Modern Science & Technology Day
May 17th, 2022
Forward-looking statements and Disclaimer

This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, including statements regarding: the potential for mRNA as a new class of medicines; the potential of Moderna’s mRNA-LNP platform; Moderna’s ability to build its mRNA platform and expand its clinical applications; Moderna’s ability to effectively utilize artificial intelligence in the development of mRNA medicines; Moderna’s ability to optimize the parameters required for LNP engineering and to develop proprietary LNPs for different delivery goals; Moderna’s collaborations with Vertex, including to develop an inhalable LNP; the potential for inhaled delivery of mRNA to address a range of unmet medical needs; the ability to improve vaccine stability by understanding mRNA inactivation pathways; anticipated timing for an IND filing and clinical development of Moderna’s CFTR mRNA program in collaboration with Vertex; Moderna’s manufacturing platform and ability to produce a robust and consistent product with a predictable stability profile; the effects of mRNA vaccination on pregnancy and fertility; the ability to use IS/ID models to predict neutralizing antibody responses across various doses and predict a priori immunogenic responses in various age groups; and the biodistribution and safety of Moderna’s intramuscular vaccine LNPs. In some cases, forward-looking statements can be identified by terminology such as "will," "may," "should," "could," "expects," "intends," "plans," "aims," "anticipates," "believes," "estimates," "predicts," "potential," "continue," or the negative of these terms or other comparable terminology, although not all forward-looking statements contain these words. The forward-looking statements in this presentation are neither promises nor guarantees, and you should not place undue reliance on these forward-looking statements because they involve known and unknown risks, uncertainties, and other factors, many of which are beyond Moderna’s control and which could cause actual results to differ materially from those expressed or implied by these forward-looking statements. These risks, uncertainties, and other factors include, among others, those risks and uncertainties described under the heading “Risk Factors” in Moderna’s most recent Annual Report on Form 10-K filed with the U.S. Securities and Exchange Commission (SEC) and in subsequent filings made by Moderna with the SEC, which are available on the SEC’s website at www.sec.gov. Except as required by law, Moderna disclaims any intention or responsibility for updating or revising any forward-looking statements contained in this presentation in the event of new information, future developments or otherwise. These forward-looking statements are based on Moderna’s current expectations and speak only as of the date of this presentation.
mRNA is a unique opportunity to change medicine forever

• mRNA is an information molecule

• mRNA had never been a drug modality, so the pace of learning about mRNA would likely follow a S curve

• Exponential increase of mechanistic understanding of disease knowledge, at the molecular level, is an incredible tailwind

The only way to maximize our impact with patients is to

• Build an mRNA platform
• Obsess over how fast we can expand its clinical applications
Obsess over how fast we can expand its clinical applications

- Better mRNA molecules
- Better manufacturing processes to make the medicines
- Ability to deliver mRNA to new cell types
Our platform has incredible independent features to expand.
Moderna is in a unique position to lead the emerging mRNA industry

- We are an mRNA-only company
- We have scale in Science: >700 platform research scientists
- We have a unique cross-functional culture: Focused in MA to increase collaboration
- We are a digital company: Now layering AI to accelerate our pace of leaning
- We have unparalleled resources: ~$19B in cash

(1) As of March 31, 2022

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Our commitment to be the best at mRNA science is core to who we are.

Mindset 4

We obsess over learning

We don't have to be the smartest—we have to learn the fastest.
This year, we will focus on our newest modality and advances in our vaccine platform. Rebranded to Science & Technology Day to reflect investments across technical development.
## Today’s Agenda

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Science & Technology Day

Melissa J. Moore, Ph.D.
Chief Scientific Officer, Scientific Affairs
Moderna’s Science and Technology Ecosystem

**Platform Science**
- mRNA, delivery system and gene editing discovery
- ~280 Employees
  - Chemistry & Formulation Discovery, RNA Science, Biological Science, Computational Science, Immunology, Nonclinical Development, mGx

**Technical Development**
- mRNA & delivery system development; manufacturing technologies
- ~450 Employees

**Therapeutic Area (TA) Research Teams**
- Program-specific science
- ~200 Employees
  - Autoimmunity, Infectious Diseases, Oncology, Rare Diseases

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An engineered T7 RNA polymerase that generates immune-silent mRNA

Athanasis Dousis, Kanchana Ravichandran, Elissa M. Hobert, Melissa J. Moore, Amy E. Rabideau

Nature Biotechnology (2022) in press
Publications from 2020 and 2021 Science Days

Impact of lipid nanoparticle size on mRNA vaccine immunogenicity

Kimberly J. Hassett¹, Jaclyn Higgins¹, Angela Woods, Becca Levy², Yan Xia, Chiao Wen Joyce Hisao, Edward Acosta, Orn Ahrnsson³, Melissa J. Moore, Luis A. Brito⁴

Article

mRNA-1273 vaccine-induced antibodies maintain Fc effector functions across SARS-CoV-2 variants of concern

Paulina Kapitane⁵, Stephanie Fischinger⁶, Deniz Cosan⁵, Yannic C. Bartels⁷, Jaweon Kang⁵, John S. Burke,⁸ Sally A. Shin⁵, Diana Daya⁵, Patrick Martin⁵, Colin Mann⁵, Fatima Armanap⁵, Boris Julg⁵, Eric J. Nilles⁵, Eion R. Muck³,⁸ And S. Menon⁵, Florian Kramer⁵, Enza Olmi-Salme⁵, Andrea Carli⁵, and Gail Alter⁵

LNP Anti-Pentamer Titer

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Today’s Agenda

- Our new inhalable LNP
- Improving vaccine stability by understanding mRNA inactivation pathways
- Biodistribution and safety of our IM vaccine LNP
- Mathematical modeling of vaccine immunogenicity and reactogenicity
Moderna’s mRNA delivery systems
Moderna combines mRNA engineering, delivery system discovery, and clinical manufacturing platforms within a single, fully-integrated organization.

We define success in our platform as achieving the following properties:

- Predictable dose response
- Reproducible pharmacology, including upon repeat dosing
- Therapeutic potency, through achieving the intended pharmacologic activity in the target tissue
- Safety and tolerability
- Scalability for development
- Highly reproducible manufacturing processes
Moderna’s 2022 Q1 investigational pipeline
mRNA-based investigational vaccines and medicines cover 7 different modalities

We have 46 programs in development (31 vaccines)
Different modalities require different routes of administration

- Subcutaneous injection
- Intramuscular injection
- Intratumoral injection
- Inhaled delivery
- Intracardial injection
- IV infusion

Routes of administration

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Most routes of administration require mRNA formulation into lipid nanoparticles (LNPs)

- **mRNA**: Codes for desired protein(s)
- **Ionizable lipid**: Interacts with RNA
- **Phospholipid**: Forms surface membrane
- **Sterol**: Enhances membrane fluidity
- **PEG lipid**: Prevents LNPs from fusing in vial

Cryoelectron micrograph

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LNP engineering requires optimization of many parameters

Each new route of administration (ROA) or target cell type requires consideration and optimization of all parameters

Components
- Ionizable lipid
- Sterol
- Phospholipid
- mRNA
- Excipient
- PEG lipid

Composition & Process
- Δ molecules
- Δ composition
- Δ process

Structure

Stability
- Chemical stability
- Physical stability

Routes of Administration

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Our strategy to determine design rules for individual mRNA delivery challenges

Components
- Structure
- Composition
- Process

Composition & Process
- Δ molecules
- Δ composition
- Δ process

PEG lipid
- Ionizable lipid
- Sterol
- Phospholipid
- Excipient

>2,000 Novel Delivery Components synthesized to date

>10,000 Composition and Process tested variants to date
We have previously developed multiple proprietary LNPs for different delivery goals

- **Prophylactic Vaccines**
  - **Cancer Vaccines**
  - Intramuscular injection
  - **IM LNP**

- **Systemic Therapeutics**
  - IV injection
  - **IV LNP 1 (MMA, PA)**
  - **IV LNP 2 (Gsd1A)**

- **Intratumoral Immuno-oncology**
  - Intratumoral injection
  - **ITu LNP**

References:
- Sabnis … Benenato (2018) Molecular Therapy
- Hassett … Brito (2019) Molecular Therapy Nucleic Acids
- Hassett … Brito (2021) Journal of Controlled Release
- Brader … Jin (2021) Biophysical Journal
We’ve also been working to develop an inhalable LNP

Vertex and Moderna Establish Exclusive Collaboration to Discover and Develop mRNA Therapeutics™ for Cystic Fibrosis

- Collaboration to explore use of mRNA Therapeutics to treat the underlying cause of CF by enabling cells to produce functional CFTR proteins in the lungs.


https://www.modernatx.com/research/product-pipeline
We now have five proprietary LNPs for different delivery goals

- **Prophylactic Vaccines**
  - Cancer Vaccines
  - Intramuscular injection
  - IM LNP

- **Systemic Therapeutics**
  - IV injection
  - IV LNP 1 (MMA, PA)
  - IV LNP 2 (Gsd1A)

- **Intratumoral**
  - Immuno-oncology
  - Intratumoral injection
  - ITu LNP

- **Inhaled pulmonary therapeutics**
  - Inhaled delivery
  - Pulmonary LNP

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Pulmonary Delivery of mRNA LNPs

Jean C. Sung, Ph.D.
Senior Director, Respiratory Delivery, Drug Product Development
Vertex collaborations to address Cystic Fibrosis

Two parallel approaches to addressing the unmet need

(Treating the approximately 5,000 CF patients who do not produce any CFTR protein)

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Inhalation of mRNA has the potential to address a range of unmet medical needs

For example:

- Cystic Fibrosis (CF)
- Alpha-1 Antitrypsin (AAT)
- Idiopathic Pulmonary Fibrosis (IPF)
- Lymphangioleiomyomatosis (LAM)
- Primary Ciliary Dyskinesia (PCD)
- Chronic Obstructive Pulmonary Disease (COPD)
- COVID-19
- Tuberculosis
Challenges for inhaled delivery of mRNA LNPs

mRNA LNP

Device

Aerosol generation & inhalation

LNPs inside of droplets

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Key questions

• Can we efficiently deliver mRNA to target pulmonary cells?

• Does the delivered mRNA express protein?

• Can we aerosolize LNPs and maintain their functionality?

• Can a commercially-viable product be realized?
Our strategy to determine design rules for individual mRNA delivery challenges

Components
- Ionizable lipid
- Sterol
- Phospholipid
- mRNA
- Excipient
- PEG lipid

Composition & Process
- Δ molecules
- Δ composition
- Δ process

Structure

>2,000 Novel Delivery Components synthesized to date

>10,000 Composition and Process tested variants to date
Early studies using luciferase mRNA and existing LNP library

Mouse intratracheal (IT) dosing
Whole body imaging

###: p<0.01, compared to PBS Group
No significant differences compared to Group 23 (positive control)
One-Way ANOVA, Dunnett's post test

![Graph showing Radiance (p/s/cm²/sr) for different groups](image)

- **PBS**
- **Naked mRNA**
- **Test Article 2**
- **Test Article 26**

# #: p<0.01, compared to PBS Group
No significant differences compared to Group 23 (positive control)
One-Way ANOVA, Dunnett's post test
Early studies using luciferase mRNA and existing LNP library

Mouse IT
Ex-vivo lung imaging

Lungs

Radiance (p/s/cm²/sr)

###: p<0.01; ####: p<0.0001, compared to PBS Group
**: p<0.01; ***: p<0.001, compared to Group 23 (positive control)

One-Way ANOVA, Dunnett's post test

Test Article (LNPs)
For most LNPs, eGFP was either undetectable or localized to alveolar macrophages with microscopic evaluation…

Test Article 2

Luciferase protein

Test Article 26

Luciferase protein

eGFP mRNA

eGFP protein

Alveolar Macrophage

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...but a few gave weak protein expression in epithelial cells

Further LNP design optimization needed:

- A mechanistic understanding of delivery limitations
- Tools to enable a deeper dive with higher throughput
Mechanistic understanding of delivery limitations needed to inform LNP design
Human Bronchial Epithelial (HBE) cell model: Higher throughput and human relevant

Lung

HBE cell model at air-liquid interface (ALI)

Transwell plate

Pseudostratified epithelium
V5-tagged, nuclear-targeted luciferase protein: Better in situ protein reporter

Mouse

| eGFP protein | Nascent Peptide Imaging (NPI) Luciferase (NPI-Luc) | V5 NPI-Luc protein |

Adapted from Yan ......Tanenbaum (2016) Cell

V5: Peptide tag for protein detection
NPI-Luc mRNA containing LNPs with dye-label: Quantification of both protein expression and LNP uptake

LNP Uptake
- % LNP positive cells
- LNP uptake per cell (rhodamine intensity)

Protein Expression
- % expressing cells
- Protein expression per cell (nuclear anti-V5 IF intensity)
Nascent Peptide Imaging (NPI) also allows us to count cytoplasmic mRNAs and assess their translational activity.
HBE cell results recapitulated *in vivo* observations and identified uptake versus protein expression differences.
mRNA reaches the endosome, but very few mRNA molecules were translationally active in the cytoplasm.
Higher throughput HBE screening enabled rational LNP design based on mechanistic understanding.

Components:
- Ionizable lipid
- Sterol
- Phospholipid
- mRNA
- Excipient
- PEG lipid

Composition & Process:
- Δ molecules
- Δ composition
- Δ process

Structure:

HBE

NPI-Luc V5 Protein Expression:
Fold Increase Relative to LNP-C

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Optimized LNP expression while maintaining cellular uptake was confirmed across systems.

- LNP Uptake
- NPI-Luc V5 Protein
- HBE

- Mouse IT
  - NPI-Luc mRNA
  - NPI-Luc V5 Protein

- Pulmonary LNP
  - NPI-Luc V5 Protein
  - NPI-Luc mRNA
Improved reporter in combination with AI algorithm allowed for high-throughput analysis of target airway epithelium across whole lung.
Aerosol delivery of LNPs showed widespread distribution of protein expression throughout the airway epithelium in rat.
Aerosol delivery in non-human primate (NHP) also showed widespread protein expression in the airway epithelium.
Demonstrated ability to develop an inhalation product

**Scale-up**
100,000-fold scale increase with retained LNP activity

**Stability**
Stable with long-term -70 °C storage

**Aerosol Delivery**
Respirable droplet sizes across devices

- **Protein Expression in HeLa Cells**
- **mRNA purity (%)**
- **MMAD (μm)**

- Milligrams
- 100s of grams
We have developed a new LNP formulation that enables efficient delivery of mRNA to and protein expression in the pulmonary epithelium upon aerosol delivery.

Our ability to do so was enabled by building in-house systems and assays for high throughput testing of rational LNP design strategies.

Our optimized inhalation LNP demonstrates high levels of protein expression localized to the airway epithelium and is well-tolerated in rats and NHPs.

This new LNP is well-suited for development, with proven scale-up, stability and ability to deliver by aerosol.
CF mRNA therapeutics collaboration with Vertex

Recent Highlights

IND-enabling studies completed for CFTR mRNA program

Key Milestones Ahead

On track to file IND in 2H 22 and begin clinical development thereafter

Vertex Pharmaceuticals First Quarter 2022 Earnings Call

https://investors.vrtx.com/events/event-details/vertex-pharmaceutical-q1-2022-conference-call
Improving Vaccine Stability

Phil White, Ph.D.

Vice President, CMC Lifecycle management
Introduction

- Moderna has invested heavily in the mRNA-LNP platform for over 10 years

- Historical and ongoing efforts to fundamentally understand the product enabled rapid scale-up for our COVID-19 vaccine

- Our obsession with science and attention to details have led to industry-leading knowledge, CMC control and consistency of our product
Personalized Cancer Vaccine (PCV) workflow

1. Screening Tissue Samples
   - Tumor (biopsy) and normal (blood)

2. Next Generation Sequencing (NGS)
   - What are the mutations and HLA type for this patient?

3. Vaccine Design
   - Automated bioinformatics algorithm

4. Manufacturing
   - One vaccine per patient rather than one drug for many patients

5. Administration
   - Every 3 weeks
   - With pembrolizumab

mRNA encoding up to 34 neoantigens

Dosing every 3 weeks for a total of 9 cycles = 6 months total

Preference to store Drug Product at 2-8°C

Started with phosphate-based buffer system
The amount of mRNA recovered appeared to decrease upon storage at 2-8°C. The method is not stability indicating so it was unclear why mRNA appeared to reduce in content upon storage.
What, based on our understanding of the platform, and the tools available to us, could be causing these observations?
New analytical method for mRNA: Reversed-Phase Ion Pair (RP-IP) High Performance Liquid Chromatography (HPLC)
Discovery of a late-eluting peak (LP) when switching to an RP-IP HPLC method for purity

A late-eluting peak was observed when mRNA is isolated from LNPs.
Characterization of the late-eluting peak (LP)

Re-analysis of the isolated LP using RP-IP showed the same profile.
Traditional analytical methods for assessing RNA quality: Capillary electrophoresis (CE) and size exclusion chromatography (SEC)
Characterization of the late-eluting peak (LP)

Re-analysis of the isolated LP using CE did not reveal differences
Characterization of the late-eluting peak (LP)

Size exclusion chromatography

The LP was shown not to be an aggregate
Characterization of the late-eluting peak (LP)

The LP increased over time and with increased temperature.
Characterization of the late-eluting peak (LP)

In-vitro expression

The LP does not express the encoded protein
Other standard analytical techniques found no differences

- NaOH digestion to nucleotides
- FT-IR Spectroscopy
- T1 digestion / Oligo Mapping
- NMR

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What is the late-eluting peak (LP) and where is it coming from?
Digestion and MS revealed lipid modifications on nucleobases

Signal counts

Mass-to-charge (m/z)

112.05
204.08
376.26
394.27
526.30

Δ132.03

Lipid-modified nucleosides

150X y-axis magnification

EIC of selected m/z in LP fraction
EIC of selected m/z in MP fraction

Signal counts

100 180 260 340 420 500 580

Binary assays with components of our vaccine LNP

Ionizable Lipid
Sterol
Phospholipid
PEG lipid
mRNA

Buffered mRNA solution
Lipid solution in ethanol
Precipitation and incubation
Extraction and RP-IP HPLC analysis

Ionizable lipid/Phosphocholine
Ionizable lipid/Cholesterol
Ionizable lipid/PEG lipid
Phosphocholine
Cholesterol
PEG-lipid
Ionizable lipid

% LP in Sample
0 2 4 6 8 10
Nucleobases are known to react with aldehydes.

Aldehyde
HCRO

NMR analyses on N-hydroxymethylated nucleobases—implications for formaldehyde toxicity and nucleic acid demethylases
Diverse toolset led us to broadly applicable mechanism for adduct

- Oxidized ionizable lipid forms large amount of adduct in binary compared to parent lipid

![Graph showing UV absorbance over time for N-oxide binary at different times.](image)
Diverse toolset led us to broadly applicable mechanism for adduct

- Oxidized ionizable lipid forms large amount of adduct in binary compared to parent lipid
- In acidic buffer used for binary and LNP precipitation, N-oxide degrades to generate aldehydes
Diverse toolset led us to broadly applicable mechanism for adduct

- Oxidized ionizable lipid forms large amount of adduct in binary compared to parent lipid
- In acidic buffer used for binary and LNP precipitation, N-oxide degrades to generate aldehydes
- Spiked binary series with two synthetic aldehyde products of N-oxide hydrolysis generated the same adduct species on the intact mRNA and single nucleoside level

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Oxidation of a tertiary amine can result in an aldehyde.

Forms an “Adduct” on the mRNA molecule.
All ionizable lipids tested gave a late-eluting peak when combined with mRNA.

100s of Moderna compounds screened, and all that contain a tertiary amine group demonstrate adduct formation.
Ionizable tertiary amine appears in other RNA LNP vaccine products

The proposed adduct formation mechanism is likely to be widely applicable to RNA LNP products.
Why is adduct formation a problem for mRNA?

mRNAs are partially single-stranded, meaning that many nucleobases are available to react.

A single modification in the coding region will stall the ribosome and inactivate the entire mRNA molecule.
Manufacturing controls required to abrogate adduct formation

- Improved Shelf-life ✓
- Higher Storage Temperature ✓
- Maintenance of Activity ✓
- Consistency of Manufacture ✓

Impact on final product

Raw Material Manufacturing Controls
- Lipid synthetic schema
- Control of oxidation
- Purification processes

Analytical Testing and Specifications
- Release methods (RP-IP)
- IPC limits on impurities
- Use and functional testing

DP Manufacturing Controls and Formulation
- Manufacturing process
- Incorporation of "Aldehyde sinks" e.g. Tris

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Tris buffer acts as an aldehyde sink and enables longer term storage at 2-8°C
Does adduct formation explain the original observation?
The amount of mRNA recovered appeared to decrease upon storage at 2-8°C.
Analysis by anion exchange (AEX) quantitation

Dramatic back shoulder in late-eluting peak (LP), split peak and poor recovery in method
In addition to adduct formation, what else do we know is driving our product shelf-life?
mRNA Integrity
A critical quality attribute and the primary determinant of shelf life

The coding region must be correct to generate intended product

A cap structure is **crucial** for mRNA translation

A PolyA tail is **crucial** for mRNA translation

mRNA must be fully intact to express the intended product
RNA is inherently unstable due to strand scission via trans-esterification

Protein expression is impacted by mRNA Integrity

- Intact mRNA readily expresses when transfected into cells in culture
- The purity of mRNA is essential for protein expression

HPLC Chromatograms

- Non-Degraded mRNA
- Degraded mRNA

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COVID-19 vaccine – establishing the shelf life

Common-slope models indicate consistent and predictable stability profiles.
Predictable stability profile across our vaccine platform

Longer mRNAs have a faster degradation rate and hence shorter shelf-life
A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems

Meredith Packer, Dipendra Gyawali, Ravikiran Yerabolu, Joseph Schariter & Phil White

Conclusions

• Deep knowledge of platform through investments in fundamental science led to key observations

• Broad analytical toolkit and state of the art methods uncovered a critical quality attribute

• Formation of adduct is a universal impurity in tertiary amine containing LNPs

• Strand scission and adduct are two key determinants of Drug Product (DP) shelf-life

Moderna has a well-characterized platform, industry-leading knowledge and detailed manufacturing and pharmaceutical controls. This ensures a robust and consistent product.
Today’s Agenda

| Introduction | Stéphane Bancel, CEO  
  Melissa J. Moore, Ph.D., Platform Research |
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Biodistribution and Safety of our IM Vaccine LNPs

Melissa J. Moore, Ph.D.
Chief Scientific Officer, Scientific Affairs
Moderna’s proprietary vaccine LNP improves local expression and tolerability without impacting immunogenicity.

Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines

Kimberly J. Hassett,1 Kerry E. Benenato,1 Eric Jacquinet,1 Aisha Lee,1 Angela Woods,1 Olga Yushakov,1 Sunny Himansu,1 Jessica Deterling,1 Benjamin M. Gellrich,1 Tatiana Ketova,1 Cosmin Mihai,1 Andy Lynn,1 Iain McFadyen,1 Melissa J. Moore,1 Joseph J. Senn,1 Matthew G. Stanton,1,2 Örn Almbransson,1 Giuseppe Ciaranello,1,3 and Luis A. Brito1

1Moderna Therapeutics, 201 Technology Square, Cambridge, MA 02139, USA

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Common questions

Where does the mRNA go after IM administration?
• What tissues and cell types?

How long does the mRNA persist?

Where is the protein expressed?
• What tissues and cell types?

Are there any concerns relative to fertility and pregnancy?
Measurement of mRNA biodistribution over time after IM injection of Vaccine LNP

100μg 6 vaccine mRNAs in IM vaccine LNP (SM-102) Analysis (tissue b-DNA assay)

Liver
Muscle
Spleen

Homogenize
Lysate

Signal enhancing b-DNA assay for detecting mRNA in tissue homogenates
Example preclinical IM vaccine distribution study

- mRNA distributes predominantly to injection site and draining lymph nodes.
- mRNA is undetectable after 120 hours (5 days)
Skeletal muscle anatomy

Cross-section of skeletal muscle fibers

- Neutrophil
- Muscle cell
- Dendritic cell
- Connective tissue
- Macrophage
- Blood vessel
- Monocyte

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Assays for detecting **mRNA** and **protein** in situ

- GFP mRNA in IM vaccine LNP (SM-102)
- Analysis

RNA Scope® (in situ hybridization) assay

- mRNA
- Protein

Immunohistochemistry (IHC)
Both mRNA and protein are readily detectable in the connective tissue at the injection site.
mRNA is undetectable at injection site after 3 days
Protein is undetectable at injection site after 3 days
Both mRNA and protein are detectable in situ in the draining lymph node.
Antigen Presenting Cells (APCs) capture, process and present foreign antigens and help activate the immune system.

Antigen capture and processing by APCs help trigger an **INNATE** immune response.

**Professional APCs**
- monocytes
- macrophages
- dendritic cells
- B-cells

- Most efficient at antigen presentation
- Phagocytosis, destruction and antigen presentation of pathogens
- Precursor to other APCs

**Specialized APC**
- subcapsular sinus macrophages
- Directly processes and presents antigen in the germinal center of lymph nodes

Antigen presentation and innate immune signaling lead to **ADAPTIVE** immune responses.
**Protein** is expressed in draining lymph node macrophages

**Draining lymph node**

- Germinal center
- Medullary sinus
- Subcapsular sinus

**protein (fluorescent)**

**CD169 (macrophages)**
Flowcytometry demonstrates that lymph node protein expression is primarily in APCs.

High-throughput multiparameter flow cytometry to interrogate cell type.
Common questions

Where does the mRNA go after IM administration?
• What tissues and cell types?

How long does the mRNA persist?

Where is the protein expressed?
• What tissues and cell types?

Are there any concerns relative to fertility and pregnancy?
• Transient injection site and systemic inflammatory effects attributable to LNP and immunological response to expressed antigens were typical of IM vaccines.

• No histopathological effects observed in reproductive organs or heart/cardiac tissue.
Example fertility and developmental study in rats

6 vaccine mRNAs in IM Vaccine LNP (SM-102) at clinically-relevant doses

<table>
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<tr>
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<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
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<tr>
<td>Day Relative to Mating</td>
<td>-28</td>
<td>-14</td>
<td>0</td>
<td>+1 (GD1)</td>
</tr>
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- No effects on mating performance, fertility, pregnancy, delivery or development of pups observed.
- Similar to toxicity study, transient effects in maternal rats at injection site resolved within 3-6 days.
- Antibodies were transferred to pups

Female Dams (Ab titers)

Pups (Ab titers)
Antibody and CD4/CD8 T-cell responses were present in pregnant and lactating persons following mRNA vaccination\textsuperscript{1-7}.
- Vaccine-elicited antibodies were detected in infant cord blood and in breastmilk.
- T cells targeting SARS-CoV-2 spike protein have also been observed in breastmilk from vaccinated lactating women.

mRNA vaccines are generally well tolerated among pregnant and lactating persons\textsuperscript{8-10}.
- A retrospective study found similar odds of recent COVID-19 vaccination among women who experienced a spontaneous abortion and those with ongoing pregnancies.

Adverse pregnancy/neonatal outcomes in vaccinated pregnant persons were similar to incidences reported in pregnant women before the COVID-19 pandemic\textsuperscript{11}.

\textsuperscript{1} Collier AY, et al. JAMA. 2021;325(23):2370-2380.
CDC recommends vaccination for people who are or may become pregnant and breastfeeding people

CDC.gov/mmwr/volumes/71/wr/mm7107e3.htm#:~:text=small%20sample%20size. - Completion of a 2-dose primary mRNA COVID-19 vaccination series during pregnancy

People who are pregnant, may become pregnant, or are breastfeeding should get vaccinated against COVID-19

https://www.cdc.gov/mmwr/volumes/71/wr/mm7107e3.htm#:~:text=small%20sample%20size. - Completion of a 2-dose primary mRNA COVID-19 vaccination series during pregnancy

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Summary

After IM administration of our vaccine LNP, the mRNA is found almost exclusively at the injection site and in the draining lymph nodes.

The mRNA is >95% gone after 48 hours.

Protein expression occurs in immune cells in the muscle interstitial space and draining lymph nodes.

No adverse consequences for pregnancy or fertility have been observed.
Modeling and Simulation to Guide Dose Selection for Vaccines
Pharmacometric models have been widely utilized for dose decisions across the biopharma industry for therapeutics

- **Pharmacometrics** is the science of interpreting and describing pharmacology in a quantitative fashion
- **Pharmacokinetics (PK)** is mathematical understanding of rate of absorption/distribution/metabolism/elimination of drug and its metabolites
- **Pharmacodynamics (PD)** is study of the kinetics of pharmacologic response at different drug dose or concentration
We developed a PK/PD model using preclinical data for Chikungunya antibody program and successfully predicted PD responses in humans.

Observed PD response in humans (shapes) overlaid on top of model prediction shows that **clinical PD response can be predicted based on PK/PD responses seen in preclinical species**.
Modeling and Simulation to Guide Dose Selection for Vaccines

Husain Attarwala

*Sr. Director, Pharmacometrics & Clinical Pharmacology*
IS/ID models are semi-mechanistic mathematical models that describe the immune response stimulated by vaccination (IS), and the resulting immune response dynamics (ID).

We adapted traditional PK/PD modeling approach to vaccines by developing immunostimulation/immunodynamic (IS/ID) models.
Typical time course of immune response following vaccination
What is an IS/ID Model?

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What is an IS/ID Model

- 10 differential equations
- 17 parameters

Adapted from Rhodes et al., Nature (2018)
Key questions IS/ID models help address

What vaccine dose should be used in clinical studies? How do we select doses for different age groups?
We can use the IS/ID models to predict dose-response relationship for clinical studies.

**IS/ID and Reactogenicity Modeling for CMV Vaccine**

**IS/ID Modeling for mRNA-1273 Vaccine**

- CMV (Pentamer & gB) → CMV Phase 3 dose selection
- COVID-19 (spike protein) → mRNA-1273 pediatric dose selection

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We used data from our CMV vaccine Phase 1 clinical study to develop our first IS/ID model.

CMV vaccine (mRNA-1647) includes 6 mRNAs:

- 5 encode the pentamer,
- 6th encodes gB antigen

Pentamer includes:

- UL131
- UL128
- UL130
- gL
- gH
- gB

Cytosol
We used data from our CMV vaccine Phase 1 clinical study to develop our first IS/ID model.

CMV vaccine (mRNA-1647) includes 6 mRNAs:
- 5 encode the pentamer,
- 6th encodes gB antigen

CMV Vaccine Phase 1 Data
Data used for model development in 2019/2020

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We used data from our CMV vaccine Phase 1 clinical study to develop our first IS/ID model.

CMV vaccine (mRNA-1647) includes 6 mRNAs:
- 5 encode the pentamer,
- 6th encodes gB antigen.

CMV Vaccine Phase 1 Data
Data used for model development in 2019/2020

Neutralizing Antibody Titer Against Fibroblast Cell Infection

- 30 µg
- 90 µg
- 180 µg
- 300 µg

Time (days)
Our model adequately described the observed data

Solid blue line = Fitted GMT; Shaded area = 90% Prediction interval
Dots = Observed data
The final model adequately described the time-course and relationship between dose and neutralizing antibody titers for our CMV vaccine.

Once the model is qualified using diagnostics and predictive checks, we then use it to perform clinical trial simulations.
What dose level is predicted to yield nAbs titers above the benchmark of natural infection in the majority of subjects?

Model was used to predict relationship between mRNA-1647 dose and nAb titers at 1 month following 3rd dose.

CMV, cytomegalovirus; mRNA, messenger ribonucleic acid; nAb, neutralizing antibody.
Model predictions: blue line = geometric mean; blue shaded area = 5th to 95th percentile prediction interval; dotted red line = GMT benchmark for natural infection.
Modeling the relationship between dose and adverse reactions
Reactogenicity modeling supported selection of 100 µg dose level for the Phase 3 clinical study

- Composite risk of adverse reactions was derived using probabilities of individual adverse reactions
  - 0 is no risk and 1 is maximum risk

- Risk of adverse reactions (especially Grade 3) is predicted to remain low at ≤100 µg dose
The 100 µg dose was selected to both maximize immunogenicity and minimize adverse reactions.

The 100 µg dose was predicted to yield neutralizing antibody titers against both epithelial and fibroblast cell infection above the seropositive benchmark of natural infection in majority of subjects (baseline seronegative) and was predicted to have minimal risk of adverse reactions.

CMV, cytomegalovirus; mRNA, messenger ribonucleic acid; nAb, neutralizing antibody.
Model predictions: blue line = geometric mean; blue shaded area = 5th to 95th percentile prediction interval; dotted red line = GMT benchmark for natural infection.
We can use the IS/ID models to predict dose-response relationship for clinical studies.
Model was developed using data pooled across various clinical studies

**Pooled primary vaccination data**
(N=2567*)

**Phase 2 Studies**
- P201 - Adults
- P203 - Adolescents
- P204 - Pediatrics

**Phase 3 Study**
- P301 - Adults

**IS/ID Structural Model**

- **Model Development**: Adapted the IS/ID modeling framework developed for CMV to COVID-19 via optimizing antigen specific parameters using clinical data
- **Evaluation**: Assessed dose and age as covariate on nAb titer response
- **Predict**: Simulated nAb titers at various dose levels across different age groups

*N=Number of subjects with immunogenicity data used for model development (Dec 2021)
Our goal was to predict which dose levels in young children and infants would yield neutralizing antibody titers that are similar to young adults.

\[
GMR = \frac{GM_{test}}{GM_{reference}}
\]

- **Pass** if the test GM is within the reference range.
- **Fail** if the test GM is outside the reference range.
Primary series at 25µg dose was predicted to meet non-inferiority criteria in young children (2-5y) and infants (6-23m)

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>Dose</th>
<th>GMR (95%CI) compared to young adults (18-25yo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants: 6m-23m (P204)</td>
<td>230</td>
<td>25 µg</td>
<td>1.28 (1.12, 1.47)</td>
</tr>
<tr>
<td>Young Children: 2-5y (P204)</td>
<td>264</td>
<td>25 µg</td>
<td>1.01 (0.88, 1.17)</td>
</tr>
</tbody>
</table>

Model Predicted GMRs in Different Age Groups

Observed GMRs in Pediatrics

Box plots show distribution of predicted GMRs
Summary

• Modeling and simulation helps drive data-driven decision making for clinical dose selection

• We adapted the traditional PK/PD modeling approach for therapeutics to develop our IS/ID models for vaccines

• IS/ID models can be used to successfully predict neutralizing antibody responses across various doses

• Our IS/ID models can also predict apriori immunogenic responses in various age groups

• We demonstrated the utility of IS/ID models for Phase 3 dose selection for our CMV vaccine and successfully used it for pediatric dose selection for our COVID-19 vaccine
Concluding Remarks and Q&A

Stephen Hoge, M.D.
President
Our platform has incredible independent features to expand.
Today’s topics offer glimpses into two modalities

**Expansion of our mRNA platform**

Developed a **new LNP** formulation that enables efficient delivery of mRNA to and protein expression in the **pulmonary epithelium** upon aerosol delivery

**Increased depth and understanding across our vaccine modality**

A broad analytical toolkit and **state of the art methods uncovered the formation of adduct** in the manufacturing process

Increased **understanding of the biodistribution of our IM mRNA vaccines** (location, half-life, protein expression)

Adapted PK/PD modeling to vaccines and can make **data driven clinical dose selections**
Our mission
To deliver on the promise of mRNA science to create a new generation of transformative medicines for patients.