticles (LNP) formulations consist of other components, a helper lipid, cholesterol and a polyethylene glycol (PEG) lipid (Fig.2). Each of these lipids have a crucial role in ensuring effectiveness of the nanoparticles. Cholesterol, is a hydrophobic and rigid lipid. It fills in the gaps between lipids within the liposomal bilayer membranes, promoting vesicle stability (Jokerst et al.,2011) [28]. Helper lipids such as 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) promote fusion of both cell and

endosomal membranes, facilitating cell uptake and endosomal release enhancing LNP efficacy. PEG lipids consist of a PEG molecule conjugated to alkyl chains that anchored into the LNP bilayer. These lipids reduce opsonization by serum proteins as well as reticuloendothelial clearance [29].

Table 1: Summary of salient features of Pfizer-BioNTech and Moderna mRNA vaccines

Key Features	Moderna (mRNA-1273)	Pfizer-BioNTech (BNT162b2)
Active pharmaceutical ingredients: Messenger ribonucleic acid (mRNA)	Synthetic mRNA encoding the pre-fusion stabilized spike glycoprotein (S) of SARS-CoV-2 virus.	Nucleoside modified messenger RNA (modRNA) encoding the viral spike glycoprotein (S) of SARS-CoV-2.
Ionisable lipids: Encapsulates & protects the fragile mRNA	Cholesterol Sphingomyelin-102(SM-102) Polyethylene-glycol [PEG]2000dimyristoylglycerol [DMG] 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC]	Cholesterol 2- [(polyethylene glycol)-2000]-N, N-ditetradecylacetamide ((4-hydroxybutyl) azanediyl) bis(hexane-6,1-diyl) bis(2-hexyldecanoate) 1,2-distearoyl-sn-glycero-3-phosphocholine
Buffer solution: for maintenance of pH level close to our body	Acetic acid Sodium acetate Sucrose Tromethamine Tromethamine hydrochloride	Dibasic sodium phosphate dehydrates Monobasic potassium Potassium chloride Phosphate Sodium chloride Sucrose
Dosing	0.5 mL (containing 100 µg vaccine), 2 doses (first priming shot followed by a second booster shot), 28 days apart	0.3 mL (containing 30 µg vaccine), 2 doses (first priming shot followed by a second booster shot), 21 days apart
Storage requirements	(−25 to −15 °C storage)	(−80 to −60 °C storage)
Stability	Stable for 6 months at −20 °C Stable for 30 days at 2–8 °C	Stable for 6 months at −80 °C Stable for 5 days at 2–8 °C
Clinical efficacy (phase- III clinical trial)	94.5%	95%

Lipid nanoparticles in vivo activity

Following administration, lipid nanoparticles are taken up into cells via endocytosis where they are deposited into endosomal compartments, here the pH range gets low about 4.5 [30]. At this low pH lipid nanoparticles positively ionize; the positive charge facilitates electrostatic interaction followed by fusion with the negatively charged endosomal membrane [31]. This fusion destabilizes the nanoparticle lipid bilayer, causing the discharge of the nucleic acid molecule into the cytoplasm [32, 33]. The ability of nucleic acid delivery systems to ionize as the pH drops has proved to be essential for the endosomal escape process. The need for the ionizable lipid nanoparticle to take on positive charge under mildly acidic conditions is emphasized by studies that report efficacious siRNA delivery only for nanoparticle with surface pKa values ranging from 5.5 to 7.

Lipid nanoparticles salient formulation constituents

The efficacy of lipid nanoparticles is however substantially affected by the relative amounts of the individual components being ionizable lipid, helper lipid, cholesterol and PEG. This was depicted from the observation made of LNP formulations designed for siRNA delivery [30] it was shown that lower amounts of ionizable lipid and cholesterol and higher amounts of helper lipid and PEG lipid are required for optimal mRNA delivery [28] .The use of helper lipid DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) commonly in siRNA LNP formulations, compared to DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) also allows for high levels of mRNA delivery efficacy into cells. This could be attributed to the ability of unsaturated lipids, like DOPE, to form unstable hexagonal rather than stable lamellar phases, leading to reduced membrane stability and enhanced endosomal escape [36] For this reason formulations are to be optimized for a given application and administration route to achieve maximum clinical effects .Other factors to be

considered include, the lipid type, size and surface charge because they have major impact on the behaviour of lipid nanoparticles in vivo.

The rapid ease with which the BioNTech/Pfizer's BNT162b2 and Moderna's mRNA-1273 COVID-19 mRNA vaccines were developed and approved for use is associated with the scientific milestone attained in optimizing the lipid nanoparticle formulations for nucleic acid delivery in the development of patisiran. Patisiran is an ionizable lipid nanoparticle vehicle for short interfering RNA (siRNA) drug indicated for polyneuropathies induced by hereditary transthyretin amyloidosis [30] It was the first siRNA-based drug to be approved by the United States Food and Drug Administration (FDA) in 2018.Clinical efficacy of (siRNA) was achieved by optimizing all the aspects of the lipid nanoparticle formulation, this included screening more than 300 ionizable lipids .extensive evaluation of the site of administration and pharmacokinetics into the cytoplasm of hepatocytes . Translational criteria, such as a size range of 100 nm or less, encapsulation efficiency, low surface charge, robustness, scalable manufacturing processes and adequate product stability were established during the development of this drug [37].

LPN mRNA COVID -19 vaccines cause of systemic efficacy

The effectiveness of the nano particulate vehicles in systemic delivery of mRNA COVID -19 vaccines is based on the following properties;

1. The ability of the nanoparticle to encapsulate mRNA and partition a series of extracellular and intracellular barriers for delivery into the cytoplasm of the target cells. [30].
2. Following parenteral administration nanoparticles can evade immune cells and circumvent renal clearance by glomerular filtration while avoiding nonspecific interactions with serum proteins [38].
3. The lipid nanoparticle carrier can cross the endothelial barrier and diffuse to the vicinity of the target tissue, of which many molecules larger than 5 nm typically do not cross the capillary endothelium with the exception of some tissues such as the liver and spleen [39].
4. Once in the extracellular matrix, the lipid nanoparticle can diffuse through a network of fibrous proteins and polysaccharides to the target cell membrane [40].Lipid nanoparticles undergo cellular uptake, by endocytosis forming an endosome, a delivery vehicle must escape the endosome before releasing its mRNA cargo into the cytosol where translation can occur [41].The endosomal escape process has been the most formidable aspect of delivery, statistics have shown that even the world-class RNA delivery materials escape the endosome only up to 2% of the time [42,43]. The lipid nanoparticle ability to ionise (positively) at low pH conditions has outmanoeuvred this challenge.

This knowledge about the properties and other key components of formulation has undoubtedly contributed to the rapid development of COVID-19 mRNA prophylactic vaccines. From a pharmaceutical science perspective, the success of lipid nanoparticle mRNA vaccines is a major breakthrough as it underlines the value of pharmaceutical science for medical advances and inspires further fundamental and applied nanoparticle research.

Conclusion and Future perspectives

The indication of mRNA in genetic medicine possesses significant promise, intercepting all challenges associated traditional DNA based gene therapies. mRNA only induces temporal protein expression, without the risk of genomic integration which can facilitate a wide range of biological processes. With clinical applications like immunotherapy, gene editing and cell reprogramming that require protein expression for only limited periods of time. Clinical progression of mRNA therapeutics has been hindered by two major factors being instability and immunogenicity of invitro transcribed (IVT) mRNA and the insufficient efficacious delivery systems. From the material science perspective, the clinically advanced materials form of lipid nanoparticles has the ability to overcome these challenges. However, because most of these delivery vehicles are proprietary, there is a necessity for more academic research to improve knowledge of scientific community on the influence of lipid structure and nanoparticle formulation on efficacy and potential applications. That being the case, there is cause for certainty that with the right delivery materials, mRNA therapeutics have the potential to transform medicine by facilitating generations of therapeutics and provides a guide to rapidly control any future pandemics.

DECLARATION

Contributors PM developed the concept in close guidance with SG. All authors have read and approved the final version of the manuscript.

Competing interests There were no competing interests from all authors in this study.

REFERENCES

1. Meo S. COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna vaccines. Eur. Rev. Med. Pharmacol. Sci. 2021; 25(3):1663–1669.
2. Shi J., Votruba A.R., Farokhzad O.C., Langer R. Nanotechnology in drug delivery and tissue engineering: from discovery to applications. Nano Lett. 2010; 10(9):3223–3230.
3. Farokhzad O.C., Langer R. Nanomedicine: developing smarter therapeutic and diagnostic modalities. Adv. Drug Deliv. Rev. 2006; 58(14):1456–1459.
4. Martinon, F. et al.Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. Eur. J. Immunol. 1993; 23, 1719–1722 .This paper is one of the earliest studies using liposome-entrapped mRNA for an influenza vaccine.
5. Zhang L., Gu F., Chan J., Wang A., Langer R., Farokhzad O. Nanoparticles in medicine: therapeutic applications and developments. Clin. Pharmacol. Ther. 2008;83(5):761–769.
6. Khurana A., Tekula S., Saifi M.A., Venkatesh P., Godugu C. Therapeutic applications of selenium nanoparticles. Biomed. Pharmacother. 2019;111:802–812.
7. Khurana I., Allawadhi P., Khurana A., Srivastava A.K., Navik U., Banothu A.K., Bharani K.K. Can bilirubin nanomedicine become a hope for the management of COVID-19? Med. Hypotheses. 2021;149.
8. Tang Z., Kong N., Zhang X., Liu Y., Hu P., Mou S., Liljeström P., Shi J., Tan W., Kim J.S., Cao Y., Langer R., Leong K.W., Farokhzad O.C., Tao W. A materials-science perspective on tackling COVID-19. Nat. Rev. Mater. 2020;5(11):847–860.
9. Tang Z., Zhang X., Shu Y., Guo M., Zhang H., Tao W. Insights from nanotechnology in COVID-19 treatment. Nano Today. 2021;36 .
10. Allawadhi P., Khurana A., Allwadhi S., Navik U.S., Joshi K., Banothu A.K., Bharani K.K. Potential of electric stimulation for the management of COVID-19. Med. Hypotheses. 2020;144
11. Nanomedicine and the COVID-19 vaccines, Nat. Nanotechnol., 15(12), 2020, pp. 963–963.
12. Tang Z., Zhang X., Shu Y., Guo M., Zhang H., Tao W. Insights from nanotechnology in COVID-19 treatment. Nano Today. 2021;36
13. Shim E., Galvani A.P. Distinguishing vaccine efficacy and effectiveness. Vaccine. 2012;30(47):6700–6705.
14. Walsh E.E., Frenck R.W., Falsey A.R., Kitchin N., Absalon J., Gurtman A., Lockhart S., Neuzil K., Mulligan M.J., Bailey R., Swanson K.A., Li P., Koury K., Kalina W., Cooper D., Fontes-Garfias C., Shi P.Y., Türeci Ö., Tompkins K.R., Lyke K.E., Raabe V., Dormitzer P.R., Jansen K.U., Şahin U., Gruber W.C. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N. Engl. J. Med. 2020;383(25):2439–2450.
15. Dai L., Gao G.F. Viral targets for vaccines against COVID-19. Nat. Rev. Immunol. 2020:1–10.
16. Knoll M.D., Wonodi C. Oxford–AstraZeneca COVID-19 vaccine efficacy. Lancet. 2021;397(10269):72–74.
17. Balakrishnan V.S. The arrival of Sputnik V. Lancet Infect. Dis. 2020;20(10):1128.
18. Livingston E.H., Malani P.N., Creech C.B. The Johnson & Johnson vaccine for COVID-19. JAMA. 2021.
19. Bouazzaoui A., Abdellatif A.A.H., Al-Allaf F.A., Bogari N.M., Al-Dehlawi S., Qari S.H. Strategies for vaccination: conventional vaccine approaches versus new-generation strategies in combination with adjuvants. Pharmaceutics. 2021;13(2):140.
20. Pardi N., Hogan M.J., Porter F.W., Weissman D. mRNA vaccines—a new era in vaccinology. Nat. Rev. Drug Discov. 2018;17(4):261–279.
21. Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020;586(7830):516–527.
22. Anderson R.M., Vegvari C., Truscott J., Collyer B.S. Challenges in creating herd immunity to SARS-CoV-2 infection by mass vaccination. Lancet. 2020;396(10263):1614–1616.
23. Schlake T., Thess A., Fotin-Mleczek M., Kallen K.J. Developing mRNA-vaccine technologies. RNA Biol. 2012;9(11):1319–1330.
24. Wadhwa A., Aljabbari A., Lokras A., Foged C., Thakur A. Opportunities and challenges in the

25. Pardi N., Hogan M.J., Porter F.W., Weissman D. mRNA vaccines – a new era in vaccinology. Nat. Rev. Drug Discov. 2018;17(4):261–279.

26. Bozzuto G., Molinari A. Liposomes as nanomedical devices. Int. J. Nanomed. 2015;10:975–999. 39. Mitchell M.J. Engineering precision nanoparticles for drug delivery. Nat. Rev. Drug Discov. 2020:1–24.

27. Guevara M.L., Persano F., Persano S. Advances in lipid nanoparticles for mRNA-based cancer immunotherapy. Front. Chem. 2020;8(963)

28. Jokerst, J. V., Lobovkina, T., Zare, R. N. & Gambhir, S. S. Nanoparticle PEGylation for imaging and therapy. Nanomedicine .2011;6, 715–728.

29. Kauffman K.J., Dorkin J.R., Yang J.H., Heartlein M.W., DeRosa F., Mir F.F., Fenton O.S., Anderson D.G. Optimization of lipid nanoparticle formulations for mRNA delivery in vivo with fractional factorial and definitive screening designs. Nano Lett. 2015;15(11):7300–7306.

30. Petsch, B. et al. (2012).Protective efficacy of in vitro synthesized, specific mRNA vaccines against influenza A virus infection. Nat. Biotechnol 30, 1210–1216.

31. Reichmuth A.M., Oberli M.A., Jaklenec A., Langer R., Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. Ther. Deliv. 2016;7(5):319–334.

32. Hajj K.A., Whitehead K.A. Tools for translation: non-viral materials for therapeutic mRNA delivery. Nat. Rev. Mater. 2017;2(10):1–17.

33. Cullis P.R., Hope M.J. Lipid nanoparticle systems for enabling gene therapies. Mol. Ther. 2017;25(7):1467–1475.

34. Sahin, U., Kariko, K. & Türeci, Ö .mRNA-based therapeutics — developing a new class of drugs. Nat. Rev. Drug Discov. 2014;13, 759–780

35. Uchida S., Perche F., Pichon C., Cabral H. Nanomedicine-based approaches for mRNA delivery. Mol. Pharm. 2020;17(10):3654–3684.

36. Billingsley M.M., Singh N., Ravikumar P., Zhang R., June C.H., Mitchell M.J. Ionizable lipid nanoparticle-mediated mRNA delivery for human CAR T cell engineering. Nano Lett. 2020;20(3):1578–1589.

37. Luo Y., Chen D., Ren L., Zhao X., Qin J. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. J. Control. Release. 2006;114(1):53–59.

38. Chiluba BC, Dube G. Descriptive review of epidemiological geographic mapping of coronavirus

39. Schwarz C., Mehnert W., Lucks J.S., Müller R.H. Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. J. Control. Release. 1994;30(1):83–96.

40. Thess A., Grund S., Mui B.L., Hope M.J., Baumhof P., Fotin-Mleczek M., Schlake T. Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals. Mol. Ther. 2015;23(9):1456–1464.

41. Heil F. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 2004;303(5663):1526–1529.

42. Oberli M.A., Reichmuth A.M., Dorkin J.R., Mitchell M.J., Fenton O.S., Jaklenec A., Anderson D.G., Langer R., Blankschtein D. Lipid nanoparticle assisted mRNA delivery for potent cancer immunotherapy. Nano Lett. 2017;17(3):1326–1335.

43. Banerji A. mRNA vaccines to prevent COVID-19 disease and reported allergic reactions: current evidence and suggested approach. J. Allergy Clin. Immunol. Pract. 2 020.