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

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Disclaimer

The views expressed are those of the presenter and should not be construed to represent FDA's views or policies

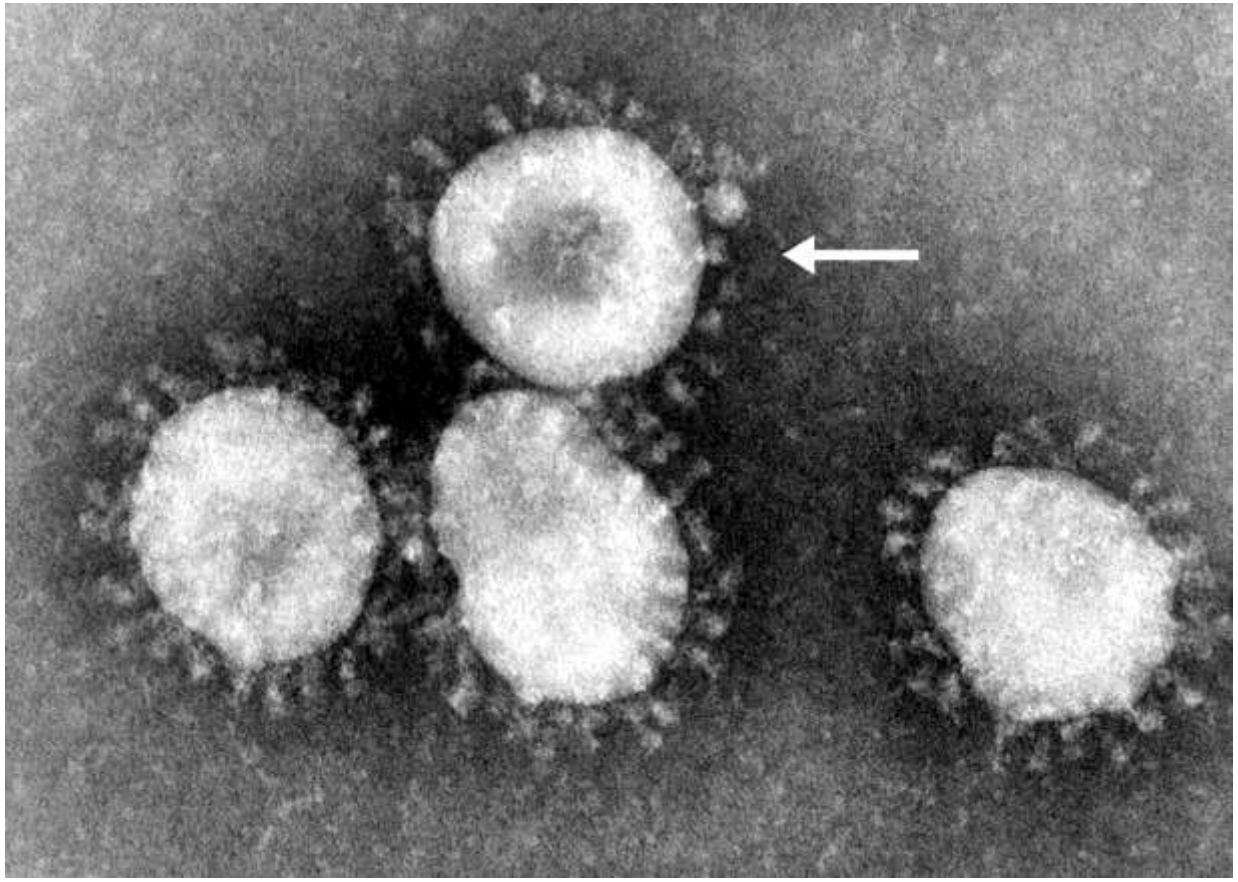
Outline of Talk

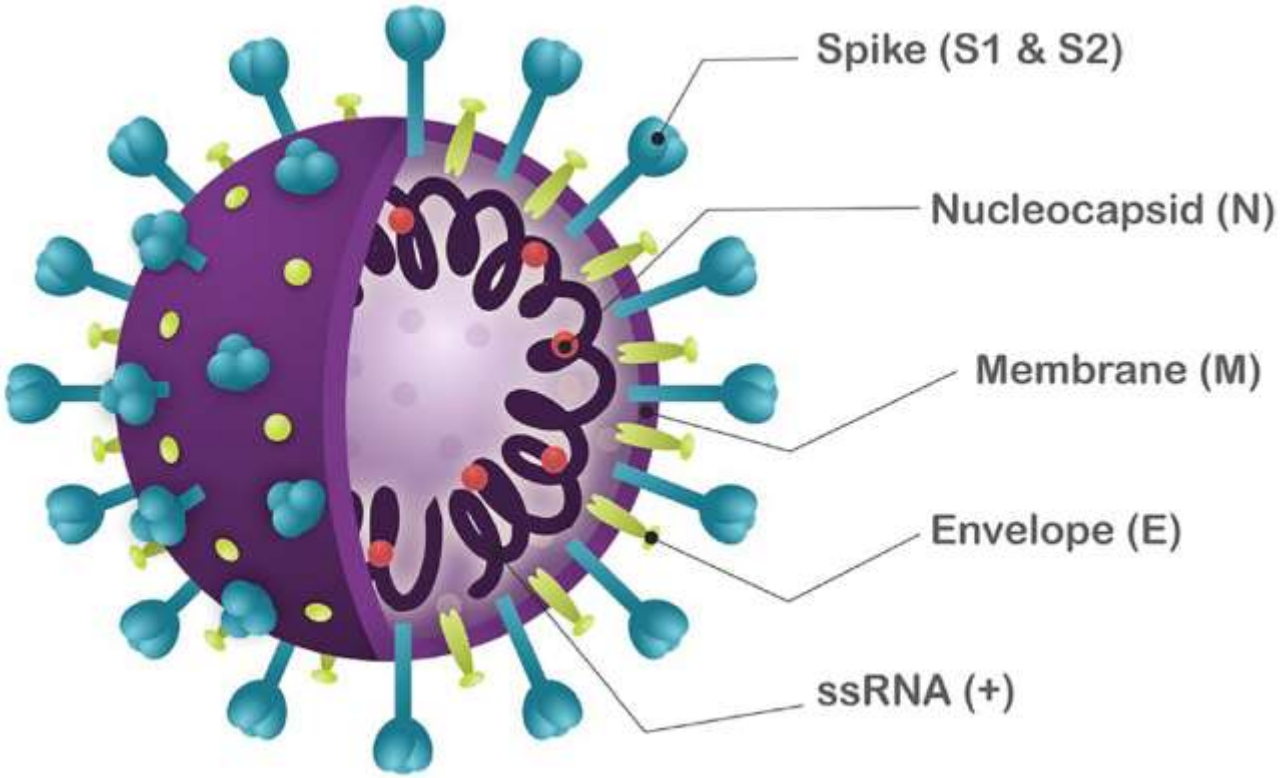
- Origins of SARS-CoV-2
- Developmental history of mRNA vaccines
- How they are produced
- Product and CMC Issues
- How to determine the potency of the vaccines
- Pre-clinical studies
- How to assess efficacy: What to monitor and what assays to use
- Is this a platform technology and what would that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

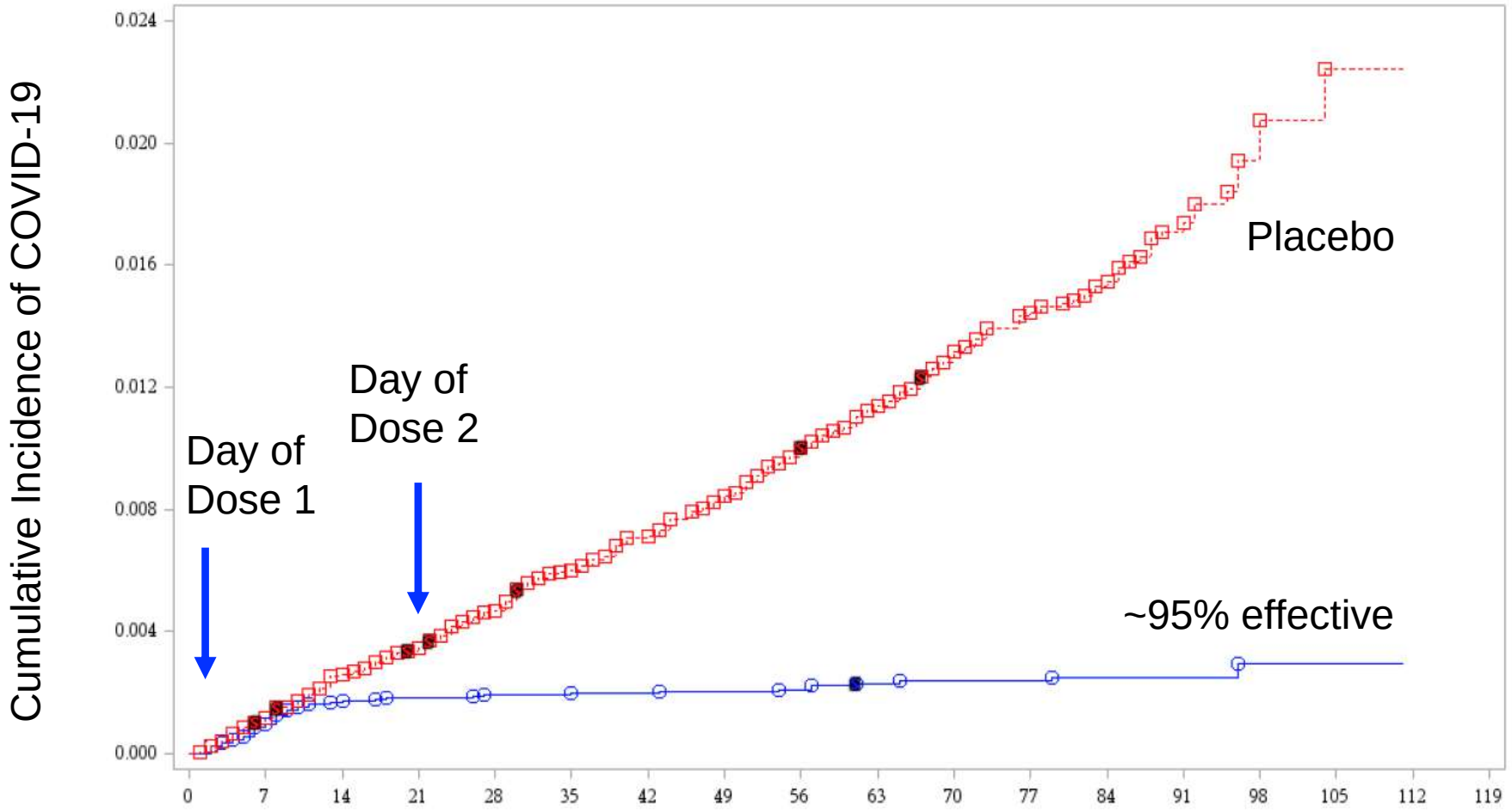
- Disease first recognized in December 2019 in Wuhan, China
- China notified WHO of a pneumonia of unknown etiology
- Virus identified as a coronavirus in January 2020
- Virus thought to have arisen by zoonotic transmission to humans
- Related to the **S**evere **A**cute **R**espiratory **S**yndrome (SARS) virus 2002 - 2004
- Termed SARS-CoV-2
- Disease is COVID-19 for **CO**rona**VI**rus **D**isease 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?

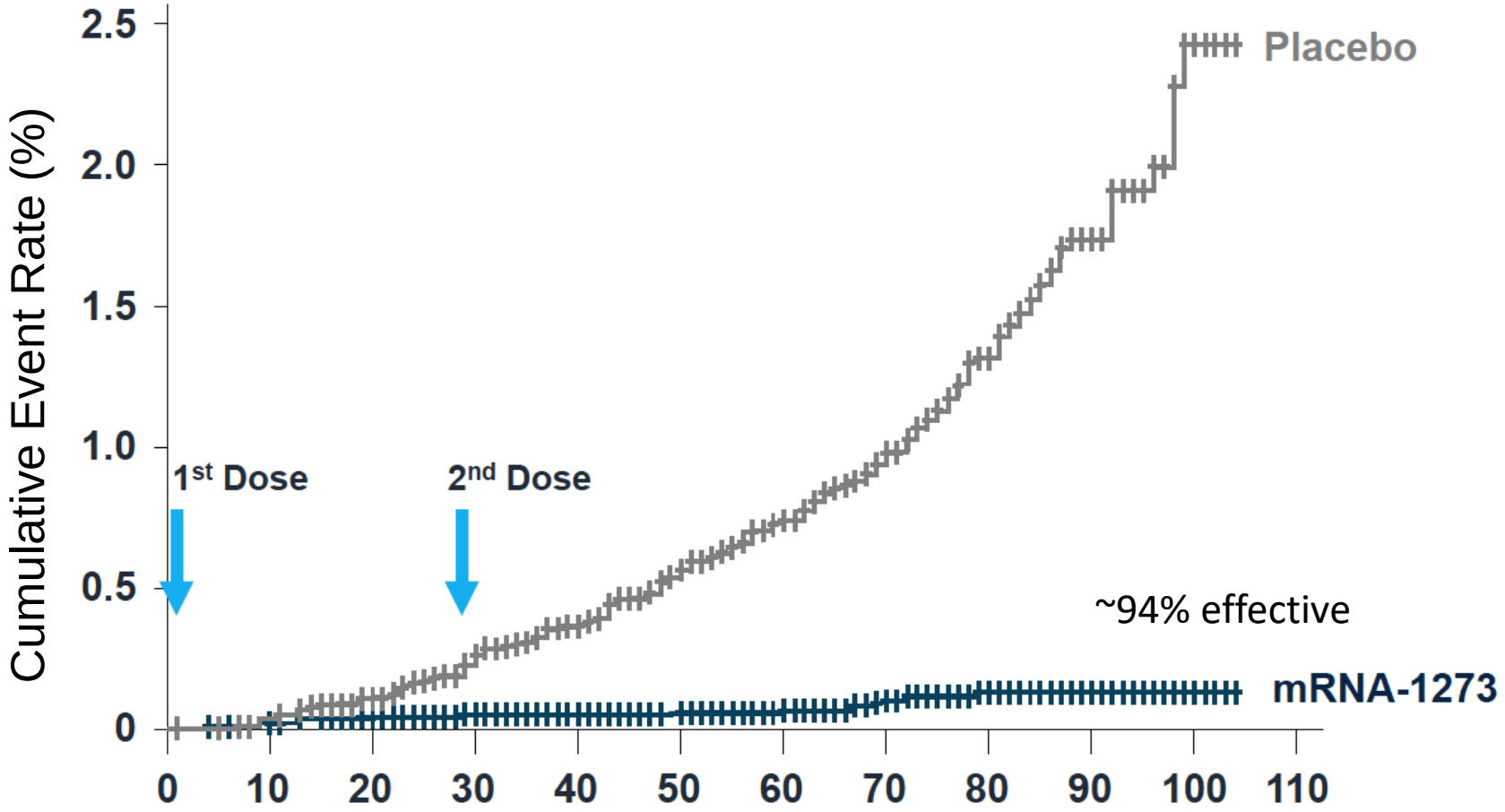
Results of Phase III Trials (December 2020)

Efficacy of Pfizer/BioNTech Vaccine BTN162b



Polack *et al.*, 2020

Efficacy of Moderna Vaccine mRNA-1273



Baden *et al*., 2021

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 N^1-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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Nano Today 28, 2019

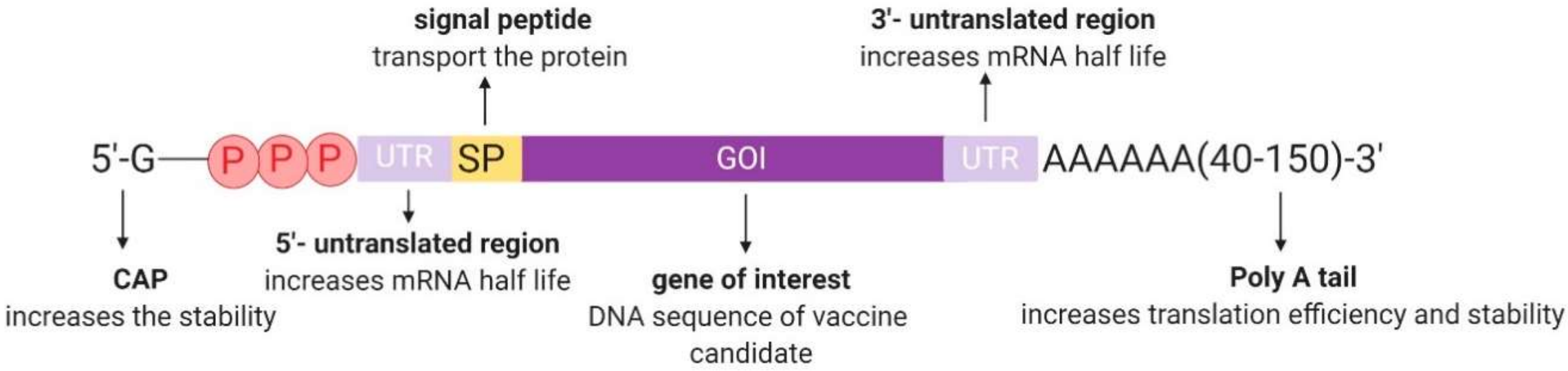
Some Challenges with mRNA as Vaccines or Therapeutics

- 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

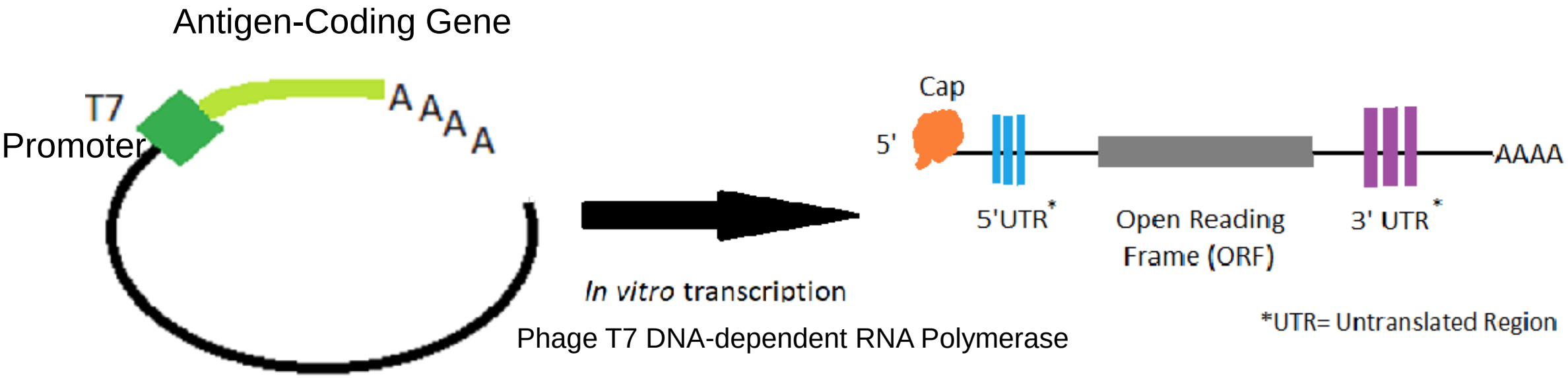
- Krieg and Melton 1984: enzymatic synthesis of mRNA from DNA template
- Wolff *et al*. 1990: Expression of proteins in mouse muscle from injected DNA and from *in vitro*-transcribed mRNA
- Martinon *et al*. 1993: mRNA in liposomes inoculated in mice induced cellular responses to influenza virus nucleoprotein
- 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

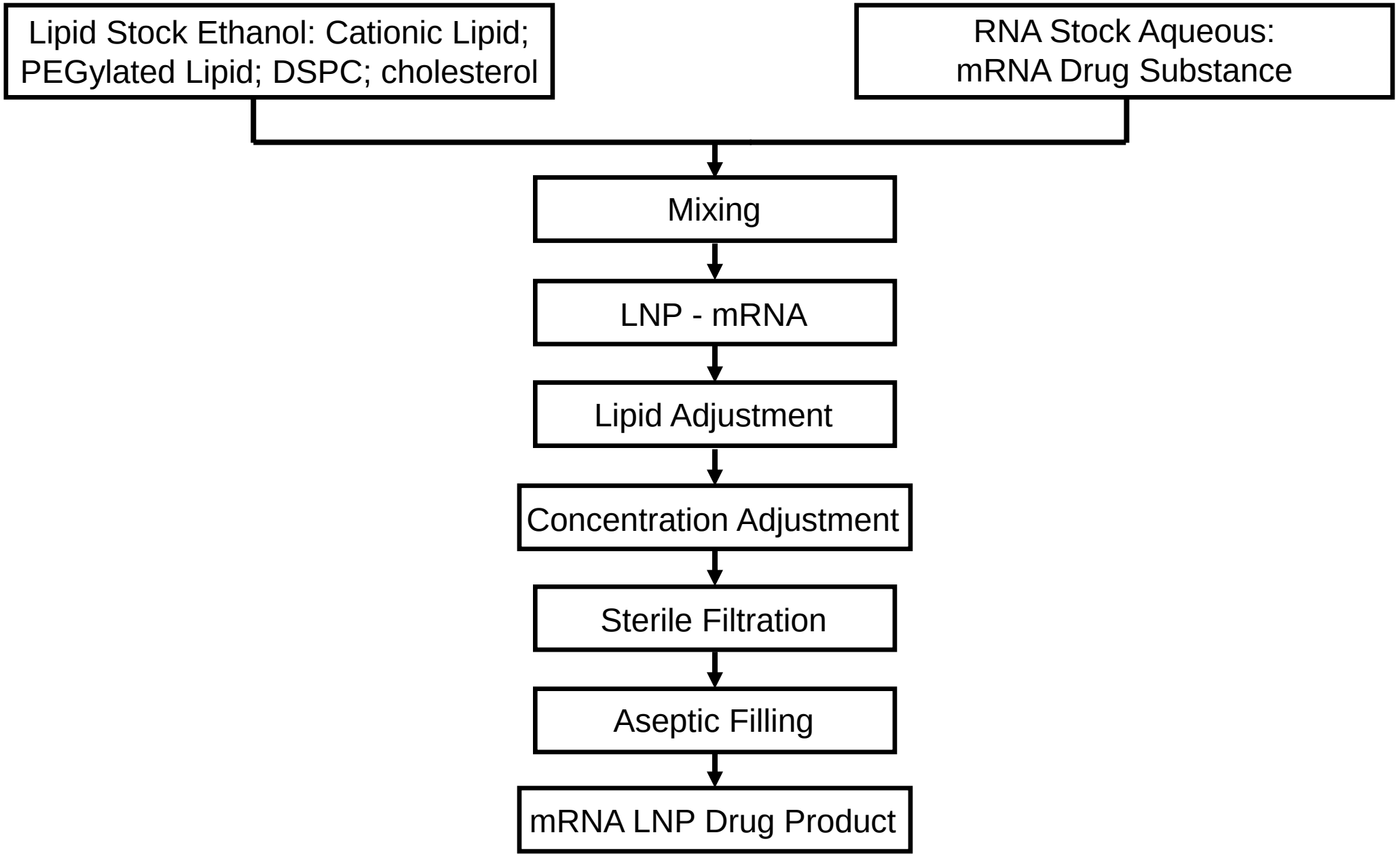


Linearized DNA Plasmid Template

In Vitro-Transcribed mRNA

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

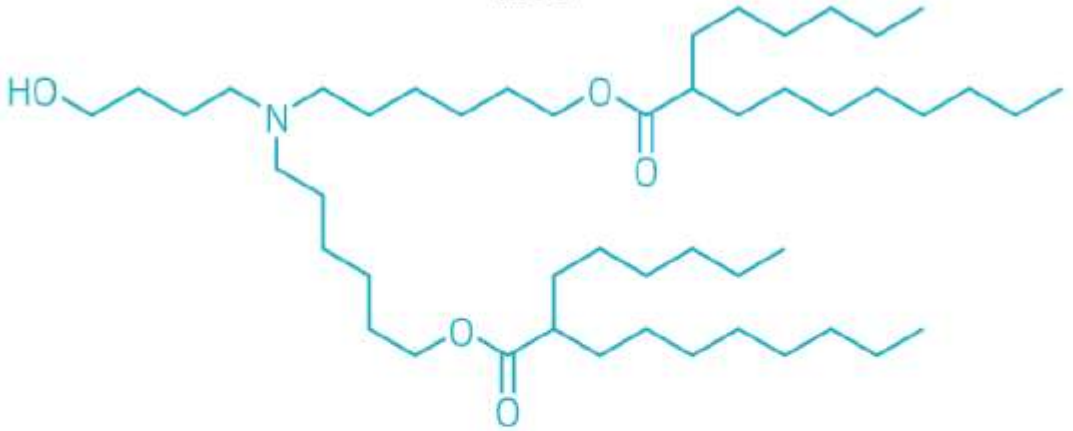
Composition and Functions of Lipids in mRNA LNPs

- 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

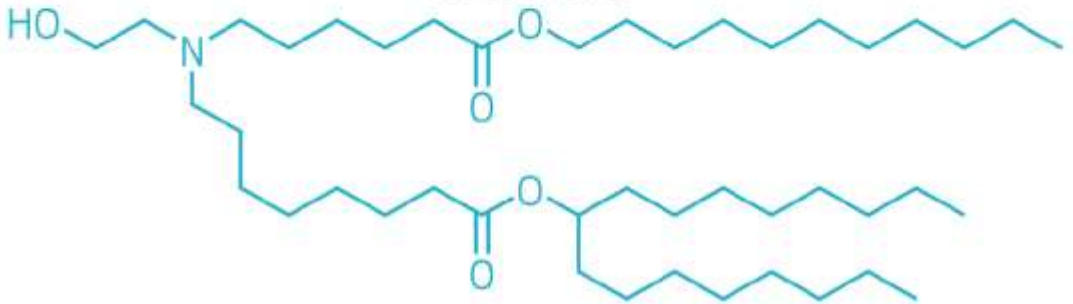
MC3

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?

CMC Issues: Drug Substance and Drug Product

- 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