

Considerations for the Quality, Safety and Efficacy of Prophylactic Lipid Nanoparticle mRNA Vaccines

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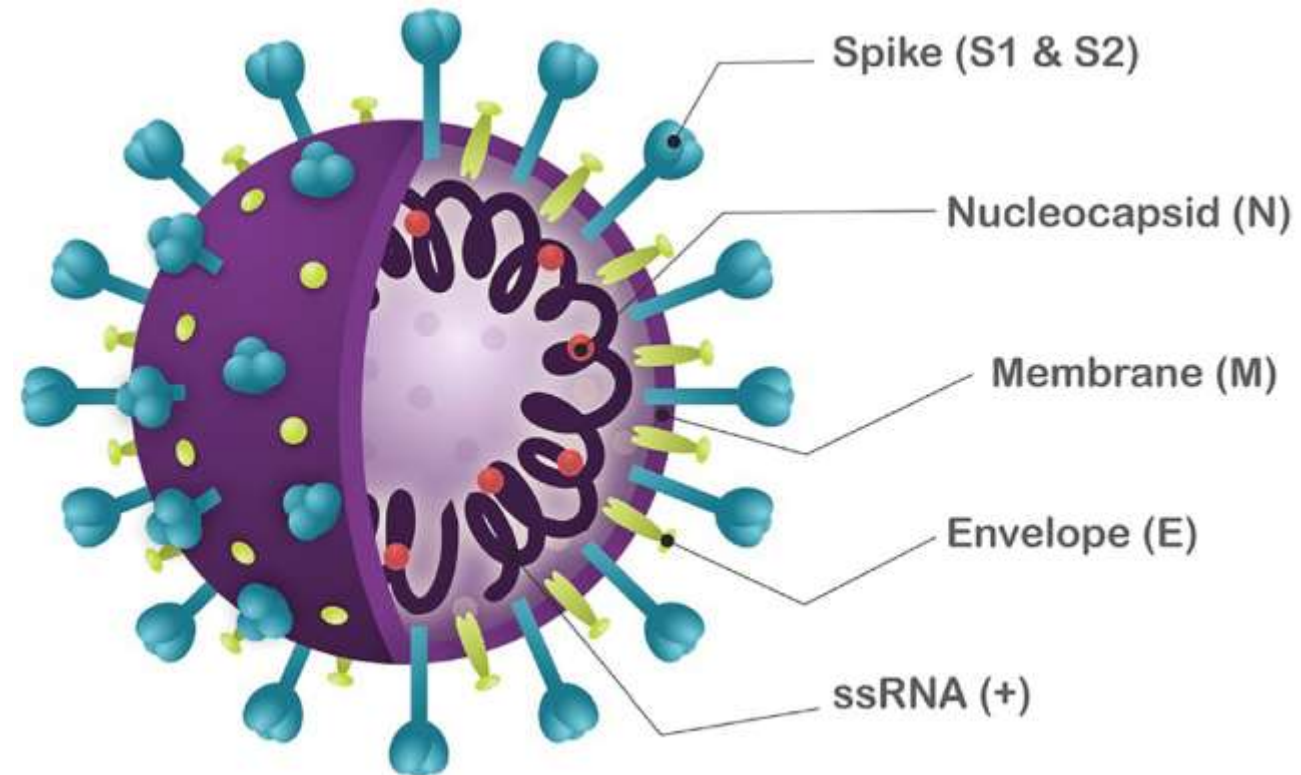
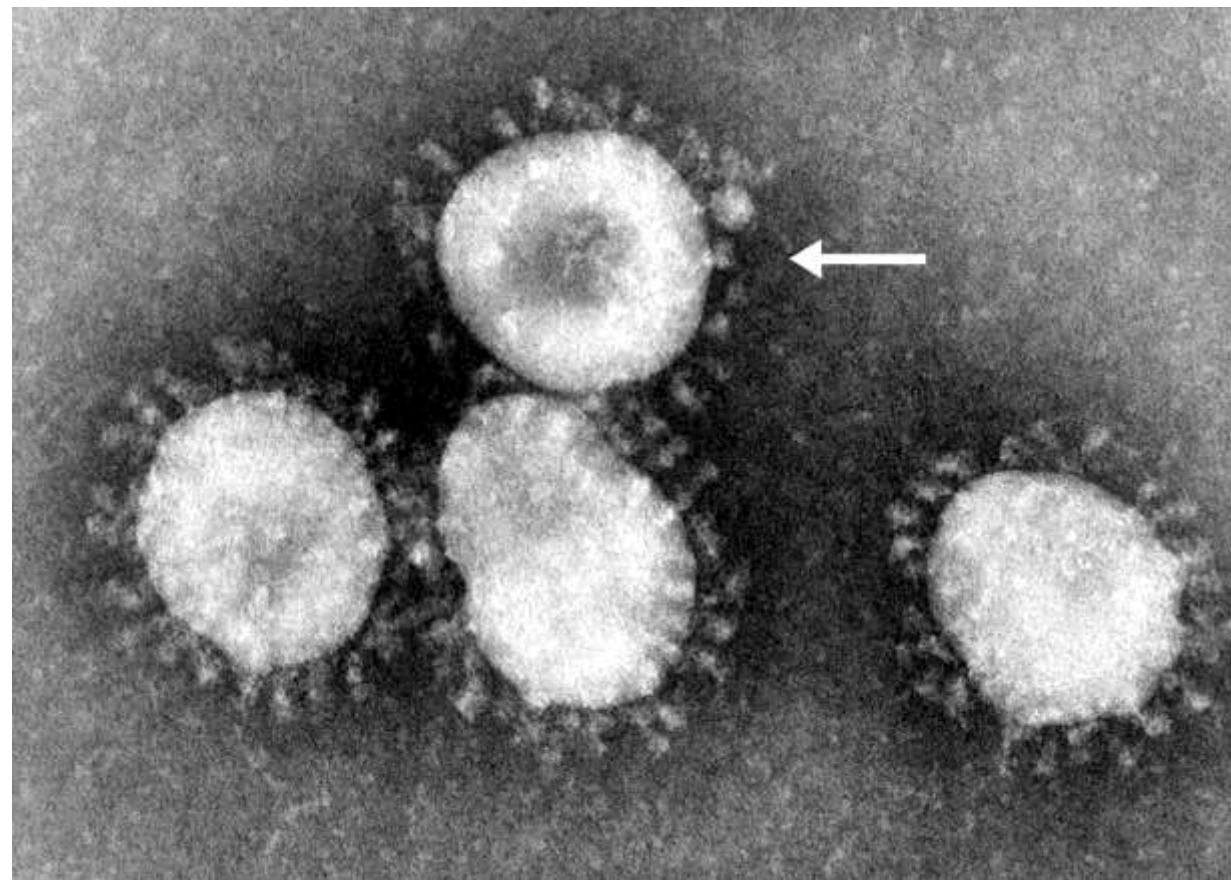
Outline of Talk

- Origins of SARS-CoV-2
- Developmental history of mRNA vaccines
- How they are produced
- Product and CMC Issues
- How to determine the potency of the vaccines
- Pre-clinical studies
- How to assess efficacy: What to monitor and what assays to use
- Is this a platform technology and what would that mean?
- Variants: the evolving nature of coronaviruses

SARS-CoV-2: Origins

- Disease first recognized in December 2019 in Wuhan, China
- China notified WHO of a pneumonia of unknown etiology (January 2020)
- Virus identified as a coronavirus in January 2020

Structure of SARS-CoV-2



SARS-CoV-2: Origins

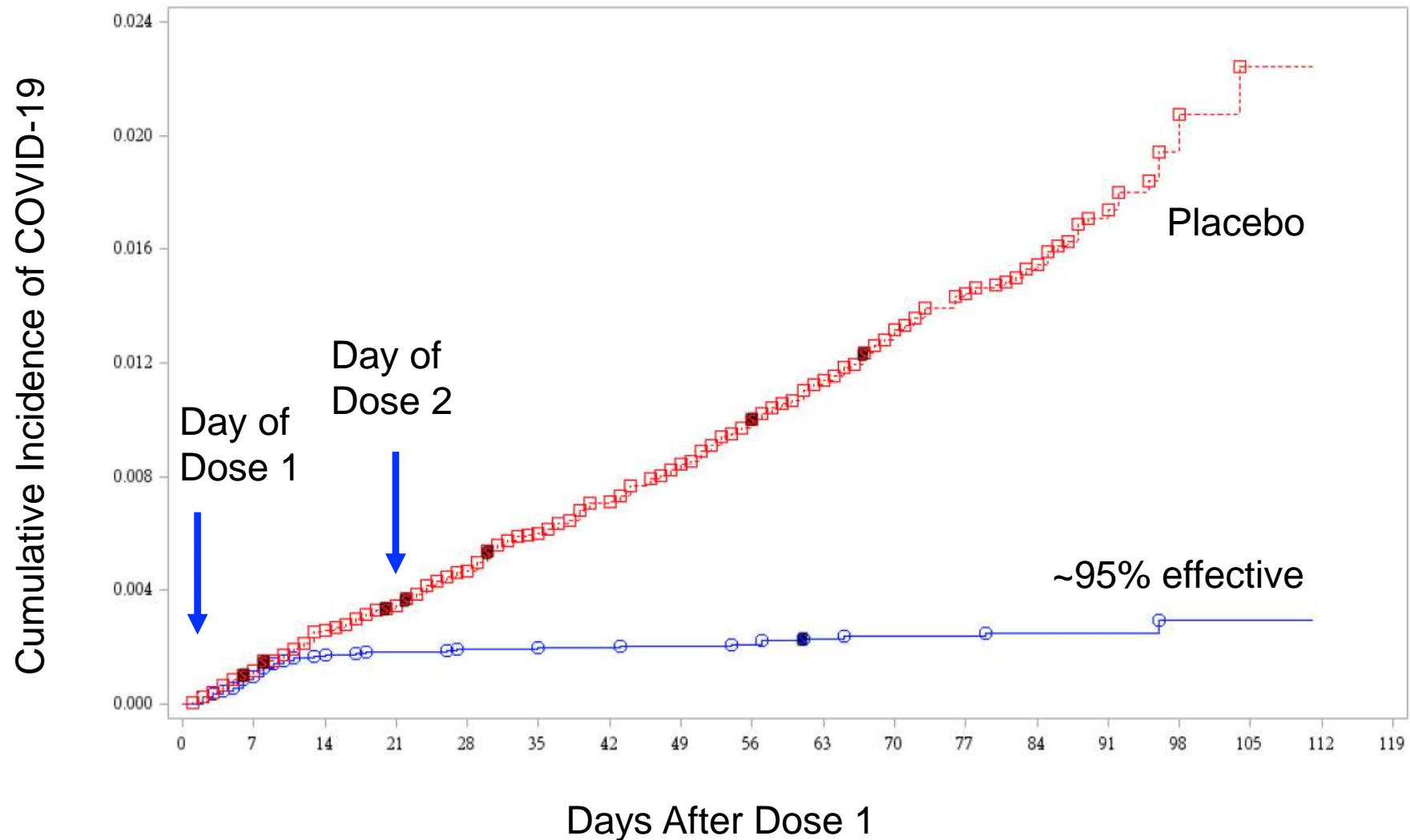
- Disease first recognized in December 2019 in Wuhan, China
- China notified WHO of a pneumonia of unknown etiology
- Virus identified as a coronavirus in January 2020
- Virus thought to have arisen by zoonotic transmission to humans
- Related to the **Severe Acute Respiratory Syndrome** (SARS) virus 2002 - 2004
- Termed SARS-CoV-2
- Disease is COVID-19 for **COronaVirus Disease 2019**
- China released the sequence of SARS-CoV-2 in January 2020
- Allowed scientists to start vaccine design
- January 30, 2020, WHO declared SARS-CoV-2 a Public Health Emergency of International Concern (PHEIC)
- March 11, 2020, WHO declared COVID-19 a pandemic

Some Initial Thoughts on mRNA Vaccines

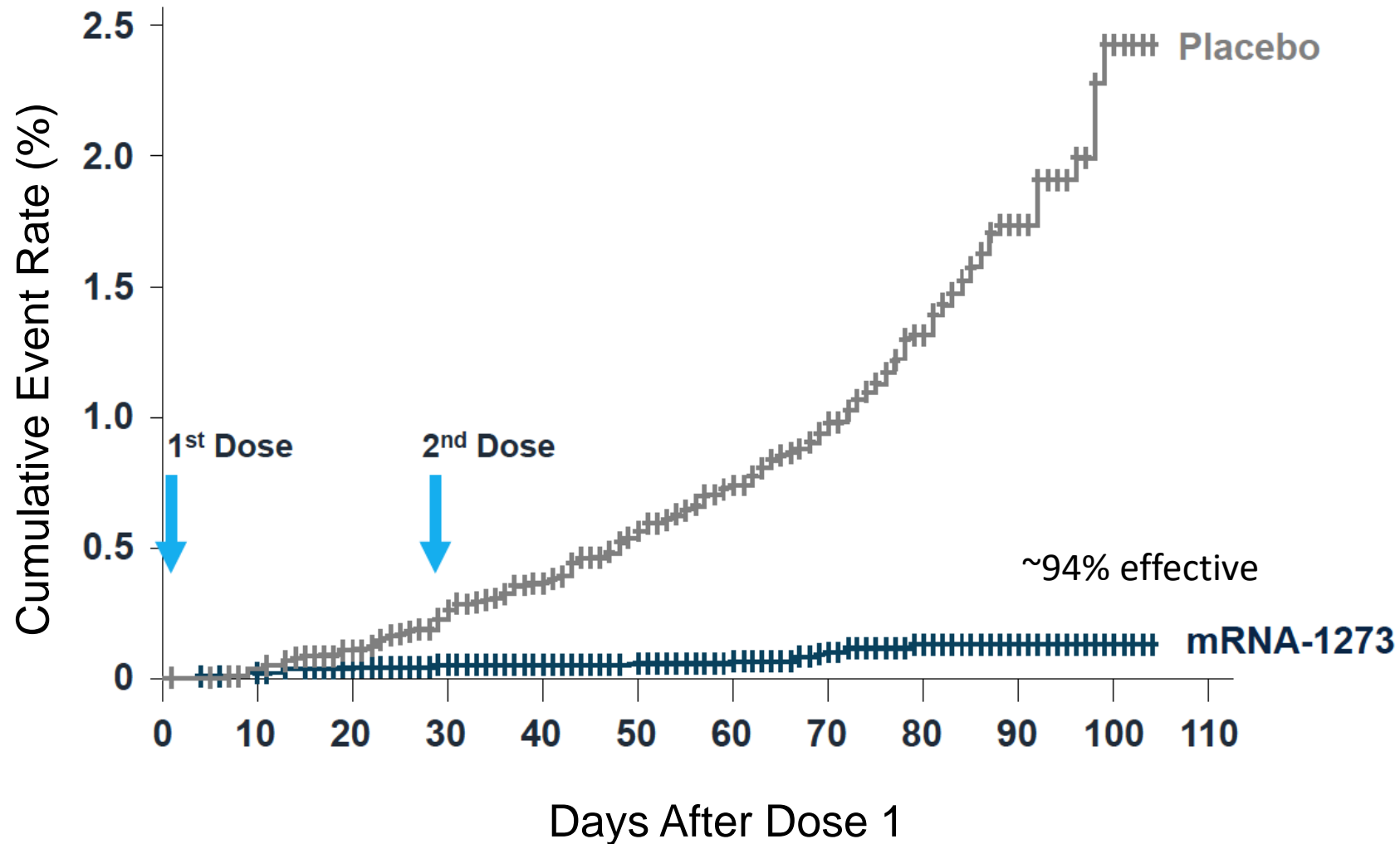
- How could a mRNA vaccine be produced in sufficient quantities to provide the number of doses needed in a pandemic?
- Would this type of vaccine be effective?

Results of Phase III Trials (December 2020)

Efficacy of Pfizer/BioNTech Vaccine BTN162b



Efficacy of Moderna Vaccine mRNA-1273



Advantages of Nucleic Acids as Vaccines

- No need to obtain or propagate the infectious agent (high containment BSL3, BSL4)
- Only requires the sequence of the antigen of interest
- Production of mRNA vaccine starts with a DNA template for expression of the antigen sequence, which can readily be produced synthetically
 - Codon optimization for optimal translation
 - Incorporation of N¹-methylpseudouridine in place of uridine; reduces immune sensing, enhances translation
 - Removal of undesired RNA secondary structures and cryptic splice sites
 - Design in desirable features, such as the P-P pre-fusion stabilizing mutations for SARS-CoV-2 spike protein
 - Rapidly adjust sequence to new variants

The Path to mRNA Vaccines

Review

Three decades of messenger RNA vaccine development

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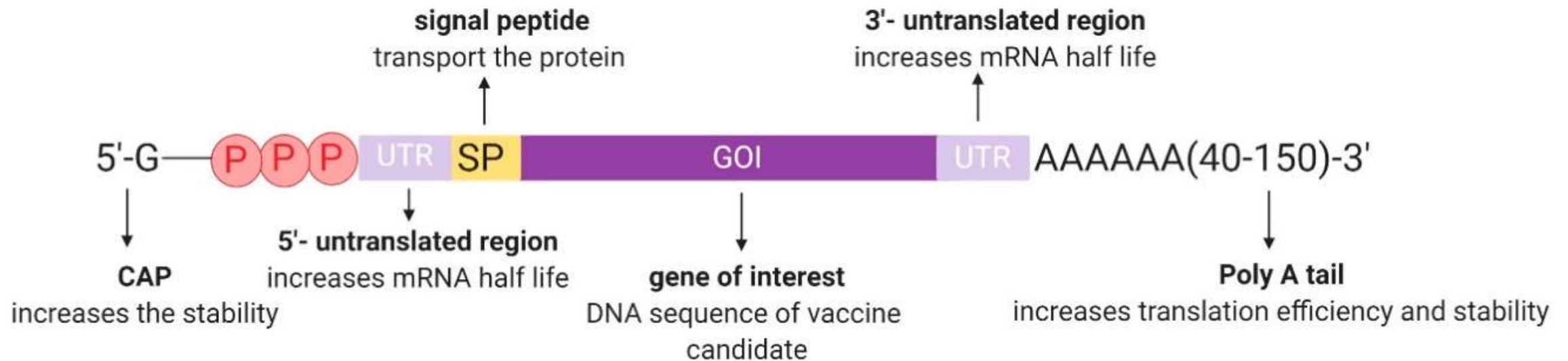
Some Challenges with mRNA as Vaccines or Therapeutics

- RNA is a large anionic molecule that is subject to cleavage by ubiquitous RNases
- Unstable at high pH
- Anionic molecules do not enter cells; require the charge to be neutralized
- RNA is reactogenic through the innate immune system
- Require a delivery vehicle that:
 - Protects RNA from degradation
 - Efficiently enters the cell
 - Shields RNA from the cell's immune sentinels
 - Releases RNA from endosome to allow translation
- LNPs have proven effective as delivery vehicles

Some Important Milestones for mRNA Vaccines

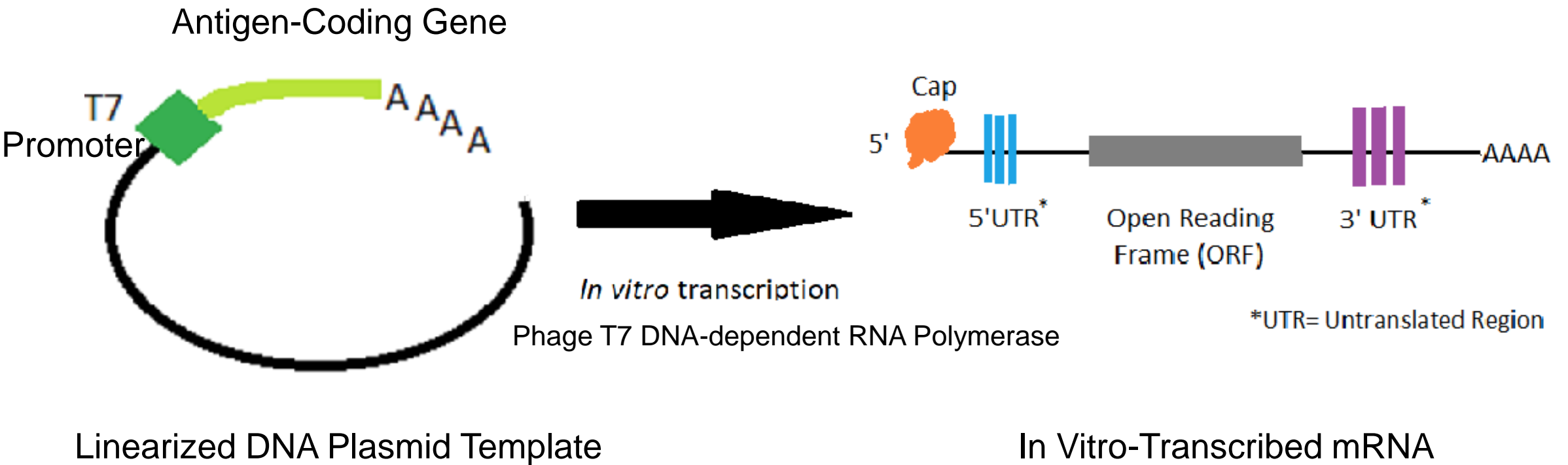
- Krieg and Melton 1984: enzymatic synthesis of mRNA from DNA template
- Wolff *et al.* 1990: Expression of proteins in mouse muscle from injected DNA and from *in vitro*-transcribed mRNA
- Martinon *et al.* 1993: mRNA in liposomes inoculated in mice induced cellular responses to influenza virus nucleoprotein
- Semple *et al.* 2001, 2010: ionizable cationic lipids and LNP
- Karikó and Weissman 2007: Controlling the reactogenicity of mRNA by use of modified nucleosides (Lasker Award 2021 for modified mRNA vaccines)
- Scaled up production of mRNA
- Evolution of LNP technology

Structure of Typical Cellular mRNA



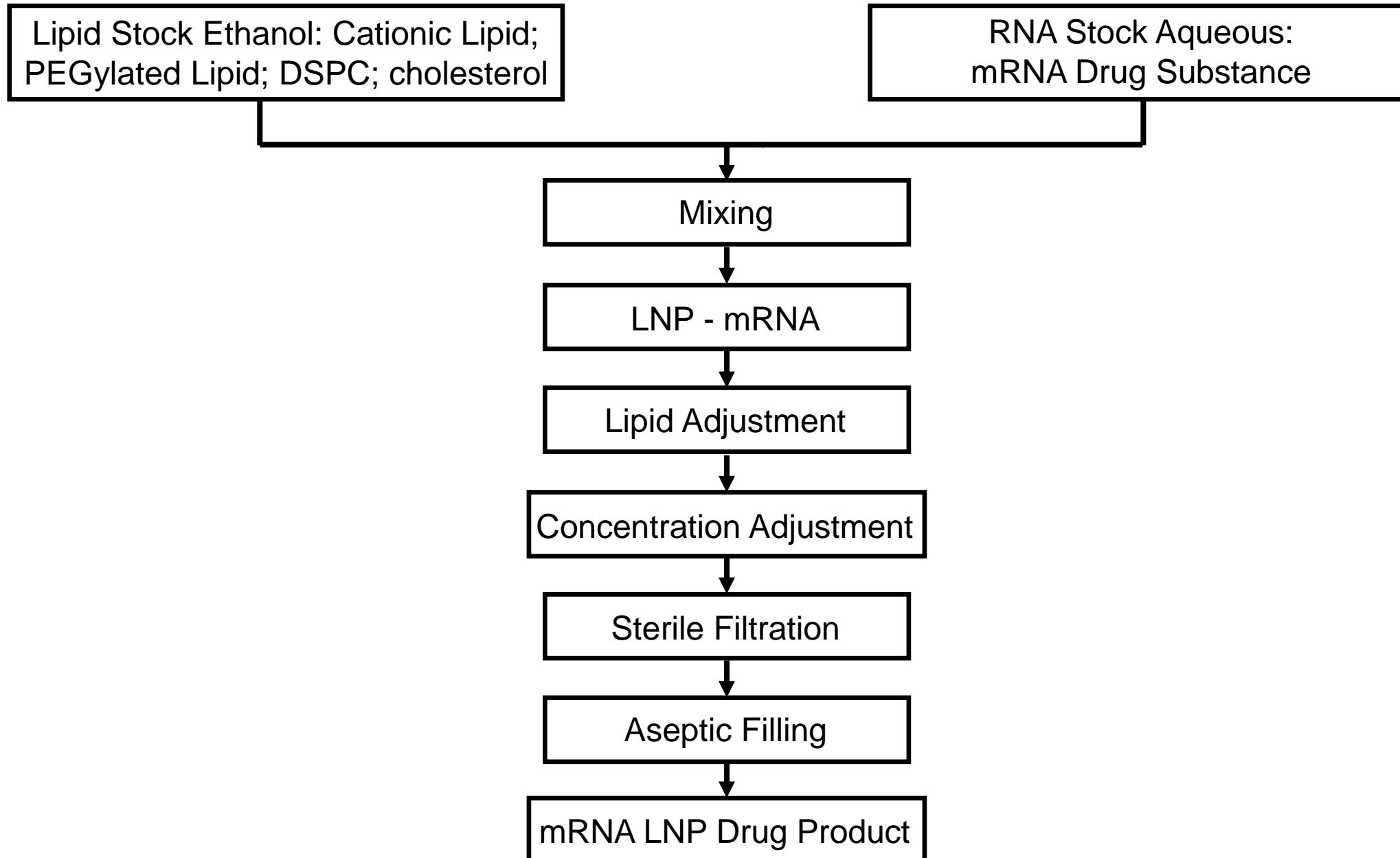
Production of mRNA (Drug Substance)

Steps Involved in mRNA Synthesis



Production of Drug Product

Typical Drug Product Manufacturing Process



Composition and Functions of Lipids in mRNA LNPs

- Ionizable cationic lipid (e.g., MC3, SM-102, ALC-0315)
 - Nucleic acid complexation
 - At pH 5 lipids are positively charged; at pH 7.4 lipids are uncharged
 - Less toxic than permanently charged cationic lipids
 - Membrane fusion
 - Endosomal release
 - Provides adjuvant activity to LNPs
- Phospholipid (e.g., DSPC)
 - Increases transfection efficiency
 - Improves structural integrity and stability
 - Ensures appropriate encapsulation

Composition and Functions of Lipids in mRNA LNPs

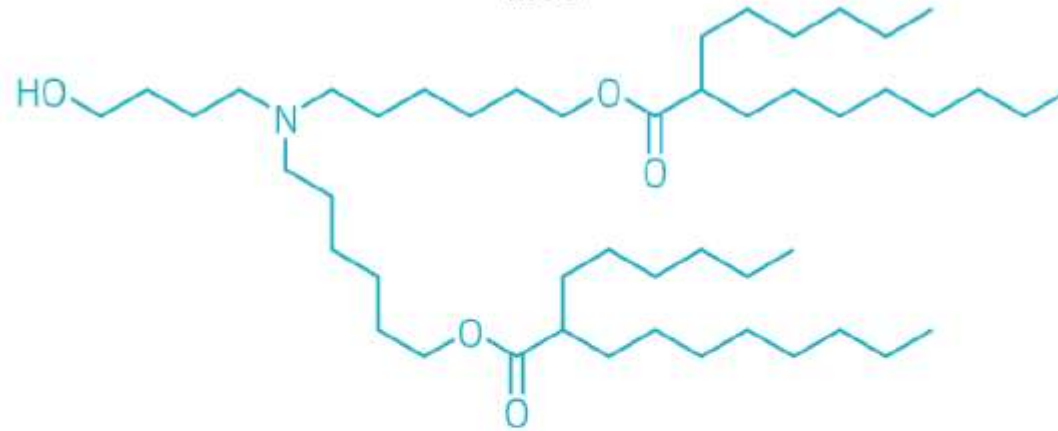
- PEGylated lipid (*e.g.*, DMG-PEG2000)
 - Two domains: Hydrophilic PEG and a hydrophobic lipid
 - Determines the size of LNPs (50 – 110 nm)
 - Minimizes particle aggregation; particles are more homogeneous
 - Prevents binding of plasma proteins; extends lifetime in blood
 - Decreases non-specific immune responses
- Cholesterol
 - Increases particle stability
 - Increases fusogenic properties of particles
 - Endosomal release

Cationic Lipids Used in FDA-Licensed LNP Products



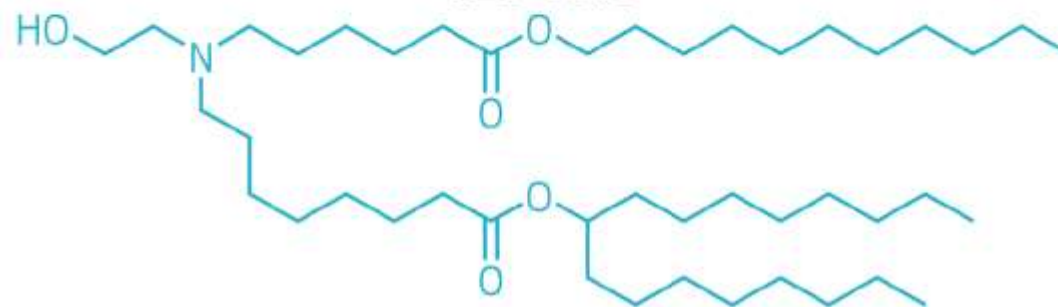
MC3

Onpattro



ALC-0315

BNT162b



SM-102

mRNA-1273

Some Possible Safety Issues

- Novel vaccines with novel components
- How to assess safety?
- What test methods should be used, and would they reveal potential safety signals in clinical trials?
- What animal systems would reveal potential toxicities?
- Should the individual LNP components be evaluated separately or as the vaccine?
- Should mRNA be evaluated separately for its expression *in vivo*?
- How should the results from biodistribution/retention studies be interpreted?

■ Drug Substance

- Assessing mRNA integrity
- Quantifying the proportion of RNA that is:
 - Capped
 - Polyadenylated
 - Full length
- Identity of mRNA (DNA template sequence, RNA sequence, PCR)
- Purity of mRNA (smaller products, dsRNA, residual components of the IVT)

■ Drug Product

- Evaluation of the LNP structure (e.g., particle-size distribution; charge)
- Determining proportion of mRNA encapsulated
- Identifying and quantifying impurities

■ Stability of DS and DP (transportation and storage)

How to Assess Consistency of Manufacture

- Non-clinical studies
- Clinical studies
- Analytical comparability using physicochemical methods

Potency Determination

- How to evaluate potency when the vaccine type is new
- Correlate of protection against SARS-CoV-2 is not known
- It is not known what type of immune response is necessary to elicit
- Potency depends on uptake of the LNP-mRNA by cells and the expression of the antigen
- Potency assays:
 - Cell-culture-based (read-outs: ELISA, western, flow cytometry)
- After demonstrating the vaccine is immunogenic, animal potency assays were not considered practical, desirable, or able to be validated
- Dosing is done by the amount of RNA

Some Issues with Multi-Valent Vaccines

- How to ensure that each DP component is manufactured consistently
 - Mixing mRNAs to produce a single LNP-mRNA DP
 - Manufacturing each DP separately and mixing the DPs
- Determining that each mRNA is present and quantifying the amount of mRNA
- How to assess potency for each vaccine component
- How to assess the stability of each component

Pre-Clinical Evaluation

- Immunogenicity in animal models
 - Required for Phase I
- Demonstration of protection in animal models
 - In what animal model? (Syrian hamsters, NHPs, transgenic mice)
 - At what stage of vaccine development?
 - Has not been required for Phase I

Clinical Assays to Evaluate Immune Responses

- Humoral Immunity
 - Binding antibodies by ELISA
 - Neutralization assays:
 - Live SARS-CoV-2: plaque-reduction neutralization test
microneutralization assay
 - Pseudotyped viruses: replication-competent or incompetent (VSV)
replication-incompetent lentivirus
- Cellular Immunity
 - Assay not used to assess efficacy in the trials but are being used to determine the complete immune responses to the vaccines
 - Likely that cellular immunity plays a role in protection and/or clearance of the virus

Is This a Platform Technology? And What Would That Mean?



- What testing would be required for an mRNA vaccine that expresses a different antigen using the same LNP and manufacturing process?
 - Once the manufacturing process is validated and experience accumulated, could a vaccine for a variant of the same virus be considered as a strain change, similar to that done with influenza vaccines?
 - What testing would be required for a vaccine expressing an antigen for a different infectious agent using the same manufacturing process?
- What non-clinical studies would be required, and which could be dispensed with, based on data from similar products manufactured by the same process?
 - Biodistribution studies
 - Toxicology studies
- How could vaccine development be streamlined for new mRNA vaccines with different LNPs?

Non-Clinical Testing for mRNA Vaccines with Different LNPs

- LNP with known lipid components but in different proportions
 - Biodistribution and toxicology studies might be dispensed with
- LNP with new lipid components for which there is no human experience
 - Biodistribution / retention studies will likely be requested
 - Toxicology studies will likely be requested

CMC Issues for mRNA Vaccines with Different LNPs

- Each product requires its own specifications, as summarized on slide 24
 - Drug Substance: mRNA integrity, percent capped and polyadenylated, identity, and purity
 - Drug Product: LNP structure, particle-size distribution, charge, proportion of mRNA encapsulated, and impurities
- There are no settled specifications
- The immunogenicity needs to be determined
- Potency assay needs to be developed
- Stability of the DS and DP needs to be determined and monitored over time

SARS-CoV-2 and its Variants

- Coronaviruses are RNA viruses
- Evolution of virus is expected
- Viruses with increased ability to replicate and to transmit more efficiently in humans will be selected for
- Immune escape could be a driver of variant generation

Reduction in Protection and Booster Doses

- Experience has demonstrated that immunity wanes over time
- Neutralizing antibodies are less effective against some variants
- Should booster doses be given and which vaccine?
- Should the vaccines be adjusted to reflect current circulating strains?

Summary

- Shown the results of Phase III efficacy trials of the Pfizer/BioNTech and Moderna vaccines, which allowed FDA to grant EUA
- How this type of vaccine is produced
- Some of the manufacturing and product issues with mRNA vaccines
- How potency of the vaccine is determined
- Special issues associated with multi-valent vaccines
- Whether mRNA vaccine manufacture is a “platform technology” and what that would mean for new vaccines
- Evolution of SARS-CoV-2 and why the vaccines may need to be modified

Concluding Remarks

- mRNA vaccines have proven to be surprisingly effective
- The safety of the vaccines have been demonstrated – hundreds of millions of doses given
- Israeli studies reported a low incidence of myocarditis and pericarditis in some populations; not known whether this is an issue associated with mRNA vaccines in general or specifically with COVID-19 vaccines – discussed at a VRBPAC meeting this year
- mRNA vaccines for other infectious-disease indications (and cancer) are in development
- It is likely that modifications to the manufacturing process and to the LNPs will occur in the future

Thank You for Your Attention
