Annual Science Day
June 2, 2020
This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995, as amended, including regarding the Company’s mRNA and encoded protein engineering efforts, the Company’s new squaramide-based LNP and its expected use with mRNA-3745 and other new development candidates, the ability of mRNA medicines to potentially modulate pharmacologic effects, the scientific and clinical potential of the Company’s newly developed lipid nanoparticles; the ability of the Company’s proprietary lipid nanoparticles to drive optimal immune response in primates, the potential for the Company’s mRNA platform to significantly accelerate early clinical trials and transform HIV vaccine science and development efforts, and the presentation of research and data at the Company’s upcoming Science Day. 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: the fact that there has never been a commercial product utilizing mRNA technology approved for use; potential adverse impacts due to the global COVID-19 pandemic such as delays in regulatory review, manufacturing and supply chain interruptions, adverse effects on healthcare systems and disruption of the global economy; and those other risks and uncertainties described under the heading “Risk Factors” in Moderna’s most recent Quarterly Report on Form 10-Q 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 hereof.

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Central dogma of molecular biology

Storage
DNA stores instructions for proteins in the nucleus

Software
mRNA is a temporary set of instructions for cells to make a protein; mRNA is made using DNA

Applications
Proteins form the basis of life by performing the functions required by every cell; proteins are made using mRNA
mRNA as a new class of medicine

Storage
DNA stores instructions for proteins in the nucleus

Software
mRNA is a temporary set of instructions for cells to make a protein; mRNA is made using DNA

Applications
Proteins form the basis of life by performing the functions required by every cell; proteins are made using mRNA
mRNA is a temporary set of instructions for cells to make a protein.
mRNA as a potential new class of medicines

1. Large product opportunity
2. Higher probability of technical success
3. Accelerated research and development timelines
4. Greater capital efficiency over time vs. recombinant technology
## Our platform

### mRNA

<table>
<thead>
<tr>
<th>Chemistry</th>
<th>Sequence engineering</th>
<th>Targeting elements</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chemistry" /></td>
<td><img src="image2.png" alt="Sequence engineering" /></td>
<td><img src="image3.png" alt="Targeting elements" /></td>
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</tbody>
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### Delivery

<table>
<thead>
<tr>
<th>Chemistry</th>
<th>Composition</th>
<th>Surface properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image4.png" alt="Chemistry" /></td>
<td><img src="image5.png" alt="Composition" /></td>
<td><img src="image6.png" alt="Surface properties" /></td>
</tr>
</tbody>
</table>

### Manufacturing Process

<table>
<thead>
<tr>
<th>mRNA</th>
<th>LNP</th>
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</thead>
<tbody>
<tr>
<td><img src="image7.png" alt="mRNA" /></td>
<td><img src="image8.png" alt="LNP" /></td>
</tr>
</tbody>
</table>

**Systemic** intracellular therapeutics

**Localized** regenerative therapeutics

**Intratumoral** immuno-oncology

**Prophylactic** vaccines

**Cancer vaccines**

**Localized regenerative therapeutics**

**Systemic secreted & cell surface therapeutics**

**Systemic intracellular therapeutics**
Our commitment to be the best at mRNA science is core to who we are: A 20-year journey
Our commitment to be the best at mRNA science is core to who we are

Continue to invest in mRNA science, delivery technology and manufacturing

We have advanced mRNA science to enable our current development pipeline

Capturing future product performance improvements requires investments:

- For the long-term
- At Scale
- With the right team
As the President of Moderna, Stephen Hoge leads our platform R&D, therapeutic area research, and pre-clinical development efforts. He joined Moderna in late 2012 from McKinsey & Company where he was a partner in the healthcare practice. Prior to McKinsey, Dr. Hoge was a resident physician in New York City.

He holds an M.D. with thesis from the University of California, San Francisco and a B.S. in neuroscience from Amherst College.
In her role as Chief Scientific Officer of Moderna’s Platform Research, Dr. Moore is responsible for leading mRNA biology, delivery and computation science research at Moderna. She joined Moderna in 2016 from the University of Massachusetts Medical School (UMassMed), where she served as Professor of Biochemistry & Molecular Pharmacology, Eleanor Eustis Farrington Chair in Cancer Research and a long-time investigator at the Howard Hughes Medical Institute (HHMI). At UMassMed, Dr. Moore was a founding Co-Director of the RNA Therapeutics Institute (RTI) and was instrumental in creating the Massachusetts Therapeutic and Entrepreneurship Realization initiative (MassTERi), a faculty-led program intended to facilitate the translation of UMMS discoveries into drugs, products, technologies and companies. Dr. Moore is an elected member of both the National Academy of Sciences (2017) and the American Academy of Arts and Sciences (2019).

Dr. Moore’s 23-year career in academic research focused on the roles of RNA and RNA-protein (RNP) complexes in regulating gene expression, and touched on many human diseases including cancer, neurodegeneration and preeclampsia. She began working on RNA metabolism during her postdoctoral training with Phillip A. Sharp, Ph.D. at MIT, where she also received her Ph.D. in Biological Chemistry working under Christopher T. Walsh, Ph.D. Dr. Moore holds a B.S. in Chemistry and Biology from the College of William and Mary.
From idea to expression

1. It starts with an idea
2. Digital sequence design based on propriety algorithm
3. Manufacturing of plasmid, mRNA, and LNP
4. Fill, finish, and QC
5. Biodistribution, cellular uptake, and protein expression
LNPs are akin to endogenous lipid transport complexes

LNP are akin to endogenous lipid transport complexes


Lipoprotein Subclasses:
- Chylomicron (CM)
- Very low-density lipoprotein (VLDL)
- Low-density lipoprotein (LDL)
- High-density lipoprotein (HDL)

Diameter (nm)

Density (g/ml)
Where does Platform Research fit in?

1. It starts with an idea
2. Digital sequence design based on propriety algorithm
3. Manufacturing of plasmid, mRNA, and LNP
4. Fill, finish, and QC
5. Biodistribution, cellular uptake, and protein expression
2018 Science Day Themes and Publications

- Development of proprietary ionizable lipids for improved systemic mRNA delivery
- Development of proprietary ionizable lipids with improved tolerability for mRNA vaccines
- Use of miRNA target sites to create mRNAs with "off switches"
- Translation initiation and "leaky scanning"
- How artificial intelligence can help us design better coding sequences

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

Sabnis ... Benenato (2018) Mol Ther

Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines

Hassett ... Brito (2019) Mol Ther Nuc Acids

MicroRNAs Enable mRNA Therapeutics to Selectively Program Cancer Cells to Self-Destruct

Jain ... Chakraborty (2018) Nuc Acid Ther
2019 Science Day Themes and Publications

- Why we incorporate modified nucleotides into our mRNAs
- Coding sequence design: Codon optimality vs RNA secondary structure
- 5'-UTR design
- The physical and computational methods we employ to understand LNP structure
- An early glimpse into our Immune LNP

Impact of mRNA chemistry and manufacturing process on innate immune activation
Jennifer Nelson\textsuperscript{a}, Elizabeth W. Sorensen\textsuperscript{a}, Shrutika Mintri\textsuperscript{b}, Amy E. Rabideau, Wei Zheng, Gilles Besin\textsuperscript{c}, Nikhil Khatwani\textsuperscript{d}, Stephen V. Su\textsuperscript{e}, Edward J. Miracco, William J. Issa, Stephen Hoge, Matthew G. Stanton\textsuperscript{a}, John L. Joyal\textsuperscript{a}

mRNA structure regulates protein expression through changes in functional half-life
David M. Mauger\textsuperscript{a, b}, Joseph C. Cabral\textsuperscript{c, d}, Vladimir Presnyak\textsuperscript{a}, Stephen V. Su\textsuperscript{e}, David W. Reid\textsuperscript{a}, Brooke Goodman\textsuperscript{a, d, e}, Kristian Link\textsuperscript{a}, Nikhil Khatwani\textsuperscript{d}, John Reynolds\textsuperscript{a}, Melissa J. Moore\textsuperscript{a, d}, and Iain J. McFadyen\textsuperscript{a, d}

Human 5' UTR design and variant effect prediction from a massively parallel translation assay
Paul J. Sample\textsuperscript{a, f}, Ban Wang\textsuperscript{a, g}, David W. Reid\textsuperscript{a}, Vlad Presnyak\textsuperscript{a}, Iain J. McFadyen\textsuperscript{a}, David R. Morris\textsuperscript{a} and Georg Seelig\textsuperscript{a, b, f, g}

Nelson … Joyal (2020) Science Advances
Mauger … McFadyen (2019) PNAS
Sample … Seelig (2019) Nature Biotech
## 2020 Science Day agenda

<table>
<thead>
<tr>
<th>Time</th>
<th>Chapter/Session</th>
<th>Presenter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00-8:10 AM</td>
<td>Introduction</td>
<td>Stéphane Bancel, CEO</td>
</tr>
<tr>
<td>8:10-8:45 AM</td>
<td>Chapter 1 Extending Pharmacology</td>
<td>Melissa Moore, Ph.D., Chief Scientific Officer, Platform Research</td>
</tr>
<tr>
<td>8:45-9:05 AM</td>
<td>Chapter 2 T7 Enzyme Engineering to Reduce dsRNA Production</td>
<td>Amy Rabideau, Ph.D., Principal Scientist, Process Development</td>
</tr>
<tr>
<td>9:05-9:25 AM</td>
<td>Chapter 3 New LNPs for Liver Delivery</td>
<td>Kerry Benenato, Ph.D., Senior Director, Platform Chemistry</td>
</tr>
<tr>
<td>9:25-9:40 AM</td>
<td>Chapter 4 Impact of LNP Size on Immunogenicity</td>
<td>Kimberly Hassett, Ph.D., Senior Scientist, Formulation Science</td>
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<tr>
<td>9:40-9:50 AM</td>
<td>Break</td>
<td></td>
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<tr>
<td>9:50-10:50 AM</td>
<td>Chapter 5 Our Progress Toward Creating a mRNA Vaccine for HIV</td>
<td>Andrea Carfi, Head of Research, Infectious Disease, William Schief, Ph.D., Scipps, International Aids Vaccine Initiative (IAVI), Paolo Lusso, M.D., Ph.D., National Institute of Allergy and Infectious Diseases (NIAID)</td>
</tr>
<tr>
<td>10:50-11:00 AM</td>
<td>Conclusion</td>
<td>Stephen Hoge, M.D., President</td>
</tr>
<tr>
<td>11:00-11:30 AM</td>
<td>Q&amp;A</td>
<td></td>
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What controls the duration of our pharmacologic effects?
Factors controlling duration of effect by traditional small molecule drugs are well understood

Modified from: Clarelli et al. (2020) Cell Mol Life Sci
doi: 10.1007/s00018-019-03376-y
Factors controlling duration of effect by traditional small molecule drugs are well understood

Drugs are administered (Drug Dosing), absorbed (Absorption), and distributed to the systemic circulation (Systemic Circulation). At the site of action (Site of Action), they bind to targets (Target), which leads to signal transduction (Signal Transduction) and ultimately drug effect (Drug Effect).

**Pharmacokinetics**
- Drug concentration vs. time
- Maximum concentration ($C_{\text{max}}$)

**Pharmacodynamics**
- Effect vs. time
- Maximum effect (response)

But what about mRNA medicines?
But what about mRNA medicines?
But what about mRNA medicines?
But what about mRNA medicines?
How mRNA and protein half-lives interplay to modulate pharmacologic effects
What are typical endogenous mRNA and protein half-lives?

**mRNAs:**
- **Human crystallin (lens protein):** $t_{1/2} \approx$ human lifespan
- **mRNAs:** median $t_{1/2} \approx$ 5 hours
  - 0 MIN
  - 1 MONTH
  - 100 YEARS

**PROTEINS**
- **Relaxin-2:** $t_{1/2} <$ 10 minutes
- **Phenylalanine hydroxylase:** $t_{1/2} = 8$ to 48 hours
- **Secreted immunoglobulin:** $t_{1/2} = 10$ to 21 days
- **Human crystallin (lens protein):** $t_{1/2} =$ human lifespan

References:
- Chen...Mordenti (1993) Pharm Res
- Chang...Milstein (1979) JBC
- Baker...Shiman (1979) JBC
- Mankarious...Wedgwood (1988) J Lab Clin Med
Examples of our therapeutic mRNA and protein half-lives ($t_{1/2}$)

<table>
<thead>
<tr>
<th>$t_{1/2}$</th>
<th>hMUT (mouse)</th>
<th>ChikmAb (NHP)</th>
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</thead>
<tbody>
<tr>
<td>mRNA</td>
<td>5 hours</td>
<td>11 hours</td>
</tr>
<tr>
<td>protein</td>
<td>24 hours</td>
<td>26 days</td>
</tr>
</tbody>
</table>

Duration of response is a function of both mRNA and protein half-life

Increase mRNA half-life 2X

Increase protein half-life 2X

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How can we extend mRNA half-life?

Increase mRNA half-life 2X

Increase protein half-life 2X

- mRNA: $t_{1/2} = 5$ hrs
- Protein: $t_{1/2} = 24$ hrs

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One way to increase mRNA half-life is to maximize codon optimality and secondary structure in the coding sequence.

**mRNA structure regulates protein expression through changes in functional half-life**

David M. Mauger1, B. Joseph Cebula2,1, Vladimir Penney3, Stephen Y. Yu1,2, David W. Rolf1, Brooke Goodman1, Kristian Lush4,5, NABD Mathoun-Desrosiers4, John Reynolds1,2, Melissa J. Moore1,2 and Ian J. McFadyen1,6*  

1*Moderna Research, Inc., Cambridge, MA 02139  
2University of Massachusetts, Amherst, MA 01003  
3Icahn School of Medicine at Mount Sinai, New York, NY 10011  
4Virginia Commonwealth University, Richmond, VA 23298  
5University of Virginia, School of Medicine, Charlottesville, VA 22908  
6CNRS, IBGP, UPR 3182, BP 126, 31055 Toulouse Cedex 9, France

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**mRNA therapeutics**  
- **mRNA** | nucleic acid | translation

**mRNA therapeutics** directly translates proteins from mRNA sequences into functional proteins. This is achieved by synthesizing mRNA with sequences that are designed to optimize codon usage and minimize down-regulation. The mRNA sequence is transcribed into a pre-RNA, which is then edited to remove unwanted sequences before being delivered to the cell. The edited mRNA is then translated into the desired protein using ribosomes.

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**Significance**

Despite widespread recognition that mRNA is inherently structural, the interplay between local and global structural information in the mRNA sequence and its translation efficiency has not been thoroughly explored. Our work and the work of others has suggested that mRNA secondary structures can influence translation efficiency and mRNA stability. In this study, we present a comprehensive analysis of multiple RNA molecules expressed in mammalian cells, demonstrating the importance of local and global secondary structures in determining mRNA translation efficiency and stability.

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**Methods**

- **mRNA synthesis**  
- **mRNA packaging**  
- **mRNA delivery**

**Results**

- **mRNA translation efficiency**  
- **mRNA stability**

**Conclusions**

- **mRNA therapeutics** can be used to optimize codon usage and minimize down-regulation, resulting in improved translation efficiency and stability.

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**Acknowledgments**

This research was supported by the National Institutes of Health (NIH) grants U01GM129425 and U10AI139457.

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**References**


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**Table**

<table>
<thead>
<tr>
<th>Codon Optimality</th>
<th>mRNA Secondary Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High tRNA concentration</strong></td>
<td><strong>Weak secondary structure</strong></td>
</tr>
<tr>
<td><strong>Low tRNA concentration</strong></td>
<td><strong>Strong secondary structure</strong></td>
</tr>
</tbody>
</table>

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**Figure**

- **mRNA structure**  
- **Codon optimality**  
- **mRNA secondary structure**

---

**Supplementary Information**

- **Figure S1**
- **Table S1**

---

**Figure Legends**

- **Figure 1**
- **Figure 2**
- **Figure 3**
- **Figure 4**

---

**Author Contributions**

- **I.J. McFadyen** designed the study, analyzed the data, and wrote the paper.
- **D.W. Rolf** and **M.J. Moore** contributed to the study design and data analysis.
- **V. Penney** and **S.Y. Yu** contributed to the study design and data analysis.
- **B. Cebula** and **K. Lush** contributed to the study design and data analysis.
- **N.B.D. Mathoun-Desrosiers** and **J. Reynolds** contributed to the study design and data analysis.
- **M.J. Moore** contributed to the study design and data analysis.
- **I.J. McFadyen** supervised the study.

---

**Supporting Information**

- **Supplementary Table S1**
- **Supplementary Figure S1**
mRNA decay in cells starts with deadenylation

m\textsuperscript{7}G

Deadenylase

Decapping complex

Xrn1

Exosome

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The deadenylase uses two active site metal ions to cleave off the terminal adenosine

Tang ... Passmore (2019) NSMB; PDB 6r9j
Adding a terminal inverted dT (idT) changes the tail geometry
A terminal idT alters the enzyme-substrate interaction and prevents deadenylation

Standard poly(A)

Standard poly(A) + idT

Scissile bond

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idT provides a ~2-fold half-life extension in cultured cells

\[
\frac{dD}{dt} = -k_1D \\
\frac{dR}{dt} = k_1D - k_2R \\
\frac{dP}{dt} = k_3R - k_4P
\]

mRNA half-life: \( t_{1/2, R} = \log(2)/k_2 \)

protein half-life: \( t_{1/2, P} = \log(2)/k_4 \)
For *in vivo* studies, we can use a luciferase reporter.

**Diagram:**

1. **Delivery of mRNA**
   - Luciferase mRNA is delivered to the mouse.
   - 24 – 96 hrs later

2. **Delivery of bioluminescent substrate**
   - Luciferin is delivered to the mouse.

3. **Acquisition of light signals and data processing**
   - Luciferin + Oxygen → Oxyluciferin
   - Oxyluciferin + Luciferase → Light
   - In *vivo* imaging

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idT luciferase mRNA gives a 4-fold enhancement of protein activity

IV delivery of mRNA

Luciferase mRNA

idT, inverted deoxythymidine; IV, intravenous

© 2020 Moderna Therapeutics
idT luciferase mRNA gives a 4-fold enhancement of protein activity

idT, inverted deoxythymidine; IV, intravenous

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Substantial enhancement in luciferase expression in both liver and spleen at 48 hours

Isolated liver and spleen show increased luciferase activity from mRNA with 3’ idT relative to unmodified mRNA.
OX40L reporter for immune cell expression

- **Day 0**: Delivery of mRNA
- **Day 1-3**: Analysis

**Flow cytometry**

- **Spleen**
- **Single-cell suspension**
- **Antibody staining**

**T cells** (CD3+): OX40L+

**B cells** (CD19+): OX40L+

**Macrophages** (CD11b+): OX40L+

**Dendritic cells** (CD11c+): OX40L+
idT also enhances protein expression in sorted splenic immune cells

At 72 h post-dose, mouse T cells, B cells, macrophages, and dendritic cells show increased mOX40L from mRNA with 3’ idT relative to unmodified mRNA.
Phenylketonuria (PKU)

- Deficiency in phenylalanine hydroxylase (PAH) → Patients fail to process the amino acid phenylalanine taken up from diet; as a result, phenylalanine can build up to toxic levels.
Stabilized PAH mRNA in PKU mouse model

WT PAH expressed from a 3’ idT-stabilized mRNA supports sustained reduction of serum phenylalanine levels
How can we extend mRNA half-life?

- mRNA: $t_{1/2} = 5$ hrs
- Protein: $t_{1/2} = 24$ hrs

Increase mRNA half-life 2X

Increase protein half-life 2X

- mRNA: $t_{1/2} = 10$ hrs
- Protein: $t_{1/2} = 48$ hrs

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How can we extend protein half-life?

Increase mRNA half-life 2X

Increase protein half-life 2X

mRNA: $t_{1/2} = 5$ hrs
Protein: $t_{1/2} = 24$ hrs

mRNA: $t_{1/2} = 10$ hrs
Protein: $t_{1/2} = 48$ hrs

mRNA: $t_{1/2} = 5$ hrs
Protein: $t_{1/2} = 48$ hrs

mRNA: $t_{3/2} = 10$ hrs
Protein: $t_{1/2} = 48$ hrs
Drivers of *in vivo* protein half-life

- Ubiquitination
- Autophagy

**Intracellular**
- Renal clearance
- Immune clearance
- FcRn recycling

**Secreted**
- Enzymatic degradation
- Ubiquitination
- Autophagy
Relaxin

Endogenous protein associated with cardiovascular re-modeling

• A naturally occurring hormone (human relaxin-2), present in both men and women, that becomes elevated in pregnant women

• Protects from vascular overwork, increases renal function, promotes cell growth and survival, and maintains vessel structure

Relaxin is a vasoactive peptide associated with cardiovascular remodeling

Concept:
IV-administered mRNA encoding relaxin peptide hormone with longer serum half-life to address heart failure
Relaxin-2 and its Achilles’ heel

Serelaxin: human recombinant Relaxin-2 protein

Requires continuous infusion over 48 hours

Techniques to increase half-lives of serum biologics

Fusion domains that bind to target tissue

Increasing resistance to proteolysis

Blood vessel

Glomerulus

Renal corpuscle

Renal filtration

Fusion domains that engage FcRn recycling pathway

Increasing hydrodynamic radius to slow renal clearance
Techniques to increase half-lives of serum biologics

- Fusion domains that bind to target tissue
- Increasing resistance to proteolysis
- Fusion domains that engage FcRn recycling pathway
- Increasing hydrodynamic radius to slow renal clearance
Relaxin-2 constructs

Relaxin

Propeptide replaced with glycosylated linker

Branched sugar chains

Relaxin fused to albumin-binding domain

B chain

Albumin Binding Domain

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Fusion and linker variants improve Relaxin-2 $C_{\text{max}}$ and extend half-life
Drivers of *in vivo* protein half-life

- Ubiquitination
- Autophagy
- Renal clearance
- Immune clearance
- FcRn recycling
- Enzymatic degradation
- Ubiquitination
- Autophagy

Intracellular

Secreted
Intracellular protein degradation

Protein unfolding/denaturation

![Graph showing protein unfolding/denaturation](image)

Ubiquitin-mediated protein degradation

![Diagram showing ubiquitin-mediated protein degradation](image)
Protein engineering to extend intracellular half-life

Objectives
- Stability
- De-ubiquitination

Considerations
- Structural models
- Evolutionary diversity

ANALYZE
SCREEN/TEST
DESIGN
MAKE
To stabilize phenylalanine hydroxylase (PAH), we needed to understand what amino acids were amenable to change.
Since PAH is a central metabolic enzyme, it's present in all branches of life.
Homology searches can identify related sequences from other species

**query sequence: human PAH**

```
MSTAVLENPGLRKLSDFGQEQTSYIDCNQNGAISLIFSLKEEVGAALKVRLRFEDVNLTHIESRPSRLKKDEYEFTTHDLKRSLPALNTNIKILLHDIGATVHELRSRDGKDTVFWFRTIQRDLRFAQILSYGAELADAMPGFKDFVYARRKQFADIAYNRYHRQFIPRVEYMEEKRTWGTVFKTLSLYKTHAYENHIFPPLEKRYCGFHEDNIPQLEDVQSQFLQTCTGFRLRPVAGLLSSRDFFLGLAVRFYHCCTYIIRHSGKMYTPEPIDCHELSSVPLSRDFSAQPSQIGLGLASLPFEYIKLATYVWTVEPGICCCQGDIKAYGMLSSSPGELO/QLSEKPLLPLLEKTAIQNYTFQFQPLYVAESNDAKEKVRNFAATIPRPFSVRYDPYQRIEVLDNTQQLILADSINSEIGILCSALQIK
```

**NCBI (NIH) BLAST database**

Human PAH

Bacterial PAH

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Sequence alignment representations

Global view: Emphasizes overall diversity

Sequence view: Shows specific amino acid letters and highlights similar chemical profiles
Mining useful substitutions from nature

BLAST homology search

4975 hPAH-like seqs

Remove “gappy” sequences

3760 seqs

Remove short sequences

2700 seqs

Remove sequences without Phe in description

690 seqs

Large gaps suggest topological differences from human

Phenylalanine hydroxylase is evolutionarily related to Tyrosine hydroxylase and Tryptophan hydroxylase
Alignment of PAH sequences

Human
Mammals
Birds
Reptiles
Fish
Invertebrates and fungi

- Human (P00439.1)
- Human (NP_000268.1)
- Human (AA78816.1)
- Chimpanzee (XP_001156919.1)
- Human (AA82651.1)
- Western lowland gorilla (XP_004053827.1)
- Synthetic construct (AK171638.1)
- Synthetic construct (AK171639.1)
- Synthetic construct (AK171640.1)
- Synthetic construct (AK171637.1)
- Northern white-cheeked gibbon (XP_003269981.1)
- Synthetic construct (XP_011790468.1)
- Crab-eating macaque (XP_005572100.1)
- Gelada (XP_025258879.1)
- Sumatran orangutan (XP_024111837.1)
- Golden snub-nosed monkey (XP_010355577.1)
- Rhesus monkey (XP_001094859.1)
- Black snub-nosed monkey (XP_017748935.1)
- Bolivian squirrel monkey (XP_003929669.1)
- White-tufted-ear marmoset (XP_002752958.1)
Amino acid changes expected stabilize hPAH folding
Remove hPAH ubiquitination sites

Wagner... Choudhary (2012) Mol Cell Proteomics

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PAH protein variants expressed from idT-protected mRNAs in PKU mouse model

Day 0 1 2 3 4 5 6 7
Biomarker ■ ■ ■ ■ ■ ■ ■

Day 0 1 2 3 4 5 6 7
PBS
PAH WT with idT
PAH WT

Phenylalanine → PAH → Tyrosine

Phenylalanine
Phenylketones

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Human PAH engineering

Predicted to stabilize the folded state

K150 ↔ T150

Removes a potential ubiquitination site

M180 ↔ T180
PAH protein variants expressed from idT-protected mRNAs in PKU mouse model

Low serum phenylalanine levels are maintained even further by combining PAH mRNA stabilization and protein engineering
Conclusions

• The duration of pharmacological effect for mRNA medicines is a function of both mRNA and protein half-life

• Understanding the biological rules governing these half-lives gives us multiple levers to engineer the desired pharmacology

• As with other mRNA engineering principles, the effects are additive
Where does Platform Research fit in?

1. It starts with an idea
2. Digital sequence design based on propriety algorithm
3. Manufacturing of plasmid, mRNA, and LNP
4. Fill, finish, and QC
5. Biodistribution, cellular uptake, and protein expression
Wild type T7 RNA polymerase makes both full-length mRNA and dsRNA impurities

T7 RNA polymerase  \( A, C, G, 1\text{m}^\Psi \)

Transcription

DNA

TOP strand

BOTTOM strand

Short RNAs

Full-length mRNA

100's of copies

Immunostimulatory impurities

Smaller dsRNA

Loopback dsRNA

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dsRNA triggers innate immune responses
HPLC purification is commonly used to reduce dsRNA content

By engineering process conditions, we can minimize dsRNA formation by wild type T7 RNA polymerase.

Highly sensitive assay for detecting dsRNA

<table>
<thead>
<tr>
<th>Legacy process</th>
<th>Moderna process</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng dsRNA/μg mRNA</td>
<td>0</td>
</tr>
</tbody>
</table>

© 2020 Moderna Therapeutics
Engineering T7 RNA Polymerase to further reduce dsRNA formation
Amy Rabideau, Ph.D.

Principal Scientist, Process Development
Wild type T7 RNA polymerase makes both full-length mRNA and dsRNA impurities

- T7 RNA polymerase makes A, C, G, and 1mΨ.
- Transcription starts from the TOP strand and proceeds to the BOTTOM strand.
- Short RNAs are produced as a result of the transcription process.
- Full-length mRNA is produced with hundreds of copies.
- Immunostimulatory impurities include smaller dsRNA and loopback dsRNA.

© 2020 Moderna Therapeutics.
By engineering process conditions, we can minimize dsRNA formation by wild type T7 RNA polymerase.
WT T7 produces short RNA transcripts

WT, wild type T7 RNA polymerase

5’-GGGAAAUAAGAGAGAAAAGAGAAUAGAAGAAUAAUAAGAGCCACCAAAAAAAAAAAAAAAAAAUCUAG-3’

5’-GGGAAAUAAGAGAGAAAAGAGAGAG-3’

Full length RNA

Short RNAs

Free \(^{32}\)P-GTP
WT T7 produces small and loopback dsRNA

WT, wild type T7 RNA polymerase

© 2020 Moderna Therapeutics
Cell-based assays for measuring innate immune responses to dsRNA

Immune response in fibroblasts
- Transfect fibroblasts with mRNA
- Measure IFN-β protein

Immune response in macrophages
- Isolate monocytes
- Differentiate to macrophages
- Transfect macrophages with mRNA
- Measure IP-10 mRNA
- Human peripheral blood mononuclear cells (PBMCs)

Fibroblast cells

mRNA

Ratio IP-10 / HPRT

Ratio IP-10 / HPRT
Transcription cycle: Unbound T7 RNA polymerase

DIRECTION OF POLYMERIZATION

Promoter

DNA

T7

T7 RNA Polymerase (T7)
Transcription cycle: Binding promoter in DNA template
Transcription cycle: Melting DNA to form transcription bubble
Transcription cycle: *De novo* initiation with 2 GTPs
Transcription cycle: Initiating short RNA transcript
Transcription cycle: Transitioning to elongation

DIRECTION OF POLYMERIZATION

T7 DNA

Template strand DNA

New RNA strand

© 2020 Moderna Therapeutics
Transcription cycle: Rapidly transcribes RNA

**DIRECTION OF POLYMERIZATION**

- **Template strand DNA**
- **New RNA strand**
- **T7**
Can we engineer T7 to favor the fast processive state?

Initiation complex

Elongation complex
Moderna computer-aided protein engineering workflow
Analyze & Design: Mutation positions in structure

Conformational mutations
Active site mutations
By engineering process conditions, we can minimize dsRNA formation by wild type T7 RNA polymerase.
Screen/test: dsRNA content and immune response

dsRNA ELISA

IFN-beta response in fibroblasts

dsRNA (\% relative to WT)

WT T7  Moderna T7

0  50  100

Cytokine response

IFN-beta (pg/mL)

WT T7  Moderna T7

0  50  100  150

MULTIPLE MUTANTS

LOQ, level of quantitation; WT T7, wild type T7 RNA polymerase

© 2020 Moderna Therapeutics
Moderna T7 also produces short RNAs

WT, wild type T7 RNA polymerase

5’-GGGAAAUAAGAGAGAAAAGAGUAGAGAGAAAUAAGAGAGCCAC-3’
5’-GGGAAAUAAGAGAGAAAAGAGAG-3’

Full length RNA

Short RNAs

Free 32P-GTP
Moderna T7 produces very little dsRNA

5’ - GGGAAUAAGAGAGAAAGAGAUAGGAAAAGAGAUCACCAAAAAAAAAAAAAAAAAAAAUCUAG - 3’
5’ - GGGAAUAAGAGAAAGAGAGAGAGAGAUCAG - 3’
3’ - CCCUUAAUGUCUUCUCUCUCUCUCUC - 5’

WT, wild type T7 RNA polymerase
Moderna T7 produces mRNA with low dsRNA without HPLC

LOQ, limit of quantitation; WT, wild type T7 RNA polymerase
Moderna T7 produces mRNA with low dsRNA without HPLC

**dsRNA ELISA**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>WT</th>
<th>WT</th>
<th>Moderna T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>–</td>
<td>+</td>
<td>–</td>
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</table>

**Legacy transcription conditions**

**IP-10 response in macrophages**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>WT</th>
<th>WT</th>
<th>Moderna T7</th>
<th>Media only</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

LOQ, limit of quantitation;
WT, wild type T7 RNA polymerase

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Modernova T7 produces mRNA with low immune response without HPLC in vivo

C57BL/6 mice
IV administration
0.5 mg/kg mRNA
Analyzed at 6 h post-dose

WT, wild type T7 RNA polymerase
Moderna now has two ways to reduce dsRNA

1. Process Optimization

2. Moderna T7
Where does Platform Research fit in?

1. It starts with an idea
2. Digital sequence design based on propriety algorithm
3. Manufacturing of plasmid, mRNA, and LNP
4. Fill, finish, and QC
5. Biodistribution, cellular uptake, and protein expression
We target different tissues via multiple routes of administration (ROAs)
Rational structure-based design of LNPs for mRNA starts with understanding the components and their molecular interactions.
New LNPs for Liver Delivery
Kerry Benenato, Ph.D.
Senior Director, Platform Chemistry
Rational structure-based design of LNPs for mRNA starts with understanding the components and their molecular interactions.

Components:
- Ionizable lipid
- Cholesterol
- Phospholipid
- PEG lipid
- mRNA

Structure:
- Δ molecules
- Δ composition
- Δ process

Function:
- Chemical stability
- Physical stability

Therapeutic Effect:
- Biodistribution
- Cellular uptake
- Endosomal escape
- Protein expression
Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

Neutral lipids
FEBS lett., 1971, 14, 95

✓ Good encapsulation of proteins
✗ Poor encapsulation of DNA

Electron micrograph of protein containing liposome
Double-stranded oligonucleotides have a negatively charged backbone
Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

Cationic lipids
PNAS, 1987, 84, 7413

Good encapsulation of DNA
Cationic lipids interact with the negatively charged backbone of double stranded oligonucleotides
Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

Cationic lipids
*PNAS, 1987, 84, 7413*

- Good encapsulation of DNA
- Good *in vitro* delivery efficiency
- Poor *in vivo* systemic delivery

Parker … Sheridan (2003) *Expert Reviews in Molecular Medicine*
Evolution to the modern day lipid nanoparticle identified the importance of ionizable lipids

**Ionizable lipids**
*Pharmaceutical Res., 2005, 22, 362*

- Good encapsulation of DNA
- Good *in vitro* delivery efficiency
- Good *in vivo* systemic delivery
Moderna has invested in development of ionizable lipids for delivery of mRNA

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

The successful development of novel therapeutic agents depends on the optimized balance of safety and efficacy. mRNA delivery systems must be developed and optimized to allow for effective and safe administration in patients. The Moderna Therapeutics mRNA Delivery System (MDT) is a novel delivery technology that can deliver a broad range of therapeutic agents, including vaccines and therapeutics, in a safe and effective manner.

Moderna has invested in the development of ionizable lipids for delivery of mRNA. These lipids are designed to improve the delivery of mRNA by enhancing endosomal escape and providing sustained pharmacology. In non-human primates, the ionizable lipids showed improved safety and efficacy compared to traditional lipids, with reduced toxicity and increased transfection efficiency.

The ionizable lipids have been shown to improve the pharmacokinetic profile of mRNA, leading to safe, sustained pharmacology. This is evidenced by the concentration profiles of hEPO (human erythropoietin) mRNA in various tissues, including spleen, liver, brain, heart, lymph node, and bone marrow. The concentration profiles show that the ionizable lipids result in higher and more sustained concentrations of hEPO mRNA in these tissues compared to traditional lipids.

**efficiency**

hEPO mRNA, 0.01 mg/kg iv infusion

- Control
- Lipid A
- Lipid B

**lipid clearance**

Tissue lipid concentrations, 12 h

**3-6 fold**

**safe, sustained pharmacology**

- hEPO Serum Concentration (ng/mL)

Day 1  Day 8  Day 15  Day 22  Day 29

© 2020 Moderna Therapeutics
Moderna has invested in development of ionizable lipids for delivery of mRNA.
Our commitment to be the best at mRNA science is core to who we are: A 20-year journey
Moderna has invested in development of ionizable lipids for delivery of mRNA

Ionizable amine

Hydrogen bond donor and acceptor

Ethanolamine head group

Moderna proprietary ionizable amino lipid

Sabnis … Benenato (2018) Molecular Therapy
Moderna has invested in development of ionizable lipids for delivery of mRNA
Moderna has invested in development of ionizable lipids for delivery of mRNA
But mRNAs are different: They have both single- and double-stranded regions.
Moderna has invested in development of ionizable lipids for delivery of mRNA
Molecular dynamic simulations allow us to gain insight into how our molecules behave.
Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA.
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Molecular dynamics simulations indicate the ethanolamine engages in transient hydrogen bonds with the mRNA.
The ethanolamine hydrogen bonds to the phosphate backbone and the nucleobases
Could we design lipids with even stronger interactions with the nucleobases?
Hydrogen atom is critical for activity
Nucleobases are too specific

Introduce nucleobases

Protein output (fold change)
Hydrogen bond donors and acceptors are critical for activity.

Introduce new H-bond donors.
Further development of structure activity relationships identified a new lead structure.
New lead structure is a squaramide ionizable amino lipid

- **Ionizable amine**
- **Aromatic**
- **Four hydrogen bond acceptors**
- **Two hydrogen bond donors**
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases.
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Squaramide ionizable lipid
Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases.
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Simulations of squaramide ionizable lipids interacting with mRNA demonstrate preferential interactions with the nucleobases.
Squaramide ionizable lipids bind mRNA via hydrogen bonds
Squaramide ionizable lipids bind mRNA via pi-stacking
Squaramide based ionizable lipids afford improved protein expression after iv administration

Squaramide ionizable lipid

Mouse
IV infusion
0.5 mg/kg
hEPO mRNA

NHP
IV infusion
0.1 mg/kg
hEPO mRNA

Log [hEPO] (µg/mL)

Time (h)

Log [hEPO] (ng/mL)

Time (h)
Squaramide based ionizable lipids afford sustained pharmacology

NHP
IV infusion
0.1 mg/kg
hEPO mRNA
Biweekly dosing

\[
\text{Log \[hEPO \ (ng/mL)\]}
\]
Squaramide based ionizable lipids efficiently deliver to hepatocytes in cyno liver

NHP
IV infusion
2 mg/kg
NPI-Luc mRNA
6 h immunohistochemistry
Glycogen Storage Disease Type 1a (GSD1a)

- Deficiency in glucose-6-phosphatase (G6Pase), encoded by G6PC gene
  → Patients fail to maintain blood glucose level and suffer hypoglycemia during fasting
Sustained improvement in fasting blood glucose with the squaramide LNP

G6PC KO mouse
IV bolus
0.2 mg/kg
G6Pase mRNA

1st Dose  2nd Dose  3rd Dose

Fasting Blood Glucose (mg/dL)

Therapeutic threshold (60 mg/dL)
Our commitment to be the best at mRNA science is core to who we are: A 20-year journey

Product Performance

- Ethanolamine ionizable lipid
- Squaramide ionizable lipid

© 2020 Moderna Therapeutics
Rational structure-based design of LNPs for mRNA starts with understanding the components and their molecular interactions.

**Components**
- Ionizable lipid
- Cholesterol
- Phospholipid
- PEG lipid

**Structure**
- Δ molecules
- Δ composition
- Δ process

**Function**
- Chemical stability
- Physical stability

**Therapeutic Effect**
- Biodistribution
- Cellular uptake
- Endosomal escape
- Protein expression
Impact of LNP Size on Vaccine Immunogenicity
Moderna’s proprietary vaccine ionizable lipid improves local expression and tolerability without impacting immunogenicity.
Generating an immune response with mRNA vaccines

1. Recruitment of immune cells to the site of administration

2. Migration of LNPs and APC to the draining lymph node

3. LNP uptake and antigen expression in cells at the injection site and in draining lymph nodes
Literature suggests particle size can impact immune response

  - Vesicle Size Influences the Trafficking, Processing, and Presentation of Antigens in Lipid Vesicles

  - Critical size limit of biodegradable nanoparticles for enhanced lymph node trafficking and paracortex penetration

- Prabha . . . Labhasetwar (2002)
  - Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles

  - Nanoparticles target distinct dendritic cell populations according to their size

- Carstens . . . Jiskoot (2011)
  - Effect of vesicle size on tissue localization and immunogenicity of liposomal DNA vaccines

  - Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns

- Benne . . . Stütter (2016)
  - Orchestrating immune responses: How size, shape and rigidity affect the immunogenicity of particulate vaccines

  - Engineered nanoparticles interacting with cells: size matters
Particle size affects lymphatic vessel entry and APC uptake

Particle size affects lymphatic vessel entry and APC uptake

Immunogens
- Soluble antigens
- Viruses
- Bacteria

Vaccine delivery system
- LNPs

A retrospective analysis shows a trend between CMV LNP size and immunogenicity.

The retrospective analysis contained 23 CMV immunogenicity studies evaluating 129 LNP formulations.
LNP size can be varied by controlling process parameters
LNP size can be varied by controlling process parameters

- Mixing Variable 1
- LNP Diameter (nm)
- Mixing Variable 2
- Buffer Exchange Variable 1
- Mix Variable 2A
- Mix Variable 2B
- Mix Variable 2C
- Secondary Processing Variable 1

Lipid mix (organic)

mRNA (aqueous)

LNP size can be varied by controlling process parameters through the following steps:

1. Lipid mix (organic)
2. mRNA (aqueous)
3. Mixing
4. Buffer exchange
5. Secondary processing

The graph shows how LNP diameter (nm) changes with different mixing variables:

- Mixing Variable 1
- Mixing Variable 2
- Buffer Exchange Variable 1
- Secondary Processing Variable 1

The graph indicates that the LNP diameter increases with increasing mixing variable values.
Optimal CMV LNP size is ~100 nm in mice

- Antibody Titer After Prime
- Antibody Titer After Boost

LNP Diameter (nm)
Anti-pentamer Titer (d21)

LNP Diameter (nm)
Anti-pentamer Titer (d36)

Analysis (blood draw)
Day 0 21 22 36

CMV LNP

Lipid mix (organic)
Mixing
Buffer exchange
Secondary processing

mRNA (aqueous)

Dose
Prime
Boost

Balb/C mice
IM administration
3 µg dose
CMV mRNA
Optimal CMV LNP size is ~100 nm in mice

LNP Diameter (nm)
Anti-pentamer Titer (d21)

LNP Diameter (nm)
Anti-pentamer Titer (d36)

CMV LNP

Lipid mix (organic)
Mixing
Buffer exchange
Secondary processing

mRNA (aqueous)

Balb/C mice
IM administration
3 µg dose
CMV mRNA
Different sized LNPs were selected for further evaluation

**Dynamic Light Scattering**

- **Small**: 64 nm
- **Medium**: 81 nm
- **Large**: 108 nm
- **X-Large**: 146 nm

**Polydispersity Index**

- Small: 0.00
- Medium: 0.05
- Large: 0.10
- X-Large: 0.15

**Nanoparticle Tracking Analysis**

- **Small**: $2.5 \times 10^{12}$
- **Medium**: $2 \times 10^{12}$
- **Large**: $1.5 \times 10^{12}$
- **X-Large**: $1 \times 10^{12}$

**Cryo-Electron Microscopy Images**

- Small: 64 nm
- Medium: 81 nm
- Large: 108 nm
- X-Large: 146 nm
CMV LNP vaccine size evaluated in mice

Mouse
Balb/C mice
IM administration
3 µg dose
CMV mRNA

LNP
Anti-Pentamer Titer

Day 0
Analysis (blood draw)

Day 21 (3wp1)
Day 36 (2wp2)

Pre-dose

Significance (p<0.01) at Day 21 and Day 36 by Tukey’s test

CMV LNP

*
CMV LNP vaccine size evaluated in mice and NHP models

Mouse
Balb/C mice
IM administration
3 µg dose
CMV mRNA

NHP
Cynomolgus monkey
IM administration
30 µg dose
CMV mRNA

Day 0 Analysis
(blood draw)

Dose
Day 0 21 22 36

Pre-dose
Day 21 (3wp1)
Day 36 (2wp2)

* Significance (p<0.01) at Day 21 and Day 36 by Tukey’s test
Conclusions

• LNP size can be controlled while maintaining lipid composition by modulating formulation process parameters

• In mice, LNP sizes below 80nm induce antibody titers dependent on particle size

• In mice, LNP sizes above 100nm consistently generate high antibody titers independent of particle size

• NHPs are more tolerant to changes in LNP particle size

• Current size range of Moderna’s mRNA vaccine LNPs is appropriate for optimal immune responses in primates
# 2020 Science Day agenda

<table>
<thead>
<tr>
<th>Section</th>
<th>Presenter</th>
<th>Time</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Introduction</strong></td>
<td>Stéphane Bancel, CEO</td>
<td>8:00-8:10 AM</td>
<td>10 min</td>
</tr>
<tr>
<td><strong>Chapter 1</strong></td>
<td><strong>Extending Pharmacology</strong></td>
<td>8:10-8:45 AM</td>
<td>35 min</td>
</tr>
<tr>
<td>• Controlling the duration of our pharmacologic effect</td>
<td>Melissa Moore, Ph.D., Chief Scientific Officer, Platform Research</td>
<td></td>
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<tr>
<td>• Enhancing mRNA and protein half-lives</td>
<td></td>
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<td></td>
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<tr>
<td><strong>Chapter 2</strong></td>
<td><strong>T7 Enzyme Engineering to Reduce dsRNA Production</strong></td>
<td>8:45-9:05 AM</td>
<td>20 min</td>
</tr>
<tr>
<td><strong>Chapter 3</strong></td>
<td><strong>New LNPs for Liver Delivery</strong></td>
<td>9:05-9:25 AM</td>
<td>20 min</td>
</tr>
<tr>
<td><strong>Chapter 4</strong></td>
<td><strong>Impact of LNP Size on Immunogenicity</strong></td>
<td>9:25-9:40 AM</td>
<td>15 min</td>
</tr>
<tr>
<td><strong>Chapter 5</strong></td>
<td><strong>Our Progress Toward Creating a mRNA Vaccine for HIV</strong></td>
<td>9:50-10:50 AM</td>
<td>60 min</td>
</tr>
<tr>
<td>• Vaccine design to elicit broadly neutralizing antibodies against HIV</td>
<td>Andrea Carfi, Head of Research, Infectious Disease William Schief, Ph.D., Scipps, International Aids Vaccine Initiative (IAVI) Paolo Lusso, M.D., Ph.D., National Institute of Allergy and Infectious Diseases (NIAID)</td>
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<tr>
<td>• NIH-Moderna HIV-1 vaccine</td>
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<tr>
<td><strong>Conclusion</strong></td>
<td>Stephen Hoge, M.D., President</td>
<td>10:50-11:00 AM</td>
<td>10 min</td>
</tr>
<tr>
<td><strong>Q&amp;A</strong></td>
<td></td>
<td>11:00-11:30 AM</td>
<td>30 min</td>
</tr>
</tbody>
</table>
Andrea Carfi
Head, Infectious Disease Research
mRNA vaccines overview
Key characteristics and differentiation

1. Large product opportunity
   - Ability to do complex antigens ✔
   - Ability to do combination vaccines ✔

2. Higher probability of technical success
   - Vaccine mechanism of action (MOA) ✔
   - B-cell and T-cell response ✔
   - Time to clinical study/market ✔
   - Uniform process allows for fast scale up ✔

3. Accelerated research and development timelines

4. Greater capital efficiency over time vs. recombinant technology
   - Lower capex ✔
   - Greater flexibility ✔

Moderna’s vaccine franchise:
- 7 vaccines for major unmet needs
- >1,500 healthy volunteers enrolled in vaccine clinical trials
- Observed neutralizing antibodies to viral antigens against all eight viruses for which we have had clinical trial readouts
HIV collaborations with BMGF, IAVI and NIH
Important part of Moderna’s commitment to public health

Commitment to public health
- Long standing collaborations with BMGF, IAVI and NIH
- HIV remains a high unmet need with 2.1 million new annual infections
- In ~30 years, only 6 HIV-1 efficacy vaccine trials

Applying mRNA to challenging biological and technological problems
- Moderna’s mRNA technology to accelerate HIV vaccine discovery by rapid production and clinical testing for novel vaccine candidates
- mRNA vaccine platform allows for rapid iterative cycles of design and human testing enabling novel vaccination strategies
- mRNA technology expands the antigen design space enabling the generation of vaccine candidates that can not be easily manufactured otherwise

1. UNAIDS, Global AIDS Update 2016
William Schief, Ph.D.
Professor, Immunology and Microbiology, Scripps
Executive Director, Vaccine Design, IAVI

William Schief is a Professor in the Immunology and Microbial Science Department at The Scripps Research Institute in La Jolla, CA, Director for Vaccine Design at the International AIDS Vaccine Initiative (IAVI), and an Associate Member of the Ragon Institute of MGH, MIT and Harvard. He is also a founder and board member of CompuVax, Inc., a startup biotech company focused on vaccine design.

William received a B.S. in Applied Mathematics from Yale University and a Ph.D. in Physics from the University of Washington. Dr. Schief’s work focuses on computation-guided and structure-based design of immunogens and immunization regimens, with the goal of inducing broadly neutralizing antibodies against HIV and other pathogens that have frustrated traditional vaccine design strategies.
Dr. Lusso was born in Torino, Italy. He received his M.D. from the University of Torino and his Ph.D. from the University of Bologna. He is board certified in Internal Medicine and Infectious Diseases. From 1986 to 1994 he worked as a Visiting Scientist in the Laboratory of Tumor Cell Biology, NCI, Bethesda, MD.

From 1994 to 2007, he directed the AIDS Research Laboratory at the San Raffaele Institute in Milan, Italy. In 2007, he returned to the US where he became the Chief of the Viral Pathogenesis Section in the Laboratory of Immunoregulation, NIAID, Bethesda.

He is an elected Member of the European Molecular Biology Organization (EMBO) and a Fellow of the American Academy of Microbiology. His research is focused on the mechanisms of HIV/AIDS pathogenesis and the development of an HIV vaccine. His 1995 discovery of the HIV-suppressive chemokines was nominated “Breakthrough of the Year” by Science magazine.
Vaccine Design to Elicit Broadly Neutralizing Antibodies Against HIV

William Schief
Professor, Immunology and Microbiology, Scripps
Executive Director, Vaccine Design, IAVI

Moderna Science Day

June 2, 2020
HIV/AIDS Continues to Devastate

- 1.8 million new infections per year (nearly 5,000 per day)
- 37 million people living with HIV – 40% not receiving ART
- 1 million deaths per year due to HIV
- 35 million people have died due to HIV

We need a vaccine.

Source: UNAIDS Fact Sheet 2018
HIV entry

Env spike = (gp120)$_3$ (gp41)$_3$
Neutralizing Abs block HIV entry.

Successful vaccines induce neutralizing Abs.

For HIV, need to induce broadly neutralizing Abs (bnAbs).
Sequence Diversity of HIV ENV Dwarfs that of Influenza HA

b) 1996 Influenza Sequences
Hemagglutinin (H3)
n=96

c) HIV-1 Single Individual (v2-C5)
Subtype B
Asymptomatic phase
Year 6 post sero-conversion
n=9

f) Democratic Republic of the Congo
1997, n=193

HIV Envelope Trimer: the Target of Neutralizing Antibodies

Structure, Glycosylation, and Sequence Variation Pose Major Challenges to the Development of Broadly Neutralizing Antibodies
Prototype HIV Broadly Neutralizing Antibodies (bnAbs)

- bnAbs neutralize diverse isolates, some up to 99% of all isolates.
- bnAbs provide sterilizing immunity in NHP models
- If vaccine can elicit bnAbs, could prevent HIV infection
Infected individual

Protective Abs (HIV: Abs=broadly neutralizing Abs; target=Env)

Molecular characterization of Ab-Env

Immunogen design and testing

combination of several immunogens = vaccine

*modified Env

HIV Vaccine Goal

Develop a vaccine that elicits sustained protective levels of broadly neutralizing antibodies (bnAbs) in humans
**Hypothesis:** An effective HIV vaccine will need to consistently induce bnAbs against 2-3 different sites on the HIV Env in most vaccine recipients.

Targeting multiple sites is necessary to provide adequate coverage against the huge diversity of global isolates.
How will an HIV Vaccine Induce Broadly Neutralizing Antibodies (bnAbs)?

- bnAb precursor B cell
  - Rare
  - Diverse
  - Difficult to activate with HIV proteins

naïve B cells → Somatic Hypermutation → plasma cells secrete bnAbs

bnAbs
Strategy to induce bnAbs: Germline-Targeting Vaccine Design

- **Vaccine Prime** "germline targeting"
- **Vaccine Boost 1** "shepherding"
- **Vaccine Boost N** "polishing"

 naïve B cells → memory B cells → memory B cells → plasma cells secrete bnAbs

Somatic Hypermutation

bnAbs
Germline-targeting nanoparticle activates bnAb germline precursor B cells, but similar nanoparticle lacking germline affinity fails to activate.
Our repertoire-guided germline-targeting approach provides a framework for priming the induction of many HIV bnAbs and could be applied to most HCDR3-dominant antibodies from other pathogens.

Steichen, et al. Science, 2019
Proof of principle for germline-targeting vaccine design: bnAbs can be elicited starting from human GL B cells

Caveats:

• BnAb precursor frequency was ~100% in this mouse model → Too easy, no competition from other B cells
• Priming immunogen had only modest affinity for bnAb precursors
• Not ready for human testing – priming immunogen needs higher affinity, broader specificity
Eliciting bnAbs in humans – importance of consistent priming

• Consistent priming of bnAb precursors is a vaccine requirement

• Consistent priming will likely require a priming immunogen that can target a diverse precursor pool for any one class of bnAb (due to human genetic diversity and antibody recombinational and junctional diversity)

➢ Priming immunogen needs appreciable affinity and avidity for diverse precursors (diverse human naïve B cells)

Vaccine Prime

“germline targeting”

naive B cells  memory B cells
Lead Project for Germline-Targeting Vaccine Design: VRC01

VRC01-class bnAbs
- bind the gp120 CD4bs
- require VH1-2 and 5AA L-CDR3 to engage CD4bs
- have diverse H-CDR3s and light chains

- Need priming immunogen with appreciable affinity and avidity for diverse VRC01-class human naïve precursors

Germline-Targeting Immunogen
- eOD-GT8 60mer
- Self-assembling nanoparticle presenting 60 copies of an engineered gp120 outer domain

- Has appreciable affinity and avidity for diverse VRC01-class human naïve precursors
- Primes VRC01-class responses in stringent mouse models
- Induces VRC01-class memory responses that can be boosted toward bnAb development in mouse models

IAVI G001 Phase I Trial: eOD-GT8 60mer/AS01B

- First-in-human test of germline targeting
- Self-assembling nanoparticle immunogen
- Trial start: Sept 2018 (1st immunization)
- Conducted at FHCRC (Seattle) and GWU (Washington, DC)
- Critical readout by B cell sorting/sequencing at FHCRC (Cohen/McElrath) and NIH/VRC (Koup/McDermott)

<table>
<thead>
<tr>
<th>Study Group</th>
<th>N</th>
<th>eOD-GT8 60mer dose</th>
<th>Week 0</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (low dose)</td>
<td>18</td>
<td>20 µg</td>
<td>eOD-GT8 60mer/AS01B</td>
<td>eOD-GT8 60mer/AS01B</td>
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<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>placebo</td>
<td>placebo</td>
</tr>
<tr>
<td>2 (high dose)</td>
<td>18</td>
<td>100 µg</td>
<td>eOD-GT8 60mer/AS01B</td>
<td>eOD-GT8 60mer/AS01B</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>placebo</td>
<td>placebo</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td></td>
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</tr>
</tbody>
</table>
Challenge: To develop a highly effective HIV vaccine, we will need to carry out many iterative human clinical trials. If we rely on GMP protein manufacture, our progress will be limited by the relatively slow pace and high cost of manufacture.

Solution: We are hopeful that Moderna mRNA will provide a rapid, economical, and highly immunogenic vaccine platform to enable expeditious iterative human vaccine optimization.
Moderna mRNA vs protein for priming VRC01-class responses in a stringent mouse model: BCR sequencing indicates that mRNA performs at least as well as protein

- VH1-2 mouse model (Tian et al Cell 2016)
- Three different immunogens tested as mRNA vs protein + adjuvant
- Immunogens are variants of eOD-GT8 60mer
- Single immunization at day 0
- mRNA (10 µg) via IM route
- protein (20 µg) + SAS adjuvant via IP route
- Readout is epitope-specific B cell sorting/BCR sequencing at d42
- >100 BCR sequences recovered in each of 6 cases
- What % of epitope-specific B cells at d42 are VRC01-class?
Moderna Collaboration with Schief/Burton/IAVI: 
Developing an mRNA-based HIV Vaccine that Induces BnAbs, through Expeditious Iterative Human Vaccine Evaluation

**Overall Vaccine Strategy**
- Germline Targeting
- Shepherding of Affinity Maturation
- Trimer Polishing

**Iterative Vaccine Design/Test Cycle**
1. Immunogen Design
2. mRNA Formulation
3. Vaccine Testing in Engineered Mice and NHPS
4. Human Clinical Trials
5. Protective Vaccine

**Vaccine Targets (bnAbs)**
- VRC01-Class bnAbs
- V2 Apex bnAbs
- N332 bnAbs

- IAVI, BMGF and NIH funding
- Animal exps with: Alt, Batista, Crotty, Haynes, Nemazee, Silvestri, Verkoczy
Acknowledgements

Schief lab (Scripps/IAVI)
Yumi Adachi
Charina Fabilane
Erik Georgeson
Bettina Groschel
Xiaozhen (Jenny) Hu
Possu Huang
Joe Jardine
Oleksandr Kalyuzhniy
Mike Kubitz
Dan Kulp
Alessia Liguori
Krystal Ma
Skye MacPherson
Sergey Menis
Nicole Phelps
Sebastian Rämisch
Torben Schiffner
Jon Steichen
Jordan Willis

Scripps/IAVI
David Nemazee
Taka Ota
Patrick Skog
Terri Thinnes
Dennis Burton
Devin Sok
Bryan Briney
Elise Landais
Ian Wilson
Jean-Philippe Julien
Anita Sarkar

Harvard
Fred Alt
Ming Tian
Sai Luo

Emory
Guido Silvestri
Diane Carnathan

La Jolla Institute
Shane Crotty
Colin Havenar-Daughton
Robert Abbott
JH Lee
Yu (Alex) Kato
Ivy Phung

Rockefeller
Michel Nussenzweig
Pia Dosenovic
Amelia Escolano
Lotta von Boehmer

Ragon
Facundo Batista
Ying-Cing Lin
Simone Pecetta
Sven Kratochvil
Eleonora Melzi
Xuesong Wang

Moderna
Andrea Carfi
Sunny Himansu
Samantha Falcone
Karina Steiger-Gonzalez
Phil White
Sophia Zendzian

IAVI
Mark Feinberg
Labeeb Abboud
Antu Dey
Olayinka Fagbayi
Khoa Le
Dominick Laddy
Dagna Laufer
Karen Tran

NIH
National Institute of Allergy and Infectious Diseases

CHAVI ID
International AIDS Vaccine Initiative

Bill & Melinda Gates foundation
The Collaboration for AIDS Vaccine Discovery

Ragon Institute
of MGH, MIT and Harvard
NIH-Moderna HIV-1 Vaccine
An mRNA-Based Approach to the Development of a Protective HIV-1 Vaccine

Paolo Lusso, M.D., Ph.D.
Chief, Viral Pathogenesis
Laboratory of Immunoregulation
National Institute of Allergy and Infectious Diseases (NIAID)

Supported by:

Information in following slides are from a third-party source. While we believe such information is reliable, we have not independently verified any third-party information, and make no guarantee, express or implied, as to the accuracy and completeness of it.
Greatest Pandemics in History

Throughout history, as humans spread across the world, infectious diseases have been a constant companion. Even in this modern era, outbreaks are nearly constant.

Here are some of history’s most deadly pandemics, from the Antonine Plague to COVID-19.

Source: Visual Capitalist (https://www.visualcapitalist.com)
Two Key Lessons Learned from Decades of HIV Vaccine Research

- A protective vaccine is feasible
- Induction of broadly neutralizing antibodies (bNAbs) is an essential condition for a protective vaccine
The HIV-1 Envelope Is a Membrane-Anchored Trimer

From: Koff & Berkley, NEJM 2010
Different Forms of the HIV-1 Envelope Utilized as Vaccines

1. Soluble, monomeric gp120 subunit

   Ineffective as vaccine

   - Elicits nonprotective antibodies
Different Forms of the HIV-1 Envelope Utilized as Vaccines

1. Soluble, monomeric gp120 subunit
   - Ineffective as vaccine
   - Elicits nonprotective antibodies

2. Soluble, stabilized (SOSIP) trimer
   - Sub-optimal for vaccine
   - Elicits off-target antibodies
Different Forms of the HIV-1 Envelope Utilized as Vaccines

1. Soluble, monomeric gp120 subunit
   - Ineffective as vaccine
   - Elicits nonprotective antibodies

2. Soluble, stabilized (SOSIP) trimer
   - Sub-optimal for vaccine
   - Elicits off-target antibodies

3. Membrane-anchored pre-fusion trimer
   - Best for vaccine
   - Same form as in real-life virus
   - Best suited for mRNA expression
1. Use membrane-anchored Env expressed in vivo by mRNA

2. In vivo production of virus-like particles (VLP) by co-formulation of Env with Gag

3. Priming with Ab ancestor-engaging transmitted/founder (T/F) Env

4. Intensive heterologous boosting with multi-clade, glycan-repaired tier-2 Envs (i.e., real-life HIV-1 strains)
1. Use **membrane-anchored Env** expressed *in vivo* by mRNA
The NIH-Moderna Approach: Key Points

1. Use membrane-anchored Env expressed in vivo by mRNA

Main advantages:

- Native antigenic state (best mimic of virion-associated Env)
- Endogenous protein processing (native glycosylation)
- Lack of "distracting" epitopes (e.g., SOSIP-trimer base)
The NIH-Moderna Approach: Key Points

2. *In vivo* production of **virus-like particles (VLP)** by co-formulation of Env with Gag
2. *In vivo* production of **virus-like particles (VLP)** by co-formulation of Env with Gag
The NIH-Moderna Approach: Key Points

2. *In vivo* production of **virus-like particles (VLP)** by co-formulation of Env with Gag

Co-formulation of Gag+Env mRNAs

mRNA formulated in lipid nanoparticles
2. *In vivo* production of **virus-like particles (VLP)** by co-formulation of Env with Gag
2. In vivo production of virus-like particles (VLP) by co-formulation of Env with Gag

Main advantages:

- Native antigenic state (closest proxy to HIV virions)
- Life size (optimal APC uptake/processing)
- Extracellular release (travel to afferent lymph nodes)
- Induction of both Env and Gag responses
The NIH-Moderna Approach: Key Points

3. Priming with *Ab ancestor-engaging* transmitted/ founder (T/F) Env

**Main advantages:**

- *Ab initio* recruitment of rare precursor antibodies for the generation of broadly neutralizing antibodies
The NIH-Moderna Approach: Key Points

4. **Intensive heterologous boosting** with multi-clade, glycan-repaired tier-2 Envs (*i.e.*, real-life HIV-1 strains)

Main advantages:

- Selective immune focusing on “shared” epitopes across different HIV-1 isolates and clades, *i.e.*, bNAb epitopes
- Exclusion of distracting, non-neutralizing epitopes, *i.e.*, glycan holes, V3 loop, bridging sheet, etc.
- Mimicking of sustained antigenic stimulation that occurs in patients who eventually develop bNAbs
VPS-M1 Study: Design and Rationale

Prime
(1 dose)

Clade-B T/F
OPEN

Engage unmutated bNAb precursors
VPS-M1 Study: Design and Rationale

**Prime**
(1 dose)

Clade-B T/F
OPEN

Engage unmutated bNAb precursors

**Boost 1**
(2 doses)

Clade-B T/F
AUTOLOGOUS
CLOSED (tier 2)

Selectively expand tier-2 epitopes
**VPS-M1 Study: Design and Rationale**

- **Prime**
  - (1 dose)
  - Clade-B T/F OPEN
  - Engage unmutated bNAb precursors

- **Boost 1**
  - (2 doses)
  - Clade-B T/F AUTOLOGOUS CLOSED (tier 2)
  - Selectively expand tier-2 epitopes

- **Boost 2**
  - (4 doses)
  - Clades A+C HETEROLOGOUS MIXED (tier 2), GLYCANT-REPAIRED
  - Focus on “shared” bNAb epitopes

*
Species: Rhesus macaques (*M. mulatta*), juvenile, A01⁻, B08⁻, B17⁻ 4 (n = 4 animals each)

**Species:** Rhesus macaques (*M. mulatta*), juvenile, A01⁻, B08⁻, B17⁻ 4 (n = 4 animals each)

**Study arms:**
- Arm 1: mRNA prime, SOSIP boost, WT Envs
- Arm 2: mRNA prime, SOSIP boost, 113-429 locked Envs
- Arm 3: mRNA only, WT Envs
- Arm 4: mRNA only, 113-429 locked Envs

**Inoculation:** Intramuscular

**Env mRNA or protein:** WITO (clade B), BG505 (clade A), DU422 (clade C) all glycan-repaired and 375Y-mutated

**Gag mRNA:** SIVmac239

---

**Slide 240**

**Autologous Env**

- Env+Gag mRNA 400μg (ΔN276)

**Heterologous Env**

- Env+Gag mRNA 240μg
- Env+Gag mRNA 240μg or SOSIP protein 100μg + Adj
- Env+Gag mRNA 450μg
- Env+Gag mRNA 450μg or SOSIP protein 100μg + Adj

**Low-dose rectal SHIV challenges**

- Env+Gag mRNA 400μg or SOSIP protein 100μg + Adj
- Env+Gag mRNA 240μg or SOSIP protein 100μg

---

**Zhang et al., manuscript in preparation**
Induction of Broadly Neutralizing Antibodies
Progressive Induction of Autologous HIV-1 Neutralization in Macaques during the Course of the Immunization Phase

Zhang et al., manuscript in preparation
Appearance of Heterologous HIV-1 Neutralization* in Macaques after Repeated Heterologous Immunizations

*Neutralization of HIV-1 JR-FL (tier-2)
Broad-Spectrum Heterologous Tier-2 Neutralization in Macaques at the End of the Immunization Period (Wk. 58)

Montefiori’s global HIV-1 Env panel

Global HIV-1 isolates:

AD8, JR-FL, 398F1, 246F3, CNE8, CNE55, TRO11, X2278, BUOXO, CH119, 25710, CE1176, CE0217, X1632
Heterologous Tier-2 SHIV Challenge
VPS-M1: Study Design

- **Autologous Env WITO (Clade B)**
  - Env+Gag mRNA 400μg
  - Env+Gag mRNA 240μg

- **Heterologous Env BG+DU (Clade A+C)**
  - Env+Gag mRNA 240μg
  - Env+Gag mRNA 450μg

- **Auto (B)**
  - Env+Gag mRNA 400μg
  - Env+Gag mRNA 240μg

**Challenge phase**

**Low-dose rectal SHIV challenges**
Protection from Pathogenic Tier-2 SHIV (AD8) Challenge in Immunized Macaques

Infection-free survival

Percent survival

Days

WT Env (n=7)
Locked Env (n=7)
Naïve controls (n=4)

p = 0.009

Zhang et al., manuscript in preparation
Correlates of Protection
Antibodies to the CD4-Binding Site (VRC01-Like) Detected in Serum from a Protected Animal

Macaque #9, week 58

Zhang et al., manuscript in preparation
Antibodies to the CD4-Binding Site (Probe M49) Correlated with Protection from SHIV-AD8 Infection in Vaccinated Macaques

**Week 37**

- **eODGT8.M49 antibodies**
- $r^2 = 0.243$
- $p = 0.202$

**Week 58**

- **eODGT8.M49 antibodies**
- $r^2 = 0.49$
- $p = 0.008$

Zhang et al., manuscript in preparation
Single-Cell Sorting of eOD-GT8.M49-Binding B Cells from a Protected Macaque (#9)

Pre-immune

Week 60

Single-cell sorting:
BG505-SOSIP+
BG505-CD4bs KO-eOD-GT8.Mut49+
Single-Cell Sorting of eOD-GT8.M49-Binding B Cells from a Protected Macaque (#9)

Pre-immune

Week 60

Zhang et al., manuscript in preparation
Prevalence of Heavy Chain Variable Gene (VH) Usage among CD4-BS-Specific Antibodies Cloned from a Protected Macaque (#9, week 60)

Heavy Chain

Kappa Chain

Lambda Chain

Total=64

Total=85

Total=49

Zhang et al., manuscript in preparation
CDR3 Length Distribution in Heavy Chain Variable (VH) Genes of CD4-BS-Specific Cloned Antibodies

**HCDR3 length:** 9-53 aa. (average 18.4)

Zhang et al., manuscript in preparation
1. Use membrane-anchored Env expressed \textit{in vivo} by mRNA

2. \textit{In vivo} production of virus-like particles (VLP) by co-formulation of Env with Gag

3. Priming with Ab ancestor-engaging transmitted/founder (T/F) Env

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4. Intensive heterologous boosting with multi-clade, glycan-repaired tier-2 Envs (i.e., real-life HIV-1 strains)

Close mimicry of real-life infection with native virus
1. Repeat/expand the first pre-clinical NHP study to:
   - Confirm initial results
   - Further optimize immunogens
   - Streamline protocol

2. Design first-in-human clinical trial with an mRNA-based HIV-1 vaccine
Acknowledgments

**LIR, NIAID**
Peng Zhang, Ph.D.
Yin Lin, Ph.D.
Qingbo Liu, Ph.D.
Hana Schmeisser, Ph.D.
Samuel Owusu, B.Sc.
Denise Rogers, B.Sc.
Mit Patel, M.Sc.
Tson Andine, B.Sc.
Huiyi Miao M.Sc.
Ferzan Uddin, B.Sc.
Alice Kwon, M.Sc.

**Modern Inc.**
Elisabeth Narayanan, Ph.D.
Andrea Carfi, Ph.D.
Guillaume Stewart-Jones, Ph.D.
Isabella Renzi, Ph.D.
Vlad Presnyak, Ph.D.
Sayda Elbashir, Ph.D.
Giuseppe Caramella, Ph.D.
Mike Watson, Ph.D.
Sunny Himansu, Ph.D.
Kapil Bahl, Ph.D.

**VRC, NIAID**
Madhu Prabakaran, Ph.D.
Adrian McDermott, Ph.D.
Jason Gorman, Ph.D.
Hui Geng, Ph.D.
John R. Mascola, M.D.
Peter D. Kwong, Ph.D.

**San Raffaele Institute**
**Milan, Italy**
Francesca Sironi, M.Sc.
Mauro S. Malnati, M.D.

**University of Kansas, Lawrence**
Heather Desaire, Ph.D.
Eden P. Go, M. Sc.

**FNLCR, NCI**
Yaroslav Tsybovsky, Ph.D.

**BCBB, NIAID**
Michael A. Dolan, Ph.D.

**Bioqual Inc.**
Deborah Weiss, VDM
Jonathan Misamore, VDM
Conclusion
Our commitment to be the best at mRNA science is core to who we are: A 20-year journey
2018 Science Day Themes and Publications

- Development of proprietary ionizable lipids for improved systemic mRNA delivery
- Development of proprietary ionizable lipids with improved tolerability for mRNA vaccines
- Use of miRNA target sites to create "smart" mRNAs
- Translation initiation and "leaky scanning"
- How artificial intelligence can help us design better coding sequences

A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Non-human Primates

Staci Sabnis, E. Sethurajith Kumaranasinghe, Timothy Salerno, Cosmin Mihaí, Tatiana Ketova, Joseph J. Senn, Andy Lynn, Alex Bubley, Iain McFadyen, Joyce Chan, Örjan Almarna, Matthew G. Stanton, and Kerry E. Benenato

Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines

Kimberly J. Hassett, Kerry E. Benenato, Eric Jaquinet, Aisha Lee, Angela Woods, Olga Yuzbakov, Sunny Himanshu, Jessica Detertling, Benjamin M. Gelich, Tatiana Ketova, Cosmin Mihaí, Andy Lynn, Iain McFadyen, Melissa J. Moore, Joseph J. Senn, Matthew G. Stanton, Örjan Almarna, Giuseppe Ciaramella, and Luis A. Brito

MicroRNAs Enable mRNA Therapeutics to Selectively Program Cancer Cells to Self-Destruct

Ruchi Jain, Josh P. Frederick, Eric Y. Huang, Kristine E. Burke, David M. Mauger, Elizaveta A. Andrianova, Sam J. Farlow, Sunimar Siddiqui, Jeffrey Pimentel, Kahlin Cheung-Ong, Kristine M. McKinney, Caroline Köhler, Melissa J. Moore, and Trintha Chakraborty

Sabnis ... Benenato (2018) Mol Ther
Hassett ... Brito (2019) Mol Ther Nuc Acids
Jain ... Chakraborty (2018) Nuc Acid Ther
2019 Science Day Themes and Publications

• Why we incorporate modified nucleotides into our mRNAs

• Coding sequence design: Codon optimality vs RNA secondary structure

• 5'-UTR design

• The physical and computational methods we employ to understand LNP structure

• An early glimpse into our Immune LNP

Impact of mRNA chemistry and manufacturing process on innate immune activation

Jennifer Nelson<sup>1</sup>, Elizabeth W. Sorensen<sup>1</sup>, Shrutika Mintri<sup>1</sup>, Amy E. Rabideau, Wei Zheng, Gilles Besin<sup>2</sup>, Nikhil Khatwani<sup>3</sup>, Stephen V. Su<sup>4</sup>, Edward J. Miracco, William J. Issa, Stephen Hoge, Matthew G. Stanton<sup>4</sup>, John L. Joyal<sup>2</sup>

Nelson … Joyal (2020) Science Advances

mRNA structure regulates protein expression through changes in functional half-life

David M. Mauger<sup>5</sup>, E. Joseph Cabral<sup>6</sup>, Vladimir Presnyak<sup>6</sup>, Stephen V. Su<sup>4</sup>, David W. Reid<sup>5</sup>, Brooke Goodman<sup>6</sup>, Kristian Link<sup>7</sup>, Nikhil Khatwani<sup>3</sup>, John Reynolds<sup>8</sup>, Melissa J. Moore<sup>9</sup>, and Iain J. McFadyen<sup>4</sup>

*Mediixome Research, Moderna, Inc., Cambridge, MA 02139

Mauger … McFadyen (2019) PNAS

Human 5’ UTR design and variant effect prediction from a massively parallel translation assay

Paul J. Sample<sup>10</sup>, Ban Wang<sup>10</sup>, David W. Reid<sup>2</sup>, Vlad Presnyak<sup>6</sup>, Iain J. McFadyen<sup>8</sup>, David R. Morris<sup>7</sup> and Georg Seelig<sup>10</sup>++

Sample … Seelig (2019) Nature Biotech
mRNA platform science builds our foundation

Themes from today’s presentations

• How we are extending pharmacology

• T7 enzyme engineering to reduce dsRNA production

• New LNP’s for liver delivery

• Impact of LNP size on immunogenicity

• Progress towards creating a mRNA vaccine for HIV

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