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EDITED TRANSCRIPT

MRNA.OQ - Moderna Inc Virtual Science Day

EVENT DATE/TIME: JUNE 02, 2020 / 12:00PM GMT



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PRESENTATION

Operator

Good morning, and welcome to Moderna's Annual Science Day. (Operator Instructions) Please be advised that this call is being recorded.

At this time, I'd like to turn the call over to Lavina Talukdar, Head, Investor Relations at Moderna. Please proceed.

Lavina Talukdar - Moderna, Inc. - Head of IR

Thank you, operator. Good morning, everyone, and thank you for joining Moderna's Third Annual Science Day. Today's presentations will highlight advances in platform science and innovative vaccine research. We issued a press release this morning with an overview of today's topics. The press release and slides accompanying today's presentation can be accessed by going to the Investors section of our website.

Speaking on today's call are Stéphane Bancel, our Chief Scientific Officer -- excuse me, Chief Executive Officer; Stephen Hoge, our President; Melissa Moore, our Chief Scientific Officer, Platform Research; Moderna scientists and key opinion leaders in the HIV vaccination field.

Before we begin, please note that Science Day presentation will include forward-looking statements. Please see Slide 2 within the presentation and our SEC filings for important risk factors that could cause our actual performance and results to differ materially from those expressed or implied in these forward-looking statements. We undertake no obligation to update or revise the information provided on this call as a result of new information or future results or developments.

I will now turn the call over to Stéphane for introductory remarks.

Stéphane Bancel - Moderna, Inc. - CEO & Director

Thank you, Lavina. Thank you, Lavina. Good morning or good afternoon, everybody, and thank you so much for joining us this morning. The team and I are thrilled that you decided to spend the next few hours with us.

Today is Science Day 2020. It is the third Science Day we have hosted to give the chance to our investors and analysts to look under the hood. As you know, we started the company believing that mRNA is the software of life, and if we could find a way to create medicines out of mRNA, we could have a profound impact on patients.

On Slide 4. The most powerful aspect of our technology is that mRNA is a temporary set of instruction. mRNA is an information molecule. That is a total disruption versus small molecules used by the traditional pharmaceutical industry or large molecules used by the biotech industry. We use human DNA information as a raw material to create our therapeutics. We use viruses' genetic information of either the DNA or the mRNA, depending on what type of virus it is, to create our infectious disease vaccines. We use genetic information as a raw material, which we code in our mRNA molecules, which carry that information into human cells. That is why mRNA is such a transformation in the R&D process of making medicines.

On Slide 5. Software is based on information encoded in the binary system of 0s and 1s. Information in biology is encoded in a quaternary system of 4 nucleotide molecules. For mRNA, these 4 building blocks are AUCG. Messenger RNA is a class of software-like molecules in biology that convert the information stored as gene in DNA into the protein that cells need to operate. The ribosome, represented in orange on Slide 5, convert the information encoded in mRNA molecule into an amino acid protein chain.

Based on this powerful scientific framework, we believe mRNA has a potential to be a new class of medicines with 4 important value drivers: a large product opportunity; medicines with a higher probability of technical success from drug concept to launch because, one: MRNA is a platform; and two, we use information coded in human DNA or viruses' genetic information to encode our products; a greater speed of research and clinical development versus traditional medicine; better manufacturing capital efficiency and lower cost of goods than injectable recombinant.

On Slide 7. mRNA is an information molecule, so each component of that system creates a platform that can be used for all of our medicines. There are many attributes of the mRNA molecule we can choose and improve: chemistry, sequence engineering and so on. The manufacturing process, in green, which we use to make the mRNA molecules, impact the biological properties of our medicines. The delivery system has many attributes, amongst others: chemistry, composition, surface properties. The manufacturing process, in green, which we used to formulate our lipid nanoparticles, or LNPs, impact the biological properties of our medicine.

An important part of Moderna is a commitment we have as a company to science. Since the early days, that is what we believe as a management team. We're extremely committed to doing amazing science. We have believed and been guided since the beginning of the company in the power of the S curve. Every technology that men have worked on has always gone through an S-shaped learning curve. It usually takes around 20 years to go from bottom performance to plateau.

We believe in investing at scale. There's a critical mass of talent and capabilities to do this right. There's a critical mass of investments to be able to interrogate science in higher preclinical species like nonhuman primates. We believe in investing for the long term. Our scientific investments are, by nature, multiyear, which is why having a strong balance sheet with multiyear runway has been a critical part of our corporate strategy.

We believe in investing with the right team. We believe in the importance of a right scientific culture, of high-quality, broadness, collaboration, curiosity and relentlessness. We believe in investing in digital tools, including machine learning and AI. We believe this is a 10- to 20-year journey. We continue to invent and learn every day. We continue to launch faster than most and to file IP to protect our inventions. We use our scale and pace of learning to continue to lead the field. We are not aware of anybody else who can do this at this scale with this focus at this speed. Our lives are centered around mRNA science.

On Slide 9. As the company has grown through its development phase from a research-only company to a company focused on research, manufacturing and clinical development, to now a company focused on research, manufacturing, clinical development and commercial, we remain



as committed as on day 1 about investing in science, to continue to be the leading company in mRNA science to make transformative medicines for patients.

I had a chance to meet Stephen Hoge in 2012, and later that year, he accepted to join us in this journey. We were around 20 employees. I am thankful to have called him my partner for almost 8 years now. He is the president of the company and has been leading on mRNA platform research as well as therapeutic area discovery. Stephen will close the session today and take your questions with the team.

While a professor of biochemistry and molecular biology at the University of Massachusetts and a long-term investigator at the Howard Hughes Medical Institute, Melissa Moore joined Moderna Scientific Advisory Board to help guide our science. We had the chance to get to know her, and she got a chance to see our science by looking under the hood. We thought she will take our science to the next level. And Stephen, in a very Moderna-like relentlessness manner, convinced Melissa to leave academia as a full-time professor and Howard Hughes fellow to join a risky biotech company in 2016. We are thankful for her leadership and are delighted that she is leading our platform research as its Chief Scientific Officer. Since joining Moderna, Melissa has been elected at the National Academy of Science in 2017 and the American Academy of Art and Science in 2019.

With that, I will now hand over to Melissa.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

Thank you, Stéphane, and thanks, everyone, for joining us this morning. It's a pleasure to be here for our third Annual Science Day.

So to get us started, I thought that maybe it would be helpful to get everyone on the same page. And I'm often asked, what does it take to make an mRNA medicine? So I know this slide is a little busy, but it really shows every step in the process, and I want to take you through it.

So if we start up in the upper left-hand corner, it all starts with an idea, an idea to make a new therapeutic protein. If we -- once we decide to make a new protein, that, obviously, we know the amino acid sequence for that protein, and we need to then back translate or -- that sequence into the sequence of mRNA, because, of course, amino acids and proteins are in a different language, the language of amino acids from the language of nucleic acids. And so in step 2, once we've decided what protein we want to make, we feed that sequence into our proprietary computational algorithms that we've developed over the years. And that then gives us an mRNA sequence that would encode that protein.

Once we have the sequence, we then send that off to our manufacturing group who creates, going now down to the middle row, a circular DNA, known as a plasmid. This is created in bacteria and grown and purified to scale. That plasmid is then linearized, that's cut with an enzyme that just cuts in a single place. And that linear DNA is then mixed with enzymes and nucleotides that enable the synthesis of many, many copies of the mRNA.

Once the mRNA is purified, it is mixed with lipids to form lipid nanoparticles. These are then, in step 4, put into vials where they're filled, finished and quality controlled. And then ultimately, for our clinical applications, these are then -- they're administered to humans, where your own body then receives these instructions in the form of the mRNA and becomes the manufacturing center for the protein that we originally had the idea for.

Now one of the other things that I'm often asked is, what are these lipid nanoparticles? Are they artificial viruses really? What are they? The answer is they're not. In fact, what they are, are -- we're using lipids, lipids or fats, and we're using lipids to clothe our mRNAs and to make them look to the body very much like lipid transport complexes. Now many -- almost all of you have heard about LDL and HDL with regard to cholesterol. These are the lipid protein complexes that your body uses to transport fats around the body. And basically, all we're doing is we're making a lipid nanoparticle that, instead of containing proteins, contains our RNA. And the function of the lipids is to hide the RNA from digestive enzymes in biological fluids until the RNA can get to its final destination. The other function of the lipids is to tell the body where that particular lipid nanoparticle needs to go. And so we are creating lipid nanoparticles that look very much like lipid transport complexes.



Now let's go back to our first slide. I'm the Chief Scientific Officer of Platform Research. And so what does that mean? Where do we fit in? So platform research, what we -- our mission is to constantly be upgrading our platform and evolving it. And our platform, of course, is our knowledge and know-how of making the best RNAs and making newer and newer delivery vehicles.

And so what I've done here, in pink, the areas that platform research fits in are in helping the therapeutic areas determine what amino acid sequence they want to use for creating a new therapy, working on our computational algorithms for designing new message RNA sequences. We also, as you will see as part of today, work on our manufacturing enzymes and trying to optimize that. And then finally, we have a substantial investment in creating new delivery vehicles.

So today, as I said, is our third Science Day. I just want to go over a little -- remind those of you who joined us for our previous 2 Science Days and those of you who didn't, talk to you a little bit about what we reviewed on previous Science Days.

So in 2018, we covered 5 different main topics in our Science Day. One was development of our proprietary ionizable lipids for improved systemic mRNA delivery. Similarly, we talked about development of proprietary ionizable lipids to improve tolerability for our mRNA vaccines. We talked about using microRNA target sites in our mRNAs to create off switches, so the mRNAs would not be translated in certain cells where we didn't want them translated. We talked about translation initiation and leaky scanning of the ribosome, and then how we're using artificial intelligence to help us better design our coding sequences. And what you can see on the right is that there have been 3 peer-reviewed research articles that we have published to date based on the information that we told you at 2018 Science Day.

Now for those of you who are more familiar with scientific publishing, you'll know it's a long and arduous process to get things published. We fully intend to publish all of the science that we talk to you about on Science Day. And so some of those publications go faster than others. So we expect that the other topics that we talked about in 2018 will eventually be published in research articles.

Similarly, in 2019, we had another 5 topics, which were, why do we incorporate modified nucleotides into our RNAs? How we design our coding sequences, the effect of codon optimality versus RNA secondary structure. We talked about 5' UTR design. We talked about the physical and computational methods we employed to understand LNP structure, and we gave you an early glimpse into our immune LNP. And again, these -- many of these topics have already resulted in a number of peer-reviewed articles that I'm showing here on the right.

So the topics for today, we have 5 new topics. We are going to talk -- first, I'm going to talk to you about what controls the pharmacology of our mRNA drugs? In other words, how long do they last? And how we can enhance that pharmacology by increasing the half-lives of mRNA and proteins. Then Amy Rabideau is going to tell you about how we've engineered T7 RNA polymerase to reduce double-stranded RNA production. The third chapter will be given by Kerry Benenato, and she'll be talking about novel lipid nanoparticles for liver delivery. And chapter 4, we will have a talk by Kimberly Hassett, who will be telling you about the impact of LNP size on immunogenicity of our vaccines.

We'll then take a break and then come back and Andrea Carfi, who's the Head of Research for our infectious disease therapeutic area, will be welcoming 2 outside speakers to talk about our progress toward creating an mRNA vaccine for HIV. And then at the end of the day, we will -- Stephen Hoge will come back and sum everything up, and then we'll have a question-and-answer session.

So let's get to it. So first topic, what controls the duration of our pharmacological effects? If we think about small molecule drugs, it's quite well understood what controls the -- how long they last or their pharmacology. Basically, when you take the medicine, it is absorbed by the bloodstream, by the digestive system and the bloodstream and then that small molecule eventually reaches its site of action, which is usually interacting with a protein, and then produces its effect.

So the things that control the duration of the pharmacological effect are the pharmacokinetics. That's the kinetics with which the drug enters the body and is available, and then the pharmacodynamics which is dependent on the drug concentration at the target. And the two of those things together combined, at the bottom, to give you the effect duration. And so you can see very obviously, that the effects at the beginning comes up very rapidly and then over time goes slowly away.



Now mRNA drugs are different because there's many more steps in the process. So let's think about mRNA medicines. So with mRNA medicines, we are generally applying them by injection into, in this case, I'm showing into the bloodstream. The lipid nanoparticles then are in circulation. They eventually reach the liver. And what this -- the little cubical cell that I'm showing you there is a hepatocyte in your liver. The RNA is taken up by the liver cell and then released into the cytoplasm of that cell where it encounters the ribosomes. Those are the factories for making proteins. It's translated, and we get the proteins, which are ultimately our drug product. That is our -- the effect of our medicines is the synthesis of a protein with, and the activity of that protein.

So there are 3 basic things that control the duration of our pharmacological effect. One is the rate of the delivery, how quickly the lipid nanoparticles reach their target organ? That happens very quickly. And we're not going to be talking so much about that today, because in terms of the main rates, it's much faster than the other things I'm going to talk about.

The second thing is how quickly the mRNA is released and proteins are then translated from the RNA, and then, of course, the protein. But what is really key is how long these things last. So mRNA in your cells is meant to be a -- not a permanent molecule. It is meant to come and go because that's how your cells regulate gene expression. It's by turning on genes, making messenger RNAs and then they go away over time. And so things go off. So our MRAs only last a certain amount of time. Similarly, proteins also only last a certain amount of time, and they're eliminated or degraded. And so really, the -- what controls the pharmacological effect of our drugs is how long the mRNA and proteins last, their half-lives. So they interplay to modulate our pharmacological effects.

So let's look at, well, what is a typical half-life for an mRNA and protein? So in your body, so endogenous mRNAs, there have been a lot of published literature about how long these things last. So typically, at the top there, you'll see that the mRNAs have a median half-life, so $t_{1/2}$ means half-life. That means the amount of time it takes for half of the mRNA to disappear. The median half-life is about 5 hours. The range in half-lives is anywhere from just a few minutes up to 30 hours.

Now with proteins, it's a much different story. They're much larger dynamic range. Let's start with relaxin, which is a signaling molecule. We're going to talk about that a little later. It has a half-life in the blood of less than 10 minutes, very quickly degraded once it's made, because its job is to give a very brief signal in the body. Another protein would be phenylalanine hydroxylase -- we're going to, again, talk about that today. That is an intracellular enzyme. And it has a half-life somewhere between 8 and 48 hours. Then secreted immunoglobulins, those are the antibodies that give you immunity. They circulate anywhere from 10 to 21 days. So they're much longer half-lives. And then the most amazing protein in terms of half-life is the crystallin protein, which makes up the lens of your eye. And that -- actually, that protein lasts your entire lifespan. So this is why people get cataracts when the crystallin gets damaged because it cannot be easily replaced. So again, proteins can span from -- their half-lives, can span from just a few minutes to your entire lifetime.

So how have we been doing at Moderna in terms of what are the typical half-lives that to date we have been achieving? So what I'm showing here are published data from 2 preclinical studies, where we either gave mRNA to mice where -- that encoded the MUT protein, which is -- we are investigating to treat methylmalonic acidemia. So it encodes the methylmalonic CoA mutase protein. And you can see from our modeling of the half-lives that the mRNA in this case lasted about 5 hours and the protein lasted about 20 hours.

Now the red line shows the pharmacodynamics of our chikungunya monoclonal antibody in nonhuman primates. And here, that mRNA lasted for about 11 hours and the protein lasted for 26 days, so a much different protein.

So obviously, one of the things that we would really like to do for -- in particular, for treating rare diseases where we're having to give proteins -- or having to give mRNAs over and over again to encode proteins that would encode enzymes to treat the rare disease, we would like to extend the pharmacology of our drug products. And so let's look at how we might do this.

So here, I'm showing you 4 different graphs that model the duration of both our mRNA and proteins. And starting in the upper left-hand corner, I'm using the numbers that -- going back 1 slide, these numbers for the hMUT in mice, so 5 hours for the mRNA and 24 hours for the protein. And the dotted line that is going across each of the graphs toward the bottom there would be the level of protein that we need to get above to have a pharmacological effect. And so you can see that, in this case, in our modeling, with a half-life of the mRNA of 5 hours and a half-life of the protein for 24 hours, we would have a pharmacological effect of about 3 days.

Now if we could increase the half-life of the messenger RNA, so I'm -- by going across to the -- on the right-hand side, we're just now taking the messenger RNA to 10 hours, leaving the protein half-life the same. Now you can see that the protein is above the line for a pharmacological effect for over 6 days. So again, just that small change can make a big change in the duration of our pharmacological effect. We'll come back and talk about changing the half-life of the protein a little bit later.

So how can we increase the half-lives of our mRNAs? Well, last year, we -- one of the stories that we talked about and it's now published in this article in the Proceedings of the National Academy of Science is the relationship between codon optimality and secondary structure in the reading frame or in the coding region of our mRNAs. And what we showed is that, if we both give the highly optimal codon -- so that would be in the upper left-hand corner there, and if we maximize the secondary structure, which will be in the lower right-hand corner, that those 2 things combine to really increase the half-lives of our mRNA. So some of the increase in half-lives, we can do by sequence engineering.

So -- but today, I want to talk to you about a different means that we use to increase half-lives and that is by considering the decay pathways of our mRNA. So here, what I'm showing you is the -- an mRNA depicted as a rod. Now it's not really a rod-like molecule. This is just a cartoon that we use to -- because it's easy to look at. But what I'm showing you are the -- all of the enzymes, the protein enzymes that are involved in degrading mRNA, getting rid of it when it's no longer needed. And the very first step in that degradation pathway is the little Pacman on the right, the aquamarine Pacman, and that is an enzyme called a deadenylase. And its job is to start at the 3' end of the RNA and chew off those -- that -- what we call the poly (A) tail, the adenosines. So because this is the first step in the process -- excuse me. I need a drink of water there. Because this is the first step in the process, if we can slow this down or stop the deadenylase from acting, then we would expect that, that would stabilize the mRNA.

So let's take a look at the structure of that deadenylase. So here, I'm showing you a -- both a zoomed-out view of the crystal structure of the deadenylase from humans and the -- a more zoomed-in view of the poly (A) tail of those adenosines in the active site of the protein. So the active site of an enzyme is where the enzyme does the catalytic cleavage. And in this case, what the arrow is pointing to is the scissile bond. That is the bond that's going to get cleaved. And you can see that's a bond between the penultimate or next to the last adenosine and the last adenosine.

The other thing I want you to notice is that there's 2 metal ions close to that scissile bond. There's a manganese and there's a zinc. And these metal ions are crucial to the mechanism of this particular type of enzyme. Now in the right panels, what you can see at the top is the dotted lines are connecting the metal ion, the zinc, to the phosphate bond that is going to be cleaved. And so -- and then in the bottom-right corner is, after cleavage, you can see that those bonds have been broken. So what is particularly crucial for the deadenylase to be able to do its job is to position the phosphate perfectly next to those metal ions.

So what we have done to thwart the deadenylase is something very subtle. We have developed a method that allows us to -- instead of having an adenosine on the end of the RNA, as depicted in the left-hand panel, we have now an inverted deoxythymidine. So deoxythymidine is a nucleotide that's in your DNA. And so this is a perfectly natural nucleotide. We've incorporated this now at the end of the RNA and flipped it around. So instead of making the normal so-called 3' to 5' linkage, it makes a 3' to 3' linkage. So again, we're putting just one nucleotide on the end and just -- and putting it in an opposite orientation to what it would normally be there.

So what does this do in the active side of the enzyme? So what you can see here on the left is what I showed you before with our standard poly (A) coming into the active site of the enzyme. And now what I've done is I've put an oval, a green oval around those 2 metal ions, so you can see how that phosphate bond is positioned perfectly between the 2 metal ions. On the right-hand side is -- we have modeled in what it would look like with this deoxythymidine in the active site. And now you can see -- again, it's very subtle, but that phosphate bond is just not quite in the right position to be associated with the metal ions. And so therefore, this prevents the enzyme from cleaving that bond or at least slows it down dramatically.

So what does this do for our mRNA half-lives? Well, obviously, I wouldn't be telling you about this today if it didn't have a desirable effect. So here, what we're showing is 2 different mRNAs that encode green fluorescent protein or GFP. And so at the top, you can see that the 2 mRNAs differ only because one of them has this inverted dT or idT on the end. And we can use these mRNAs to put them into tissue culture cells, and I'm going to show you a movie here. And over time, we can see those tissue culture cells become green. And then the green goes away as the protein decays. And so we can convert those images that I just showed you, that movie into a digital form where we can measure the total green fluorescence over time. And so what you can see in the middle is that the RNA that only had the poly (A) tail, it had an mRNA half-life of about 5 hours. In this case,



the protein is much -- half-life is much longer, about 27 hours. But then when we just add that one little nucleotide to the end, now we've gone from a 5-hour half-life to a 12-hour half-life. So we've increased the half-life of the mRNA by twofold just with that one change.

Now that's in tissue culture cells. What about in animals? So another reporter system that we use for our in vivo studies is firefly luciferase. So this is the enzyme that makes fireflies blink in the night and they were able to make light. And we can use this. It's very commonly used to detect protein expression in animals. And the way that we do this is we deliver our mRNA. We allow the protein to get made. We then give the mice a substrate for the luciferase that allows them to make the light. And then we can follow with living mice over time how much light they're emitting.

And so here are pictures of -- again, these are living mice. These are the same mice that are pictured at 24 hours, 48 hours, 72 hours and 96 hours. And you can see when we don't protect the tail or we don't protect that -- we only have the 100 adenosines on the tail. We can see light coming out of the area of the liver in these mice at 24 hours, but really don't see anything after that. But now if we add that inverted dT to the end, very simple, now we can see light at 24 hours, at 48 hours, at 72 hours and even a little bit at 96 hours. And so in terms of the, how much protein we got expressed? We often talk about that as the area under the curve or the AUC. We got 4x as much protein expressed by making this one small change to the mRNA.

Another way we can do this is to measure the amount of light coming out of either the liver or the spleen, because in the body, the liver and the spleen are really next to each other. And you can see again here that the amount of light -- and this is at 48 hours, is substantially more, over 2 orders of magnitude more than with the idT added than without the idT.

Okay. So what about other cell types? Here's another reporter that we use. This is reporter protein. It's called OX40 ligand. OX40 ligand is a protein that is a membrane protein and is expressed on the outside of cells. So it allows us to access immune cells and query the immune cells for how much protein they are making on their surfaces. And we can separate different immune cells from the spleen by using other surface markers. So we can look at T cells, B cells, macrophages and also dendritic cells. And what you can see here -- I don't think I need to go through the data in very much detail. But in every case, when we have the idT added to the end of the RNA, we're getting much more protein on the surface. And this is at 72 hours post dose. So it's really quite a long time point.

Okay. So what about some protein that we might care about for a -- treating a disease? So what about phenylketonuria or PKU. So PKU is caused by a deficiency in phenylalanine hydroxylase or PAH enzyme. And patients who lack this enzyme fail to process the amino acid fat phenylalanine. And as a result, phenylalanine can build up to toxic levels. And so one of the programs that we're investigating is to supply the mRNA that encodes PAH or phenylalanine hydroxylase so that we can -- those patients can now process the phenylalanine and reduce the toxic levels.

So we have a mouse model for phenylketonuria where the mouse is lacking the PAH protein. And what you can see here is if we look at the phenylalanine levels of these mice, so in gray, you can see the gray line at the top, you can see that the mice have very high levels of phenylalanine if we do nothing. The gray bar at the bottom would be the range at which would be therapeutically efficacious. Now if we give the -- an mRNA that just -- that encodes phenylalanine hydroxylase but does not protect it on its tail, we do get some effect, but it only lasts for a day or 2. And if we simply add that one idT to the end of the tail, now we're able to extend the pharmacological effect out to 3 days. So again, can make a big difference.

All right. So we've been talking now for quite a bit about how we can extend mRNA half-life. What about extending protein half-life? So here, going down from top to bottom, what we're modeling is what happens if we, instead of extending the mRNA half-life, we extend the protein half-life by twofold from 24 hours to 48 hours. How do we do that? So there are multiple drivers of in vivo protein half-life. And the rules for what causes proteins to be degraded or eliminated are different depending on whether the protein is secreted or intracellular. So in both cases, proteins can be degraded by enzymatic degradation. But for secreted proteins that are going off into the bloodstream, they're either ultimately cleared through the kidneys, they are cleared by the immune system, and there is this process called FcRn recycling that can affect their -- how long they stay in the bloodstream.

For intracellular proteins, it's a different -- completely different situation. They are degraded by a process called ubiquitination, and they can also be degraded by a process called autophagy, which means self-eating. I'm not going to get into that today. But I do want to talk about how we use knowledge of the different rules for RNA degradation or -- excuse me, for protein elimination to design different modified enzymes or modified proteins to increase their half-lives.



So the first one I want to talk about is this protein relaxin. We talked about this at the very beginning. It has a very short half-life. And it is known to be involved with cardiovascular remodeling. So one of the concepts that we've been investigating over the years is to administer an mRNA-encoding relaxin peptide that has a longer serum half-life to address heart failure.

What I'm showing here is the structure of relaxin. It starts out as a long, what is called a propeptide, which is then cleaved, and ultimately, you end up with 2 chains, the alpha chain and beta chain. And this has been made as a biologic called serelaxin that can be infused into the body over time. And you can see here, this -- what I'm showing is data from a paper in the Journal of Clinical Pharmacology from 2015. And what you can see is as long as the infusion is taking place, the serelaxin serum concentrations are quite high. But as soon as that infusion stops, they drop precipitously. And I want you to note that the y-axis is a log scale, so every tick mark is a tenfold drop in the concentration of the serelaxin.

So methods to increase the serum half-life of secreted proteins have been very well worked out by the biologics community because, of course, all biologics are proteins that need to work in the bloodstream. And so it's very well-known, all of the different pathways that are being used by the body to eventually eliminate proteins and then how we can slow down those pathways. So we're not making up anything new here. We can use the rules that are already known.

And for relaxin, we've tried 2 different methods. One is to put in a fusion domain to engage with these FcRn recycling pathway. We also can think about increasingly just the hydrodynamic radius to slow the renal clearance. And so 2 ways that we did this: on the left, we added a linker that puts then -- that linker becomes attached to a lot of sugar chains or glycosylations to increase the hydrodynamic radius. And then on the right, we fused relaxin to an albumin binding domain to help with that recycling reaction. And as you can see here, this is a preclinical study in mice with the wild type protein. And here, we're supplying the mRNA, not the protein. We're supplying the mRNAs that encode these proteins. And you can see that with the wild type protein, we get an initial burst of protein that rapidly goes away. Again, note that the y-axis is a log scale. The protein that has the glyco linker, that has the branched sugars on it, has a longer half-life. But then when we put the albumin binding domain on the protein, much longer half-life. So it's -- the engineering principles for how we can engineer secreted proteins are very well-known, and they work in our hands when we are making those proteins from mRNA.

So let's go back now and think about intracellular proteins. So intracellular proteins are subject to degradation by 2 main pathways. One is it has to do with how well they're folded or unfolded. And so as you may know, protein molecules are long polymers of amino acids, and they fold up into these complex 3-dimensional structures. But they also can unfold and aggregate. And that unfolding rate is often a function of how stable to thermal denaturation they are. And so if you can move the curve in the left-hand side, if you can move that curve over to the right, you can make your protein more stable and so, therefore, less prone to degradation.

The other main pathway for degrading intracellular proteins is ubiquitin-mediated protein degradation. And for ubiquitin-mediated protein degradation, what happens is that the protein becomes attached -- so many molecules of another very small protein called ubiquitin become attached to the protein, and those ubiquitin tails are a signal that, that protein needs to go in the trash can. The trash can, in this case, is the proteasome, which I'm showing you there on the right. And the proteasome literally is a -- on the inside of that structure, it -- are degradation machines for proteins. And so they're literally like a decomposer or the disposal in your sink. They just chew up the protein. And so the ubiquitins are attached to the protein by another series of enzymes that we are showing as the E1, E2 and E3. But the important thing to know here is that the place that the ubiquitin chains are attached is on the outside of the protein to lysine residues. So lysines are an amino acid that are particular sites where the ubiquitin can be attached.

So if we go back to -- I'm sorry. So in order to do the protein engineering then to extend intracellular half-life, we have the objectives of both stabilizing the protein and decreasing the rate of ubiquitination. Now the things that we need to consider when doing that, of course, is that we need to not mess with the structure of the protein, but we can use evolution what -- the lessons that evolution has taught us to be able to do this. And the technique that we generally use for engineering proteins at Moderna is our virtuous cycle of analyze the protein, think about what mutations we might be able to make, design those mutations, make the mutations, screen those for activity, and then find the answer and then do it all again until we finally arrive at the mutant form of the protein that we -- is best for us.

So in thinking about phenylalanine hydroxylase or PAH, what we need to know is we need to be able to understand what amino acids are amenable to be changed. Now the thing about phenylalanine hydroxylase, it is a central metabolic enzyme. And so for those of you who took biochemistry



in college, you will have remembered seeing this metabolic chart here that I have on the left. And this is all of metabolism. And I know some of you are probably tortured by this. I used to teach biochemistry, so I used to torture my students by making them memorize various parts of this. But what you can see is that phenylalanine hydroxylase occupies a central position up there in the upper right-hand corner. And because it is a metabolic enzyme that is central to all metabolism, all different animals, plants, bacteria, all living organisms on planet earth have a PAH enzyme. And so we have -- we can really learn a lot from evolution.

Now one of the things that has been happening over the last 25 years is the tremendous increase in genetic information. And so now we have thousands and thousands and thousands of different genomes that have been sequenced from all different kinds of organisms. So one of the things that we can do is take the sequence of the human PAH enzyme, and that's shown there on the left, and that's the sequence of amino acids. And we can feed that sequence into a search algorithm called BLAST, and this is at NCBI, which is part of NIH. And what BLAST does is allow us to -- it lines up the sequence that we put in, the human PAH at the bottom, and you can see, at the bottom, it's comparing that to a bacterial PAH. Now that bacterial PAH actually has very different amino acid sequence because of the distance in evolution between humans and bacteria. But on the right-hand side, what you can see is we've overlaid the crystal structures of human PAH with bacterial PAH. And you can see, at the structural level, they're almost identical.

So what we can learn from this is, what amino acids or what parts of the sequence are important for the enzyme to fold up and do its job versus what are things that are just specific to different organisms? And so what we're showing here in the middle is thousands and thousands of these sequences that we have lined up using this BLAST algorithm. And so as we move over to the right-hand side, you'll see that the human sequences at the top, this is just a small part of the human sequence. And now we're comparing it to things that are very closely related. So if you look down through that list, you'll see that it's all primates or monkeys. So organisms that are very closely related to us. And if you look at the colors going up and down, you'll see that they are very, very similar. But as you go further down in the alignment on the left, you'll start to see gaps and different colors. And so that's as you're getting into more of the bacterial sequences.

So the way that we then mine this information, we did, first a BLAST search with the human PAH protein. That gave us almost 5,000 different sequences. Now a lot of those sequences had big gaps in them or they were incomplete. So we removed those "gappy" sequences to get down to a smaller number. And then some of the -- as I said, some of the sequences are incomplete. We removed those to get down to 2,700. And then it also turns out that phenylalanine hydroxylase is evolutionarily related to a couple of other enzymes that work on amino acids, tyrosine hydroxylase and tryptophan hydroxylase. So we needed to make sure that we weren't comparing to those. So we got rid of all those and eventually got down to 690 different sequences. And each one of these sequences is from a different organism.

So when we align these 670 (sic) [690] sequences -- and now I've changed the color scale to emphasize what's the same and different. When we align them, what you can see is that in green are all of the amino acids that are identical between different species; and in red are the amino acids that are different. And what I want you to focus on, if you go into the middle part of the slide at the bottom, you'll see the numbers 140 through 180. At position 180, what you can see is there's a red T, and above that is a green M. So the M, which stands for methionine, is what is the amino acid in the human sequence. But in some of our closest relatives, this amino acid is actually a threonine. We're going to come back to that. But that's telling us that we can change that amino acid from methionine in the human sequence to a threonine in -- because our close relatives clearly have functional PAH enzymes. And so that mutation must not affect function.

So by doing this kind of analysis over and over again and looking through all these sequences, we were able to identify many different amino acid residues on the -- in PAH that we expected to stabilize the folding of PAH. We also were able to identify a number of many different sites on PAH that would be potential sites of ubiquitination. And so we could then mutate these and see what the effect is.

Now you've already seen this slide. I showed it to you when we talked about extending the mRNA half-life by adding an idT to the end of the RNA. But now what I'm going to do is show you what happens when we combine that with some of these mutant proteins as well.

So the 2 mutants that I'm going to show you are: first, on the right, that methionine 180 to threonine 180, this was actually predicted to -- by that making that change, it's predicted to stabilize the folded state. And then on the left, there is a K, which stands for lysine, at position 150. We've changed that to a threonine, and this removes a potential ubiquitination site. And so when we combine these 2 amino acid changes with the idT tail on the mRNA, we get an additive effect. So now instead of being in the therapeutic range for 3 days, we're now in the therapeutically efficacious

range for 4 days. So of course, you might be asking, well, what if you make both mutations in the protein? And that's something that we're investigating now to try to get out further.

So in conclusion to this part, what I want hopefully to have convinced you and you have learned is that the duration of our pharmacological effect for mRNA medicines is a function of both mRNA and protein half-life. And that by understanding the basic biology governing these half-life rules, we can use those to engineer the desired pharmacology because it gives us multiple levers to push. And then as with other -- our other engineering principles that we've talked about in previous years, effects are additive. So in other words, if we increase both the mRNA half-life and the protein half-life, then we get additive effects.

Okay. So that's the end of that story. And next, I want to turn to our synthesis of mRNA. So going back to our slide that I showed you at the beginning about, where does Platform Research fit in, what I've been talking about for the last 30 minutes or so has been the pink area at the top where, how do we design our new proteins and then how do we create mRNA sequences.

What I'd like to turn to next is our efforts to come up with better ways to actually make our mRNA. And so that's the little pink square there that says enzymes and nucleotides. Now to understand this, I need to tell you a little bit more about how our synthesis process works. So the way that we synthesize our mRNAs, as I said, we are -- we start with a circular DNA plasma that we linearize and add enzymes to. The enzyme that we add is T7 RNA polymerase. And we add T7 RNA polymerase in -- plus the various nucleotides, A, C, G, and it should say 1-methyl pseudo U there. As we've told you in previous years, and you can look in our publications, we do a complete substitution of U in our RNAs with 1-methyl pseudo U to decrease the innate immune response to our RNAs. So when we add the polymerase and those 4 nucleotides, the polymerase -- I'm sorry, I missed a step there. The polymerase transcribes or copies the DNA into RNA, and it can do this hundreds of times and make many copies of the full-length mRNA.

But just like any other manufacturing process, there are some undesirable side products that are made. One of them is a series of short RNAs that the polymerase -- when it starts doing transcription, it sometimes stutters before it goes into full elongation mode and so produces these short RNAs. And these are not particularly troublesome, but what is troublesome is that both these short RNAs and the full-length RNA can also be substrates for the polymerase and it can transcribe the opposite strand to create double-stranded RNA. And these come in 2 flavors: these smaller double-stranded RNAs; and then what we call loop-back double-stranded RNAs, where the polymerase is using the full-length RNA and creating even longer molecules of double-stranded region.

So why are these double-stranded RNAs problematic? The reason they're problematic is that they -- double-stranded RNA will trigger innate immune responses. And so when an mRNA medicine or a lipid nanoparticle is taken up by cells, cells have in them many ways to detect double-stranded RNA. And the reason that cells -- that we do this, our cells do this naturally is because many viruses are RNA viruses. And in order for RNA viruses to copy themselves or to replicate, they have to, at some point during their life cycle, create double-stranded RNA. And so we -- our bodies have evolved very strong defense mechanisms to recognize double-stranded RNA and say, "Hey, something's wrong here. I'm being affected by a virus. Let's do something." And so if we're creating RNA medicines, particularly in the case where we are going to be chronically treating a, let's say, a rare disease, we need to not have this response happen.

And so there are 2 main types of innate immune triggers that recognize double-stranded RNA. One are the Toll-like receptors that are in the endosomes, and the other are the RIG-I and MDA-5 proteins that are in the cytoplasm. And these different mechanisms or triggers can have different ultimate downstream biological effects. One is to -- one effect is to increase the production of IFN-beta and IFN-alpha, so the interferon responses; and the other is to create pro-inflammatory cytokines, so proteins that tell the immune system that's a danger signal. And so it's really important, again, that we are able to eliminate or to minimize double-stranded RNA in our -- particularly in our therapeutic mRNA applications.

So how have other people dealt with this? Here's a paper, a foundational paper from 2011, showing really what has become the standard in the field. And the standard is to -- after you do the transcription reaction is to use HPLC, so high-performance liquid chromatography, to purify the full-length RNA away from these double-stranded impurities. And so these are -- what I'm showing here are figures from this paper. And in the middle is the trace coming off of the HPLC. We use absorbents at 260 nanometers to detect RNA because RNA absorbs at that wavelength. And what you see in the middle, in fraction II, is a big peak. That's the full-length RNA. And then if you go over to the right, and we're looking at here IFN-alpha response in an immune assay, you can see that when the RNA was transcribed with uracil, there was a big response. But when it was

transcribed with pseudo U or 5-methyl-C and pseudo U, there's absolutely no immune response. This is exactly the reason why we put -- modify nucleotides into our RNA, to decrease that immune response. Because even when you get rid of the double-stranded RNA, if your RNA only has Us in it, you have an immune response. But fractions I and III, which contain either the smaller RNAs or the loop-back RNAs, there is an immune response regardless of whether you have a modified nucleotide or not.

So the problem though with doing HPLC purification, and we do HPLC purification in our manufacturing facility, is that it is very costly and it is hard to scale. And so we have asked ourselves, well, what if, instead of trying to purify away the mRNA, we can engineer a polymerase that does not make this RNA. But before I tell you about that, I also have to tell you about what we have been doing at Moderna prior to this engineering effort. So at Moderna -- and this is something that I told you about last year, was that we can minimize the amount of double-stranded RNA that's made by the wild type polymerase by changing the process conditions. So in other words, the transcription reaction conditions, we can really minimize the amount of double-stranded RNA that's made. And the way that we detect double-stranded RNA in our transcription reactions is through using this ELISA assay that I'm showing you here. An ELISA assay is a sandwich assay where you've got 2 different antibodies that recognize the same molecule, in this case, double-stranded RNA. And then if you -- if the sandwich is made, you get a chemical reaction that can be detected. And this can be done in a high throughput manner and is highly quantitative.

And so what you can see in the graph at the bottom is that, using a legacy process for making RNA, we get very high levels of double-stranded RNA. But with the Moderna process, and again, this is with the wild-type T7 enzyme, we are able to minimize that RNA.

But as I said on the last slide, what we were particularly interested in is instead of trying to always purify away this double-stranded RNA, what if we could just not have it made in the first place. And so the next chapter is about engineering T7 RNA polymerase to further reduce double-stranded RNA formation.

And to tell you about this, is Amy Rabideau, who is a principal scientist in our Process Development department. So Amy, take it away.

Amy Rabideau;Principal Scientist

Great. Thank you, Melissa. So as Melissa has already shown, the transcription reaction using T7 RNA polymerase produces full-length RNA, along with several impurities, including double-stranded RNA. Today, I'm going to tell you how we engineered T7 to reduce double-stranded RNA formation in our transcription reaction. But first, I need to tell you about the assays that we use to detect double-stranded RNA so that you can understand how we did this.

One assay that we use is the double-stranded RNA ELISA that Melissa just previously mentioned. This is very sensitive and high throughput, which allows us to quantify the amount of double-stranded RNA present in an RNA sample. Another way to detect RNA impurity is to radiolabel the RNA transcript produced in a transcription reaction and run them through a polyacrylamide gel, which separates the RNA based on size. So here, what we've done is use a DNA template that makes a very short RNA product. This short RNA product has a number of keys, which are radiolabeled, or in blue in the schematic above. This labeling allows us to detect both full-length RNAs as well as short abortive, like the one shown below the full-length transcript image in the schematic. As you can see in this gel, wild-type enzymes makes full-length RNA from the top strand, as indicated by the dark band circled in blue at the top of the gel. Also visible are the short, abortive RNA below the full length band as well as larger species above the full length band at the top of the gel.

In order to detect the opposite or bottom strand that is only present in double-stranded RNA, we labeled the Cs rather than Gs in the transcription reaction because G base pairs with C. Now you can see these short double-stranded RNAs below the full-length RNA band, as indicated in red, as well as the longer species, which are the loop-back, double-stranded RNAs.

The last assays I want to tell you about are our cell-based assays. For these -- our -- we use fibroblasts, which secrete IFN-beta in response to double-stranded RNA, or human peripheral blood mononuclear cells or PBMCs, that have been differentiated into macrophages. Macrophages are professional immune cells that upregulate IP-10 mRNA in response to very low levels of double-stranded RNA. So collectively, we have an array of different assays to help guide our protein engineering effort.



Next, I need to tell you about how T7 RNA polymerase work. So to start, here's a picture or crystal structure of wild-type T7. Initially, it encounters the double-stranded DNA template in a sequence called a promoter region. The promoter is a unique sequence that tells the enzyme where to start transcription.

Here's a picture of T7 bound to the promoter. You can see the DNA here, which is colored in orange. Once bound to the DNA, T7 separates the 2 strands of DNA to create a bubble. In order for transcription to begin, the incoming RNA nucleotides must base pair with the DNA, which is the purpose of the bubble.

The next thing that needs to happen is that 2 molecules of GTP bind in the active site. And these are going to be the first 2 nucleotides transcribed by the preliminary and the first 2 nucleotides of the RNA transcript. These GTP nucleotides are then joined together, creating a very short RNA, as indicated in pink. After the initiation event, the polymerase starts incorporating more and more nucleotides. And as the RNA transcript grows, the polymerase must change shape in order to accommodate the growing molecule.

Once the RNA is long enough to fully emerge from T7's exit channel, the enzyme enters into its final confirmation, the elongation state. In this state, the enzyme can rapidly transcribe full-length RNA. Once it reaches the end of the DNA, it falls off, releasing the full-length transcript and then starts the process all over again. So if you were watching closely, you may have noticed that the protein was changing confirmation throughout the different steps of the transcription cycle.

Here's a picture of all the states that I showed you side by side, with the initiation complex on the far left and the elongation complex on the far right. Now hopefully, you can see that the enzyme has to go through several structural rearrangements in order to make an RNA transcript. Since the elongation state is the state that makes full-length RNA, we hypothesized that if we could somehow stabilize that state by making judicious mutation, perhaps that would decrease the enzyme's propensity to make undesirable side products, such as double-stranded RNA.

In order to engineer T7 RNA polymerase, we took our tried-and-true Moderna protein engineering approach, which is first to identify what position might be amenable to mutation while still preserving function. We then designed those mutations, made the protein and tested them in our assays. Then we iterate this process to ultimately come up with our final reengineered enzyme.

On the right, I'm showing you again the picture of T7 with the positions that we chose to mutate highlighted and sphered. About half of the mutations are highlighted in light blue, and these are expected to affect the confirmation state of the enzyme because they occur in regions that change most drastically going from initiation to elongation. The remaining mutations are shown in dark blue. These are in and around the active site and could affect the chemistry of polymerization. Once we made and purified our desired T7 mutant, we first assessed their ability to make RNA. The ones that weren't able to make RNA obviously weren't further evaluated.

Now in order to determine how much double-stranded RNA our mutants made, we needed to use conditions where the wild type enzyme makes a lot of double-stranded RNA. So just as a reminder, the condition that we use is the legacy process, shown in light gray on this slide, not our optimized Moderna process.

Once we screen for the mutants that were active and made RNA under the legacy condition, we next ran our analytical tests for double-stranded RNA. For this, we used our ELISA and our cell-based fibroblast assay. As you can see in these bar graphs, over the course of our engineering efforts, we were able to identify an enzyme, indicated in red, that produced extremely low amounts of double-stranded RNA as was tested by the ELISA. This same RNA sample also generated almost no innate immune response in the fibroblast assay. We dubbed this reengineered enzyme Moderna T7.

Next, we want to look at the impurity population generated by T7 -- by Moderna T7 in more detail. The question we asked here was, in creating Moderna T7, how did we change the impurity population of the RNA product we generate? So we went back to our gel-based assays that I discussed before. The lanes on the left are what I showed you before with the wild-type enzyme. Here on the right is our Moderna T7 sample. When we labeled the Gs in the top strand, the population of short RNA is similar in abundance and distribution between wild-type and Moderna T7 RNA samples. In other words, Moderna T7 still makes short abortive RNAs. However, as you can see at the top of the gel, Moderna T7 produces very little of the longer transcripts. Where we really saw a difference was when we labeled the Cs in the RNA, which detects the bottom strand present only in

double-stranded RNA. Remarkably, we observed no small or double-stranded RNA nor any loopback impurities. Thus Moderna T7 made no double-stranded RNA by this assay.

So if we're making very little double-stranded RNA, do we still need to use HPLC purification with Moderna T7? For this evaluation, we again used our sensitive double-stranded RNA ELISA assay. Under the legacy transcription commission, wild-type T7 makes quite a bit of double-stranded RNA, as seen in the light gray bar. HPLC purification is capable of reducing that level of double-stranded RNA, but is still not able to fully eliminate it as seen in the dark gray bar. However, the mRNA sample made with Moderna T7, in red, has even less double-stranded RNA without HPLC purification. We observed a similar trend with our very sensitive macrophage assay. RNA samples produced by wild-type T7 elicit a very high IP-10 cytokine response. And this response is reduced somewhat by HPLC purification. Now note the break in the scale of the y axis. The RNA sample made with Moderna T7, even without HPLC purification, produces an immune response fairly over the background levels observed for the media-only control.

Finally, we formulated the mRNA, injected them into mice intravenously and analyzed IP-10 protein levels in the blood. As you can see, the wild-type enzyme without HPLC purification, you get very high levels of IP-10 protein. HPLC purification brings that down. Moderna T7, however, brings those levels completely down to baseline, defined by the PBS control. Taken together, these results indicate that we have created an enzyme that produces little to no immunostimulatory impurity.

So in conclusion, I've shown you that at Moderna, we now have 2 ways to reduce double-stranded RNA. The first is through process optimization using wild-type T7. And the second is through protein engineering, which yielded Moderna T7. These strategies make it possible for us to produce therapeutic mRNA which we want to have low immunostimulatory content to allow for repeat dosing in a highly efficient manner.

So with that, thank you for listening. And I'll turn it back to Melissa.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

Thank you, Kim (sic) [Amy].

Okay. So now we have talked about so far the engineering of proteins and mRNAs. We've talked about how we are engineering our process enzymes and our process for making the RNA. And next, I'd like to, for the last 2 chapters, turn to our lipid nanoparticles and our discovery efforts there.

Now for our lipid nanoparticles, we use, at Moderna, a number of different routes of administration to target different tissues. And for these different routes of administration, we need different delivery vehicles that are optimized for each route of administration. And in order to find these optimized delivery vehicles, we have taken a rational structure based design approach that really starts, again, with the understanding of every aspect of the molecules and the structures that we're designing.

And so starting on the left, our lipid nanoparticles generally contain 5 components. One is, of course, the mRNA. Then there are 4 different kinds of lipids, the ionizable lipid, and the ionizable lipid will be the -- very much the topic of the next talk. We use cholesterol, another phospholipid and then a PEG lipid. The purpose of the PEG lipid is to give physical stability to the lipid nanoparticles when they're stored in the vial.

Now we can change the structure of our lipid nanoparticles by changing the chemistry or the exact chemical nature of the lipids by the composition, meaning what -- in what fraction or ratio do we mix the various lipids together, and also, the process by which we make our LNPs. And then we are very -- we measure every possible thing that we can. So we think about chemical stability, physical stability in the vial, and then, of course, biodistribution, cellular uptake, endosomal escape of the mRNA and protein expression. And our typical approach at Moderna is that we really try to understand the basic principles that operate at all levels and then engineer based on that knowledge.

So what the next chapter in our Science Day today is going to be, how we have engineered new LNPs for liver delivery. And to tell you about this is Kerry Benenato, who leads our Platform Chemistry team. Kerry.

Kerry Benenato;Senior Director

Thank you, Melissa.

Thank you, Melissa. So as Melissa referred to, at Moderna, we have invested heavily in the development of a lipid nanoparticle platform for the delivery of our mRNA. And over the course of these studies, what we've identified is the critical role the ionizable lipid plays in both the structure and function of our particles.

And so today, what I'd like to tell you about is how we have further developed our understanding of the design principles for ionizable lipids specifically suited for mRNA and how we've been able to use this information to develop a new series of ionizable lipids specifically for mRNA.

So before we begin, I thought we would start by just defining what is an ionizable lipid and why are they so important for lipid based delivery of oligonucleotides. So if you go back about 50 years, it was first identified that you could encapsulate biomolecules in lipid based systems. These lipid based systems were made up of a mixture of neutral lipids, and it was first very successfully applied to proteins. And here, in the bottom corner of the slide, just showing an early electron micrograph of one of these early lipid based particles. However, this new technology, they found when they try to apply it to oligonucleotide like DNA, just was not successful. And so in thinking about why and how to solve this, we turn to the structure of DNA. Now we're all very familiar with the famous double helix structure of DNA. And now this structure is held together by 3 very important molecular interactions and molecular bonds, and I'd like to take some time to speak about those as they're very important for the story that I'm going to tell.

To start, the 2 complementary DNA strands are held together by Watson Crick base pairs. And now Watson Crick base pairs, what unites these are hydrogen bonds. And what a hydrogen bond is a positive electrostatic interaction between a hydrogen on one electro-negative atom, such as a nitrogen, an oxygen or a sulfur. And it interacts with another electro-negative atom. So very simply put, you can consider what a hydrogen bond is, is it's 2 electro negative atoms sharing a hydrogen.

Another very important stabilizing bond that stabilizes DNA is pi-stacking. And what pi-stacking is, is a favorable interaction of 2 aromatic -- planar aromatic systems. In DNA, the aromatic systems are the nucleobases. And so here, again, very simply put, you can consider what pi-stacking is, is it's the sandwiching of these aromatic systems provide stabilization to the molecules. And now what the combination of hydrogen bonds and pi-stacking does is it forces these Watson Crick base pairs, these nucleobases to be on the interior of the double helix, and on the exterior is your phosphate backbone. Now your phosphate backbone is made up of negatively charged phosphates. And so to stabilize these negative charges, they require a counter ion or a positive charge. Here in this figure, are representing the positive charge as a metal atom, for example, a sodium atom. And what this stabilizing charge-charge interaction is called, these are ionic bonds. So all together, the hydrogen bonds, the pi-stacking and the ionic bonds all work together to stabilize this double helix structure. And so what this tells us is that in order -- if we want to encapsulate DNA and bind lipids to DNA, we're going to require a positive charge. And so positively charged atoms are referred to as cations. And so how do we introduce a cation into a lipid? Well, one way you can do that is you can readily convert tertiary amines or nitrogen containing compounds into cations. Now amines, in their most stable state, only make 3 bonds to other atoms. However, you can introduce a fourth substituent causing the nitrogen to be positively charged in a cation. Now it is important to note that this is not -- this is an irreversible process. So once you form that those cations are stable and they cannot be reconverted back to the tertiary amine.

So now with these cationic lipids, they were able to now form and encapsulate DNA very efficiently. And this is because the positively charged cation performed as stabilizing ionic bonds with the negatively charged phosphate. Now these new particles were very efficient for cell-based experiments, so in vitro experiments outside of the body. However, they found that they were not viable for systemic administration. And the reason is, as I had mentioned, the formation of a cation is an irreversible process, so these are permanently positively charged molecules. And typically, when you form these lipid-based systems, it is done with a large excess of the cationic lipid relative to the DNA cargo. So ultimately what you end up with is a positively charged particle. And now these positively charged particles, when delivered systemically, were seen as foreign by the immune system and readily cleared. And so the big step forward was, when it was recognized, you could replace the cationic lipid with ionizable lipids. And so what does it mean to be ionized? Well, an ionizable amino lipid is a tertiary amine, which at neutral pH 7.4, so physiological pH, is neutral or uncharged. However, if you introduce this molecule into an acidic environment, that molecule becomes protonated or positively charged. Now in this case, this is a reversible process. So when that positively charged molecule is reintroduced into neutral pH or physiological pH, it is no longer charged. And so it was found that you could efficiently encapsulate DNA with ionizable lipids at low pH. So when the nitrogen was protonated



or positively charged, it could very efficiently form those stabilizing ionic interactions with the DNA and forming very stable particles. However, because this is -- this ionization process is reversible, ultimately, when these particles were delivered systemically, these are now neutral particles. And so as Melissa introduced in the introduction, these now very much look like our natural lipoprotein particles. And so these particles are very well tolerated with -- under systemic administration and really enabled delivery of oligonucleotides.

Now over the past 5.5 years at Moderna, we have been heavily invested in the discovery and development of novel components, specifically for delivery of our mRNA. In 2018, we published a peer review research article highlighting the discovery of one of our lead amino lipid series. What we're able to show is that with these lead new amino lipids, they were very potent. They were rapidly cleared. And we could repeat dose without any loss of potency. Now this new series of ionizable lipids is the basis of our clinical formulations, and we are very excited to see that they are showing to be safe and effective in humans.

However, as Stéphane referred to is that we are never satisfied and we are never done at Moderna. And so really what we thought about is how, as excited and continue to be about this new series of ionizable lipids, how could we make them better? How could we improve our systemic administration formulation? And so to do that, we went to the structure and examined the structure of the molecule. Now this new class of molecules is unique relative to anything that had been reported previously for DNA and siRNA because of this ethanolamine head group. Now the head group has the requisite ionizable amine, which is required in order to make that form a stabilizing ionic interaction with the oligonucleotides. However, it also has this alcohol functionality, and this alcohol functionality has the potential to engage in hydrogen bonds.

And so considering this, as a medicinal chemist, the criticality of this alcohol was something that was always in the back of my mind. Very early on, our structural activity relationships told us that the ethanolamine was superior relative to anything else we had looked at, and then we focused much of our optimization on the lipid tails and did not revisit the head group. We knew that if we remove that alcohol and only had an ionizable amine or if we replace the alcohol with a second ionizable amine, these compounds were much less effective than the ethanolamine. And so this was indicating that potentially, for mRNA, only interacting with the mRNA via ionic bonds was not enough. And so if we consider the structure of mRNA, so mRNA, similar to si and DNA, has significant double-stranded regions, where you have your Watson Crick base pairs being stabilized by the hydrogen bonds and pi-stacking and the negatively charged phosphate on the exterior. But what mRNA has is it also has significant single-stranded regions. So now not only are the negatively charged phosphates available for interacting with the ionizable lipids, but also the bases.

And so we asked ourselves, could it be that the criticality of this ethanolamine for the efficiencies of delivery of our mRNA is because we are engaging in hydrogen bonds with the mRNA. And so to interrogate this, we turned to computational chemistry. And what computational chemistry is relative to synthetic chemistry, so a synthetic chemist like myself, I can use my knowledge of chemistry and laboratory skills to design synthetic routes and ultimately produce the desired molecules. Now what a computational chemist can do is they can use their knowledge of chemistry and their knowledge of coding and different computational methods to interrogate and ask detailed questions about our molecules. Now in our case, the system we're dealing with is very mobile. We have these mobile amino lipids, and we want to understand how they're interacting with the mRNA. So to do this, we turn to molecular dynamic simulations.

Now what molecular dynamic simulations, what we're looking at here is we have a large excess of our ionizable amino lipid -- a lipid in combination with a short RNA strand. And now these simulations are run at low pH, so we can assume that the ionizable amine is protonated.

Now as I play this simulation, I'd like you to focus your eyes on the 2 areas that I've circled under -- that are -- within these blue circles. And basically, what we're seeing is we're seeing that the lipid, there's a lot of mobility, and the lipids are in [CC] interacting with the mRNA via hydrogen bonds, but they seem to be transient. They do not seem to be that stable.

And so as we zoom in on different snapshots of these simulations, what we see is, in fact, that ethanolamine is engaging in hydrogen bonds with the mRNA. In this example, you have a hydrogen bond between the alcohol of the ethanolamine and the phosphate, the oxygen and the phosphate. And here, you can see a hydrogen bond between the alcohol and one of the nucleobases. And so we wondered, as the simulations indicated, these are forming, but they do not seem to be that stable. So could we design compounds that would improve the robustness of these interactions with the RNA? And if we were able to do that, would that afford a more active particle? And so we initiated a discovery effort aimed at doing this. And what we're trying to do is design new compounds which potentially would be able to mimic some of the interactions you see, for example, in the Watson Crick base pairs. So the process we used to do this is we would design -- generate our hypothesis and design a series of molecules. These



molecules are synthesized in our labs, and then each is formulated into its own lipid nanoparticle. We tested these particles in vivo. And based on the levels of protein output that we observed with the different particles, we're able to use that information to go back to the design board and then further develop our structure activity relationship.

So one of the first series of compounds which we looked at was really to challenge ourselves, is that hydrogen critical for activity? And so what we did is we synthesized a series of compounds where we removed the hydrogen. However, we maintained the electro negative atom. I had forgotten to mention back when we were discussing hydrogen bonds that, in those bonds, the hydrogen is referred to as the donor atom and the electro negative atom it's interacting with is referred to as the acceptor atom. So these molecules maintain the acceptor, but there's no donor atom. And as we can see, relative to our original control, we saw significantly less levels of protein expression.

Next, another series of compounds which we looked at, was we asked ourselves, well, if we're trying to mimic the interactions that you have in Watson Crick base pairs, what happens if we actually introduce nucleobases into our ionizable lipids? However, as you can see, this was not a viable option as these were very low potency.

And so next, we turn to series of compounds or we introduced functionality where we reintroduced hydrogen bond donors and additional hydrogen bond acceptors. And so the functionality we looked to is well-known in organic chemistry and in small molecule drug development, and these functionality are known to engage in very strong hydrogen bonds with themselves as well as with proteins. And now with this series of compounds, we're now observing levels of protein relative to our control. And so to us, this showed us that potentially we were on -- we we're definitely on the right track, and so we were able to use these first hits to further develop the structure activity relationship.

Now this whole process went through many, many iterations. Over the course, we were able to synthesize molecules, and we were able to learn what chemical modifications improves potency as well as what chemical modifications diminish potency. And all together, we're able to combine this information and ultimately identify 1 compound that was significantly more potent than every other lipid nanoparticle we had tested. And what this new ionizable lipid is, it is a squaramide ionizable lipid. Now squaramides, as an organic chemist, squaramides are just super cool structures. They are 4-membered carbo cycles substituted by 2 amines. Like our ethanolamine, the squaramide ionizable lipids have the requisite ionizable amine. But as opposed to only having 1 hydrogen bond donor, the squaramides have 2 hydrogen bond donors. And in addition to that, they have 4 hydrogen bond acceptors. Another really unique property of squaramides is that because of their pi character and their planar nature, squaramides are actually aromatic. And so it is known in the literature that squaramides are able to pi-stack with each other as well as with other aromatic systems.

And so with this new molecule I identified, we turned back to our molecular dynamic simulation to see, in fact, does this molecule have stronger interactions with the mRNA relative to our ethanol amine? And now what we're seeing in this simulation is that the lipids are -- there's a lot of mobility, but as the lipids come into contact with the mRNA, they're really not moving away. They're not -- it doesn't seem to be seeing those transient interactions which you saw with the ethanolamine. Now zooming in and looking at the same simulation, now what you can see is that these squaramides seem to be forming hydrogen bonds with the mRNA and these hydrogen bonds seem to be stable. They don't seem to be moving away like what we saw with the ethanolamine. And so zooming in, we do, in fact, see a number of examples where the squaramides are hydrogen bonding with the mRNA. However, as opposed to the ethanolamine, which is only able to make 1 hydrogen bond because it only has 1 donor and acceptor atom, the squaramide, because of the multiple donors and acceptors, we see many examples within these simulations of the squaramide making multiple bonds to the mRNA and really causing a nice stabilization effect.

For example, in this right side of the slide, what you can see is the squaramide oxygens are interacting with the proton in one of the nucleobases. And the 2 donors, 1 donor is interacting with another nucleobase and the donor atom here is interacting with a phosphate backbone.

Now I'd mentioned that something that's really cool about squaramides is that they're aromatic. And in fact, we saw a number of examples of the squaramides engaging in pi-stacking interactions. Pi-stacking interactions with themselves as well as with the nucleobases. And you can see how the squaramides are able to sandwich themselves in between the nucleobases, offering a lot of stabilization to these particles.

And so now the question is with these -- now what we think are much more robust in these -- ionizable lipids are much more strongly balanced than mRNA, does that enable a higher -- a more active and potent lipid nanoparticle? And so after optimization of the formulation, we found that,



indeed, the squaramide lipid nanoparticle does afford higher protein expression relative to our original ethanolamine. Now in these studies, we used -- employed human erythropoietin mRNA. And for our discovery efforts, this is just a really nice tool mRNA for us because it's readily detected in circulation and we have very robust assays.

Now what was nice to see, what we observed in mouse also translated to primate. And so relative to our ethanolamine, we saw really nice and robust levels of expression of human EPO after 1 -- 0.1 mg per kg dose in IV infusion and really nice sustained expression. Now for mRNA therapeutics, it's been mentioned a number of times before, for a number of our therapeutics, we were going to be -- need to be able to dose chronically. And so it was really nice to see that with these new squaramide lipid nanoparticles, that nice sustained expression we observed in primate after a single dose was also observed in a multi-dose experiment.

Now another really important aspect of our lipid nanoparticles is a number of therapies that we'd like to target are hepatocyte specific therapies. So therefore, they require us to deliver the lipid nanoparticles in the mRNA selectively into the liver hepatocytes.

And so to interrogate how well the squaramide lipid nanoparticles performed. We delivered 2-milligram per kilogram dose of our squaramide lipid nanoparticles with an mRNA encoding for an intracellular protein. And now we're able to use immunohistochemistry to stain for that protein and see localization of protein expression. One thing to note, with this intracellular tool recorder, it has a nuclear localization sequence. And what that means is that the mRNA, once it -- when it produces the protein in the cytosol, that protein is directed into the nucleus. And this really enables just an increased sensitivity of the readout because what you see is a darkened nucleus in any cell that has made protein. So relative to an untreated control, you can see we've seen very high levels of protein expression and very consistent across the entire tissue. As you zoom in, you can see that we're transfecting almost every hepatocyte. And based on the darkness of the nuclei, we have very, very high levels of protein in each cell.

And so this is really exciting to see. And so then we asked ourselves, with these properties, with a sufficient high level of delivery in hepatocyte selective, could these lipid nanoparticles be employed in a therapeutic setting and for hepatocyte-specific disease? And one of those such diseases is glycogen storage disease type 1A, or GSD1a. Now what this is, this is -- these patients have a deficiency in glucose 6 phosphatase. And without this enzyme, they are unable to metabolize glucose 6 phosphate into glucose, resulting in low blood glucose levels. And these patients often suffer hypoglycemia during fasting. Now current standard of care involves rigorous dietary measures, including frequent feeding in food supplements. And only curative of measure is liver and kidney transplants. And so we asked ourselves if we could deliver an mRNA encoding for this enzyme, could we be able to rebound and improve the blood glucose levels?

And so to study this, we looked into an animal model which is lacking this enzyme. And as you can see, comparing the blue and the gray line at the beginning of the experiment before receiving because the mRNA, you can see the differences in blood glucose levels. However, after just a single dose of the mRNA, you see rapid increase in the blood glucose levels, above the therapeutic threshold. And now we were able to maintain those glucose levels up to 9 days, which then, upon a subsequent dose -- dose, we were able to, again, rebound the blood glucose levels. And so for this, this is a really exciting example for us to see that, yes, that these new novel squaramide ionizable amino lipids can be applied for new therapeutics.

And so while we are very excited and still are excited about our ethanolamine ionizable amino lipids, which are showing to be safe and effective in the clinic, we've been able to use different methods and computational methods to further understand how these molecules interact with mRNA and then improve upon it. And now we have this new series of ionizable lipid based on these squaramides have very robust interactions with the mRNA.

And so as excited as we are about this new series, as Stéphane has mentioned, we are not done, and we will continue to innovate and try to further develop our lipid nanoparticle technology.

And so at this point, I'd like to turn it back over to Melissa. Thanks.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

Thank you, Kerry, for that story.

So now we're coming to the last part of the Platform Research presentation. And what Kerry was talking to you about was the -- our efforts to engineer new ionizable lipids for -- specifically for mRNA. But as I said at the beginning of this section, we also very much care about thinking about all of the biophysical properties of our lipid nanoparticles and how they might affect their therapeutic efficacy.

And so for the last chapter of our presentation today from Platform Research, we want to talk about the impact of LNP's size on vaccine immunogenicity. And to tell this story, we would like to invite Kimberly Hassett, who is a senior scientist in our Formulation Science department. Kim?

Kimberly Hassett; Senior Scientist

Thank you, Melissa, and good morning, everyone.

I'm excited to talk to you today about the work that we've been doing on how LNP's size impacts vaccine immunogenicity.

In the last talk, Kerry did a really great job explaining the importance of ionizable lipid in our delivery system. When designing LNPs to deliver mRNA, the ionizable lipid is central to improving potency. Last year, we published a paper describing our screening efforts to identify an optimal ionizable lipid for intramuscular delivery of our mRNA vaccine. In this paper, we showed that our vaccine ionizable lipid is capable of enhancing local expression and local tolerability. But the ionizable lipid is not the only factor that can impact vaccine potency.

Let's take a minute to think about how mRNA vaccines generate an immune response. Initially, our vaccine LNPs are injected intramuscularly. Shortly after administration, immune cells are recruited to the injection site. Then the LNP itself or antigen presenting cells, a.k.a. APCs, that have taken up the LNP, travel to the draining lymph nodes. At both the injection site and in the draining lymph nodes, APCs take off the LNP and express the antigen of interest. Once the antigen is expressed, an immune response is created in a similar fashion to a natural viral infection. The big difference, of course, is that we're only supplying mRNAs that encode viral structural protein. Because we don't include the rest of the viral genome, there is no possibility of infection.

As you've already seen in the previous talks, our approach at Moderna is to evaluate every possible attribute that affects potency and then engineer each variable to achieve an optimal response. Given that our LNPs travel through vessels in the lymphatic system upon intermuscular administration and they interact with various cell types along the way to generating a robust immune response, we thought that LNP size could play a role in mRNA vaccine potency.

On this slide, there is a small sampling of published papers illustrating that particle size assess how particles interact with cells and affects the immunogenicity for a wide range of nanomaterial. In fact, particle size can impact particle distribution within the body, including drainage in the lymphatic system, cellular uptake in key immune cells, and the magnitude and quality of the immune responses generated for other types of vaccines. Despite the wealth of literature, there are currently no published papers to evaluate how particle size influences the effectiveness of mRNA vaccines.

Why does nanoparticle size matter? Well, if we look at the size of immunogens, we see that they span a very large size range, from few nanometers for soluble antigens, all the way up to thousands of nanometers for bacteria. The immune system has evolved to keep very large things like bacteria out of the lymphatic system, but allows smaller things like viruses to drain into the lymphatics and get taken off by APCs.

If we now overlay the size range of typical LNPs on this plot, we see that they fit well within the range of particles that are able to enter lymph vessels and be taken up by antigen presenting cells. But there is a range from approximately 10 to 200 nanometers, where we have both efficient entry into lymph vessels and efficient uptake by APC. So with that in mind, we set off to determine whether LNPs within this size range all work equally well or if there is an optimal size. Since we have been working to optimize our vaccine platform over several years, we had accumulated a wealth of biophysical characterization data that we could link back to LNP performance. To address the particle size question, we undertook a retrospective analysis of 23 cytomegalovirus, CMV immunogenicity studies, spanning 129 different LNP formulations. The only fixed variables for the retrospective analysis were the animal model, dosing schedule, including the mRNA dose level administered, and the LNP construct in the LNP.



On these graphs, you will notice different color data points that represent unique formulations. Different colors may have different lipids with the composition, process variables or ratios of ionizable lipid to mRNA. For this data set, we dosed mice intramuscularly twice 3 weeks apart with the same amount of mRNA. Three weeks after the prime and 2 weeks after the boost, blood withdrawn and analyzed for antibody titers against the CMV pentamer. Each symbol on the graph represents the geometric mean titer for 1 group of animals. On the left graph, after the prime dose, antibody titers tended to be higher with larger particle sizes. When we look at the response after the boost, on the right, you'll notice a similar trend. As sizes less -- as sizes less than 80 nanometers, the antibody titer was highly variable. But at LNP sizes larger than 100 nanometers, the antibody titer leveled off. Even though this retrospective analysis suggests a strong effect of particle size on immunogenicity, I want to remind you that this analysis contains multiple variables. To really truly assess the impact of size alone, we needed to find ways to change the size of our LNPs without changing their composition.

So let's talk for a minute about how LNPs are made. To produce LNPs, we mix lipids in an organic stream with mRNA in an aqueous stream. Once these streams are mixed, the materials self-assemble into LNPs. From there, ethanol is removed through buffer exchange. After buffer exchange, we can further manipulate the resultant LNPs with a secondary processing step if desired. Over the past several years through process development, we have learned that particle size can be modulated in various steps during the process. For example, if we increase Mixing Variable 1, particle size, as measured by dynamic light scattering, increases. Alternatively, if we increased Mixing Variable 2, particle size decreases. If we change Mixing Variable 2 in conjunction with the buffer exchange variable, the particle size increases further. Lastly, we can modulate particle size by adding a secondary processing variable. These methods enabled us to produce a wide range of LNP sizes while keeping the lipid composition content.

Using the methods I just showed you, we were able to create a large set of LNPs ranging in size from 50 to 200 nanometers in diameter. We next evaluated the immune titers generated by the particles of different sizes. The color coding of these symbols here matches the process set that was modulated. For example, yellow dot show immunogenicity when a mixing variable was changed. Orange dot for a buffer exchange variable and red for a secondary processing variable. We used the same dosing schedule as the retrospective analysis, but here, each dot represents the response of an individual animal. Just like the retrospective analysis, we again observed an effective LNP size. When particle size was less than 80 nanometers, antibody titers tended to be lower and were more variable. We determined an optimal particle size to be around 100 nanometers. This study really showed us that the difference in immunogenicity seen in the retrospective analysis was primarily due to size and not due to other secondary variables. When we overlay the retrospective analysis with this study, we can see the trend is consistent.

Next, we wondered whether these findings were also true in primates. For this, we chose to simply prepare 4 formulations containing a small, medium, large and extra-large particle. The upper left panel shows dynamic light scattering results for all 4 samples. Dynamic light scattering measures the average particle size based on the intensity of scattered light. Larger particles will scatter more light, where smaller particles will scatter less. For this technique, we measured the LNP diameters at 64, 81, 108 and 146 nanometers. For all samples, the Polydispersity Index was below 0.2, indicating a monodispersed particle population. We also used an alternate method to evaluate particle size, follow nanoparticle tracking analysis, as shown in the upper right panel. Nanoparticle tracking follows individual particles and then calculates particle size based on the rate of Brownian motion. Similar to dynamic light scattering, the nanoparticle tracking analysis revealed a gradual shift from smaller to larger particles. You'll notice that the height of the peaks decrease in particle count as we increase particle size. For each formulation, we used the same input amount of mRNA and lipid. So as particle size increases, there are simply fewer particles for a given input amount of mRNA and each particle contains more and more mRNA molecule. This is even easier to see in the false colored cryo-electron microscopy images shown at the bottom, where the extra-large particles are more than twice the size of the small particles.

When we tested these 4 well-characterized samples in mice, we again observed a strong effective LNP size on immunogenicity, with the medium, large and extra-large particle sizes yielding a statistically greater amount of antibody titers than the small LNP. In nonhuman primates, however, that impact was not as dramatic. Although there was a slight upward trend in antibody titers with increasing particle size, no size was statistically significant from another.

So to conclude, I showed you that we can control LNP particle size without changing lipid composition through modulation of formulation process parameters. In mice, LNP sizes below 80-nanometers yield significantly reduced immunogenicity, whereas LNP sizes above 100 nanometers consistently generate high antibody titers independent of particle size. Nonhuman primates, however, appear to be more tolerant of LNP particle size changes. Based on this analysis, we are confident that the current size range of Moderna's mRNA vaccine LNPs is appropriate for optimal immune responses in primates.



Thank you for listening today.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

All right. Thank you, Kim.

So that ends the portion of the Science Day that's being brought to you by Platform Research.

And as you can see, given the agenda, we've gotten a little bit behind. So we're going to take a 5-minute break before we start up again with our -- Andrea Carfi talking about our progress toward making an HIV vaccine. So be back at 10:05. Thank you.

(Break)

Andrea Carfi; Head of Research, Infectious Disease

Hello. Good morning, everyone. This is Andrea Carfi. I'm head of research for infectious disease at Moderna. In this section, we will highlight our research on HIV vaccine with our collaborators at the Bill & Melinda Gates Foundation, BMGF; International AIDS Vaccine Initiative, IAVI; and the National Institute of Allergy and Infectious Diseases, NIAID.

In a couple of minutes, you will hear from 2 leading scientists and key opinion leaders in the HIV vaccine research space who are using novel approaches in developing HIV vaccine that leverage on the opportunities offered by the mRNA technology.

But before then, I want just to give you a quick overview of our vaccine platform and the key characteristics of the mRNA technology that make it well suited for these exciting novel vaccine approaches and to tackle complex and unsolved vaccine problems, such as developing an effective HIV vaccine.

Starting with the ability to make vaccines against a highly complex antigen, as demonstrated by our human cytomegalovirus vaccine candidate. Our CMV vaccine, mRNA-1647, is composed of 6 messenger RNAs and encoded for 2 viral antigens. One of those antigens is the CMV pentamer complex, which is composed of 5 distinct proteins. These proteins are made in the cell and come together to form a properly assembled and functional protein complex that is presented to the immune system, and this is what mRNA-1647 is achieving. The ability to make vaccines against complex antigens like CMV is one of the features of the mRNA vaccine platform that hasn't been simple to achieve with recombinant protein technology.

mRNA also allows for a combination vaccine. An example of such combination is our pediatric vaccine against 3 different respiratory viruses: hMPV, PIV3 and RSV. Together, these viruses cause over 3 million hospitalizations in the pediatric setting and are a major unmet medical need as there aren't any licensed vaccines to address these 3 infections. Having 1 single vaccine against all 3 of these viruses in 1 vial is possible with our mRNA technology and would have a great impact on preventing these respiratory diseases in a fragile population such as the infants.

Now turning to the mechanism of action of our mRNA vaccines. In brief, our vaccines, like many other vaccines, are administered as an injection in the shoulder muscle. The vaccine from there drains into the nearby lymph node, where antigen presents itself, pick up the mRNA, encapsulate it in the LNP, in the lipid nanoparticle, insulate the mRNA into viral protein. These are recognized as foreign proteins by cells like B cells and T cells. So engagement of B and T cells result in a robust immune response against the virus. So generation of antibody from B cells and also cell-mediated responses with activated CD8 plus T-cell response. Then so the mRNA this way has the ability to engage both sides of the adaptive immunity, B and T cells, increasing the probability of success.

Our mRNA vaccine platform has also demonstrated accelerated research and development time line with a shortened time to the clinic and potentially to market. And a clear example of this is the mRNA-1273, our vaccine against the SARS-CoV-2, where it took only 63 days to go from antigen design and sequence, actually, to first-in-human clinical trials.

So by last Friday, we had announced the start of a Phase II clinical study, and we expect to enter a Phase III efficacy study in July. This ability to manufacture quickly using processes that are independent of the specific vaccine being manufactured is part of the story behind the accelerated time line to clinic and potentially to the market.

Another feature of our mRNA vaccines platform is greater capital efficiency over time and compared to recombinant technology, let's say. We use the same input, and by using the same input, meaning the nucleotide composed mRNA and the LNP, and the same manufacturing processes for all our vaccine programs allows for higher capital efficiency, lower capital intensity, greater flexibility and, as I mentioned, speed.

So these attributes of our vaccine platform and Moderna commitment to public health led us to exciting collaboration with our long-standing partners at the Bill & Melinda Gates Foundation and IAVI to develop HIV vaccine.

So the HIV remains an unsolved problem, and HIV vaccines are really a big medical need. There are still more than 2 million infections every year occurring. And in the last 30 years, only 6 vaccines have made it to efficacy trials and have all failed.

So the collaborations aim to apply Moderna mRNA technology to accelerate a vaccine -- HIV vaccine discovery and to address a complex biological vaccine problem by rapid production and clinical testing on novel vaccine candidates.

As our speakers will show, our platform and streamlined manufacturing capabilities allows for rapid iterative cycles of design and testing, enabling novel vaccination strategies that weren't possible before. In addition, the mRNA technology expands the antigen design space, enabling the generational vaccine candidates that cannot be manufactured easily otherwise.

So it's a pleasure for me to introduce the 2 speakers today that will take you through these novel approaches.

So the first speaker will be Dr. Bill Schief, who's Professor of Immunology and Microbiology at the Scripps Research Institute and Executive Director of Vaccine Design, IAVI. Bill is also an associate member of the Ragon Institute of MGH, MIT and Harvard. And here, we see that he has a Bachelor's Degree in Applied Mathematics from Yale University and a Ph. D. in Physics from the University of Washington.

His focus, the focus of his work is on computation-guided and structure-based design of immunogens with the ultimate goal of inducing broadly neutralizing antibodies against HIV and other pathogens that have frustrated traditional vaccine design strategy.

After Bill, you will hear from Dr. Paolo Lusso, who is the Chief of Viral Pathogenesis at the Laboratory of Immunoregulation at the NIAID. Paolo received his M.D. from the University of Torino and then his Ph. D. from University of Bologna. He is board certified in internal medicine and infectious disease. After a few years in the U.S. for training, then he moved from '94 to 2007 to Milan, where he was a -- where he directed the AIDS research laboratory at the San Raffaele Institute (sic) [San Raffaele Scientific Institute] and then returned to the U.S. to assume his current position in the NIAID. Paolo is an elected member of the European Molecular Biology Organization, EMBO, and also Fellow of the American Academy of Microbiology.

And his research focuses on the mechanism of HIV/AIDS pathogenesis and the development of an HIV vaccine. I would like to highlight that in 1995, the discovery on the HIV-suppressive [control with] chemokines was nominated Breakthrough of the Year by the Science magazine.

So it's really my pleasure and an honor to have both Bill and Paolo to present today these exciting collaborations. And with that, I will turn it over to Bill.

William Schief;IAVI;Executive Director of Vaccine Design

Well, Andrea, thanks. This is Bill Schief. I'm really happy to be here and talk to the listeners about our work and our collaboration with Moderna on trying to make an HIV vaccine. I'm just going to try to be quick to help keep up.

So in the time where we're facing the SARS-CoV-2 epidemic is changing everyone's lives, it's important to remember there are a lot of other health problems that we still have to deal with, and it's incredible how many there are, and HIV/AIDS is one of them. We're still -- as Andrea mentioned,



there's nearly 2 million people getting infected every day. Still, there are 37 million people living with HIV. And even though in the United States and in the Western, developed world, we have antiretroviral treatment that makes HIV infection largely a manageable disease and people don't die from it, 40% of the people infected currently are not receiving ART due to various economic and social factors. And so those people are -- their survival is unlikely. And this problem is not going away. There's 1 million people dying every year due to HIV. And there are a variety of different strategies to try to solve this problem, but I think most people agree that the most sustainable solution and long-term sustainable solution would be if we could make a vaccine. So we need a vaccine.

So you've heard a lot about the SARS-2 spike protein that is a part of the Moderna vaccine and a part of many other -- the Moderna-NIH vaccine and a part of many other vaccine candidates. HIV has a very similar spike, as shown in the bottom of this graph. There's 2 of them shown, and they're glycosylated -- large glycosylated proteins -- trimeric proteins actually, 2 different trimers joined together, heterotrimeric proteins and HIV, in order to inject its DNA material into a human target cell and infect the cell, the spike binds to a receptor protein called CD4, on human CD4 T cells, and starts undergoing conformational changes and then binds also to another cell surface receptor called CCR5 or CXCR4. And after engaging those 2 proteins, it -- the conformational changes in the spike cause the membrane of the virion to fuse with the membrane of the target cell, and the RNA gets injected into the target cell, and off to the races, that cell now becomes a factory to make new HIV particles. So that's HIV entry, and that's what a vaccine needs to prevent.

And so now the slide is showing you neutralizing antibodies, a schematic of what they might look like. And neutralizing antibodies will bind to the spike and prevent it from infecting a target cell. So -- sorry. So most licensed vaccines induce neutralizing antibodies certainly against viral -- against viruses.

And the big challenge for HIV and one of the reasons that it's so very different and more difficult than making a flu vaccine or making a SARS-CoV-2 vaccine is that the spike protein is -- the surface of it is incredibly variable from 1 virus to the other. In fact, HIV is not really 1 virus. It's really like 100 million different viruses that are infecting different people and changing within each person that's infected all the time. And so in order to prevent all of those different viruses from infecting their target cells, we need to elicit antibodies that are called broadly neutralizing antibodies. That means they can bind to many, many different spikes, many different forms of the HIV spike and prevent all the different virion strains from infecting target cells.

And to give a schematic for how kind of big that challenge is, this is a diagram representing the diversity -- the sequence diversity of the HIV envelope spike on the far right. And these are different viruses, HIV viruses from -- recovered from 1 year in 1 country in Africa. And in the middle are 9 different HIV viruses recovered from 1 infected person. And on the left is representing the sequence diversity of about 100 different influenza viruses recovered from different people. And you know how difficult it is to make a flu vaccine that works year in and year out. As you can see, we can't even make a vaccine that protects against the diversity that's shown on the left panel. And what we're saying is we need to make a vaccine that protects actually against the diversity that's shown on the far right panel. It gives you an idea of the scale of the challenge.

And this is just showing you -- this movie is showing you a model for the HIV spike. It's rotating around and the blue structures are glycans that are added by the human cells. So they look a lot like cells, and they don't elicit antibodies very well. And we've colored the protein in this spike yellow and red. Red is where the surface is highly variable and yellow's where it's not perfectly conserved. And I just wanted to show you how difficult it is. And you can imagine, if you want to make a vaccine and you're going to use the spike protein, you've got to try to elicit antibodies that don't bind -- they shouldn't bind to the red regions because antibodies binding to the red regions will -- they may neutralize the virus that shares the envelope in your vaccine, but they're unlikely to neutralize other viruses or, at least, it's very difficult to have them do that. And even antibodies binding the yellow regions will be challenged to be broadly neutralizing.

But through the work of a lot of different investigators in the field, broadly neutralizing antibodies have been discovered from infected individuals. A small percent of infected individuals make broadly neutralizing antibodies. And this slide shows that the field has characterized different antibodies that bind to different locations on that spike. These are electron microscopy -- representations of electron microscopy data showing where different antibodies can bind to that same spike that was just rotating around. And these broadly neutralizing antibodies do neutralize diverse HIV isolates, some of them up to 99% of all isolates. And many of them had been tested in nonhuman primate challenge experiments, and they can provide sterilizing immunity. If an antibody is present in an NHP before viral challenge, if there's enough of the antibody present, it can completely block

any HIV infection. And so that is kind of the proof of principle that if we could design a vaccine that would elicit broadly neutralizing antibodies, and on the slide, we're calling them bNABs. If we could elicit bNABs, we could -- in theory, we could prevent HIV infection in humans.

And this idea of looking at antibodies from infected individuals and then using them as guides for how to design a vaccine is generically referred to as reverse vaccinology or reverse vaccinology 2.0 originally suggested by my colleague, Dennis Burton, at Scripps. And it's depicted on this slide. Basically, it's what we've just said. You find infected individuals and you characterize their antibody response and you find the rare people that make very potent and broadly neutralizing antibodies, then you characterize those antibodies and their interaction with the spike protein and use that information to try to design a vaccine. And the idea is then you would give that vaccine and you would elicit broadly neutralizing antibodies in people that have never been infected with HIV. And because the vaccine elicits broadly neutralizing antibodies, if they ever got exposed to the virus, they would be protected. That's the idea.

And so to make an HIV vaccine, our goal really is to develop a vaccine that elicits sustained protective levels of broadly neutralizing antibodies in humans.

And just a quick one sidenote. We do think -- we do hypothesize that an effective vaccine will need to consistently induce broadly neutralizing antibodies against 2 to 3 different sites on the spike in order to provide the coverage that we need against the huge diversity of global isolates. So I'm showing you this cartoon of the spike of antibodies down to multiple different places, and we think we need to elicit antibodies down to 2 or 3 different places.

So how is that going to happen? When your immune system responds to a pathogen or a vaccine, generally speaking, the vaccine or pathogen interacts first with naive B cells, and they enter a germinal center and gain somatic mutations and gain affinity, and ultimately through -- after a long process, they turn into plasma cells that secrete antibodies. And we need to do the same thing here to induce broadly neutralizing antibodies against HIV. It's just more difficult than normal because, as shown on this slide, only a very small fraction of human naive B cells can serve as starting points to end up -- to develop into broadly -- into B cells that will secrete broadly neutralizing antibodies. They're rare. They're rather diverse so it's not so easy to just target one kind of them because they have different -- they have various properties.

And finally, they're difficult to activate with HIV proteins. So normally, if you would deliver a native wild-type HIV spike, you'll turn on B cells, but the data in the field would suggest that you're unlikely to turn on the B cells that can start -- that can initiate a bNAB response. So it's hard to get the process started but also HIV broadly neutralizing antibodies are typically very highly mutated, much more mutated than antibodies against -- that are needed against other viruses. For example, there have been numerous papers that are coming out on antibodies that are potentially neutralizing against SARS-CoV-2. And many of those antibodies have 1% mutation away from the naive germline recombined state, whereas HIV antibodies are often 20% or 30% mutated. So it's really challenging to create a vaccine regimen that will, on the one hand, initiate, find and activate the right starting cells and then furthermore elicit all of the somatic hypermutation that's needed to produce bNABs.

And the strategy that we're pursuing -- sorry -- is called germline-targeting vaccine design, and that comes from the first priming step that I'm showing you here. And the idea is, as I mentioned, the cells that you need to trigger to start the process of inducing a bNAB are very rare. And as I mentioned, they don't bind very well to native HIV proteins. So we believe and we hypothesize that you've got to engineer a custom immunogen that can find the rare B cells and turn them on. And the job of that, we call it germline-targeting prime because those rare B cells have T cell receptors that are combinations of a DNA that's present in your germline. The idea is that this priming immunogen will find the right B cells and turn them on and kick them a little bit in the right direction, elicit a little bit of the necessary somatic hypermutation, as shown on the slide, and produce a pool of memory B cells.

And then we believe that we have to design a series of boost immunogens, we call them shepherding immunogens, that will interact with the memory B cells and get them to go back into a germinal center and elicit more somatic hypermutation and kick them further in the right direction. And we may need to do that several times. And then finally, we'll need the last immunogen, we call it a polishing immunogen, that will try to do the job of converting memory B cells into plasma cells that are long-lived and secrete high levels of broadly neutralizing antibodies. And so this sort of complex sequential vaccine design is -- has never really been done before. But in theory, it should be doable. And it's sort of like what happens, we believe, in natural infection. So that's the idea.

And we've got a lot of data to suggest that this is feasible and that we can ultimately do this. We and others first suggested this idea in 2013 in this paper. And I just showed -- I'm just showing one data panel from this paper. So the immunogen, as shown on the cover of Science, was a nanoparticle presenting 60 copies of an engineered antigen and, as shown in this image, interacting with B cell receptors on the surface of a naive B cell, kind of in the process of doing the job that it's supposed to be doing.

And the one data panel that I'm showing is the B cell activation data where we expose B cells, we expose these germline B cells, the starter B cells that you would need to trigger to start the process. We expose those B cells to our immunogen, and we see that it activates as in the red line. And if we expose those B cells to the same immunogen, the same nanoparticle in the same format, but it lacks the ability -- it doesn't have the right mutations, it doesn't have the affinity for the target B cells, those same nanoparticles then don't activate the B cells at all, as shown on the blue line at the bottom. And we think that this is kind of what's happened in the HIV clinical trials that have happened so far. They used native HIV proteins and they probably haven't triggered any of the right B cells they needed to trigger.

And we extended these -- the principles and sort of the practice of this technology in a paper that just came out last fall, where we showed how to extend this and generalize this to targeting kind of more general classes of precursors for broadly neutralizing antibodies. Our original method was a bit restricted to certain kinds of broadly neutralizing antibodies. But now we've shown how to try to do this -- design these immunogens for general antibodies that we think is applicable to making an HIV vaccine because we think we need to induce 2 or 3 different kinds of broadly neutralizing antibodies, but also, we think this technology is much more applicable to other pathogens now after this paper.

And I've shown you that the idea is this sort of complex sequential vaccination scheme that hasn't been done in a human before, but we have shown proof of principle that this kind of thing can work in a mouse model. And this was in work with -- in collaboration with Michel Nussenzweig's lab back in 2016. In my lab, we designed a sequence of immunogens following this general strategy, and Michel made knock-in mice that had the germline precursor B cells, and they carried out lots of different experiments to see what kinds of combinations of the immunogens would work. And one of the sequences that we designed actually did elicit broadly neutralizing antibodies. So that was really the first proof of principle that broadly neutralizing antibodies could be elicited starting from germline -- human germline like B cells and we could elicit a lot of somatic hypermutation and we could elicit plasma cells that secrete broadly neutralizing antibodies.

There were some caveats with this study. A lot of people asked, "Well, why don't you just go do that in humans?" And basically, although the study was a very important proof of principle, it was too low of a bar to, we believe, to justify going straight to humans. For one thing, the precursor frequency was 100% in this mouse model. So it was too easy. There was no competition from other B cells. But generally speaking, the priming immunogen needed improvement as well. It didn't have a very high affinity for those precursors. And we believe to -- actually to trigger them in humans, where the precursors will be much more rare, we need higher affinity and broader specificity.

And so -- sorry. A key issue of making a human vaccine for HIV and using this technology of germline targeting is consistent priming of bnAb precursors as a vaccine requirement. If your priming immunogen doesn't trigger the right starting B cells, your vaccine is essentially going to be dead on arrival, you're not going to be able to ultimately elicit broadly neutralizing antibodies. So you really got to do it right, right at the beginning. And to do that correctly, we believe, to get consistent priming of these precursors, we believe, will require a priming immunogen that can target a diverse precursor pool for any one kind of bnAb, and that has to do with human genetic diversity and the recombination on -- the diversity of -- inherent in antibodies. And so we're talking about this first priming step.

And ultimately, we just think that what it means is that the priming immunogen needs appreciable affinity and avidity for diverse precursors. And our lead project for that is -- for the germline-targeting vaccine design is the VRC01 project. This is 1 kind of bnAb against HIV. These broadly neutralizing antibodies, they compete -- they bind to the CD4 binding site. They compete directly with the human receptor, so it's easy to see how they prevent HIV from infecting the target cell. And they have specific structural requirements. And we don't need to go through the details, but they are very diverse. There's not -- it's not so easy to turn on all precursors for VRC01 class broadly neutralizing antibodies. You have to design an immunogen that has affinity for a wide number of different but closely related B cells.

And over the years, we've developed a nanoparticle immunogen that we believe can do that. It's called eOD-GT8 60mer, a self-assembling nanoparticle presenting 60 copies of an engineered outer domain. It's closely related to the picture that I showed you on -- from the cover of Science on an earlier slide. And this nanoparticle does have appreciable affinity and avidity for diverse VRC01-class human naive precursors. It primes

VRC01-class responses in stringent mouse models with rare precursors, and it induces VRC01-class memory responses that we've shown with collaborators that we can boost toward bNAb development in mouse models. And we've got a whole bunch of papers detailing this but I don't want to go through all of those.

We're currently conducting a human clinical trial of this molecule produced as a purified protein with the GSK adjuvant AS01B. It's a first-in-human test of germline-targeting, self-assembling nanoparticle. And I'm not at liberty to tell you the results of that trial right now. We're aiming to release the results later this year. But we're hoping to -- if the results of this trial are positive, we'd like to build on this and move into doing these kinds of things in humans with Moderna mRNA.

And so thinking about what we're trying to achieve, we need to develop a rather complicated vaccine with multi -- with multiple different immunogens given in sequence. And you can imagine that in order to develop such a vaccine will require -- and to make it work very well in humans, it's going to require many iterative human clinical trials. It's a very difficult vaccine to make. And if we're going to do that and rely on protein -- GMP protein manufacture, our progress will be limited in terms of the speed -- by the relatively slow pace and high cost of manufacture. And we're hoping the solution for us will be that Moderna mRNA will provide a rapid, economical and highly immunogenic vaccine platform that will enable expeditious and iterative human vaccine optimization. We think that's going to be critical to making an HIV vaccine, and we think that the Moderna mRNA platform is really a key aspect of our strategy.

And just for a little bit of data that we can show you about mRNA, we've been experimenting with Moderna mRNA delivery of nanoparticles, like the one I showed you. And we've been testing them out in a stringent mouse model, originally from Fred Alt's lab, in collaboration with Fred Alt at Harvard. And what this graph is showing you is we've done some experiments where we compare head to head purified protein plus adjuvants, single immunization in this mouse versus an mRNA delivery with no adjuvant in the same mouse model. And 42 days after 1 immunization, we sacrifice the mice, and we do antigen-specific sorting of the memory B cells to see, well, did we turn on a VRC01-class response or not? We need to get the sequences of those -- the B cell receptors.

And we do that, and what the plot shows is the percent of B cell receptors that are VRC01 class, on the y axis, in response to an mRNA vaccine and, on the x axis, in response to protein versus adjuvant (sic) [protein plus adjuvant]. And there's 3 different points in red, and each point is -- represents a different immunogen that we tested both via protein and adjuvant and via mRNA. And if the 2 platforms were performing equivalently, the data would be along the diagonal. And if mRNA were performing maybe slightly better than protein plus adjuvant in the way we did it, the red points would be above the diagonal as they are. So at least, we can see in this mouse model and the way we're doing the experiment, the mRNA is performing -- Moderna mRNA is performing quite well, and we're happy with that.

And so just as a final overview, we're trying to make an HIV vaccine. We believe that collaborating with Moderna is critical to being able to carry out expeditious iterative human vaccine clinical trials. Our overall strategy is shown on the left: germline targeting as the prime, shepherding -- a series of shepherding immunogens and followed by a trimer polishing.

The strategy, sort of the flow of our workflow, is shown in the middle, where we design immunogens, formulate the mRNA, test the vaccines in engineered mice and nonhuman primates. And there's an iteration loop there to make sure that they work as well as they can. And once we have something that looks like it works pretty well in those animal models, then we would do human clinical trials. There's another iteration loop there, and that's where the mRNA is particularly, we believe, critical to enabling that iteration. And hopefully, the output will be a protective vaccine. And we're pursuing at least 3 different targets that I've listed on the right.

And of course, this is a lot of work. There's a lot of support here from IAVI, the Bill & Melinda Gates Foundation and NIH. And all these animal experiments are done with many different collaborators. I mentioned Fred Alt, Facundo Batista at the Ragon; Shane Crotty at La Jolla Institute; Bart Haynes at Duke; David Nemazee at Scripps; Guido Silvestri at Emory; and Laurent Verkoczy at San Diego Research Foundation (sic) [San Diego Biomedical Research Institute].

And so I just want to close by thanking all the people that do this work: people in my lab, special call-out to Joe Jardine and Dan Kulp for developing the eOD-GT8 60mer and Sergey Menis for developing that nanoparticle and to Jon Steichen for his work on the proof of principle for germline-targeting



vaccination that was done from Michel's lab. And there's a lot of -- obviously, tons of very critical collaborators from Moderna I mentioned and the Moderna work has really been routed through IAVI and led by our colleagues at IAVI and, obviously, lots of important funding that's shown here.

So I'll stop there. Thank you.

Paolo Lusso;NIAID;Chief, Viral Pathogenesis Section

All right. So good morning to everyone. My name is Paolo Lusso. I'm glad to share with you some of the results of our effort to develop an HIV vaccine in collaboration with Moderna. And I wish to thank Andrea for the kind introduction. And I'm lucky to come after Bill, who has given you a beautiful introduction, so I have -- I can cut short of -- on the introductory part.

But I wanted to start by showing you this slide first to put things in the right perspective. As you just heard from Bill, HIV has been and still is one of the greatest calamities in the history of humanity. And if we compare HIV/AIDS with the other great pandemics in history, it is one of the worst ever. At least 25 million, most likely closer to 35 million deaths occurred since the beginning of the epidemic. While as a reference with COVID-19, we are fortunately still in the hundreds of thousand, down at the bottom.

So as Bill nicely said, with nearly 40 million people living with HIV still in 2019, the only effective means to interrupt the cycle of infection at a global level, control the pandemic will be the development of an effective vaccine. This really remains one of the most urgent public health priorities today in the world.

As you heard, HIV vaccine research is now more than 3 decades old and was disseminated of failures, but we certainly have learned some key lessons, and these are 2 of the most important: the first is an optimistic remark that a protective vaccine is feasible. Actually, we have scientific evidence for that in the face of the main challenges. And second, that in order to get a really protective vaccine, it is essential to induce the so-called broadly neutralizing antibodies you heard a lot about, bNAbs.

Don't have to spend time on this, but it's important to remark that in its native form on the surface of the virus, as you can see here on the left, HIV-1 envelope is a membrane-anchored protein, and it is a trimeric form.

So if we look historically, many different forms of the HIV-1 envelope have been attempted vaccines. And starting with the most simple form that is the soluble, monomeric gp120 subunit, unfortunately, this turned out to be ineffective as a vaccine because it induces only nonprotective antibodies, which has really been quite frustrating for many years in the field.

Then a few years ago, a breakthrough was reported with the stabilization of a soluble form of the HIV envelope, the so-called SOSIP trimer. And there were great expectations that this could really be a game-changer. Certainly, it has helped a lot in the field. But as a vaccine, it's still kind of suboptimal because it induces a lot of off-target antibodies and not enough for the protective one.

So the best form really for a vaccine is what we observe in nature: the membrane-anchored, full-length trimer that is the same form that is present in a real-life virus. However, this has been very challenging to use for many years because it's very hard to manufacture this outside the body and to scale up the production of a homogeneous form of the vaccine. And that's where the mRNA comes into the picture, and it really does make a difference because this native form of the envelope is perfectly suited for expression with mRNA. We actually have evidence in the lab that the protein comes out in the native form exactly as we want it. So it has been a long and winding road, but we got there, and we have a way to express the right form of the immunogen, and mRNA technology is very helpful in this.

So these are the key signatures of the approach that we are using to develop a vaccine. And let me go one by one. The first one, as I just mentioned, is the use of a real native, membrane-anchored envelope expressed in vivo by mRNA. What are the advantages? This is expressing the real native antigenic state of the protein, which is the best mimic of what's present on the actual virus. There is an endogenous processing of the protein, and this is really important for the HIV envelope because of that very impressive glycosylation that Bill showed you earlier that is really cell dependent, so there is no homogeneous glycosylation. We want to have the most native form to mimic the real infection and also the lack of distracting epitopes that should -- distracting system like the trimer base.



Now another key signature of our approach is the in vivo production of virus-like particles, or VLP, by coformulation of envelope with Gag. So as you know, the Moderna mRNA is formulated inside the small lipid nanoparticles that protects the mRNA and facilitates its transfer into the host cell.

Now instead of just a single mRNA, Moderna prepared for us nanoparticles containing both Env and Gag mRNA. Gag is the core antigen. And they were co-formulated inside the same particle. And we know that when Gag and Env are co-expressed inside the same cell, they assemble virus-like particles that look, by all means, like real virus, just not infectious. And this is one of the best ways to stimulate the new system. The reason being that not only the envelope is in the native antigen space, but these particles are life-size. It's a size that is optimal for uptake and processing by so-called antigen-presenting cells, that are key for the immune response. The particles are released outside the cells, and therefore, they go places. They travel to places where the action is, like the afferent lymph nodes. And importantly, instead of just 1 antigen, we're actually stimulating a system with 2 antigens, both at Gag and Env. So this is a really important aspect of our approach.

Then following Bill's model and very nice studies, we are using a similar idea of priming the immune system initially with a form of the envelope that can engage the germline, the antibody and [vectors]. And this induces an ab initio recruitment of those rare precursors that we described for the generation of broadly neutralizing antibodies.

And then we follow up with a very intensive heterologous boosting using a combination of different envelopes from different places. These are the different genotypes that circulate around the world, and these are all the glycan-repaired tier 2 for the real-life viruses. And the advantages of this heterologous boosting it to selectively focusing the system on the shared epitopes across all these different forms of envelope we are using. And to the exclusion of distracting epitopes so that we don't want to induce antibodies again which are non-neutralizing. And eventually to mimic the sustained antigenic stimulation that we have in patients who actually develop bNAbs after many years.

So to recapitulate, we have an initial priming to engage the unmutated bNAb precursor. We have an initial boosting with an autologous form but more close to selectively expand tier 2 epitopes. And then we have this intensive heterologous boosting with mixed forms of envelope of different plays. And this is aimed at focusing on the share of the bNAb epitopes. And we put this concept to trial.

So we test it based already in an initial study in rhesus macaques. I don't have time to really go into the details of the protocol. As you see, it's pretty cumbersome. But let's jump to the results and, in particular, to the induction of broadly neutralizing antibodies that is really holy grail for an HIV vaccine.

So here, I'm showing you that the mRNA vaccine was highly immunogenic. These are antibody titers that we induced over time. And these are, let's say, the lowest bar, the induction of autologous utilization against the same bar that we used for immunization, but you can see that we induced significant levels of neutralization very early. So mRNA is working very well. And this is the most challenging goal, the heterologous neutralization, which means being able to neutralize a different virus strain that the monkey has never seen before. In this, as you can see, it took much longer, but after the third heterologous dose, the green arrow on the top, we finally started to see the appearance of these growth neutralizing antibodies, and we pop the champagne bottle.

And this is a summary of how broad these antibodies are. Active, because these are a panel of highly diverse viruses coming from all corners of the world, genetically quite different. As you can see with essentially one exception, we have neutralization of most of these viruses, even though the titers on the variation are still relatively low. But this is really a result that has not been previously achieved in the field, and this was obtained with our mRNA vaccine.

So at this point, we asked another very challenging question: can these antibodies that we were able to induce protect against the heterologous virus, against a virus that was never previously encountered by these animals? And the answer is yes. As shown here, even though the protection was not absolute, the green line shows the group of animals that was protected. And this was either a delay in the infection, or in some animals, a complete protection throughout. They never got infected. So this is also very impressive results in the field because, again, this is the virus that does not correspond to the envelope data at all during the immunization with heterologous.



So to elucidate, we correlate the protection here, which is really important because they inform us about the real mechanism that mediated this protection. We used an electron microscopy approach that was developed -- created to look at the antibodies in action. You can see here these little projections that are readable by EM that indicates there are antibodies to a very important site, the CD4 binding site, which is the site used by the virus to dock to the receptor CD4. And this was confirmed by analysis using a special probe, probe N49, which corresponds to the CD4-binding site. You can see that over time, we induce the antibodies to the site that really correlates beautifully with protection.

And then we try to isolate individual cells, single cells, B cells in this case. These are the antibody factories from the protected animals. As you can see here, this cloud corresponds to the B cells that bind to our probe as well as to the full primer. And if we focus on this region, these little pink dots are the single B cells that we cloned up and used to produce monoclonal antibodies.

And here, I am showing you that we isolated a bunch of monoclonal antibodies that we are now actively studying. Many of them belong to a single gene family, the VH4. This is very interesting because it suggests there is a dominant neutralizing response. And these antibodies have the right length over a specific loop, the CDR3, that interact directly with the target. And this is another sign that we are on the right track and that these antibodies have mutated enough to reach the difficult target.

So in conclusion, we use, as you've seen, a unique combination of factors, none of which by itself would probably be sufficient. But the combination was the key, and this allowed us to successfully vaccinate macaques with an mRNA vaccine. And hit hard targets because we induced bNAbs, broadly neutralizing antibodies, although still at low titer, but more importantly, even protection from a difficult viral challenge in vivo.

And what all these factors that we use in our approach have in common is that they provide the best approximation of the real-life infection with a native virus. And this is an important point because this is really possible thanks to mRNA technology, which indeed allows us to mimic nature, as you heard from many presentations before, because we are making the immunogen, the vaccine, in the body that receive mRNA, not outside the body. So we don't provide an artificial molecule that is premade in a test tube, but we actually produce the vaccine in the recipient.

So what are the next steps? We are going to, first, repeat and expand the study in macaques in collaboration with the Gates Foundation (sic) [Bill & Melinda Gates Foundation] that is supporting us to confirm the initial results, further optimizing the immunogens, and also streamlining the protocol. And then we are very excited that we are starting to design a first-in-human clinical trial with an mRNA vaccine that will validate this concept, hopefully, in humans.

And finally, I want to acknowledge the collaborators from many different institutions, and thanks, Moderna, for their vision and courage.

Thank you for your attention.

Stephen Hoge - Moderna, Inc. - President

Thank you, Paolo, and thank you, Bill. Hey, everyone, this is Stephen.

So just -- I know we're running a little bit late. I've just got a couple of quick final closing comments, and then we'll open it up for Q&A about 30 minutes.

So as you've seen today and as we've discussed throughout our Science Days and, frankly, throughout the history of the company, we've maintained a very long-term commitment to build the very best platform of mRNA science and delivery science that we can. For many years, we've described this as a very long-term journey and Stéphane reprised that again today, one that we think takes at least 20 years and one where we still think we're just at the beginning of the really steep part of that curve, where each year of innovation and breakthroughs that we push forward actually will dramatically change in positive ways, we hope, the performance of our products and our platform.

So I hope that you've got a sense today across a wide range of topics that we covered as well as over the last few years of Science Days that we actually are progressing in the basic science across an incredibly wide waterfront. And we're very, very proud of the kinds of innovations that we've been bringing forward.

As Melissa described, our intention is always to advance these into publications and peer reviews so they're broadly available. We always use the Science Day as a chance to preview for everyone the things that we've been working on and the types of innovation that we hope to be publishing on in the very near future. But we also wanted to provide references on those previous presentations we've done.

And so as you'll recall, in 2018, we focused intensely on our Science Day on some of our work on novel proprietary ionizable lipids, some of which we've advanced on even today on their tolerability, on the way that we use microRNA sites to target things, and how we think about translation initiation and the use of leaky scanning or turning off leaky scanning to improve the performance of our drugs. Many of those publications have subsequently been published in peer review journals, and the references are here.

And in 2019, just a year ago, we continued that, talking about how important it was to use modified nucleotide in our messenger RNA drugs. And I'm quite proud to say that a quite substantial piece of work was just published in science advances, the Nelson et al referenced there. But I draw your attention to, which has some of the data that we talked about last year, but actually even much, much more characterizing mechanistically what we believe is happening and why the advantages of modified nucleotides, particularly in the therapeutic sides, are obvious to us.

We also talked a bit or quite a bit last year about further work on sequence engineering, codon optimality, 5 prime-UTR design. And a lot of that work has subsequently been published, including with a number of academic collaborators, as is that last reference.

And then some stuff that probably is not as much publication as it is know-how, but that we're happy to talk about last year, was the physical and computational methods that we use to really interrogate LNP structure and simulation. I think what you saw in Kerry's presentation today was the next glimpse of what that looks like as we bring new chemistries to bear, including how we're using molecular dynamic simulation, to characterize things that we can't see with physics alone, which is an exciting advance in our platform that we hope will continue to bear dividends.

And then this year, just building on that. The themes are hopefully much the same but also show that continued sense of progress and momentum. We had a few different topics today, as Melissa walked through. We talked a good amount about how we are extending the pharmacology of our platform. Many of the innovations that you saw described today either were combined with other sequence optimizations or UTR work or completely new improvements that we think are ultimately going to improve the pharmacologic properties and the potential for the number of diseases we can treat with our messenger RNA platform. We talked a lot about things that we sometimes treat as very confidential know-how, which is how are we making these messenger RNAs; and provided, hopefully, some good insight in how we're going beyond the state of the art in terms of purification to actually engineer completely novel enzymes that we use to make messenger RNA, which are incapable, as far as we can tell with some of these assays, of detecting -- of creating the double stranded RNA impurities that can lead to immune activation.

We also provided an update, as we often will, on lipid nanoparticle technologies and particularly our newest generation of lipids, square mid-lipid nanoparticles, base liponanoparticles in Kerry's presentation. And then the further characterization of platforms that we already have moving forward in development, in particular, the role of LNP size on immunogenicity, which is a critical thing to understand, characterize and control when you're advancing medicines.

And then lastly, as a bit of a new flavor, we wanted to provide some insight into how we're thinking about using the platform in new ways to advance new potential medicines, so things that we hope and think that messenger RNA will be able to do that other technologies are either it's very cumbersome to do, like with recombinant protein or perhaps not even possible to do because you really want to do endogenous glycosylation and presentation of antigens.

And we're incredibly grateful to both Bill and Paolo for coming and spending time with us today to share some of the work that we've been doing with them. We have a lot of work ahead, as I think they both characterized, but we're proud to be participating in their efforts to try and develop a vaccine against HIV.

Now all of that forms the foundations of our platform. Obviously, we will -- that knowledge, we continue to use to take that next step forward up the S curve. It is also a source of intellectual property. And it is all enabled by, as you would have seen at our Investor Digital Day, Manufacturing Digital Day from a couple of months ago, an approach in our platform which is fully digitally enabled. We worked very hard to make sure that information is characterized and curated in a way that it's accessible for our scientists, which avoids some of the inefficiencies of how folks have in



the past approached big challenges like those that we face in the mRNA platform. We're proud of all of that coming together, and we're really grateful for all of you for taking a day to give us a chance to talk about that.

As you'll note from the last 2 years that we've done this, this is a day where we really want to talk about our platform. We really want to provide transparency to the science of what we're doing. And we quite deliberately won't be speaking about specific programs, certainly not programs in development. And so I know that there's a lot of enthusiasm about things that we're doing in our pipeline. Please feel free to ask those questions in other fora. We do a lot. As you know, there's well over a dozen meetings a year that we do like this. This is the day where we really want to give the chance of our broader communities to see what's happening in our platform and ask us questions about how we're thinking about that part of our investment.

So with that, I'll invite the moderator to help facilitate any questions. And Melissa, I think, will join me. And of course, I hope we'll be able to ask Paolo and Bill and others any questions if they come up.

So moderator, we're open for Q&A.

QUESTIONS AND ANSWERS

Operator

(Operator Instructions) And we have a question from Matthew Harrison with Morgan Stanley.

Maxwell Nathan Skor - Morgan Stanley, Research Division - Research Associate

This is Max Skor on for Matthew Harrison. Quick question. Can you talk about the translation of the animal model data to humans, specifically the clinical trials that are ongoing? I know you're not going to talk about specific programs, but just that translation of data. And this would be regarding extended half-life and other parameters that you discussed today.

Stephen Hoge - Moderna, Inc. - President

Sure. Well, I'll invite Melissa in, in just a second to talk about some of the extended half-life stuff. I think -- thank you for the question.

In general, we have found, as you'll note from our preclinical publications on many of these things, that the relevant disease animal models and/or large animals, like primates, have generally predicted our clinical experience pretty well. Not always the same doses. Obviously, it's different as you go from small animals into, obviously, full humans. But we have -- for all the experience which we have data today, we have generally found a very good degree of translation. So we feel confident. You're never 100% sure in science, but we feel very confident that when we see something in small animals, validate them in slightly larger animals, that they're going to be translating in our human experience.

Melissa, is there anything you want to add about extended pharmacology and the degree of translation or science we've seen there?

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

I think you really hit on it, Stephen. We do, for our predicting doses in humans and pharmacology in humans, you'll remember from R&D Day last year that we talked about the chikungunya monoclonal antibody and how we were able to model what the pharmacodynamics would be based on -- and the dose based on our preclinical animal models. And we -- really, it was remarkable how well that modeling worked. So we do that modeling, and that's, so far, proven very helpful for us. I'll stop there.



Operator

And our next question comes from Geoff Meacham with Bank of America.

Alec Warren Stranahan - *BofA Merrill Lynch, Research Division - Associate*

This is Alec on for Geoff. So this will probably tie into what you presented for your approaches to reduce the LNP immunogenicity that you talked about today. But we've seen with some other COVID-19 vaccine candidates a high-frequency of vaccine-neutralizing antibodies. These are the antibodies that bind to the vaccine and lower its efficacy. And I believe that your COVID-19 vaccine uses the same LNP as the CMV vaccine. So I was wondering, just on a broad level, given your experience with both assets to date, whether you have seen any hints of neutralizing antibodies generated from this particular LNP formulation.

Stephen Hoge - *Moderna, Inc. - President*

Yes. Thank you for the question. It's a great question. So again, our chosen delivery approach, as we've said before, is lipid-based, lipid nanoparticles, as you know. And there's a number of features that we like about lipids, but one of them is that they are fundamentally amorphous on their surface. They, like in other envelopes' context, do not present a repeating epitope. And we don't have protein structure on the outside of the lipids, which is something that traditionally the immune system trains on in order to neutralize foreign envelopes like a virus.

So in our collective experience, particularly with the clinical delivery vehicles that we've been using, as we've presented here, we've always been able to repeat dose without developing a neutralizing response to the surface of our lipid nanoparticles with those delivery vehicles that we've taken into clinic. And so the same has been true for our therapeutics and our vaccines platform.

And that's just an intrinsic feature of being a messenger RNA company. We have always intended that our medicines would be repeat-dosed. For the most part, that has been intentionally focused on for the therapeutic context, but obviously, those benefits play in, in the vaccine context as well. We have no interest in developing an anti-delivery vehicle immune response. Entirely, we want the immune response to be to the protein as expressed by the messenger RNA when we put it inside of an antigen-presenting cell.

So -- and I think we've shown -- I think it was in 2018, we showed quite extensively how we'd characterized in the therapeutic context how to repeat-dose and the ability to sustain that pharmacology. And I think in everything you saw today, when you take these delivery vehicles into therapeutics, we're able to sustain that pharmacology without a decrement, which is pretty direct evidence that there's not a neutralizing response even at those high doses in therapeutics.

Operator

Our next question comes from Yasmeen Rahimi with ROTH Capital Partners.

Yasmeen Rahimi;ROTH Capital Partners;Analyst

Thank you for a really wonderful time today.

Two questions for you, both very technical. The first one is, can you help us understand how the addition of the terminal inverted IDTs to the mRNA drugs can be integrated into the manufacturing process? And then is this done for both systemic therapeutics as well as prophylactic? And then I have a follow-up question.

Stephen Hoge - Moderna, Inc. - President

Melissa, do you want to take those?

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

Stephen, do you want me take that? Yes, sure. So we are working on integrating this into our manufacturing process. We generally do not talk about details of our manufacturing process because they're our secret sauce. But we have developed methods to do this at scale, and we are proceeding to do so for some of the programs that we want to use this extended pharmacology for.

I don't know, Stephen, if you want to say anything else about that.

Stephen Hoge - Moderna, Inc. - President

No. I think that's right. There was a second part of Yasmeen's question, if I remember correctly.

Yasmeen Rahimi;ROTH Capital Partners;Analyst

Yes, whether it's designed for systemic versus prophylactic, the IDT incorporation.

Stephen Hoge - Moderna, Inc. - President

Yes. I think the -- sorry, thank you for reminding me. The way we would choose whether we want to extend pharmacology or not is really just a function of what we're trying to achieve in the program. So I wouldn't describe it as therapeutic versus prophylactic. We can all imagine therapeutic context is where you want a relatively short-acting protein and you want it to clear away relatively quickly, for instance, in some cases, with cytokines in cancer. And we can all imagine the situation where the opposite is true. We showed some today.

I think the same would apply in the prophylactic space. I think you'd want to make a decision about whether the duration of antigen expression in the case of, let's say, a vaccine is beneficial or not. And we will always be directed at a program level by which technologies to pull into that specific program usually based on how it performs in preclinical disease models or protection models. So there's not a hard-and-fast rule on that, Yasmeen. I think we'll be directed by the data.

Yasmeen Rahimi;ROTH Capital Partners;Analyst

And then one last quick question. Is the incorporation of this ionizable lipid, is that only designed for the hepatic LNP delivery? Or is that also utilized for other tissues?

Stephen Hoge - Moderna, Inc. - President

So like all -- it will be a similar answer, but like all things, we advance the science and then we do characterize how it performs. The place where we have seen -- as you saw Kerry present today, a really important step change is in the hepatic, but it's not exclusive for that. We'll -- there's other examples that Kerry presented, for instance, which was some secreted reporters like EPO, where you saw a substantial extension of the AUC protein that was expressed. And so you could imagine it being useful in the secreted context.

I think we will always want to be judicious about how we deploy new technologies, particularly in cases like with our secreted and cell surface modality, where we already have a clinically validated modality. Melissa referenced the CHIK mAb mRNA 1944 study. Because we have that well worked out and because we have confidence and experience with it, that's one where we felt we may or may not want to make a change. And I



think, again, we're just going to be data directed on a program-by-program basis. As new programs move forward, we want to understand whether -- what the best technology we have in our hands looks like at that moment and whether it makes sense to put it into a program.

So the first program, as we've disclosed today, in which that new delivery vehicle is going to show up, is the hepatic program. It is GST 1, as we said. But I don't think that's a guarantee that all will be that way or that all programs in the future would use the square lipid nanoparticle.

Operator

(Operator Instructions) Our next question comes from Hartaj Singh with Oppenheimer.

Hartaj Singh - *Oppenheimer & Co. Inc., Research Division - Research Analyst*

Great. Thank you for the questions. And again, a really elegant presentation. Just a couple. I just want to follow-up on Yasmeen's question, Stephen. One is that when you're thinking of the sort of iterations of development that you're doing on the preclinical side and the science side, the research platform, do you see that sort of helping you tackle kind of new diseases or areas where your current formulations that are either in the clinic or close to being in the clinic cannot get you to? Or do you see this more as sort of like a life cycle management, where you have products that hopefully get approved, and then after that, you follow up with better, more improved sort of product characteristics? Or is it a combination of both? And then I just got a follow-up question.

Stephen Hoge - *Moderna, Inc. - President*

Yes. Great, great question, Hartaj. Thank you. It's something that we wrestle with a lot. And I think the short answer is it's going to end up being a combination of both. For the most part right now, our investments in platform research are trying to expand the utility of the platform, right? And so we measure that in pharmacology in ways that we can modify diseases by putting messenger RNA medicines into animals and people. And as we make improvements, we can do new things pharmacologically that we couldn't do before. We can dose less frequently. We can achieve higher levels of protein. We can perhaps transact nutritions in new ways.

And so that innovation gets pushed into, as you just said well, new diseases that previously may not have been as accessible. And sometimes, it will probably show up in opportunities for life cycle management, where we have products that have moved forward, hopefully, to approval and where we can make them even more patient-friendly and by bringing those sorts of technologies in.

Again, we're going to be data-directed. But I think we all feel a sense of responsibility for the patients and the diseases that we're advancing programs in that we bring the best of what we can do over time to all of those diseases to help as many people as possible. And so I think it will end up feeling like a bit of both, if there's room for improvement. In some cases, there just may not be room for improvement. What we have may in fact be more than enough.

Hartaj Singh - *Oppenheimer & Co. Inc., Research Division - Research Analyst*

Great. That helps a lot. And then another question I have is that part of the allure of using mRNA is that the regulators we've interacted with have -- you presented lots of preclinical data across your 20-plus clinical candidates. When you present -- as you're modifying your mRNAs to have greater half-lives or protein expression that's greater half-life, how much additional work do you have to do to bring IND? Because I imagine right now, the regulators are getting pretty comfortable with a lot of the data they've seen in the preclinical trial. But these new formulations, do they add additional work or additional steps?



Stephen Hoge - Moderna, Inc. - President

Yes. Well, great question again. And I think the answer is even independent of regulators, anytime we bring something new forward, we feel a deep obligation to characterize it robustly and feel like we understand it. And so to the extent that there is additional work that we do, of course, there's a lot of additional work we do in preclinical development, in platform research, as we're advancing a new technology in the clinic. And it's always a little more work or a lot more work the first time you bring a new technology into clinical testing.

The second half is also true of that statement, which is the third, the fourth, the fifth, the tenth time, in some cases, in our vaccines context that you do something, it does start to become, obviously, much more efficient because you can rely on that large amount of prior experience, both preclinically and clinically.

I do think that, that has been reassuring, hopefully, to everybody who sees our files, whether they're patients or investigators or the regulators. And so we build the scientific support for everything that we do, and over time, we hope that repeating -- repeatedly using the same technology and platform does provide a high degree of confidence. But whenever we move something in for the first time, we have the obligation for ourselves first and then for all of those other stakeholders as well to robustly characterize it. And so you can count on us doing a lot more when it's something new.

Operator

And our next question comes from George Farmer with BMO Capital Markets.

George Farmer - BMO Capital Markets Equity Research - Analyst

Very interesting presentation today. I'd like to ask you about how you manage the formation of these shorter transcripts that are generated following a transcription with T7. It looks like from the gels that you saw that there are quite a few there. And how do you think about purifying the full length for the purpose of scaling up and bringing the drug to market? And is there an extra HPLC step involved in your manufacturing currently? Or do you use other ways to purify?

Stephen Hoge - Moderna, Inc. - President

So go ahead, Melissa. I was going to kick it to you.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

So I was just going to say that when -- these small RNAs, there's a lot of filtering steps that go on during our process for manufacturing the RNA. And the small RNAs are -- generally leave because of the filtering. We also have some steps where we can take advantage of the poly (A) tail on the long mRNAs to specifically purify those. And those are not on the small RNAs. And so they generally are not a problem for us. Stephen, you wanted to say something?

Stephen Hoge - Moderna, Inc. - President

No, I was just going to say that. I mean, I think the very, very small -- the shorter borders that you're referencing, again, it's just important to recognize that they can be sometimes 100x smaller than the other molecule. And so separation there does not require HPLC, per your question.



George Farmer - BMO Capital Markets Equity Research - Analyst

Okay. Great. And then how do you think about using the different LNPs based on the indications, whether it's used for prophylaxis or for therapeutics? Is there -- what are the nuances that go into whether, say, targeting something for hepatocytes or for the marrow or for draining lymph nodes?

Stephen Hoge - Moderna, Inc. - President

So I'll go first and then Melissa can just add anything if I miss. We -- so I think if you harken back to in 2018, we do talk about the fact that we have different delivery vehicles, lipid nanoparticles, for our vaccines modality and for the immune portions of that than we do for the systemic liver therapeutics. And the way we think about it is, the surface of the nanoparticle really does a lot to determine where the particle's going. And in some cases, you want to end up in the immune system, and in other cases, you want to end up in hepatocytes.

And so what we will do in our vaccine's modality, by way of example, is that we will make sure that the lipid nanoparticle really wants to drain into the lymph nodes and doesn't transfect the muscle or anything locally and then ultimately find its way into the immune compartment, which is where we want to deliver that messenger RNA so that we can educate the immune system about a virus, for instance.

You really want to do something quite different in the context of a therapeutic, where you want to kind of avoid the immune system and end up looking more like an LDL or apolipoprotein and find your way in the hepatocyte, let's say, in a rare liver disease therapeutic context. And then there are a range of different things in between. The way we do that is optimizing the different components. And so as we've talked about, I think, in 2018, is if you look at some of our published literature, you'll find that we will use different amino lipids as well as other changes to the surface chemistry of the lipid nanoparticle, how it's organized as a solid ball, if you will, that help effect that preference one way or the other.

Melissa, anything you'd add to that?

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer & Member of Scientific Advisory Board

No. I think you gave a pretty complete answer. Thank you, Stephen,

Operator

And I'm showing no further questions at this time. I'd like to turn the call back to Stephen Hoge for any closing remarks.

Stephen Hoge - Moderna, Inc. - President

Yes. Well, look, thank you all for taking the time again to spend it with us hearing a bit about our basic science of our platform.

As Stéphane said at the beginning, this has been the first and longest-term strategic commitment of Moderna, first, because when we started a decade ago, it was the only thing we were doing. But we're quite proud, and hopefully, you see we're quite consistent in the language we use to describe this strategic commitment, that we will continue to invest in the state of the art of mRNA science in our platform for the purposes of expanding what we can do with this platform over time. And we will do that, we believe, consistently for perhaps the next decade to build on the decade we've already done.

We think the opportunity to do that creates huge returns in terms of the pharmacology of what we deliver and continues to ensure that Moderna is going to be at the forefront, and hopefully, continues to be the leader in all aspects of this really exciting way of making medicine.

So we will often publish, but we want to make sure that we maintain the commitment that at least once a year we share with the investment community, our broader stakeholders, a sense of what that commitment looks like, what that investment looks like and why we think that there's still more than enough evidence that we should continue to make it.

So thank you all for taking a few hours of your day to hear about these basic science investments (inaudible) really want to thank our speakers as well as Paolo and Bill for taking the time to share their views with us today.

And with that, we wish you all a very good day.

Operator

Ladies and gentlemen, this concludes today's conference call. Thank you for participating. You may now disconnect. Everyone, have a great day.

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