

Formulation approaches of RNA vaccines and drugs

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 Phospholipid Research Center

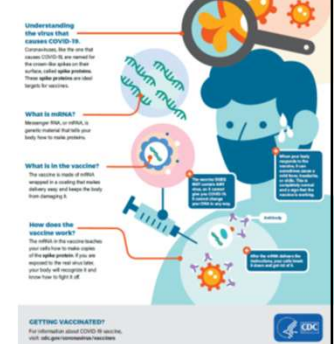
18. May 2022 | ONLINE

1

Content:

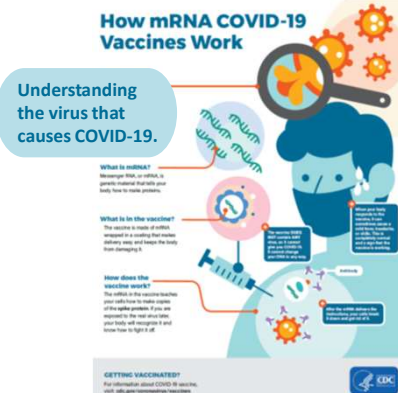
- Introduction – Basics
- mRNA Vaccines
- Lipid Nanoparticles
 - Excipients
 - RNA drugs
- Manufacturing
- Outlook – Next steps

How mRNA COVID-19 Vaccines Work



2

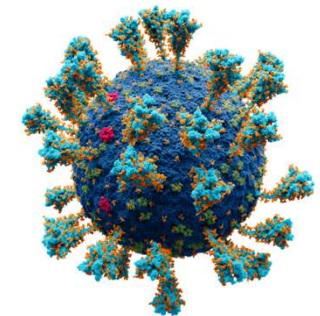
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3

Introduction: Basics

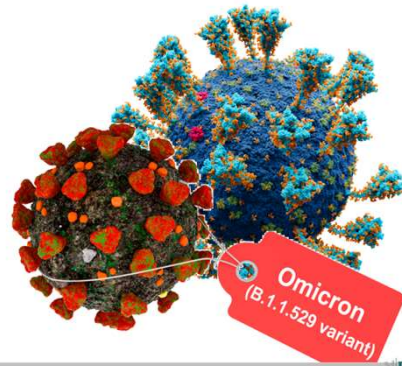
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
 - SARS-CoV-1 and MERS
 - Four common cold human corona viruses
- Enveloped, positive-sense, single-stranded RNA virus
- WHO declared the outbreak on 30. January 2020
- Pandemic claimed on 11. March 2020



4

Introduction: Basics

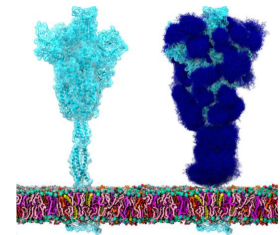
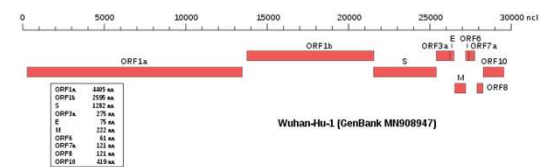
- Several variants of concern (VOC)
 - Alpha (B.1.1.7 UK)
 - Beta (B.1.351 South Africa)
 - Gamma (P.1 Brazil)
 - Delta (B.1.617.2, India)
 - Omicron (B.1.1.529, South Africa)
 - BA.1 / BA.2 / BA.3
 - BA.4 / BA.5
 - Deltacron (France)
 - Delta+BA.1 (XD), BA.1+BA.2 (XE), Delta+BA.2 (XF)
- Deltacron since March 2022



5

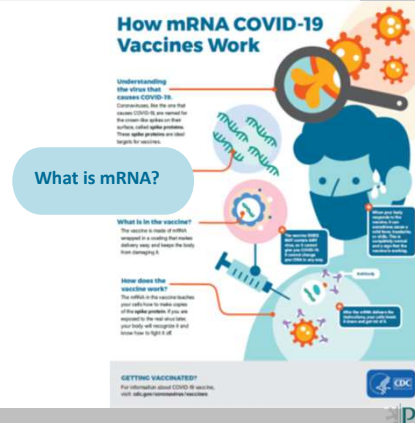
Introduction: Basics

- Virus cell uptake via the angiotensin converting enzyme 2 receptor (ACE2)
- Spike protein 1,273 aa residues in SARS-CoV-2
- 3821 nucleotides encoding full-length SARS-CoV-2 spike (S) glycoprotein



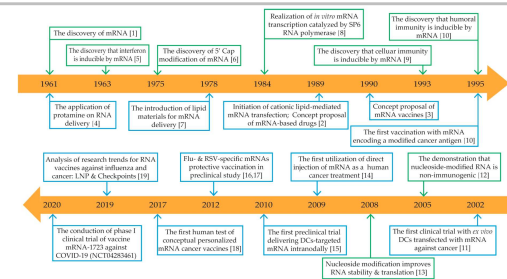
6

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7

Introduction: RNA discovery



- RNA technology BioNTech in 2008 and Moderna in 2010 (Uridine modified RNA)
- 02 December 2020, the UK's MHRA approved Pfizer–BioNTech vaccine
- 11 December 2020, the US FDA issued an emergency use authorization for the Pfizer–BioNTech vaccine and a week later for the Moderna vaccine

8

Vaccines: mRNA

- **COMIRNATY** Pfizer-BioNTech
- Nucleoside-modified messenger RNA (modRNA) sequence with 4,284 nucleotides
 - N1-Methylpseudouridine
- Two proline substitutions K986P and V987P 2P-Design



- Nucleoside-modified messenger RNA (modRNA) compound codenamed mRNA-1273 (**Moderna - Spikevax**)
- 4,101 nucleotides that encodes the full-length SARS-CoV-2 spike (S) glycoprotein with two mutations (K986P and V987P)

9

Vaccines: mRNA

- 35 SARS-CoV-2 vaccines approved
- mRNA vaccines approved as SARS-CoV-2 vaccines in up to 130 countries world wide
 - Pfizer-BioNTech: BNT162b2 (Comirnaty)
 - Moderna: mRNA-1273 (Spikevax)
 - Takeda: TAK-919 (licensed Moderna Spikevax)
- mRNA vaccines in clinical trials
 - Walvax COVID-19 vaccine (ARCoV, Phase III)
 - Chulalongkorn University: ChulaCov19 (Phase II-III)
 - Pfizer-BioNTech: BNT162b2SA – Beta variant (Phase II)
 - Moderna: mRNA-1273.211 (double compound, alpha and beta variants); mRNA-1283 / mRNA-1273.529 (Omicron) mRNA-1273.213 (Beta/Delta)
 - CureVac/GSK: CV2CoV (Phase I/II) (double compound, alpha and beta variants)



10

Vaccines: mRNA

- mRNA vaccines not longer in clinical assessment:



- CureVac: CVnCoV (Phase III)
- Sanofi Pasteur: MRT5500 (Phase I/II)
- Imperial College London LNP-nCoVsaRNA



11

How mRNA COVID-19 Vaccines Work

Understanding
After virus that causes COVID-19. Consequently, the virus that causes COVID-19, are normal for the virus the surface of their surface, other spike proteins. These spike proteins are other targets for vaccines.

What to understand?
Messenger RNA, or mRNA, is a genetic code that tells your body how to make proteins.

What is in the vaccine?
The mRNA in the vaccine teaches your cells how to make copies of the spike protein. You are exposed to the real virus later, your body will recognize it and know how to fight it off.

GETTING VACCINATED?
For information about COVID-19 vaccines, visit www.cdc.gov/coronavirus.

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12

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GRA

- Guerrero et al. Front. Chem. 8:589959 (2020)



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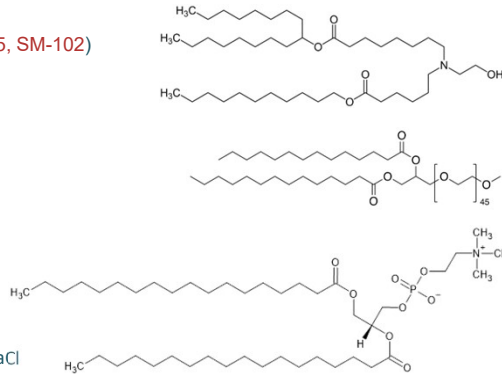
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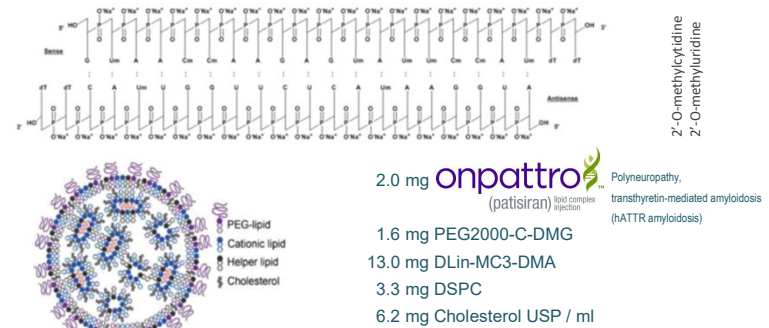
Vaccine-Formulation: quantitative LNP composition

- 48% Cholesterol
- 40% Cationic Lipids (ALC-0315, SM-102)
- 10% Zwitterionic Lipids
 - (DSPC)
- 2% PEG Lipids
 - (PEG2000-DMG)
- Molar Charge ratio (+/-)
 - Cationic Lipid : mRNA
2:1 up to 5:1
- Diluent (Pfizer-BioNTech)
 - 0.45 ml + 1.8 ml 0.9% NaCl



17

Lipid Nanoparticle (Complex) Formulation: onpattro



18

Vaccine-Formulation: CureVac

- RNActive[®] vaccines containing both free and protamine-complexed mRNA
- Weight ratio of 2:1 (free RNA / protamine complex RNA)
- Modification of mRNA sequence
 - Stabilized mRNA with an increased G/C content and optimised Codon
 - Non-modified Uridine



19

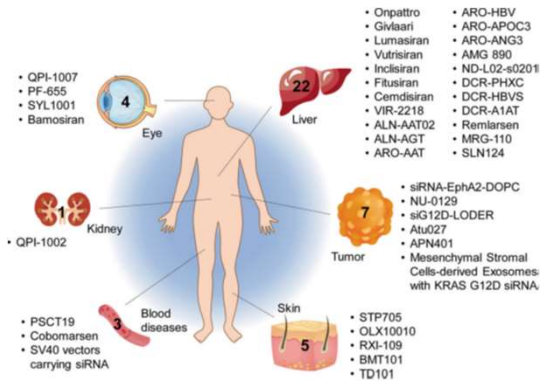
siRNA-Drugs:

Alnylam[®] PHARMACEUTICALS

- 7 drug candidates in clinical trials
 - LNP
 - GalNAc (N-acetylgalactosamine)
- Drug Delivery Systems:
 - Polymer Matrix System / CPP
 - Gold Nanoparticles
 - Exosomes
 - Liposomes (DOPC)
 - Virus Like Particles (VLP)

20

siRNA-Drugs:



Hu, B. et al. J. Gene Med. 21, e3097 (2019)

21

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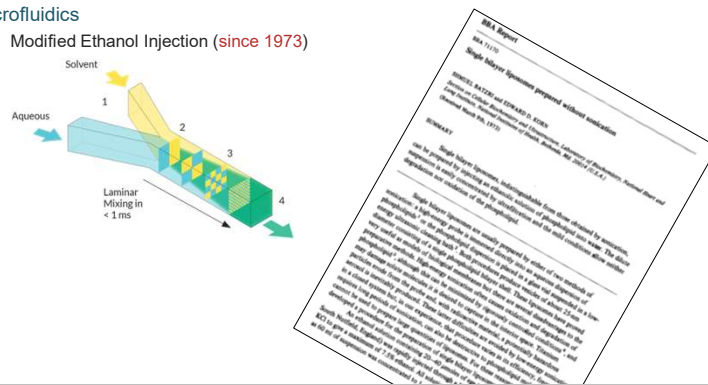
Preparation, Application and How does it work?

GETTING VACCINATED?
For information about COVID-19 vaccines, visit www.hhs.gov/covid19

22

Manufacturing: Lipid Nanoparticles

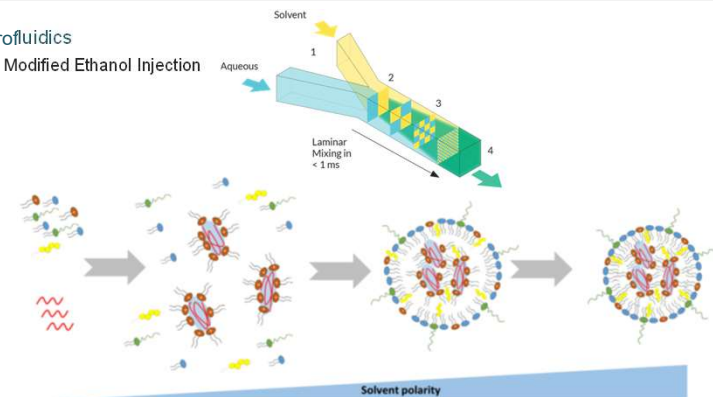
- Microfluidics
 - Modified Ethanol Injection (since 1973)



23

Manufacturing: Lipid Nanoparticles

- Microfluidics
 - Modified Ethanol Injection

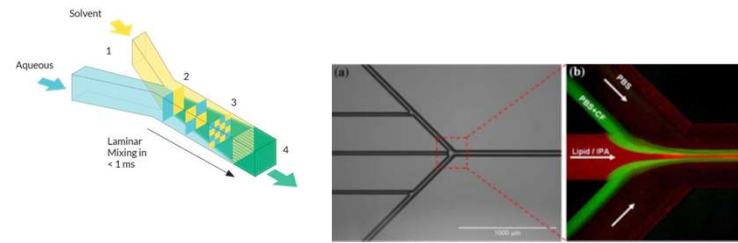


Guevara et al. Front. Chem. 8:589559 (2020)

24

Manufacturing: Lipid Nanoparticles

- Microfluidics
 - Modified Ethanol Injection

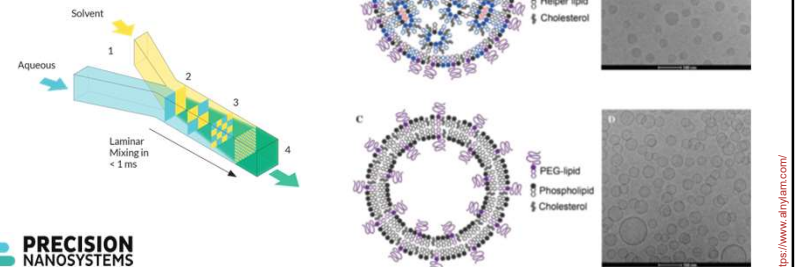


Jahn et al. J Nanopart Res 10, 925–934 (2008)

25

Manufacturing: Lipid Nanoparticles

- Microfluidics
 - Modified Ethanol Injection



26

Manufacturing: Lipid Nanoparticles

- Microfluidics
 - Mixing
 - Dilution
 - Dialysis (Buffer exchange) / Filtration (concentration)
 - Sterile Filtration
 - Fill & Finish



PRECISION NANOSYSTEMS

27

Manufacturing: Lipid Nanoparticles

- Microfluidics
 - Pilot Plant: 200 mL/min.
 - 50 mg mRNA



28

Manufacturing: Lipid Nanoparticles

- Microfluidics
 - GMP Production

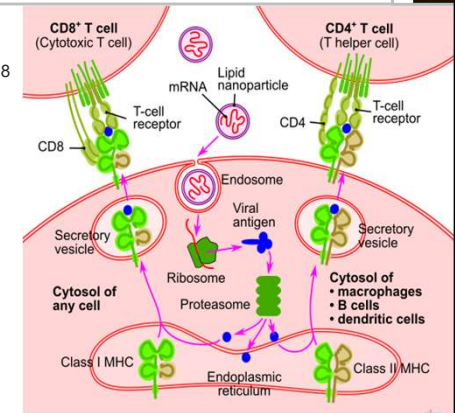


POLIMUN
SOLUTION

29

Lipid Nanoparticles: in vivo Mechanism

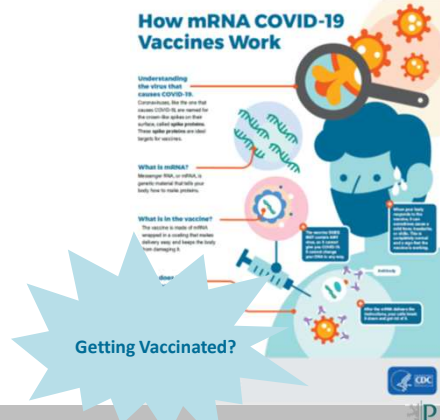
- Opsonization
 - pK_a for immunogenicity between 6.6 and 6.8
 - DSPC and cholesterol enriched at the surface
 - ApoE3 trigger redistribution of the lipids
- Endosomal Uptake
 - Escape crucial for efficacy (<10%)
- Expression of Spike Protein
- Presentation to CD8⁺ T cells and/or CD4⁺ T helper cells



30

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31

Vaccines: mRNA next Generation

- mRNA vaccine / Lyophilization
 - Pfizer- BioNTech: stable at room temperature
- mRNA vaccine
 - LNPs have been shown to be effective adjuvants
 - Pfizer- BioNTech: 10-30 μg / dose
 - Moderna: 50-100 μg / dose

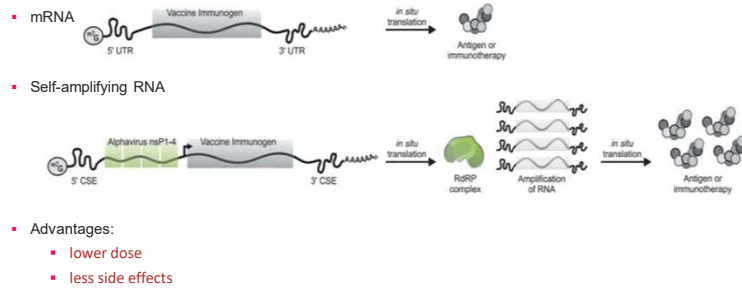


Al et al. *bioRxiv preprint* (2020) <https://doi.org/10.1101/2022.02.10.478967>

32

Vaccines: mRNA next Generation

• saRNA vaccines



- Advantages:
 - lower dose
 - less side effects

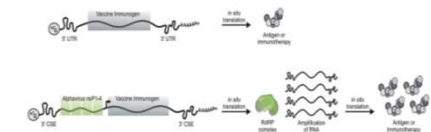
Bloom et al., Gene Therapy (2021) 28:117–128

33

Vaccines: mRNA next Generation

• saRNA vaccines

- Arcturus Therapeutics **ARCT-154** and **ARCT-165** (LNP, Phase II)
- Elixirgen Therapeutics **EXG-5003** (intra dermal, only receptor binding domain, Phase II)
- HDT Bio **HDT-301** LION – saRNA (Phase II)
- Ziphius Vaccines and Ghent University (preclinical): **ZIP-1642** (multi compound saRNA, Delta and Lambda variants)

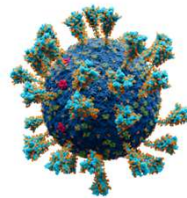


Bloom et al., Gene Therapy (2021) 28:117–128

34

Conclusions:

- mRNA vaccines are under investigation since 1990
- > 30 years basic research on nanotechnology / lipofection
- Formulation technology (LNP) was approved by FDA/EMA before
- RNA and DNA technology will be expanded
- Future:** huge options in oncology / neuro degeneration / Influenza / HIV, etc.



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35

Acknowledgements:

All Team Members and PhD Students



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36