International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 5 Sep-Oct 2021, pp: 1165-1167 www.ijprajournal.com ISSN: 2249-7781

mRNA Vaccine: Its Formulation and Clinical Trials

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Date of Submission: 15-10-2021 Date of Acceptance: 30-10-2021

ABSTRACT
Vaccine are one the most important tools in public health and plays and important role in infectious diseases control. Owing to its precision, safe profile and flexible manufacturing, mRNA vaccine are reaching the stoplight as the new alterative form of the conventional vaccine. In Fact, in the Covid-19 pandemic, it was choice of preference for many of the companies and is the first technology approved by both united states and Europe union as a prophylactic treatment. In this article we focus on understanding of the mRNA vaccine, its mechanism, delivery system. Significantly, we move towards its application in preventing various disease. Within the end, we discuss about the challenges faced while manufactures.
KEYWORDS: mRNA, vaccine, formulation, immunity, transcription, translation.

I. INTRODUCTION

Ribonucleic acid (RNA) being a single strand DNA is highly unstable making its therapeutic use a provocative idea. Despite, its sensitivity today we have mRNA advocated at a vaccine level. Convectional vaccines usually contain inactivated disease-causing organisms or proteins made by the pathogen(antigen), which work by mimicking the infectious agent. They stimulate the body's immune response so it is primed to respond more rapidly and effectively if exposed to the infectious agent in the future.

RNA vaccine use a different approach that takes advantage of the process that cells use to make proteins. Cells uses DNA as the template to make mRNA that is messenger RNA which are then translated to build proteins. An RNA vaccine consist of an mRNA strand that codes for a disease specific antigen. Once the mRNA strand in the vaccine is inside the body, the cells use the genetic information to produce the antigen. This antigen is then displayed on the cell surface and recognised by the immune cells.

II. DESIGNING AND FORMULATION

Messenger RNA vaccines apply IVT mRNA as a blueprint to produce vaccine antigens in vivo, in a patient. The translated pathogen-specific antigens will induce a specific immune response, depending on the type of the cell that was transfected, and the immunogenicity of both the mRNA product and the encoded antigen.

Steps to Design mRNA

1) Cloning of cDNA, then purified and amplified.
The target antigen is clone into a DNA plasmid and its subsequent linearization, although PCR products and synthetic oligonucleotides can also serve as templates for a cell-free in vitro transcription reaction with recombinant RNA polymerase and nucleoside triphosphates

2) Linearized cDNAtemplate is removed using RNase-free DNases to form mRNA

3) Capping and tailing of mRNA.

- In order to increase mRNA stability and translation efficiency, the transcriptional product is enzymatically capped by 7-methyl-guanosine triphosphate (m7G) to protect against RNase. Because of the presence of two free 30 -OH on both guanine moieties of the cap structure, approximately one-third of the mRNAs have a cap incorporated in the reverse orientation to solve this issue anti-reverse cap analogs (ARCAs) is used. ARCAs have only one 30 -OH group, which inhibits the incorporation in the reverse orientation seen with cap analogs
- A protein-encoding open reading frame (ORF) flanked by two untranslated regions (UTRs) is also added, to support translation. A signal peptide (SP) may be added to the ORF to facilitate the secretion of the encoded vaccine antigen candidate.
- A 30 poly(A) tail is added to improve intracellular stability and translational efficiency. It has been shown that increase in poly(A) tail length generally enhances the efficiency of polysome formation, leading to improved protein expression

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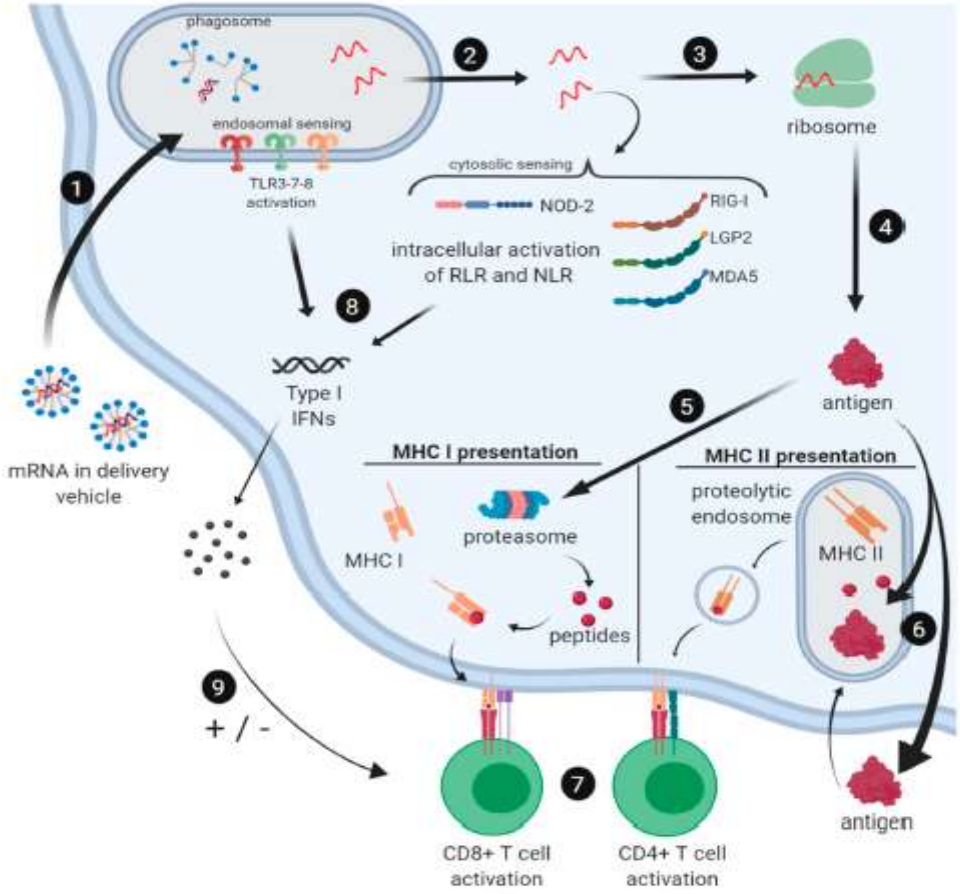
4) The mRNA product is purified to remove any remaining DNA template, double-stranded RNA, and other contaminations by HPLC and tested for stability, integrity, identity, and homogeneity.

5)Delivery of mRNA

- Complexing agents are added.Only a fraction will get into the cytoplasm and most of the internalized mRNA will get entrapped and degraded in lysosomes hence complexation may enhance uptake by cells and/or improve delivery to the translation machinery in the cytoplasm therefore it is often complexed with either lipids or polymers
- Specific mRNA delivery vehicles and transfection systemshelp the exogenous mRNA escape from the endosome into the cytoplasm before being degraded in a lysosome. The most used systems are: - in vivo electroporation, protamine, cationic Nanoemulsion, modified dendrimer nanoparticles, cationic liposomes, cationic polysaccharide particles, cationic polymers, and different versions of cationic lipid nanoparticles (LNPs)

MECHANISM OF ACTION



(1) mRNA encapsulated in the delivery vehicle is taken up by the host cell. After the delivery vehicle is digested, mRNA is recognized by Toll-like receptors (TLRs) and/or escapes from the phagosome

(2) Different cytosolic pathogen recognition receptors can then recognize the mRNA

(3) mRNA is translated by the host's ribosome and antigen is formed.

(4) After the antigen is formed, it can be processed through different pathways.

(5) The antigen is broken down to peptides by the host proteasome; peptides are accepted by major histocompatibility complex class I (MHC I). The MHC class I-peptide complex then travels to the cell membrane where it is presented to the immune system.

(6) The antigen is secreted and ingested by an endosome or alternatively enters the endosome

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without secretion, achieved by adding signaling molecules and sequences. The antigen is then degraded by endosomal proteases and peptides are bound by major histocompatibility class II (MHC II). The MHC class II-peptide complex then travels to the cell membrane where it is presented to the immune system.
(7) CD8+ and CD4+ T cell activation can be achieved through the presentation of the peptide on MHC class I and MHC class II, respectively. Co-stimulatory molecules and cytokines need to be present for successful activation.
(8) TLR3-7-8 and NOD2, RIG-I, LGP2, and MDA5 can be activated by mRNA, subsequently triggering the production of type I interferons.
(9) Secreted type I interferons can have a positive or negative effect on T cell activation. The activation level of the type I innate immune response triggered by mRNA can be controlled by the application of modified nucleosides, improved RNA purification, and low-immunogenic delivery system

CLINICAL TRAILS

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Argos Therapeutics	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	• NCT00672191 (II) • NCT01069809 (II) • NCT02042248 (I)	• Completed[105] • Completed; results NA • Completed; results NA
CureVac AG	RNActive viral Ag mRNA (i.m., i.d.)	Rabies virus	NCT02241135 (I)	Active[56,91]
Erasmus Medical Center	DC loaded with viral Ag mRNA with TriMix (i.nod.)	HIV-1	NCT02888756 (II)	Recruiting
Fundació Clinic per la Recerca Biomèdica	Viral Ag mRNA with TriMix (NA)	HIV-1	NCT02413645 (I)	Active
Massachusetts General Hospital	DC loaded with viral Ag mRNA (i.d.)	HIV-1	NCT00833781 (II)	Completed[104]
McGill University Health Centre	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	NCT00381212 (I/II)	Completed[102]
Moderna Therapeutics	Nucleoside-modified viral Ag mRNA (i.m.)	Zika virus	NCT03014089 (I/II)	Recruiting[85]
		Influenza virus	NCT03076385 (I)	Ongoing[22]

Clinical trials with mRNA vaccines against infectious diseases

III. CONCLUSION

mRNA vaccines have great potential and offer advantages over conventional vaccines like it is non-infectious, non-integrating, naturally degraded, stimulate of innate immune response by induction of T and B cell immune response. It is the growing body of preclinical and clinical results demonstrates that prophylaxis and therapy with mRNA promises to be useful for preventing infectious disease and treating tumours and that mRNA vaccines are safe and tolerated in animal models and humans. Still it has some concerns regarding its instability, immogenencity, production cost and adverse side effect. Although, will future funding and research on it improvements in increase antigen-specific immune responses, the magnitude of memory immune cell responses, including memory B and T cell responses will be noticed.

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