mRNA Vaccines -- A new era in vaccinology

Nan Zhang July. 16th. 2022

Content

Vaccine & mRNA vaccine

> IVT mRNA design

> Delivery system

> mRNA vaccine vs. other vaccines



Vaccine

Vaccine: a biological preparation that provides acquired immunity to a particular infectious disease.





mRNA Vaccine

- > 1987, mixed mRNA with DOTMA (cationic lipid)
 - Transfection NIH3T3 mouse cells
 - It might be possible to "treat mRNA as a drug"



N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA)



Robert Malone "inventor of mRNA vaccines"

Promoted misinformation about the safety and efficacy of COVID-19 vaccines



Proc. Natl Acad. Sci. USA 1989, 86, 6077-6081.

Evidence for translation of rabbit globin mRNA after liposomemediated insertion into a human cell line

Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes

Nature **1978**, *274*, 921–923.

Nature 1978, 274, 923-924.

mRNA Vaccine



Influenza A (in mice)



> 2012, 1st mRNA vaccine in LNPs tested



> 2020, COVID-19 mRNA vaccine



Founded in 2010

mRNA Vaccine



COVID-19 mRNA Vaccine

Lipid nanoparticle mRNA CGAGΨΨCGΨGΨΨΨΑΑ... The vaccines made by Moderna and Pfizer-BioNTech use mRNA that has been chemically modified to replace the uridine (U) nucleotide with pseudouridine (Ψ). This change is thought to stop the immune system reacting to the introduced mRNA. To help the body mount an effective immune response to later SARS-CoV-2 infections, the mRNA sequence is adapted to stabilize the spike protein in the shape it uses when fusing with human cells. Lipids mRNA The fatty nanoparticle around the mRNA is made of four types of lipid molecule. One of these is 'ionizable': in the vaccine, many of these molecules have a positive charge PEG-lipid* Phospholipid and cling to negatively charged mRNA, but they lose that charge in the more Cholesterol Ionizable lipid alkaline conditions of the bloodstream. reducing toxicity in the body. *Lipid attached to polyethylene glycol

In vitro Transcribed (IVT) mRNA

> 1984, Synthesized mRNA by bacteriophage SF6 RNA polymerase in lab

➤ 1990, Jon A. Wolff & Robert W. Malone

> β gCAT β gA_n RNA & β gLuc β gA_n RNA





1: control; 2: 5% sucrose; 3: 0.005 unit CAT standard; 4: 0.05 unti CAT standard;

5-8: 100 μ g β gCAT β gA_n RNA in 5% sucrose;

9-10: 20 μ g β gCAT β gA_n RNA with 60 μ g lipofection reagent

Nucleic Acids Res. 1984, 12, 7035–7056. Science 1990, 247, 1465–1468.



Translation Initiation



Balance between translation and mRNA decay

mRNA Design



(synonymous codons & GC content) Optimized base usage to reduce endonucleolytic attack (uridine to pseudouridine)

Modulation of Immunogenicity

Innate immune receptors

Toll-like receptors (TLRs)



Fast protein liquid chromatography (FPLC)



- Single-pass membrane-spanning receptors
- Recognize structurally conserved molecules derived from microbes.
- TLR3, TLR7, TLR8, TLR9 located in intracellular vesicles (as sensors of nucleic acids)

Nucleoside Modification



Immunity 2005, 23, 165–175.

Direct Injection





1: control; 2: 5% sucrose; 3: 0.005 unit CAT standard; 4: 0.05 unti CAT standard; **5-8**: 100 μg βgCATβgA_n RNA in 5% sucrose; **9-10**: 20 μg βgCATβgA_n RNA with 60 μg lipofection reagent

Cancer patient

Science 1990, 247, 1465-1468.

Delivered by macrophages & immature dendritic cells



mRNA Delivery System



Cationic Peptides and Polymers

Cationic peptide – protamine – Arginine-rich nuclear protein





> Toxicity & high poly dispersity index (PDI)

Cationic Nano-emulsion (CNE)

> MF59 -- oil-in-water emulsion-based delivery systems



Expert Rev Vaccines. 2013;12, 13-30.

Lipid Nanoparticles (LNPs)

- Fused with lipid bilayer of endosomes
- Against RNases
- Components:
 - Cationic ionizable lipids
 - PEG lipids
 - Phospholipids
 - Cholesterol
- Formulation
 - pH < 4.0 aqueous
 - Microfluidic mixing
 - RNases free



Cholesterol

PEG lipids

Helper phospholipids



Cationic ionizable lipids

mRNA



Lipid Nanoparticles (LNPs)

> Cationic ionizable lipids



T Follicular Helper Cell Response



Safety

- Theoretically safe vaccine format
 - No toxic chemicals & cell culture
 - Few opportunities to introduce contaminating microorganisms
 - Do not infection or integration into host cell DNA





Protamine-based rabies candidate CV7201 Severe adverse events in 78% participants CV7202: dose < 1 µg



LNPs-based COVID-19 vaccine: mRNA-1273 Anaphylactic reactions: 2.5 per million 2 folds more than traditional vaccines PEGylated lipid used in LNPs LNPs-based COVID-19 vaccine: BNT162b2 Anaphylactic reactions: 4.7 per million 4 folds more than traditional vaccines PEGylated lipid used in LNPs



No pain, no gain

mRNA Vaccines in development

SARS-CoV-2



Influenza virus



Zika virus



prM-E

Neuromalformations during pregnancy²⁵⁵, antibodydependent enhancement¹⁵⁶

Spike protein, spike protein

receptor-binding domain

pancoronavirus vaccine²¹²

Emerging variants²⁵²

Multivariant booster8.

Maternal vaccination²²⁵, vaccine encoding monoclonal antibody¹⁵⁷





Ebola virus



Fusion protein

VAED¹⁷³, no approved vaccine, multiple late-stage clinical trial failures¹⁷⁴

Target prefusion F conformation for neutralizing antibodies¹⁷⁹

Glycoprotein

Current FDA-approved vaccine requires -80 °C storage²⁵⁴, no mRNA vaccine in clinical development

Thermostable vaccine



Glycoprotein

Near 100% fatality after infection, setbacks in clinical trials¹⁸⁸

Optimization of delivery vehicles

Plasmodium gametocyte (malaria parasite)



PMIF, PfGARP

Lack of surface antigens, complex life-cycle of parasite²⁵⁸

Target infected cells¹⁹³, prevent immune evasion¹⁹²

Strategies

mRNA Vaccines vs Others

- ◆ Inactivated: killed to destroy disease-producing capacity; influenza, rabies, HPV
- Attenuated: reduce the virulence, but still alive; polio vaccine, smallpox vaccine
- Toxoid: inactivated toxin with immunogenicity; tetanus toxin
- Subunit: contain purified parts of pathogen; hepatitis B, pertussis vaccine
- Viral vectors: use a viral vector to deliver genetic materials; Ebola, COVID-19
- DNA: a specific antigen-coding DNA sequence; antivenom sera.
- mRNA: use mRNA to produce immune response. COVID-19



Summary

