mRNA vaccines : present scenario, challenges and future.

Falguni Pradip Sapkal
Waghire College Saswad.

Abstract: The mRNA vaccines supplement our conventional vaccines (live-attenuated and inactivated vaccines) in reality by their extremely safety, efficacy, potency, and low manufacturing cost. These many years, the administration of these mRNA vaccines was immensely limited due to their instability and low efficiency in vivo delivery. However, at present, some of the problems have been solved through recent technological advances. The positive outcomes of mRNA vaccines were proved by the conducted studies with animals and humans to show the success. This study presents you with a thorough introduction of mRNA viral vaccines and assesses the current points of view and future advancements.

Key words: mRNA vaccines , lipid nanoparticles, virus , cancer .

INTRODUCTION

The era of mRNA vaccinations is upon us and it can be attributed to the fact that there was research done in the past. Recently though, it has been just over a decade that work on the quality of mRNA itself or its many aspects has begun to flourish focusing on (i) upscaling its stability by capping, tailing, engineering point mutations and using better purification techniques; (ii) its packaging using lipid nanoparticles; and (iii) its wide-scale adopts as a vaccine due to decreased immunogenic effects through the use of modified nucleotides.

mRNA vaccines offer several key benefits over traditional vaccines, such as live and attenuated pathogens, subunit-based vaccines, and DNA-based vaccines. They can be summarized as follows: (i) safety, as mRNA does not integrate with host DNA and is non-infectious; (ii) efficacy, where modifications in the mRNA structure can enhance vaccine stability and effectiveness while reducing immunogenicity; and (iii) manufacturing and scalability, given that mRNA vaccines are produced in a cell-free environment, facilitating rapid, scalable, and cost-effective production. Furthermore, mRNA vaccines have the capability to encode multiple antigens, thereby strengthening the immune response against resilient pathogens. Also, the half-life of mRNA can be influenced within a cell through the incorporation of modifications onto the mRNA molecule. Nucleic acid-based vaccines emulate an infection with live pathogens and, in doing so, elicit an immune response characterized by the activation of follicular T helper cells and germinal B cells.

Here we review the different types of mRNA vaccines , delivery of mRNA vaccines, challenges in developing mRNA vaccines and upcoming mRNA vaccines.

1. DIFFERENT TYPES OF mRNA VACCINES.

Currently, Now, there are two types of mRNA vaccines which are the self-amplifying mRNA and the non replicating mRNA vaccines (Modified and unmodified mRNA (NRM)). mRNA vaccines are produced via a process of in vitro enzymatic transcription where mRNA is synthesized in vitro. This includes linear plasmid DNA, RNA polymerase, and nucleoside triphosphates. NRM vaccines are constructed in such a way as to be composed of an open reading frame (ORF) coding for a target antigen (TA), with untranslated regions (UTRs) binding at both ends and terminal poly A tail sequences.18 They facilitate the expression of the antigen once inside the body. mRNA without non modified lipids becomes degraded when injected into the body. However certain chemically emphasized nucleosides that are naturally present like pseudouridine and 1-methylpseusouridine have been show to allow efficient RNA translation response.[5]

Another type of mRNA vaccine is based on a positive strand virus known as alphavirus. In these vaccines, there is an exchange of the structural proteins and instead the desired gene is used. This mRNA that is self amplifying can enable the self replication of the RNA dependant RNA polymerase complex which causes many copies of the antigen encoding mRNA to be realised .They produce elevated levels of the

heterologous gene when introduced into the cytoplasm of host cells. By mimicking the antigen production of viral pathogens in vivo, they elicit both humoral and cellular immune responses. Self-amplifying mRNA vaccines demonstrate superior antigen levels compared to non-replicating mRNA vaccines. Importantly, both self-amplifying and non-replicating mRNA vaccines do not integrate into the host genome. Overall, mRNA vaccines are considerably safer than other vaccine platforms and show significant promise for effectiveness against infectious pathogens.

2. DELIVERY OF mRNA VACCINES.

The clinical translation of naked mRNA vaccines has not yet been achieved due to several significant challenges. The delivery, uptake, and endosomal escape of naked nucleic acids is inherently restricted. Firstly, the negatively charged phosphate backbone of mRNA hinders passive diffusion across a cell's phospholipid bilayer. Secondly, the susceptibility of mRNA to degradation by RNases, coupled with its rapid clearance by the reticuloendothelial system, limits its availability in vivo for the promotion of gene expression. Additionally, the administration of RNA may elicit immunogenic responses, activating both intracellular innate and extracellular immune pathways, which in turn can lead to the degradation of mRNA molecules. Lastly, the escape of naked mRNA from endosomal and lysosomal compartments is limited due to its charge and eventual degradation within lysosomes. Lipid nanoparticles (LNPs) offer a promising solution to surmount some of these barriers.[4]

Majorly there are two types of LNP systems used in the delivery of mRNA vaccine , viz cationic lipid system and ionizable cationic lipid system . Cationic lipid-based delivery systems have been linked to toxicity and immunogenicity in both in vitro and in vivo studies. For instance, the intravenous administration of cationic liposomes has been shown to induce liver damage and provoke a significant interferon-γ response in murine models, leading to inflammation. Additionally, positively charged lipids such as DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium-propane) and DOTAP (1,2-dioleoyl-3-trimethylammonium-propane) may be neutralized by anionic serum proteins, resulting in increased toxicity and diminished therapeutic efficacy.[3]

Ionizable lipidic systems, on the other hand, have been created that lessen the toxicity caused by cationic particles while maintaining their beneficial transfection properties. In particular, efficient ionizable lipids are generally neutral at physiological pH (for less toxicity post injection) but positively charged at low pH (which facilitates RNA complexation when conducted in acidic buffer). Additionally, nanoparticles are deposited into endosomal compartments through cellular uptake via endocytosis, which gradually lowers their pH from roughly 6.8 to 4.5 as they transform into lysosomes. The endosomal escape process appears to depend on the nanoparticles' capacity to ionize as the pH falls. It is believed that the lipid's positive charge promotes its electrostatic contact and eventual fusion, albeit the exact mechanisms are still unclear.The lipid bilayer is destabilized by this fusion, which causes the cargo of nucleic acids to leak into the cytoplasm. Studies that show effective siRNA delivery exclusively for lipid nanoparticle chemistries with surface pKa values ranging from 5.5 to 7 highlight the significance of nucleic acid delivery systems' capacity to assume a positive charge in slightly acidic environments.

Lipid nanoparticle (LNP) formulations usually contain a polyethylene glycol (PEG) lipid, cholesterol, and a helper lipid in addition to a charged or ionizable lipid . In liposomal membranes, cholesterol, which is stiff and hydrophobic, fills in the spaces between lipids to increase vesicle stability. By encouraging fusion with both cell and endosomal membranes, helper lipids such 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and DOPE improve LNP efficiency by enhancing cell uptake and release in endosome.Lastly, PEG lipids are made up of an alkyl chain conjugated to a PEG molecule that attaches itself to the LNP bilayer. PEG lipids decrease reticuloendothelial clearance and opsonization by serum proteins, which is especially significant for systemic distribution.

CHALLENGES IN DEVELOPING mRNA VACCINES.

1. Manufacturing of mRNA

The work of producing mRNA vaccines includes three general steps: synthesis, purification and formulation, each of which consists of a number of subtasks. These steps, however, have not yet been incorporated into a single unit operational system. Specifically, during the stage of mRNA synthesis, the

amp and the plasmid that contains the template are stored and separated, tested, and then utilized to manufacture a DNA template for in vitro transcription (IVT). After this, the transformed DNA is stored at this temperature and is sent to the site of mRNA synthesis. Afterward, the mRNA undergoes a process where it is made, purified, evaluated and stored in an environment lower than minus twenty degrees Celsius before being dispatched elsewhere. During the last stages, after the mRNA has been incorporated with lipid nanoparticles (LNPs), the material is introduced into vials and some samples are sent back for further analysis. Current technologies permit production of several million doses, and in the case of a pandemic, these vaccines will not help about 6 billion people in the world.[7]

Moving towards a continuous manufacturing type process can indeed prove the effectiveness of mRNA vaccine development. Such a concept is already being applied in the chemical and pharmaceutical sectors as an effective, flexible and cheap manufacturing strategy. First, all the three facilities may be integrated with a fluidic system where the IVT, purification and the formulation of m RNA with LNPs are done automatically. Transportation between the three states would then be eliminated and would save operational time and advances the automation technologies while improving the quantity and the quality. Second, a process embedded with continuous manufacturing could also assist in recovering and reusing the raw materials like enzymes or NTPs [Nucleotide triphosphates]. It is also possible that continuous manufacturing process may be a viable option in terms of cost efficiency in bulk scale manufacturing of mRNA vaccines.

2 . Stability during storage and transportation

Keeping things steady while they are stored or moved around To ensure vaccines work as they should it's crucial they're kept at the right temperature from when they're made to when they're given to people. Keeping vaccines cold enough through their journey from creation to use is a must and for mRNA vaccines this means they might need to be kept even colder. When looking at different vaccines that are fine sitting at a temperature between 2 and 8 degrees Celsius for many months the ones called BNT162b2 and mRNA-1273 stand out because they need much colder spots. BNT162b2 has to stay colder than minus 80 degrees Celsius and mRNA-1273 needs to be under minus 20 degrees Celsius . Keeping mRNA vaccines super cold is tough to do. The need for keeping mRNA vaccines at very low temperatures comes from how unstable the LNP-mRNA combo tends to be. Many research efforts have been directed at understanding how stable mRNA molecules are on their own. Meanwhile research on how well mRNA holds up when it is made into a formula and stored has not been looked into much. When it comes to keeping an mRNA-protamine mix that fights rabies effective lyoprotectants used in freeze-drying have proven to keep the levels of antibodies that can block the disease and its protective effects steady. Many patents have been filed for a method that keeps mRNA-protamine mixes stable at room temp for a long time. This method involves freeze-drying them and adding protective substances like lactate mannose and trehalose among others. A study not long ago looked at how well freeze-dried LNP-mRNAs did when they were mixed with various protective substances like sucrose trehalose and mannitol compared to LNPs that hadn't been freeze-dried. Researchers found out that adding 20% sucrose or trehalose to LNPs before freeze-drying helped them keep their ability to deliver mRNA well in lab tests but this method didn't work well inside the body. They think the reason might be that the freeze-drying and putting back together process changes the tiny structure of the LNP-mRNA. This alteration could mess up how LNPs interact with blood plasma leading to a drop in how well they deliver mRNA when tested in living organisms. Up until now the challenge of finding a way to store and move mRNA vaccines in extremely cold conditions hasn't been solved. This problem could seriously limit how much these vaccines can be used on a big scale in times to come. There's a pressing need to gather.

3 .Safety

mRNA vaccines have a higher risk of side effects than traditional inactivated vaccines, particularly grade 3 adverse reactions. The widespread use of the mRNA COVID-19 vaccine allowed for a thorough investigation of the adverse effects of mRNA vaccines. However, the causes of some severe adverse reactions to mRNA COVID-19 vaccines remain unknown, and understanding them is critical for future mRNA vaccine optimization. Though the overall risk of anaphylaxis from an mRNA COVID-19 vaccine is extremely low, the mechanisms underlying this reaction should be investigated and considered for further optimization of the LNP

formulation. The PEG in the LNP formulation is considered a possible allergen for anaphylaxis.

Because of the increasing number of cases of myocarditis and pericarditis following mRNA vaccine administration, the FDA continues to assess the risk . A comprehensive review of a case of suspected myocarditis following the second dose revealed an increase in the number of a specific subset of natural killer cells as well as increased expression of several autoantibodies when compared to controls . In contrast to the upregulation of IL-17 in the development of conventional myocarditis, myocarditis caused by mRNA vaccines did not exhibit this phenomenon, indicating a distinct vaccine-associated immunophenotype. However, the potential mechanisms remain unclear, necessitating more research with a larger sample size.

Rare reports of severe adverse events after vaccination include cytokine release syndrome and cerebral venous thrombosis .The presence of severe adverse events with unclear mechanisms highlights the need for increased caution in future clinical trials.

4. Mutation in targetted antigens

The emergence of breakthrough infections caused by pathogens and the immune evasion of cancers due to mutations in targeted antigens continue to pose significant challenges in the quest for effective vaccines. Infectious pathogens, particularly RNA viruses, can exhibit varying mutation rates during transmission. The ongoing COVID-19 pandemic has led to the identification of several variants of concern, including B.1.1.7 (alpha), B.1.351 (beta), B.1.617.2 (delta), and P.1 (gamma). Moderna has recently reported on the neutralizing efficacy of mRNA-1273 against 16 emerging variants, revealing a reduction in neutralization titers ranging from 2.1 to 8-fold compared to the D614G variant. A similar decline in neutralization titers was noted for BNT162b2, indicating a potential decrease in protective efficacy in humans. Since the initial submission of this manuscript, the B.1.1.529 (omicron) variant has swiftly supplanted delta globally. The omicron variant possesses 32 mutations in the spike protein relative to the original Wuhan-hu-1 strain, leading to a more than tenfold reduction in neutralizing antibody titers. Although a third dose of BNT162b2 has enhanced neutralization against the omicron variant (with geometric mean titers increasing from 1.11 after the second dose to 107.6 after the third), the neutralization capacity remains fourfold lower than that observed against the delta variant. Consequently, innovative strategies are necessary to address the rapid mutation of the virus. A combined vaccination approach utilizing two mixed mRNAs encoding the spike proteins from the B.1.351 and D614G lineages has demonstrated a notable increase in neutralization titers specific to both Wuhan-hu-1 and B.1.351. However, it remains uncertain whether the manufacturing processes for mRNA vaccines can keep pace with the swift emergence of new viral variants.

The mechanisms underlying immune evasion in cancer are significantly more intricate than those associated with breakthrough infections caused by pathogens. The primary factors contributing to this complexity include the loss or mutation of targeted antigens and the presence of immunosuppressive tumor microenvironments. While the identification of neoantigens has ushered in a new era for cancer vaccines, the neoantigens produced by rapid mutations are susceptible to further alterations and can be influenced by immunoediting processes, potentially diminishing or completely negating the therapeutic effectiveness of neoantigen-based vaccines. To counter this challenge, neoantigens that are expressed in genes essential for the survival of cancer cells may serve as optimal targets for the development of future mRNA vaccines. Furthermore, combining mRNA vaccines with agents that can reverse immunosuppression and inhibit immune checkpoints has demonstrated greater efficacy than the administration of vaccine therapy alone.

Upcoming mRNA vaccines.

With the success of mRNA COVID vaccines compaines are exploring the mRNA technology for other use . It is given in fig 1.

Virus /Cancer	Institution /Company	Vaccine ID	Antigen	Clinical trial phase.
Influenza[flu]	National Institute of Allergy and Infectious Disease[NIAID] [May 2023]	H1ssF_3928	Hemagglutinin stem	Phase 1
	Pfizer [expected to complete in 2024]four strains		Hemagglutinin	Phase 3
	Moderna [5 mRNA flu vaccines]	mRNA 1010	Hemagglutinin	Phase 3
		mRNA1020	hemagglutinin and neuraminidase	Phase 2
		mRNA1030	hemagglutinin and neuraminidase	Phase 2
		mRNA1011	Hemagglutinin	Phase 2
		mRNA1012	Hemagglutinin	Phase 2
	Sanofi [4 strains]	MRT5400 and MRT5401	Hemagglutinin	Phase ½
Respiratory synctical virus	Moderna [18 – 59] high risk	mRNA1345	F glycoprotein	Approved by FDA
HIV	Moderna	mRNA1574	envelope protein gp140	Phase1
		mRNA1644	gp120 , gp41	Phase 1
	NIAID	BG505 MD39.3 mRNA	Envelope protein	Phase1
		BG505 MD39.3 gp151 mRNA	Envelope protein	Phase 1
		BG505 MD39.3 gp151 CD4KO mRNA	Envelope protein	Phase 1
Cytomegalo virus	Moderna	mRNA 1647	HCMV gB and pentameric complex	Phase 3
	Sanofi Pasteur and Chiron corporation [now GSK]	gB/MF59	Glycoprotein B	Phase 2
Cancer	Moderna [Individualized neoantigen therapy adjuvant – Melanoma]	mRNA4157		Phasse 3
	INTl adjuvant non small cell lung cancer .. colorectal	mRNA 4157		Phase 3
	INT – Cutaneous squamous cell carcinoma	mRNA4157		Phase 3
	BioNtech non-small cell lung cancer (NSCLC).	BNT116,		

Fig no: 1. Upcoming mRNA vaccines under clinical trials

REFERENCES

[1] Chandra, S., Wilson, J. C., Good, D., & Wei, M. Q. (2024). mRNA vaccines: a new era in

vaccine development. Oncology research, 32(10), 1543–1564. https://doi.org/10.32604/or.2024.043987

[2] Gote, V., Bolla, P. K., Kommineni, N., Butreddy, A., Nukala, P. K., Palakurthi, S. S., & Khan, W. (2023). A Comprehensive Review of mRNA Vaccines. International journal of molecular sciences, 24(3), 2700.

[3] Pilkington, E. H., Suys, E. J. A., Trevaskis, N. L., Wheatley, A. K., Zukancic, D., Algarni, A., Al-Wassiti, H., Davis, T. P., Pouton, C. W., Kent, S. J., & Truong, N. P. (2021). From influenza to COVID-19: Lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. Acta Biomaterialia, 131, 16–40. https://doi.org/10.1016/j.actbio.2021.06.023.

[4] Buschmann, M. D., Carrasco, M. J., Alishetty, S., Paige, M., Alameh, M. G., & Weissman, D. (2021). Nanomaterial delivery systems for mrna vaccines. Vaccines, 9(1), 65. https://doi.org/10.3390/vaccines9010065

[5] Nitika, Wei, J., & Hui, A.-M. (2021). The development of mrna vaccines for infectious diseases: Recent updates. Infection and Drug Resistance, Volume 14, 5271–5285. https://doi.org/10.2147/IDR.S341694.

[6] Jones, K. L., Drane, D., & Gowans, E. J. (2007). Long-term storage of dna-free rna for use in vaccine studies. BioTechniques, 43(5), 675–681. https://doi.org/10.2144/000112593.

[7] Koppu, V., Poloju, D., Puvvala, B., Madineni, K., Balaji, S., Sheela, C. M. P., Manchikanti, S. S. C., & Moon, S. M. (2022). Current Perspectives and Future Prospects of mRNA Vaccines against Viral Diseases: A Brief Review. International journal of molecular and cellular medicine, 11(3), 260–272.

[8] Wang, B., Pei, J., Xu, S., Liu, J., & Yu, J. (2023). Recent advances in mRNA cancer vaccines: Meeting challenges and embracing opportunities. Frontiers in Immunology, 14, 1246682. https://doi.org/10.3389/fimmu.2023.1246682

[9] Greenwood, B. (2014). The contribution of vaccination to global health: Past, present and future. Philosophical Transactions of the Royal Society B: Biological Sciences, 369(1645), 20130433. https://doi.org/10.1098/rstb.2013.0433

[10] Sahin, U., Karikó, K., & Türeci, Ö. (2014). Mrna-based therapeutics—Developing a new class of drugs. Nature Reviews Drug Discovery, 13(10), 759–780. https://doi.org/10.1038/nrd4278.

[11] Persano, S., Guevara, M. L., Li, Z., Mai, J., Ferrari, M., Pompa, P. P., & Shen, H. (2017). Lipopolyplex potentiates anti-tumor immunity of mRNA-based vaccination. Biomaterials, 125, 81–89. https://doi.org/10.1016/j.biomaterials.2017.02.019

[12] Hassett, K. J., Benenato, K. E., Jacquinet, E., Lee, A., Woods, A., Yuzhakov, O., Himansu, S., Deterling, J., Geilich, B. M., Ketova, T., Mihai, C., Lynn, A., McFadyen, I., Moore, M. J., Senn, J. J., Stanton, M. G., Almarsson, Ö., Ciaramella, G., & Brito, L. A. (2019). Optimization of lipid nanoparticles for intramuscular administration of mrna vaccines. Molecular Therapy - Nucleic Acids, 15, 1–11. https://doi.org/10.1016/j.omtn.2019.01.013

[13] Reichmuth, A. M., Oberli, M. A., Jaklenec, A., Langer, R., & Blankschtein, D. (2016). Mrna vaccine delivery using lipid nanoparticles. Therapeutic Delivery, 7(5), 319–334. https://doi.org/10.4155/tde-2016-0006

[14] Oberli, M. A., Reichmuth, A. M., Dorkin, J. R., Mitchell, M. J., Fenton, O. S., Jaklenec, A., Anderson, D. G., Langer, R., & Blankschtein, D. (2017). Lipid nanoparticle assisted mrna delivery for potent cancer immunotherapy. Nano Letters, 17(3), 1326–1335. https://doi.org/10.1021/acs.nanolett.6b03329.

[15] Schlake, T., Thess, A., Fotin-Mleczek, M., & Kallen, K. J. (2012). Developing mRNA-vaccine technologies. RNA biology, 9(11), 1319–1330. https://doi.org/10.4161/rna.22269.