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MRNA.OQ - Moderna, Inc. - Special Call

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PRESENTATION

Stéphane Bancel - Moderna, Inc. - CEO & Director

Good morning, everybody, and thank you so much for joining us this morning. The team and I are very thrilled that you decided to spend the next few hours with us, and I know some of you are even going to spend the afternoon with us going to Norwood. So today is Science Day 2019. And we started last year [the rhythm of] doing an annual Science Day where we give a chance to our investors and analysts to look under the hood.

And I think you won't be disappointed with what Stephen, Melissa and the entire team are going to be sharing with you. What we'll try to do, given there's so much around the company, is to do a Science Day in the spring, to talk about the platform and all the advancements in the science. And then to do an R&D day in September, so that we can talk about the pipeline, and the different candidates and human data.

So today, we're talking about science. Of course, I have to start by reminding you that we're going to be making forward-looking statements, that there are many risks involved in the company. You can find those on the SEC website or on our company website. And I welcome you to go have a look at those.

Eight years ago, we started the company with the idea that if we could make mRNA safely expressed protein in humans, we could be the new class for medicines. That was really the driving force that has been guiding us in the last 8 years. We were very excited about the idea that we could do not only secreted protein vessel for intracellular and transform brain protein. Because mRNA is an information molecule, which is quite unique in the pharmaceutical space, we believe that the probability of technical success of our drugs over time should be materially higher than the probability of technical success of traditional medicine. We also believe, because mRNA is an information molecule, that if we invested in IT, in robotics, we should be able to go much faster than traditional medicine both in the labs, preclinical, but also getting to the clinic faster. And we believe that over time at commercial scales, the cost of manufacturing of mRNA should be a very attractive [best use] recombinant.

So with that, we set the mission for the company that we have to deliver on this promise to make transformative medicines for patients. And I think it's an important piece to understand about the Moderna team, is a sense of mission that exists in all the colleagues that we have. We believe that we can help as we advance the science, millions of peoples from kid with rare genetic disease to the elderly with (inaudible) vaccines that don't exist today. And we really see that as our duty to make this work, make this technology work. The thing that is quite amazing about this technology is that there is not one secret sauce. People always ask us, what is the one thing that makes mRNA work? And as we've learned over time, this is not one thing, this is technology with dozens of vectors.



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First, around the molecule of mRNA, a message. The clever chemistry which shows a macro mRNA of a sequence and many, many more. This is just for (inaudible) important decisions that have to be made for every molecule.

And then there is how you make it. The manufacturing process, as we know, for especially new technologies is really important to make high-quality products so that both in the labs but also in a clinic you can do a high-quality science. And then the formulations and delivery. The components, the chemistry, the composition, how much of each of those chemistry you put together, the surface property and many more. And then there is how you make mRNA, how you get mRNA inside the formulation, and you will hear some interesting things again today. So with science, we've a lot of vectors enabling us to improve and innovate on each of those vectors. And the other nice thing about having so many vectors is that you have a lot of freedom to operate as you observe in the labs or in the clinic things working, things not working. You have a lot of freedom that you can operate across all those levels.

And the last thing I want to share with you in this intro before I leave the floor to my colleagues is the commitment that we have as a company about science. Since the early days, and this is not changing an inch since the early day, this team is extremely committed to doing amazing science. We have believed since the beginning of the company in the power of the S-curve, that every technology that men have searched has always gone for an S-curve. And if we invest for the long term and are willing to make multiyear investments because science doesn't take weeks, unfortunately, it takes years. But if you are willing to play the long game, if you have a capability to invest at scale -- that's the other thing that I think is quite unique about Moderna and the team will talk about it, the scale of that team and the capital that we have to invest in science and the strong alignment from the board to the management team that we want to keep investing in science. We are not done, we just think we are still at the beginning, and of course the amazing team that we have. But I think a point that is sometime not fully captured about our technology because it's a platform is, every time we have a new discovery in science, that new discovery can be used for all the drugs moving forward. If you think about traditional medicine, when the pharmaceutical invest in [restructuring] long program, that investment goes toward that one molecule. And if that molecule doesn't make it to market, which is a case of most molecules, that investment won't get the return. In our case, every time the team give us a new tool in the toolbox to make new medicines, we can apply that tools to all of our molecule looking forward. So that's a very, very powerful proposition that we have.

So with this framing and this welcome, I would like to call my partner in crime, Stephen, for him to introduce the team and the agenda for today.

Stephen Hoge - Moderna, Inc. - President

Thank you, Stéphane. So welcome, everyone. The agenda for today. So setting an agenda for Science Day at Moderna is quite a challenge, with over 200 scientists dedicated full-time to just the agenda of advancing our platform science. You can imagine we have a tremendous amount of progress that we make across all of the areas we're investing, which makes it quite challenging to select, To try and represent that in a single day. What we do, rather than try and run through everything we're doing and what we hope to accomplish today is we select a few vignettes, a few choice examples to give you a flavor of the things that we've been doing. And have -- give you a sense of why we're so passionate about investing in platform science and perhaps even the progress we've been making.

Now there are a number of publications we've pushed out every year, over 13 in the last year alone, as well as about 25 over the last couple of years. And the binders in front of you include a compilation of just of some of those presentations. Going through that itself would probably take more than the 3 hours we have today. So we'll just try and do this selection. As with last year, today's Science Day we divided into 2 broad categories aligned to what Stéphane described at the beginning of the opening. The first is mRNA science topics, things where we're advancing the potency and safety of our messenger RNA. And my partner in crime throughout the day is going to be the Chief Scientific Officer of our platform, Melissa Moore, and I'll introduce her in just a second. So Melissa and team will walk through those for the first roughly hour and a half of today, covering foundations in the immune -- of immune silence to drive the safety of our platform, how we've been improving potency, and then some advanced physics that we used in creating our nanoparticle technology.

After a brief break, we'll come back and we'll introduce some delivery science, the second big theme from our day. And specifically, I will be discussing -- I'll be back to discuss our advancements, creating an immune system delivery vehicle. And a few topics that we want to cover there. First, why the immune system? Why are we so passionate about this? Why do think it's important to talk about? What is the performance of our lead immune nanoparticle? And then we'll be joined by an academic colleague and one of our SAB members, Uli von Andrian from Harvard Medical



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School, who will describe some of the important features of the immune system and how you might apply this. And hopefully, that will be very exciting to talk about.

We'll close the day with a brief Q&A with any questions you have. So with that, I want to prepare to turn over to Melissa. Melissa is -- has been my partner in leading the platform. She's our Chief Scientific Officer for the research platform, for all the science that you'll see today. And she joined us a few years ago after a storied career in academia at UMass and Brandeis and other institutions where she led the academic community in many aspects of messenger RNA, molecular biology, including the spliceosome in translation. For that work, Melissa was elected to the National Academy of Sciences a couple of years ago, the highest honor you can achieve as a scientist in many respects. And we're incredibly grateful that she joined us and has been leading and advancing the state-of-the-art of our science. And I think over the next hour, you'll get a sense of the type of impact she's been having since she's joined.

So Melissa, would you come up.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

Stephen, and thanks all of you for joining us today. So I'm really excited to be able to tell you along with 2 of my colleagues over the next hour and half about some of the truly remarkable science that goes on at Moderna. But if nothing else, I will hope that you'll leave at the end of this section with 2 things. One is a realization that we care and dig very deeply into the basic science behind the structural function relationships that affect our mRNA potency. And secondly, that we are not ever satisfied with the status quo. So no matter how much, how good we are today, we are going to be better tomorrow.

So the first vignette that I want to talk about is a question that we often get and that is this question of, why do we incorporate modified nucleotides into our mRNAs and are they really necessary? And I can tell you, the answer for me is yes. But you don't have to believe me, so we're going to look at some data. And so I'm going to show you a set of data that we're currently writing up as a manuscript to be published in peer-reviewed literature. And this is the work of a large team of individuals across the platform, including cell biologists, immunologists, bioinformaticians and nucleic acid chemists. So the first thing that you need to remember about our messenger RNA drugs is that in nature, all endogenous mRNAs are made in cells in the nucleus. And they come out into -- out in the cytoplasm to be translated into proteins. But what we're trying to do, of course, is hack into the system, and we're trying to deliver mRNAs from the outside. And when we deliver mRNAs from the outside, we actually are delivery vehicles to the cell, look a lot like RNA viruses. And RNA viruses, which are the most common families of viruses that you're exposed to, the flu virus, the cold virus, Zika, those are all RNA viruses. Let's go through their life cycle a little bit. So initially, they will bind to the cell, they get taken up into endosomes, then they are released from endosomes, their RNA, their mRNA is released into the cell. The viruses have to do 2 things, they have to make proteins from their RNA, but they also have to replicate that RNA. And so they have a viral replication complex, which results transiently in double-stranded RNA because you have to make the second copy. And then the virus is able to then replicate and be released from the cell.

So there are 2 features, general features of this replication cycle that are very different from what is happening inside the cell normally. And those 2 features are RNA and the endosome. So our -- over evolutionary time, our cells have developed mechanisms and sentinels to detect RNA molecules coming in through the endosome because that's not something that the cell would see normally. And these RNAs and the endosomes are detected by these things called Toll-like receptors, we'll come back to those in a second. The other sentinel -- the sentinels, not a sentinel -- detect double-stranded RNA in the cytoplasm because, again, this is not something that is seen from mRNAs coming out of the nucleus. And so molecules like RIG-I and MDA-5 are very good at detecting RNAs.

Now what happens when these immune sentinels detect either RNA in the endosome or this double-stranded RNA? Well, they trigger the activity of the innate immune system. So the innate immune system is one of the 2 halves of the immune system. And you're going to get a treatise on this later today, so I won't go into too much detail. But what I want to just remind you is that the innate immune system, which is involved in recognizing common features of pathogens, interacts with the adaptive immune system, which is your immune system for adapting to new pathogens that your body hasn't seen before. And so part of kicking up the adaptive immune system is the innate immune system telling the adaptive immune system there is trouble coming. And so it -- their -- the adapt -- innate immune system activates the adaptive immune system.



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So when the toll-like receptors and the double-stranded RNA receptors recognize that there's incoming RNA, 2 things happen. One is translation in the cell is shut down for all intents and purposes. Well, this is of course not good for us because we want to get our mRNAs in and have them be translated. But the other thing that happens is that the innate immune system signals to the adaptive immune system and says get ready. And so it cranks up and you start to get inflammation.

And so let's talk about these toll-like receptors for a minute. So this is where -- it's been well established that the toll-like receptors, the key part of RNA that they recognize in, for RNAs coming from outside the cell are the uridines, so the individual U basis. And I -- and this is well established in the literature, I'm just showing you one paper here from the literature. This is a foundational paper, it has over 3,000 references. And I think it's particularly easy to understand.

And what you can see here in the lower left-hand corner is that the researchers took macrophages from mice, and macrophages are part of the innate immune system, they're the sensors, and they incubated those macrophages with different polymers of different nucleotides. And the only polymer that caused an interferon-alpha response, and interferon-alpha is a signal that is -- signals activation to the innate immune system, was the poly-uracil, so poly(U).

So none of the other nucleotides, none of the other -- polynucleotides gave any signal. The other thing that you can see on the lower right hand corner is that when they incubated those macrophages with either flu RNA or mRNA encoding GST or poly(U), again, the wild-type cells, which are the black bars, got -- had lots of activity. But then if they knocked out one of the toll receptors, that completely knocked out their response. So I think this is very good evidence that the toll receptors are involved in this response and also that uracil is the key molecule. So let's take a little closer look.

So here is a structure of another toll receptor -- toll-like receptor. And toll-like receptors are monomeric molecules that dimerize. And you can see a picture of a dimer here. And they dimerize based on binding of uracil at the interface of a dimer.

And so this is a -- on the right-hand side is a picture of how the uracil base sits into the dimer interface of the toll receptor. So we can combat that interaction by actually just making the uracil base a little bigger, just putting a bad bump. So now the toll-like receptor cannot bind because of the bad bump on her molecule. And the particular modification that I'm showing you here is 1-methylpseudouracil or 1-methylpseudo(U). And that's what I'm going to be talking about for the rest of the time because this is now our preferred modification.

So here is that experiment where we took a (inaudible) nucleotide composed of either 19 copies of U or 19 copies of 1-methylpseudo(U). And we injected that into mice and then looked at the frequency of the activated B cells. So if you remember, I told you the innate immune system talks to the adaptive immune system and activates the adaptive immune system. And what you can see here is that if we put in the 19 nucleotides of U, and that's fully synthetic U, then we do get a response. But if we put in 19 -- the polymer of 19 1-methylpseudo(U), it's absolutely no response, it's clean. It's the same level as PBS control. Now we can go one step further by taking the spleens out of these mice and using a -- analyzing the gene-expression profiles from those in the spleen. So now we're looking at the changes in a bunch of different genes that are showing on the right-hand side. And the way to read this heat map is shown in the color key. But the key here is that the 1-methylpseudo(U)19 looks exactly -- the pattern there looks exactly like the PBS. Each of one of those columns is an individual mouse.

Okay, so what about mRNA? Now mRNA, we cannot make by a full chemical synthesis. It's just physically impossible to do because our mRNAs are thousands of nucleotides long. And so the state-of-the-art for making our mRNAs is that we make first a DNA copy, a DNA plasmid, and then we take that plasmid, add an RNA polymerase and the 4 nucleotides, so either ACG and U or ACG and 1-methylpseudo(U) and do what's called a transcription reaction. It's called transcription because we're copying the DNA into many, many copies of the RNA. And in this case, our desired RNA is coming from the top strand. So that means the RNA polymerase is moving left to right. However, just like any manufacturing product -- process, there are often -- there are sometimes undesired byproducts. And so occasionally, the polymerase will go in the wrong direction, it'll go right to left. And we'll end up with a few molecules of the bottom strand. And that bottom strand can then hybridize with the top strands and create double-stranded RNA.

Now if you remember, this is a problem because this is what viruses do. And the cells have sentinels to recognize this double-stranded RNA. So getting -- making sure that we're not making double-stranded RNA, our process is something that's very important to us. So we have developed



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highly sensitive assays to detect double-stranded RNA in our RNA molecule, in our RNA preparations. And I'm just showing you one here, this is called an ELISA assay, it utilizes 2 different antibodies that -- both of which recognize double-stranded RNA and can give out a signal of how much double-stranded RNA is in any sample.

And so what I'm showing here is the amount -- using this ELISA assay, the amount of double-stranded RNA that we -- is found in transcribed mRNAs using a legacy process. And by the legacy process, we mean what is in the scientific literature, so what people typically do. So it would be essentially what I used to do in my academic lab. And there was a lot of double-stranded RNA. Not huge amounts because it's nanograms per micrograms, so it's a very small quantity. However, at Moderna, what we've done is, over time, we have honed our synthesis and purification processes for our mRNAs to basically eliminate all of the double-stranded RNA.

So when we then take this, our double-stranded RNA and, in this case, transect Human Peripheral Blood Mononuclear Cell, or PBMCs, with our mRNA, and now here we're looking at another marker of innate immune activation, IP10, you can see that the legacy process, again, has very high activity of the innate immune system. Our Moderna process makes it better if we have U, but not quite all the way down. But if we combine our Moderna process with 1-methylpseudo(U), now we have almost no innate immune activation at all.

Going in vivo now, again, I've shown you this data before when I -- we were looking at the little polymers, the 19 nucleotides.

So now if we you add in our messenger RNA, you can see that our messenger RNA that is made with 1-methylpseudo(U) and by our process has innate -- has a activated B cell frequency that's very similar to the PBS control.

And finally, if we then look once again at our most sensitive assay, which is looking at the changes in gene expression within the spleen, what we can see is that, on the left, the legacy process red, it really has a lot of gene expression changes. But if we compare the 1-methylpseudo(U) Moderna process with, again, PBS control, there's -- the patterns are very similar.

And so I think, I hope that these data have convinced you of 3 things. That in order for mRNA therapeutics to be successful, they must avoid the innate immune sensors. And that global substitution of U with 1-methylpseudo(U) results in immune silent mRNA. And that also we have developed processes that result in highly, highly pure mRNA. So I hope that this vignette has put this question to rest.

Yes, we do need modified nucleotides. We believe in building belts and suspenders into our mRNAs to increase their safety, increase their potency. And I've shown you the data why.

Okay. So onto the next vignette. So next I want to turn to our engineering efforts on mRNA. And -- so in order to understand this part, and I know some of you have biology backgrounds and some of you I might have even taught biochemistry to back in the day. But as a professor, my job is to make sure that we're all on the same page. So first, I just want to give everybody a little mRNA anatomy lesson.

So this rod structure that you're seeing here is a common way that we represent our messenger RNAs. And they -- this is to show you that they have different parts. And the main -- the really important part of an mRNA is what's called the coding sequence. And of course, that is the region of the mRNA that encodes the protein that has the instructions for -- that the ribosome uses to make the protein.

On either side of the coding sequence are the so-called untranslated regions, or UTRs. Now we call them 5 prime and 3 prime because that's our lingo for the beginning and the end of nucleic acid. So 5' is the beginning, 3' is the end. It doesn't matter for this talk why we have that crazy nomenclature, but that's what it means.

And these untranslated regions, as we'll talk about a little later, are important for regulation of the expression of the protein. And then at the very ends of the RNA, at the 5' end, there's a thing called a cap; and at the 3' end, there's a poly-A tail. And these features are important for protecting the RNA from Pacman degradation that will degrade the RNA, as well as helping the translation apparatus join the RNA.



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So when we think about engineering our RNAs, we of course -- what we -- one of the things that's really important to us is that the ribosomes start at the right place. The translation always start at the right place, and we call this translation initiation fidelity. And then of course we want the coding sequence to be faithfully decoded and the ribosome translation needs to stop at the right place.

In general, we want our mRNA medicines to be as potent as possible, so we want our mRNAs to make as many copies of protein per unit time. And the numbers here are pretty amazing. So endogenous RNAs are translated. So 1 mRNA molecule can generate anywhere from just a few protein copies up to over 10,000 protein copies. So they translate it over and over again. So of course to make the most potent medicines, we'd like to have our mRNAs translated over and over again, and they should last as long as possible. So we want to increase their functional half-life. Then we also want them to be translated in the desired cell type, and we often do this by changing some features in the UTRs, I'll talk about that a little later. And then tailored to the particular protein type. So different proteins had to go different places in the cell. And it turns out that we can manipulate the RNA sequence to preferably put our proteins in the right place in the cell.

So this next vignette I want to tell you about is a manuscript that's currently in bio archives on coding sequence engineering and involves a multidisciplinary team from the computational sciences department, from molecular biology and from our technical development department.

So again, I want to remind you just so we're on the same page about translation. So why do we call it translation? The reason we call it translation is because it's the process of going from one language to another. So the -- in RNA, the language of nucleic acids, proteins are the language of amino acids. And just like English has different -- has a structure to the language, the language of RNA has a structure. So for example, the start of an English sentence is a capitalized word. The start of a protein is the first AUG downstream of the cap. So AUG means start.

Now in nucleic acid language -- I should say in English language, the words then are different lengths of groups of letters, but they're separated by spaces. There are no spaces in nucleic acid. So in nucleic acids, all of the words are the same length, they're all 3 nucleotides, they all have 3 letters. And so the ribosome, once it starts translating, just goes 3 letters at a time. And each of those words then encode a particular amino acid, and those words are called codons. And then obviously, the translation machinery needs to know where to stop. In the English language, we know that the end of a sentence is a period. In the language of nucleic acids, it's one of the 3 codons called the stop codons.

Okay, so when you think about the various features, how long is the coding sequence going to be? Well, that's pretty simple because however long the protein that we're going to make is in amino acids, the coding sequence is exactly 3x that in nucleotides. And what I'm showing you here are all of our experimental medicines, mRNAs that we put on our website and their -- the length of their coding regions. And so you can see that they range in size from around 400 nucleotides up to well over 3,700 nucleotides. So that's the length, and that's easy to figure out.

But the problem becomes how do we decide what sentence to use. Because of course, in English, we can use [many] synonyms that [mean] the same thing. And this is also true in nucleic acids. (inaudible) 20 amino acids in proteins, but there are 61 codons left to encode those amino acids. So there are synonyms [that mean] the same amino acid.

So for example, trying not to point, it's really hard to not do that. If you look at proline there, it has 4 different codons that all mean proline. Whereas lysine only has 2 different codons or synonymous codons that mean lysine. So you could use any of those sets of codons. And so just in this simple 5 amino acid stretch, there are actually 128 different combinations that would result in making the same protein. So you can see then that the number of codon choices is a function of the number of codons that -- or number of synonyms for each amino acid times the number of times that amino acid appears in the protein.

So how big do these numbers get? Well, just to give you a sense, the typical human protein has a medium length of 416 amino acids. If you do the math, that means that there are 10^{201} possibilities. Now that's a big number. But how big is that number? The estimated number of atoms in the known universe is only 10^{80} . So it's really a huge issue of how do we pick the best coding sequence.

So how do we pick the right coding sequence? So do we randomly choose synonymous codons, or we just use synonyms at random?

If we did that in English, it wouldn't really sound good, right? Do we weight this synonymous codon choice by the used frequencies [in] natural human mRNAs? So we can just say, okay, well, this is what nature does, let's repeat nature. Or maybe we should use only the 20 most optimal



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human codons. And you can think of this as, in the English language, you can just use always the same synonym and never vary your sentence structure, but use the one that's used most often. And this is what happens when -- as people have used Word and the thesaurus in Word, Microsoft Word, it only has about 200,000 different words in it. And so the English language utilization is actually getting smaller because people are using Microsoft Word's thesaurus. So I actually suggest and push people to use the one -- the other thesaurus on the web. It's much better.

What about secondary structure? So should we minimize it, maximize it or something in between? So now I need to tell you about 2 other features and that is optimal codons and secondary structure.

So let's talk about codon optimality first. So every time that the ribosome has to put in a new amino acid, the way that it goes about that is it has to sort through a bunch of molecules called transfer RNAs. And these transfer RNAs, the bottom part of the transfer RNA binds to the messenger RNA and when it's correctly paired, makes a connection. And then that allows the top of the tRNA, the transfer RNA, which holds the amino acid, to then transfer that amino acid onto the growing chain.

And so you can see here, we've -- I'm representing the abundance of 2 different transfer RNAs, a blue one and a red one. And you can see that there are many more copies of the blue one than there are of the red one.

And so it should take a shorter time for the ribosome to find a blue one than it will take to find a red one, right? Because it's just randomly sorting through these things. And so a blue codon we can imagine is an optimal codon, and in general the ribosome will tend to go more quickly when it has optimal codons. But when there are red codons, which are suboptimal, the ribosome takes longer time to sort through the tRNAs and so will go slower.

So one of the things that I've just been showing you is I'm showing you a ribosome. But I want to point out that in most cases, the mRNAs have multiple ribosomes on them and -- at a time and so these are called polysomes. And so here is a picture of a polysome that was taken in 1989. It's just an EM picture. The line that connects all the ribosomes is the messenger RNA. The blobs are the ribosomes and these chains coming off are the proteins that are getting made.

So you can imagine that one of the challenges for polysomes is very much like traffic going on a one-lane highway. So if you have a lot of cars going down a one-way road, then those cars need to make sure that they're properly spaced apart so that they don't crash into each other. And if you're driving on a one-lane road, you have the advantage that you can see the tail lights of the car in front of you. But the ribosomes can't tell whether the ribosome in front (inaudible) fast it's going or not.

So when there are slow stretches with -- like with a bunch of slow codons, you can get a ribosome traffic pileup, a collision. And in fact, here is a structure of collided ribosomes. And once ribosomes collide, the first one cannot go forward, the other one -- they can't go forward or back. So it is a stuck complex. And so cells have many mechanisms to resolve these but one of the key mechanisms is to just cleave the mRNA to then release the ribosomes from the mRNA. But of course, that's undesirable for us, because it destroys our mRNA and results in a lower half-life of our mRNA.

So we would like to avoid ribosome collisions. We need to space -- to find ways to space out the ribosomes to have them going as fast as possible, but space them out so that they don't collide with each other.

So that brings us -- me to another key feature of RNA, which is its secondary structure. And so this -- going back to our little cartoon of the messenger RNA, what I'm doing now is showing you just a zoom-in on 4 of the nucleotides that make up this messenger RNA which could be 3,000 nucleotides long. So this is a close-up. And the nucleotides, we've talked about the backbone, which is the sugar-phosphate backbone, that's shown in red here. And then the bases, which are the parts that are the -- or the information-containing parts of the molecule.

So RNA doesn't like to just hang out with the bases, just in contact with water. They really prefer to interact with one another. They're very social. And so what happens is that these bases will find other partners elsewhere within the molecules to base pair with and form secondary structures. And these secondary structures can be quite complex.



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Now what I'm doing here in going from the bottom left-hand corner to the middle, is going to a different representation of the secondary structure. Because if we -- if I keep showing you all the atoms, we're quickly going to -- it's going to get too complicated. So we tend to draw simple secondary structures like this, where the bases are these lines and the ones that are pointed at each other are base paired with each other.

But this is only a little piece of this mRNA. In fact, the entire mRNA, which in this case is only 760 nucleotides, would -- actually will form this structure. And here, the secondary structures are represented as the sort of rods and then things that are not interacting with each other, so not base paired, are single-stranded regions. And you can see the loops there. Okay.

So if you think about this -- and this is really what the ribosome sees. It sees this -- it doesn't see this linear molecule, it sees this highly structured molecule. And so -- in -- back to our analogy of driving down the road -- driving down a single-lane road, this is like driving on a mountain road. And driving on a mountain road with twists and turns, you've got to go more slowly. And so this structure of an mRNA -- this secondary structure of an mRNA, can also control the speed of the ribosomes.

And so what we wanted to know is what's the right balance for maximal protein output? So do we try -- do we maximize codon optimality? Or do we maximize or minimize the structure of the RNA? And maybe there is some combination of the 2 that's really best. Now one of the things to note is that the structure of the RNA, the structure to adopt is a function of its sequence.

So -- but also the codons and what sentences you're using are also a function of the sequence. So by changing the sequence, we're changing both the codon optimality and the structure.

So -- but we can calculate these things. So for the structure -- excuse me, for the codon optimality, here's a table of codon optimality. So there, you can just think of this as the more blue it is, that's a faster codon. The more red it is, it's slower. Not all amino acids have really fast codons. So there are some amino acids that are naturally slow or naturally just medium speed. But behind this table, there are numbers that we can assign to codon optimality. And we can also calculate what's called the minimum free energy structure for any particular mRNA sequence. And so here, what I'm showing you are 3 different mRNAs that encode the same protein, but they have different sequences for encoding that protein and so, therefore, adopt different structures. The lowest structure, you can see lots of loops; whereas the one with the high structure, you see many more sticks and bars and fewer loops. And the way that we can quantify this folding energy is by something called delta G, which is a negative number. So the more negative delta G is, or take-outs per mole, the more structured something is.

So we decided to explore the relationship between codon optimality and folding structure. And the way that we did this is to calculate 4 GFPs. So this is green fluorescent protein. It's a relatively small protein, but remember that I told you that there are -- just wait, there are many, many possibilities. So what we did is calculate for 250,000 different variants. If we used an algorithm that just randomly chose codons, then we ended up with a cloud of molecules that were essentially at relatively low structure in about the middle of the codon optimality scale. If you instead use an algorithm that weights the codons that you choose to the human genome, you get a little more optimal -- that makes sense, but not too much structure. But it really doesn't allow you to explore the entire space. And so what our computational scientist did was to tweak our algorithm to really push the -- it in as far out as we could. And we ended up with this cloud where we've now fully explored the limits of codon optimality and RNA structure.

So in order to then assess how the codon optimality and RNA structure related to protein output, what we did is we picked 6 different locations within this cloud and we made 5 different mRNA sequences for -- in each of those locations. And so at the bottom left-hand corner, you can see the brown square shows you the slow codons and low structure. And the top right-hand square, which is green, shows you optimal -- where you're really up in that top right-hand corner, most optimal codons, highest structure.

So the question is, what's going to give us the most protein output? So in order to measure this, we are using a -- we're doing this in a cell-based assay and we're doing kinetic expression in cells. And so we've taken GFP, green fluorescent protein, and we've added a little tag onto the end of it that recruits the proteasome. We did this because it makes the GFP protein very unstable. So it quickly goes away. And so here, you can see on this movie, this is a 72-hour time lapse movie of that eGFP expression and HeLa after we apply our mRNA. And so what this allows us to do, by using some fancy math, is to reduce the GFP expression to an up -- to a curve. And we can then model this and calculate the translation rate, which is the upshift or the first part of the curve and then the mRNA half-life, which is essentially the downslope of the curve.

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And so we can do that for RNAs that -- also another RNA that also encodes the eGFP, but now it's a different sequence, a different coding sequence. And as you can see here now, if we compare these 2, the one on the bottom has a shorter half-life than the one on the top. And again, we can convert that to a graph that we can then take these numbers from.

So if we do that -- and what I'm showing you here in the bottom right-hand corner are those -- one of those curves. And what I'm comparing here is the -- on the Y-axis is the relative area under the (inaudible) amount of protein that was produced to the initial rate of translation. And what you can see is that there's really not very good correspondence between that initial rate of translation for these different -- all these different RNAs and the amount of -- total amount of protein that's produced. But what -- where we do get incredibly good correspondence is in the half-life of the messenger RNA, how long the messenger RNA lasts. And so by varying the amount of structure and the codon optimality, we can vary half-life of the mRNA by almost 15-fold here.

So when we look at total protein, and now let's go back to our colors, what you can see on the lower left-hand corner, the low and slow really gives the least amount of protein. That is undesirable for us. Where we got the most amount of protein produced always was when the structure was the highest. So putting those speed bumps in there, those curves really gives us a much higher amount of protein. And so we now -- we utilize this information in our algorithm to design new sequences because we're now always maximizing the structure and generally having the codons be either mid or optimal.

So in summary for this section, we've used the computational design to thoroughly explore the relationship between codon optimality and secondary structure. And as I said, this is a manuscript that is available by bioRxiv. And we now have a new tool for dialing in the desired protein output.

Okay. So I've been telling you about the coding sequence and how we're optimizing the coding sequence. But next, we're going to switch to talking about the untranslated regions. And Ruchi Jain, who's a Principal Scientist in our Molecular Biology Department, is going to tell you about that -- her work there. But first, I want to tell just briefly what these untranslated sequences do.

So the untranslated sequences contain key [regulatory] information. The -- you can think of the 5' UTR as the ribosome on-ramp. So that's where the ribosomes initially load and Ruchi will show you an animation of this. And the 5' UTR can be relatively unstructured. It could be structured. And you can imagine that those -- unstructured versus structured, it's the same deal. It's going to affect the rate of ribosome loading. Also, there are regulatory proteins that bind in the 5' UTR to modulate the rate of ribosome loading in different cell types and under different conditions. In the 3' UTR, one of the features that is important in many mRNAs are microRNA binding sites. And microRNAs are small RNAs that regulate both the translation and the decay rate of messenger RNAs. And different cell types have different repertoires of microRNAs. So this is a key regulatory area. Another thing that can happen in the 3' UTR is that there can be structures that bind particular proteins. I'm showing you a cartoon here of a protein that binds kinesin. Kinesin is a molecular motor that runs up and down the microtubules in cells and actually can transport mRNAs to different locations in cells. So this does not in any way cover all of the things UTRs do, but I just wanted to give you a flavor of that.

So now I'm going to invite Ruchi up to the stage to tell you about UTR optimization.

Ruchi Jain - Moderna, Inc. - Principal Scientist

Thank you, Melissa. So now all the advances I'm going to show you today are collaboration between molecular biology, computational science and (inaudible) teams. And I'm very grateful to them for all the work we've been able to do together.

You just heard from Melissa how we are putting a lot of effort into optimizing our coding sequences to make the most amount of protein. Today, with our sequence engineering platform, we are already seeing substantial boost over the native coding sequences and we look at a given mRNA expression. That being said, at Moderna, we are always wondering can we do even better? To that end, now I would like to draw your attention towards the untranslated regions of our messages.

Before I get started, I just want to show you how translation actually begins. How translation begins is at the small sub-unit of the ribosome, which is half of the ribosome, [stats that scan] along the 5' UTR looking for an AUG or the start codon to start protein synthesis. Once it recognizes this



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AUG, the other half of the ribosome is recruited and protein translation begins. This protein translation now continues until you encounter the stop codon, where the functional protein is then released.

Now over the years, a lot of work has been done around the 5' UTR and we understand at least some aspects of what makes a good 5' UTR. That being said, our understanding today is incomplete. So what we did is we initiated a collaboration with Georg Seelig lab at University of Washington, and together we built a library of hundreds of thousands of different mRNA variants. All these variants encode the same protein but differ only in the 5' UTR. After making this library, we translated the entire library in a cell and evaluated translation efficiency of all the sequences together using a technique called polysome profiling. Polysome profile looks something like a -- sorry, polysome profiling is a technique which allows you to count the number of ribosomes that associate with a message and -- sorry, I'm just going to go back. I'm sorry -- okay. We're back. So polysome profiling is a technique that allows you to count the number of ribosomes that associate with a given message. And in these polysome profiles, the messages that associate with less number of ribosomes are found on the left, while the messages that associate with more ribosomes are found on the right. So what you can do is look at all this data from the polysome profile and then [seed it] to train a neural network and then ask this neural network to predict or evolve new and perhaps better 5' UTRs that engage with higher number of ribosomes. During this, what we are able to do is assess that this evolved 5' UTR from the neural network actually outperformed the random library on which they were trained as well as a selection of human 5' UTRs that we evaluate them.

So what did we learn here? We learned that it's possible to evolve 5' UTRs that associate with many ribosomes. But is that all that matters? No, that's not all that matters. It's also important that we always make the right protein from our messages. Now it's been known for years that translation initiation is not always perfect. It's possible that while the small ribosome subunit is scanning a lot of the 5' UTRs, it misses the first AUG and continues scanning downstream. This is a process called leaky scanning, where the small ribosome subunit might recognize the downstream AUG and then start translation there. As you can imagine, what would happen in this case is you could have -- make a truncated and likely nonfunctional protein. Every time you're making a nonfunctional protein out of your message, you're losing out on the potency of your messenger RNA.

So what we at Moderna have done is taken a 2-pronged approach to try and solve for both getting more protein and always getting the right protein from our messengers. The way we went about this is we need a library of 2 billion 5' UTR variants and evaluate them simultaneously for 2 features. We select 5' UTRs that give us both high translation and high initiation fidelity, and then we combine our learnings from this library with another smaller rationally designed library, which learns from literature experiences and our own Moderna-enhanced experiences. We assess all of these 5' UTRs extensively in a lot of cell-based assays and then we choose the winners from these lead sequences and evaluate them in vivo in rodents. Finally, we want to see how our UTRs are performing in disease-relevant animal models before we select them.

Now this endeavor generated a wealth of data, which I've tried to condense very simply into a graph. What you're looking at here is data from 2 different cell sites, HeLa, that's human cancer cells; and mice liver cells. What's [shocking] is on the X-axis, we are looking at expression. On the Y-axis, we are looking at initiation fidelity. All the data is normalized to a standard 5' UTR. What you can see is the standard 5' UTR already does quite well. In most cases, it's giving you significantly high expression and significantly high initiation fidelity. Also, a lot of times when you start to [regain] some expression, it actually comes at the cost of fidelity. All that being said, we were very happy to identify a few select UTRs in the upper right-hand quadrant, which is suggesting both high translation initiation fidelity and high expression.

Now that I've told you a little bit about the 5' UTR screening effort, I also want to draw your attention to the 3' prime untranslated region. Unfortunately, I won't have time to actually go into an entire screening strategy here, but I would like to show you a little bit of what we've been able to do. Of the many features that the 3' UTR can confer that Melissa already mentioned, today's talk, the focus is going to be only potency.

Now what I can show you is with our 3' UTR leads in cell-based assays shown here, we are already seeing improvements in overall protein expression compared to Moderna standard sequence. Notably, the data looks very nice, especially when you start to look at the activity where the gains over Moderna standard sequence, the magnitude is even higher than what you see for protein expression.

Now what does this actually mean for future medicines? To this end, now I would like to show you data in diseased mouse models and show you what our UTRs can do here. So in this particular case, we are showing you a study where we dosed mice twice on day 0 and day 14. The first thing I'm going to show you is protein expression data from day 15. Again, you see that the new, improved Moderna sequence outperforms Moderna standard sequence in terms of overall protein output.



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To truly appreciate the magnitude of the benefit though, you have to see what happens after the first dose. So what I'm showing you here is what happens to the biomarker that we care about in this disease. The clinical goal here is to reduce the biomarker to at least 40% over mice treated with controlled, nonrelevant mRNA. What you can see is that the standard sequence, we are already appreciably below the 40% required for clinical efficacy. With the new Moderna improved sequence in blue, we also reached that significant level. Notably, with the new, improved sequence, this duration of efficacy is extended out significantly further. And even on day 7, you maintain appreciable reduction in the biomarker levels.

And finally, the last data set I want to show you is what happens when we start to combine our 5', 3' UTR leads in disease models. In this case, I'm showing you a study where we dose mice once on day 0 and then follow them for a month and then again dose them on day 31. And 24 hours later, we look at RNA, protein and activity. Looking at all these, you can already see that Moderna-improved sequence is giving you about a twofold boost at all these levels.

Now I want to show you what happens to the biomarker. The first data point I'm showing you is about 2 weeks after we dosed. Again, the goal in this study is to lower the biomarker level. What you can see is with both the Moderna standard sequence and the improved sequence, we maintained this lowering of biomarker for at least 2 weeks. The Moderna improved sequence actually reduces it even further even at this early 2-week mark. [Excitingly], this benefit actually extends at least up to a month. That's the last time point we took, where again you see that the improved sequence actually gives you an increased duration of efficacy. This is perhaps most evident when you start to see what happens to the body weight of these mice. You can see that controlled mice treated with a nonrelevant RNA rapidly lose body weight, where the standard sequence maintained body weight for 2 to 3 weeks, and with the improved sequence at least after a month if not even more.

I just want to close by saying that when you look at our messages, the coding sequence, the untranslated regions, there is a universe of agents to choose from. I think by asking the right questions, by checking billions of variants if we have to, we can incorporate desired features in our messages. Today, what I've shown you is [by optimizing] along 3 specific parameters, we are able to increase the potency of our drugs and extend the duration of efficacy. We are moving towards lower doses, lower dosing frequencies and better patient experiences.

Thank you.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

Well, thank you, Ruchi. So I again emphasize that we go to tremendous lengths. You heard her talking about first hundreds of thousands of different sequences and then millions of different sequences that we sorted through to find the very best.

So now I want to change gears. So we've talked about coding [sequences], we've talked about UTR, and our last vignette today in this long lecture -- I'm proud of all of you for staying with us, is going to be about our delivery vehicles. So (inaudible) we have different routes of administration for different modalities. Most simple route of administration, which is shown on the bottom left-hand corner, is intracardial injection. This is the route of administration that we're using for our VEGF program with AstraZeneca, injecting directly into the heart muscle with naked RNA. The other routes of administration that we use are IM injection for vaccines, intratumoral injection for some of our immuno-oncology products, and then of course injection for targeting the liver for rare diseases. Now for all of these latter 3, because they are not going -- somehow (inaudible) able to take up mRNA -- naked mRNA. But for all of the other delivery mechanisms, we need to encapsulate the RNA in a delivery vehicle so that the RNA (inaudible) destroyed before it gets to our desired cell types and to direct it to our desired cell types.

And so our delivery vehicle of choice are lipid nanoparticles. And lipid nanoparticles are composed of 5 different molecular components, and ionizable lipid; a sterol, usually cholesterol; a phospholipid; a PEG lipid; and then of course our messenger RNA. And in the past year, we have published peer-reviewed papers, which are contained in your books that you were given, showing that we have developed different lipid nanoparticles that are optimized for liver delivery and, in the one case, for rare diseases and then also an optimal LNP for IM delivery for vaccines. So we have -- we actually invest a tremendous amount of resources into developing our new delivery vehicles. In fact, sometimes I think we're more of a delivery company than we are of a RNA company. As you'll see in a minute, we do some crazy things.

And so a huge fraction of the research platform, the Delivery Sciences and the Discovery Chemistry Departments, are devoted to making new delivery vehicles. How do we do this? So we do this by changing the molecules. So we can change the chemical structure of any of those molecules.



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We can change the composition of the molecules. So we're mixing -- how much ionizable lipid we mix in per mRNA molecule, for example. And then we can also change the process, in what order do we add things, how do we mix them together, what pH, what buffer, et cetera, et cetera. And so by changing all these vectors, we can then end up with new lipid nanoparticles that have desired features. And of course, our desired features are that the lipid nanoparticles be chemically stable, they be physically stable, that they have the desired biodistribution. And as Stephen will be telling you in the next half, we have a new nanoparticle that targets the immune system, for example, that they're efficient at cellular uptake, that they will release the mRNA from the endosomes, and that they will of course allow for as much protein expression as possible.

So as we're changing all of these potential variables, obviously, the reason that function is different is because we're changing the structure of the lipid nanoparticles. And so the gray cartoon is a structure that we've been showing for the last couple of years, and it really is an artist rendition of what a lipid nanoparticle might look like. But is this what they really look like? So maybe they're completely unstructured and completely randomly organized. Or maybe the lipids form a bilayer on the upside, and then inside of that, it's more or less unorganized. Or maybe they're multilamellar structures that -- where the RNA is sandwiched in between multiple layers of lipid bilayers. So we are very interested obviously to know what the structure of our lipid nanoparticles are and then how are the changes in our processes changing the nature of our lipid nanoparticles.

So how are we tackling this LNP structure? And it gives me great pleasure to be able to say we do a lot of physics. So we do much more physics than -- and invest tremendous resources into looking at the physics of our LNPs. We do cryo-electron microscopy, and we take images of our LNPs. We use atomic force microscopy. This is a physical technique where it's kind of like the old LP record where you have a needle and it just feels the shape of things. So this can give us information about the surface of our LNPs. We use -- we have our own NMR instrument. And this is -- I'm showing you a proton NMR spectra of one of our LNPs. We bought ourselves our own x-ray gun. So that is our small-angle x-ray scattering machine, or SAXS machine. And this could give information about how the certain molecules are arranged on the surface of our LNPs. But that's not enough. We actually even go down to Brookhaven National Laboratory to the synchrotron and submit our LNPs to small-angle neutron scattering to get even more information. And so by combining all of these different types of physical analysis of our LNPs, we're really developing a very comprehensive picture now for when we change the -- either the chemical makeup or the composition or the process, how that changes the structure of our LNPs. And then we're building a large database that will then allow us to relate those structural changes to functional changes. And so then that should allow us to make -- ultimately do rational design of LNPs to get the functions that we desire.

But as we have been studying the ultimate structure of our LNPs, what we realized is that so much of how the LNPs actually function and their structure in the end is dictated by the process by which they are made. So how is it that these molecules that I'm drawing over on the left actually assemble into these large structures on the right? And that's been a black box. Now why is it a black box? Well, here's another physical technique that we use to study our LNPs, and this is dynamic light scattering. What dynamic light scattering does is it allows you to look at a population of particles in a sample and it will tell you the size of those particles by how much they scatter the incident light. And so you can see on the bottom is time in minutes. And so using this technique, we can watch over time as our lipid nanoparticles grow in size. And we -- so one of the things that we do in manufacturing our LNPs is that we can stop this process at any time along it in order to modulate, to control the size of our LNPs, so we can stop it by changing the buffer conditions, for example. But if you look right beside where the molecules went in, so at 0 time, you can see there's a blank space there. And that's because the earliest time point that we can get with this technique is just a few minutes. So we're blind to what's happening in those first few minutes.

And so now if we change the time scale from a linear scale to a logarithmic scale, and now what we're showing here is going all the way back to the first picoseconds, that's 10^{-12} second, after we mix the RNAs together -- or the components together, this is where our black box is, where we really don't -- we haven't had vision into that because it's just -- the time frame is just too short. And so this is the time frame in which molecular simulations can actually teach us a lot about the dynamics of what is going on.

And so what I'm going to do next is ask Michelle Lynn Hall, who is a Principal Scientist in the Computational Sciences Department, and she is a computational chemist, to come up and tell you about our efforts in coarse-grained modeling dynamics.



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Michelle Lynn Hall - Moderna, Inc. - *Principal Scientist of Computational Chemistry*

All right. Thank you very much, Melissa, and thank you guys as well (inaudible) for sticking it out. Before I begin, yes, I am from the Computational Sciences Department, but this has been extremely multidisciplinary and collaborative across different departments, including with our Delivery Sciences Department and our Technical Development Department as well.

Now Melissa introduced the idea of a lipid nanoparticle, and this is an artistic rendering of what those look like. And what I want to drive home is that these are extremely complex and dynamic molecules. I'm showing in the top right just one of the many lipids, the many components that make up that lipid nanoparticle to drive home precisely how dynamic just that one component is. But let's think about this in context. That lipid is but one component of this very large molecular assembly. Lipid nanoparticles are much, much larger, so 2 nanometers versus 80 nanometers, and they are an accumulation of about 60,000 different molecules.

So that lipid is one, the lipid nanoparticle assembly is 60,000, not including water, not including ions.

And so when we think about this holistically as an ensemble of all its different components, you really begin to appreciate how extremely complicated and dynamic a lipid nanoparticle may be. And those dynamics are precisely what we want to study, as Melissa told you, using molecular simulation. What the foundation of everything that we do is fundamentally quantum mechanics, and the reason for that is I think really well encapsulated by one of my favorite quotes -- because I'm a nerd, by this physicist named Paul Dirac that was written over 100 years ago. And what he essentially says is all chemistry is known. It's known. We already have the answers. The problem is the equations are too complex to be solved. And that equation of course is the Schrödinger equation. Now this is not just another physics equation. This is, in my very biased opinion, the physics equation. And the reason for that is it is literally the most physically complete description of a system that is possible. So over 100 years ago, we knew this equation, and here I am standing talking to you because this problem is analytically unsolvable for anything but the most trivial system.

So what do we do? We're interested in understanding this equation for things like lipid nanoparticles so that we can understand their structure, their function, their dynamics, their biodistribution, et cetera. But we can't solve it analytically. So what we end up doing are making approximations. For example, Melissa talked to you earlier about nucleotides. Those are relatively small on a molecular scale. So we'll study those using quantum mechanics. As we transition to larger systems, for example, RNAs, proteins or really large lipids, we'll transition to a more approximate method, all-atom molecular dynamics, and that's the simulation that I showed you earlier about single lipids. But we began to be curious about whether or not it would be possible to study even larger systems. Systems, for example, like a virus capsid, which, as you heard earlier, is really similar in size, shape and function to a virus. And while it's technically possible to study lipid nanoparticles using all-atom molecular dynamics, it's impractical to do so if you want any realistic turnaround time, which is what we wanted. So what we had to do was bring in a completely new method in-house, and that method is called coarse-grained molecular dynamics.

Now I want to emphasize that we're not inventing a new method here. Coarse-grained MD has been used in other applications in the field of soft matter physics. What we're doing is applying this technology in particular to the study of lipid nanoparticles and drug delivery, really pushing the boundaries of what we think is possible in an industry setting for rapid turnaround and development of these lipid nanoparticles for delivery.

Now I want to explain a little bit about the secret sauce of how this works. Imagine your standard lipid. What I'm showing here is called an all-atom representation. This looks just like a ball-and-stick model you might have built back in organic chemistry class. Now what we do in coarse-graining is simply preserve all the qualitative characteristics of that molecule, but subsume some of the atoms into what we call pseudo-atoms or beads. So you can see we're effectively reducing the complexity of the system without compromising too much on the qualitative characteristics, allowing us to simulate larger and more complex systems but without enduring insane computational cost as a function. And this has been used to study other biologically relevant systems, including biomembrane fusion, biomembrane curvature and deformation, as well as lipid droplet formation and association with proteins. And so with all of this foundational work being laid, we were really excited to apply it to this problem of lipid nanoparticle discovery, in particular casting a lens on the very early stages of what this looks like, as Melissa showed -- mentioned earlier since we don't have those insights at present.

So what I'm going to show you here is one of our simulations. Before I begin, I want to just lay out what you're about to see. All of the different components of the system are shown as colored on the right. There are water and ions present in the simulation, but they are not shown just for simplicity. And what we're going to do is watch the simulation from p equals 0 until to the end of the simulation, which is only 6 microseconds.



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Now 6 microseconds does not sound like a long time, but in a simulation standpoint, that's basically an insane amount of time. And this took 700 hours to run, and that was only possible because we were able to put this in the cloud and run it through Amazon Web Services. And I'll say one final thing before I begin, which is that you may notice that we are not using a full-length mRNA here. We are using an RNA oligomer. And that's for a technical reason, but we're already starting to evolve to larger and more complex RNA.

So let me walk you through this. What we have right now looks like a random assembly of molecules, completely random. And that's because it is a random assembly of molecules. We have intentionally started the simulation randomly because we don't want to bias it. We want to allow physics to drive this according to the forces that govern those molecules and tell us what is happening. In that way, we're testing a molecular microscope and these same giant reactors that you're going to see when you visit Norwood later, allowing us key insights into those early stages.

So I'm already stopping. We're only at 300 nanoseconds. And at 300 nanoseconds, you can see we have already started to see lipid coalescence, and so particles that resemble that, which I'm showing you in the top left. We can watch this develop further. And here we are at 1 microsecond. So 1 microsecond. That's as fast as the fastest enzyme reaction we know. That is 6 orders of magnitude faster than protein folding, and look at what's already happened. This particle already looks a lot like a mature lipid nanoparticle. That tells you that the fate of the lipid nanoparticle is decided [snaps fingers] like that. And what I want you to watch as we go forward is this one RNA that I've highlighted in orange. Right now, it's sitting on the outside of the lipid nanoparticle, but as this progresses, it's going to get sucked into the lipid nanoparticle. So there you have it. We're at only 1.3 microseconds. We already have evidence of RNA encapsulation.

So this will proceed and you will see that the hydrophobic effect will take over and lipids will continue to coalesce into larger and larger protoparticles. And these protoparticles will start to interact transiently, as governed by their intermolecular forces. And what you can see is really the meat of this evolution has slowed down, right? Really so much of it was dictated by those first few microseconds. But you can see every now and then, the particles will start to associate in a way that is less transient. Here, interestingly, it appears to be bound through electrostatic interactions of the RNA itself. And then as this progresses, you can see that the particle will continue to evolve until, at the very end, we're getting particles that are reasonably sized and in agreement with experiment showing RNA encapsulation.

And so I want to just show you one more snapshot from that same simulation where we're going to focus in particular on how RNA is encapsulated. So watching this RNA, you can see that as the particle develops and grows in size, the RNA gets sucked in because the particle will start to fold over on itself, thus pulling the RNA inside. And so it's insights like this that really gave us some clues about how RNA encapsulation was occurring, things that we didn't really fully appreciate or understand before because we hadn't employed techniques like this.

And so I want to return to the analogy of the black box. Melissa told you that there is so much that's happening before we can even understand this via experimental techniques a few minutes out, and we think what we're doing is shedding insight into these very early assembly time scales, giving us insights into what we're doing, what lipid nanoparticles we're making and how so that we can ultimately use these insights to help us drive towards lipid nanoparticles with the structure and function we want. And what we have touched on so far is here, and so going forward, we're looking at applying those insights towards the rational design of lipid nanoparticles for maximum efficacy and potency.

Thank you. I'll pass it back to Melissa.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

All right. Well, thank you, Michelle. So -- wow. You're still all here. That's great. Well, I don't know about you, but my mind is spinning after that. So this is the end of this section of the Science Day, which is the -- some window into several of our basic research efforts. And I think -- what I hope that we've convinced you and something I said at the beginning is that we really, at Moderna, go above and beyond to understand the very basic biology, chemistry, physics that can help us make better drugs for better medicines for our patients and we will continue to do so. We are continuing to invest in basic research at the same level that we've been doing for a number of years now. And we believe that the end is not yet in sight, that there are many more things that we can learn from applying state-of-the-art and beyond state-of-the-art techniques.

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So now there will be a 15-minute break for you to clear your heads and get ready because Stephen and Uli are going to tell you about the immune system. And if you thought that what we told you was complex, then the immune system is really taking it up a notch. So enjoy your break and we'll be back.

Thank you.

(Break)

Stephen Hoge - Moderna, Inc. - President

All right. Welcome back, everyone. Hopefully, a restful break. For the rest of the session this morning before Q&A, we're going to pivot to talking about some of our investments in delivery sciences and recent advances there. Now just like in messenger RNA, it's nearly impossible for us to try and represent all of the things that we're actually doing in delivery science to hopefully open up new areas for messenger RNA therapeutics. And so what we've tried to do is just select 1 program, 1 program that we're particularly excited about that we think demonstrates many of the efforts and types of progress we're trying to make.

This program is in the immune nanoparticle space. The goal -- the objective of the program is to develop a delivery vehicle that allow us to deliver messenger RNA to a broad swath of the immune system, particularly lymphocytes in vivo.

But before I go into that program in too much detail, I do need to recognize the incredible team and effort that made this come together. Developing new delivery technologies is without a doubt the most multidisciplinary and multifunctional thing we do at Moderna. It's an incredible number of teams that have to come together, ranging from chemistry, immunology, delivery science, in vitro biology, pharmacology and technical development. It is a massively complicated effort to make one of these things work. I hope some of the talks this morning gave you a sense of some of those pieces, those functional pieces of how they come together and what that can do.

Now by necessity, the talk today is really going to focus on why we're interested in immune system therapeutics and what our lead nanoparticle can do. So I'm not going to spend a lot of time talking about the technology itself, the very basic components of it, but I do want to provide you with just some generic sense of it before we do just so you have a mental picture, if you will, before we get in.

So this is a novel nanoparticle technology. It's analogous in size to our LNPs in terms of -- in size and activity. It does have new chemical composition that we've scaled up and has an established safety profile. I won't be going into that data today. And the intention for this -- your mental model should be this is an injectable standard drug like our other lipid nanoparticle programs, so injectable through multiple routes of administration. Most of the data I'm going to be showing you today is IV, but it can actually -- you'll see some data through other routes of administration subcutaneously.

And then last, we do intend to publish on the structure and mechanism of action. It's an incredibly exciting story. And those manuscripts are in preparation and so we'll point you to those at the appropriate time once they're out there.

So what has us most excited and the thing that we want to represent today is that we do think that this lead nanoparticle technology that we'll talk about can form the basis of a very large number of messenger RNA-based immune system therapeutics in the years ahead. Now a good place to start is why do we think it's immune system therapeutics. We're actually very specific in selecting that. And what I need to do to give you a sense of that is probably take about 10 minutes upfront of the hour we have today and provide a little bit of context for the immune system. Many of you maybe have deep background in immune system, there may be some PhD immunologists in the room, but others might not. And so I'll try and actually, as Melissa modeled very well, provide a broad sort of common foundation in terms of some of the words we'll be using and context we'll be using as we go ahead.

So why? Why are we interested in the immune system? The immune system, let's talk about what that is. The immune system is often analogized to the police or the military. It's something that defends our bodies from invaders, whether those are infectious pathogens or cancer. But that's an inadequate description because the immune system, just like our government, actually employs more people in sanitation and teaching than it



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does in the police. In fact, a better analogy than the police for the immune system is actually all levels of government. And by all levels, I mean local government, regional government and national government all working together to maintain a system.

Now part of the problem is immune system is really misnamed. It's called the immune system, but it should be called the homeostasis system because that's what the system does: it tries to maintain balance. And when things get out of balance, the primary function of the immune system is to try and figure out what sorts of responses are needed, what sorts of actions need to be coordinated so that we can resolve and get back to balance and homeostasis.

Now that level of coordination requires a tremendous amount of communication between all the parts of government and the immune system. But the problem for the immune system is the immune system can't pick up a cell phone the way, for instance, a branch of government could and communicate with others. So how does that communication happen? Immune system communication happens a lot of different ways, but a lot of it happens in something called immune synapses. These are direct cell-to-cell contacts between cells in the immune system or sometimes other cells in the body, which exchange massive amounts of information. If you zoom in, in these synapses, what you see is a lot of that information is actually through direct cell-to-cell contact of proteins. Proteins on one hand -- on one cell interacting directly and communicating information with proteins in another cell. It's a 2-way process. There are also cytokines and secretive factors and other things going on, but a tremendous amount of information is exchanged in these synapses.

You can think of these as sort of secret hand signals because you can't really see them. They're inside that synapse, but these hand signals actually communicate tremendous amount of information. If you need any evidence of that, those who know the biotech and pharma field just can think about checkpoint inhibitors. So PD-1 and PD-L1 antibodies primarily function by excluding those hand signals, specifically just 1 axis, the PD-1/PD-L1 axis, from the immune synapse. And it's been a revolution in how we treat cancer. Clearly, these are incredibly powerful things.

Interestingly, if you look at those drugs, KEYTRUDA and IMFINZI as examples, it doesn't matter which side of the system -- of the synapse you blocked, you excluded. Block the PD-1 or block the PD-L1, both work and have tremendous effects on cancer. That's just one of these handshakes. It's an incredibly powerful, powerful system in the synapse.

Now synapses is a pretty loaded word in biology. When I first say synapse, these are actually called immune synapse. When I first say synapse, probably most folks in the room think of actually the central nervous system. Because the central nervous system actually is organized as a bunch of neurons in your brain and elsewhere that perform many of the functions that we think of as [higher-order life, cognition motion.]

But the scale of these 2 things is dramatically different. In fact, if the nervous system was smaller, there are 120 billion neurons in your brain on -- approximately, often forming thousands of connections with a neuron in a pretty fixed web, right? If you think of neurons as largely fixed in terms of physical location. There are 5 trillion white blood cells in your body. One trillion white blood cells weighs about 1 pound. You think about 5 pounds of these individual cells in your body crawling all over. And what's amazing about these synapses that those white blood cells are making is that they're moving all over the body. In fact, they are creating networks in local or regional situations to resolve an infection or address a cancer and then they're just -- they're breaking those networks apart and going to other parts of the body. So a massively more complex system in many ways.

And that system has huge impacts into these. So I already referenced the way checkpoint inhibitors have revolutionized what we're doing in cancer. In fact, a lot of cancer is now thought to be and understood to be an immune-escape disease. The immune system does a great job keeping us in control until one day it loses control for whatever reason.

But that's not it. There are other diseases. Neurodegenerative diseases, Parkinson's and Alzheimer's are actually diseases of that homeostasis system. And more and more we're discovering that a lot neuro-degeneration has a substantial immune system component at its core.

And then of course there's the classic diseases. Things like autoimmune disease, where the immune system stops to be able to be really discerning in figuring out what's self and nonself and starts attacking self like it's foreign. And there are horrible manifestations in many, many instances.



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Uli von Andrian, who you'll hear a little bit later, is fond of saying -- has said to me on several occasions, you really should think about the primary disease of the immune system is aging because most of what happens as we age correlates with the degradation of the homeostasis system, the immune system's ability to maintain homeostasis and balance.

So where do we begin talking about a system like this? Clearly, it's powerful in so many diseases. But what are the key features that we need to understand so we can understand and develop a delivery technology? So let's start with the fact that there's an incredible diversity of cell types. That it's very nontraditional anatomy. This is not a system that's in an organ like your liver. It's a system that's everywhere. And lastly, that there are highly coordinated and interdependent actions between those cells, and I showed you an example of the synapse.

So what I need to do before we get into the delivery technology is talk about for the next 5 minutes that diversity of cells, that physical location and anatomy and some of those interactions.

What are the main types of the immune system? Now here's where I'll ask for forgiveness from the immunologists. There are incredible richness of cells in the immune system, but probably I'm going to boil it down to the big 6. Big 6 cells. And they fall into 2 categories, myeloid cells and lymphoid cells. Now in the myeloid compartment, there are 3 big buckets, macrophages, monocytes and neutrophils and I'll say more about them in a second. And the lymphoid, the ones we traditionally think about, T cells, B cells and innate lymphoid cells.

So let's talk about where this all begins. All of those cells arise from a process called hematopoiesis in our bone marrow. It's those 2 main lines. I'm going to just deal with the nucleated cells. For anybody who's interested in platelets and red blood cells, I apologize. It's a necessary economy. But in the myeloid compartment, you're dealing with the 6 cell types I described here, broadly speaking. 90%-plus of cells. The dominant population in myeloid are macrophages, and macrophages really are the local government of the immune system. They do all the road building and maintenance, they're the power lines, they clean things up, they initiate and resolve inflammation. There are over 2 trillion in your body, 2 pounds of these things, crawling around all of your tissues. In fact, they're so abundant that we often name them for the tissue they're from. In the liver, we call them Kupffer cells; in the brain, we call them microglia; in the bone, we call them osteoclasts; but these are macrophages all -- through and through.

Macrophages and there -- another cell in that myeloid, [the neo] -- the dendritic cell, perform another important function which I mentioned, which is initiating and resolving inflammation. And they do this through a process called antigen presentation. This is where they take foreign material, and that foreign material either could be a pathogen or it could be a diseased cell, and present it to other cells in the immune system, often through antigen presentation. Presentation on 2 different molecules, MHC class I and II molecules or lipid antigens are presented on a molecule called CD1. Doesn't get a lot of attention, but it's an important mechanism of presentation of antigen as well.

So those are the cells in the myeloid cell, the phagocytes. There's one last cell type on there that I have to talk about, it's monocytes. And what are monocytes? We hear a lot about them. Monocytes are predominantly the replacement parts for the other two. There's about 3.5 billion of them in the blood, first in the 2 trillion macrophages out there. And what they do is they circulate in the blood looking for, hey, where do we need to replace either some dendritic cells or macrophages? After about a day or two, they'll move out of the blood into those tissues and repopulate. So for simplicity's sake, we're just going to limit it to macrophages and monocytes.

Let's go to the other half of the myeloid compartment, granulocytes. Granulocytes are so named because they have these little punctate granules of toxins really. In their lysosomes, they store enzymes and other materials that help them kill things, particularly invading bacteria and viruses. The most abundant granulocyte, the big kahuna here, 80-plus percent, are neutrophils. Neutrophils are the primary cell in our blood and the primary granulocyte. And neutrophils are remarkable in their capability. They're really the first response of the system. We're showing here a video from the 1950s by David Rogers where it actually showed a neutrophil chasing a bacteria in the blood. Those red blood cells are the other things around it. There's 1 neutrophil at the Staphylococcus aureus bacteria in this blood sample. They are relentless. They're the first responder firing, they charge at everything, they never give up, they will go everywhere. It's like you have a sentient being in your blood. And as soon as they catch, they fuse their granules to that bacteria and they kill it. A neutrophil can do this max a half a dozen times, 3, 4, before it actually starts digesting itself with those granules. These are the first responders of the immune system, the predominant blood you have -- cells that you have in your blood.

Now there's other granulocytes, eosinophils and basophils that are there, much, much less frequent and we will go into much detail.



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So that's it. Three big cell types I need you to think about on the left-hand side of the ledger, the myeloid side.

Let's now move to the lymphoid side. People usually understand the lymphoid side because we've been talking about immunology forever. T cells and B cells are kind of part of what we think and know. There is a third population of lymphoid cells I'll get into a second, but let's start with the T cells.

So T cells are hugely abundant. There are 150 billion of them in the body. In fact, they're second most common cell type. And they're really subdivided into a group -- a set of names that many of us already know. There are helper cells, which are both providing protection, but also teaching other cells. There's 100 billion CD4-positive T cells in the body.

Second major population are the regulatory cells. These are the cells that actually are critical for homeostasis and autoimmunity. What they do is resolve inflammation and they make sure that the whole inflammatory process of our immune system doesn't get out of whack. Also called CD25-positive. There's about 10 billion of them in the body. There's one of them -- there's 10 effector cells for every one of those regulatory cells.

Next population is the cytotoxic T cells. It's got a great name. You can already tell what it does. It kills cells. These are CD8-positive, there's 40 billion of them and they make up a large fraction of our cell-mediated immunity in our blood predominantly by something -- what's happening inside of cells and killing them.

And the last are the NKT cells, about 3 billion of those. These cells are the most bipolar cells in the body. They actually send lipid antigens and at the same time regularly are secreting inflammatory cytokines and anti-inflammatory cytokines. So NKT cells are a particularly interesting bunch of cells. They are also unfortunately named. Natural killer T cells do not kill, if anything. They just look like natural killer cells and that's how they picked up that name. So that's the T cell compartment.

Let's go to B cells. B cells are the antibody-secreting cells of the blood. They actually create massive amounts of antibodies that protect us from infections that we've previously seen and help us resolve infections we're getting. Now as a broad family, we think of them as B cells but they actually evolve relatively quickly over time and we're constantly creating new (inaudible). They arise as stem cells, as I said, in the bone marrow and then move out into the blood as immature B cells. These are B cells that have not yet seen an antigen. They don't have permission to replicate or secrete antibodies. Once they've encountered their antigen, I'll talk about where and how in a second, they move into -- become more mature B cells and eventually into plasma cells -- plasmablast or plasma cells, professionally secreting antibody that makes up our (inaudible) immunity.

Let's talk about that last population, natural killer cells. This is the sixth of the big 6. Natural killer cells, there are about 5 billion in the body and these cells are unique. They are judge, jury and executioner at the same time. They are appropriately named. Natural killer cells walk around when activated, not when they're inactivated, but once activated looking for reasons to kill cells. And there's one thing that primarily stops them. That thing that stops them is whether the cell they encounter has MHC class I molecules on their surface, whether it's presenting antigens to the rest of the immune system. As long as you're being transparent about who you are, I'm okay, I'm not going to kill you. But if you're not being transparent about who you are, a natural killer cell instantly can lead to -- can lead to killing of that cell type. Think of them as a fail safe, right? The natural killer cells are the ones that are working around making sure that you're being honest or at least the cell is being honest in presenting its antigens.

So that's it, the big 6: macrophages, monocytes, neutrophils, T cells, B cells and natural killer cells are the innate lymphoid cell types. All of them have distinct functions and massively multifunctional. But you can often think of the myeloid compartment as local government and maintenance: neutrophils as first responders, T cells as an interesting mix of teachers and healers and police and then the natural killer cells and B cells that we know.

What do they look like in terms of numbers? As I said, overwhelmingly a macrophage story with substantial populations of these other -- of the big 6 cells.

Now I've had to skip past a lot of subpopulations and I start by asking for forgiveness on that from everybody. But if we just focus on these subpopulations and say what do they look like in the body, and here I've tried to represent that as a distribution based on physical size. The story, as you could see, is overwhelmingly macrophages followed by T cells and then a whole bunch of secondary populations. What do they look like



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in the blood? The blood is a totally different picture. In fact, in the blood, macrophages are really, really low population and neutrophils make up a huge proportion, those first responders that we talked about.

So again, in the body, it looks like the thing on the left. In the blood, it looks like the thing on the right. Clearly, location matters. And it does in the immune system more than any other system because the immune system is not defined by anatomy. As we already talked about, macrophages make up 90% of the myeloid system and are actually tissue resident. Over half of the lymphoid system is actually in something called mucosa-associated lymphoid tissue. These are nonencapsulated, self-organizing groups of lymphoid tissue in most of our barrier tissues. That includes our guts, respiratory, they're primarily focused on monitoring and defense and immune homeostasis. And that's where you'll find, as you can see on the represented picture, large numbers of our T cell, particularly T cell memory, as well as B cells. So the immune system doesn't really live in an organ if 90% of the myeloid tissues are tissue oriented and 50% of the lymphoid cells are actually mucosal associated.

But there are organs in the immune system. And the first and most prolific is the lymph node, which folks have probably thought about and heard about a number of times. Lymph nodes, there's about 500 in the human body and they drain a tremendous amount of antigens and volume of material from tissues. I'm showing you example of just one. And in those lymph nodes, an incredibly important process happens. That process is antigen presentation. Dendritic cells, in this case I'm showing interacting with the a T cell, will present the antigen to a T cell. And when they find a match, as you saw in the video, they will cause that T cell to become activated and clonally expand often in that environment. And there's other things happening in these lymphoids as well. There's an analogous process in B cells through something called follicular DCs where B cells are created. And then lymph nodes are also a final home, if you will, for a number of components of memory, including plasma cells, which live in an area called the medullary cord, indicated here, where they will just sit there and for years secrete antibodies to maintain memory. The lymph nodes are an important organ in the immune system.

And then the second organ I want to talk about is the spleen. Unfortunate name in some ways because in medieval literature it is described as bad humor. But the spleen is actually an incredibly large lymphoid, very analogous to the rest of the lymph nodes except it doesn't filter a tissue or an organ location, skin, it actually filters the blood. The blood pumps into the spleen and functions similarly to many of the lymph node functions I just described. There you'll see actually a pretty broad swath of the immune system, just looking at the cartoons at the bottom you get a sense. There are myeloid cells, there's T cells, there's B cells, there's even natural killer cells. It's an incredible repository.

So there are processes in follicles presenting antigens, very analogous to what I described in lymph nodes. There are also areas that store memory cells, including plasma cells and B cells. And then there's whole banks of cells called monocytes and other things that really actually just take up residence in the immune -- in the spleen. The spleen really represents the best place to sample a broad swath of the immune system.

Lastly, the bone marrow where most of hematopoiesis goes on and there are some long-lived cell niches that are pretty important.

So what about the blood? I'm talking about immunity and I didn't talk about the blood. That's because the blood is pretty inaccurate. Only about 3% of your immune -- of your non-tissue-resident cells are present in the blood at any given time. And mostly these are neutrophils, 60% to 70% of the blood are those first responders circling around. Next big fraction is monocytes, which only really live in the blood as replacement parts. And the rest are lymphocytes in transit. They're literally going -- using the blood as a road to go from one place to the other. The best analogy I have for describing the role of the blood as a road is probably to say it's a -- when we measure the blood to try and figure out what's happening in the immune system, it's equivalent to going down the road here, taking a picture of the Mass Pike and trying to figure out whether the Red Sox have a home game. If you happen to do that, at 6:45 p.m. and you see a bunch of traffic going the other direction with a bunch of Red Sox flags, it's probably true. There's probably a bunch of people going to Fenway and there's a Red Sox home game. But you go any other time of the day or any other day, you're not going to learn anything. It's not terribly useful because it's just a snapshot in time. You have to catch that 3% or so at that moment. But unfortunately, for translational reasons, we have to rely on the blood alone. And so a lot of the data we'll walk through will be the blood. But as you look at things like the blood versus the spleen, you really want to think of those lymph nodes and the spleen as best representatives of what's happening in the immune system and the blood as a snapshot of the Mass Pike.

So where does that leave us at the end of talking about the immune system? A network of diverse cell types, over 1 trillion, interacting mostly through cell-to-cell synapses and dramatically different cell populations in different functions -- in different locations that are central to driving function.



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Now how do we go about creating the particle that I'll talk about in a second, an mRNA-based immune system therapeutic? We think the system is important because all of that interaction I described, all of those sub -- cell types play critical roles in health and disease. And so if you can't access a broad swath of them, you're going to miss a huge portion of the story.

So we started where we always start as a messenger RNA company, saying we have to show dose-dependent in vivo pharmacology to all major cell types, all the big 6 I talked about.

Now why -- let me [peek] that apart just a second. What do we mean by dose-dependent in vivo pharmacology? A defining feature of our platform is that it is dose-dependent. If we give you a dose today, you have a response. If you get twice that dose tomorrow, you should have twice that response. If a week from now, you get another dose again, it should be the same response again. And that should be predictable at an individual level just like it is for other drug platforms.

That's very different from a lot of genomic medicines where you don't see that sort of dose response, but it's critical to our platform that we always demonstrate that. So dose-dependent in vivo pharmacology. Has to be systemwide hopefully for an obvious reason, which is there are just so many different cell types communicating through the synapses that you can't actually just go and play on one dimension.

Now second feature we described that we thought was going to be essential before we could define a lead nanoparticle system was that we had to have -- be able to use mRNA software to select the cell types and ensure safety. And I'll talk to an example of that, but mRNA is a programmable software system we can ensure that we express proteins only in our desired tissues and particularly not in off-target tissues both within the immune system and across and between. And that's a feature of many of our drugs as we presented at last year's science day and I'll reprise that in just a second.

Third feature we defined is that we needed the transient nature of our protein expression to be able to confer cells with new functions and phenotypes, either reprogramming the cells in new directions or causing them for a period of time, days, not years, to act in a specific way because messenger RNA, one of its desirable features is the ability to do transient protein expression.

And then lastly, to drive cell trafficking. The immune system, I hope we've convinced you, is all about location and cell types and getting both of those features right is pretty important for driving phenotypes and ultimately defeating disease. So we needed to show that we can drive cells to organs and tissues to facilitate the desired cell-to-cell interactions.

All right. So last little bit before we get into some data. That dose-dependent in vivo pharmacology point. How much expression is the right amount of expression? Turns out that's not an easy question to answer at the beginning of a journey like this. But we went and looked for inspiration from a range of different biologies. The most prolific virus that our immune systems are fighting as a species is probably cytomegalovirus, CMV. CMV, we -- about more than half the people in this room, more than half the people in this country are seropositive for it, meaning we've got exposed to it and it's in our bodies and it's replicating. It's co-evolved with us over hundred millions of years. It's fantastic at replicating and staying alive, keeping itself replicating in our blood. And so the immune system spends a tremendous amount of time fighting -- in fact, the most amount of time fighting this one virus than anything else. In seropositive healthy adults in this room, on average, about 5% or 10% of your peripheral CD8-positive T cell pool, your effector T cell pool, is actually responsive to CMV. It exists to try and control that infection.

So how about that? The most prolific viral infection of all time is dominating our immune systems right now in terms of attention, taking ten, hundredfold more attention than anything else we ever faced, 5% to 10% of CD8-positive cells. So maybe 5% to 10% is the right number.

What about in cancer? Well, in cancer, we have an interesting cell therapy composition, which is CART therapy. Approved CART therapy is usually infused about 300 million T cells, which is about 1% of that CD8 fraction, so maybe 300 million and obviously that had dramatic and incredible effects to benefit a patient. So maybe 300 million is the right number.

How about autoimmunity? Well, the Treg compartment, as I talked about, particularly the natural T regs, the FoxP3-positive T regs, comprise about 5% of 10% of your CD4 fraction. As we talked about, that's about 5 billion cells. All of homeostasis, all of prevention of autoimmune disease is -- when it's controlled by T regs is controlled by that roughly 5% to 10% population. So we look at that and a dozen other comps and we say, well,



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seems like 5%. 5% is a lot of the immune system. 0.5% is a lot of the immune system. 0.05% is a lot of the immune system. But 5% seems like a really big number, the most prolific virus that's attacking humans, the ability to control autoimmunity and recently approved T cell approaches for cancer. So we said we wanted to shoot for that as the objective.

Let me introduce the lead immune nanoparticle system. I'm going to walk through some data and I'm going to start with the blood. In particular because in human PBMCs we can run a very outbred experiment. What we can do is we can take blood from samples like representative in this room, a range of different ages, immune backgrounds, disease states where we take that sample and it gives us a good sense of what's the extremity of the immune response that we can achieve and control for. So we collect those multiple donors, we expose them to a new nanoparticle. This is the same concentration we achieved when we do an IV infusion that includes both in the blood of humans, but also preclinical species. It's about 100 nanograms per 200,000 cells. And then we use an mRNA reporter encoding a transmembrane protein, in this case a mouse protein for which we have highly specific reagents and we won't find it otherwise. It doesn't exist in the background [as you'll see.]

Now again, I'll just remind you the blood doesn't exactly look like everything else in the immune system. Most of the desired cell types are there except for one. These cells are mostly naive cells in the blood. Now there's a lot of mature cells there circulating, but mostly when B cells are fully matured, as I described, they take up residence in the lymph nodes, in the spleen, in the bone marrow. They're not running around in the same way that T cells are looking for their antigen or the other cells are. So there's going to be a unique feature of this, which is the B cells actually aren't really transect-able for that reason.

Okay, so negative controls. If we -- I'm going to show some FACS plot here. It's Science Day after all. I apologize to those of you who don't like looking at FACS plots. We happen to love looking at them. A FACS plot is a technique for measuring the amount of -- the number of cells and the amount of protein expressed in those cells in immunology.

Just to orient you to one of these, I'm going to point to a version on the left-hand side, which is a T cell negative control. What you're seeing is that gray cloud is a bubble of cells. Often there's hundreds of thousands of cells in that cloud. And what we're doing is we're sorting those cells from left to right based on how much protein they're expressing, which ones are positive. There are tons of dots. We show these as density plots because the amount of dots would overwhelm the presentation system. So looking left to right, what you can see is across all the different cell types, and we've gated for the different cell types, the amount of expression we set at a gate for is defined as less than 1% or in this case less than 0.5%, right? There's negative, negative, negative. They are not expressing the (inaudible) for the ligand protein. And you can see the gate is that little window. It's that rectangle for each of those cell types.

So let's start with T cells. How did we do? Across those 4 donors, comparing it to negative control, we saw a substantial shift to the right and you can see that. Generally a large shift to the right, meaning these cells are expressing proteins. We try to be conservative in setting that gate and so we set it farther to the right than we probably had to. But ultimately, when you quantify those in terms of the numbers of cells that are positive, about 28% are cells. We also confirmed this through imaging, through microscopy and other means as well, but I'm just going to show you the facts stated here. 28% of T cells in a single dose equivalent to what we can do in the blood.

How about the different T cell compartments that we mentioned before? And again I'll just draw your attention to the FACS plot to the bottom for your helper, regulatory, cytotoxic and NKT cells, a substantial shift in all of them. If you want to go quantify that, it looks roughly like what we saw for the overall population, 28% of helper cells, 18% of regulatory cells, 26% of cytotoxic and 19% of NKT.

So that's the T cell compartment. How about the B cell compartment? Now remember, B cells in the blood are naive cells. And there, we didn't do so good. Only about 3% of B cells -- naive B cells in the blood were actually transect-able. Now I'll come back to that as we move in vivo because that situation doesn't arrive as you go look in other organs.

NK cells. NK cells, those judge, jury and executioner cells, 19% were positive, a similar distribution. How about neutrophils? 11% positive of neutrophils in the blood. And in the myeloid compartment -- now I'll remind you these are the phagocytes, they eat a lot and they express a lot. Monocytes in the blood, we were able to get into 66%; and dendritic cells, 58%. So as we started out by saying we wanted to get 5 -- minimum 5% in all of the above populations. We did incredibly well.



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So how does repeat dosing work? What's the pharmacology in this? So we started -- I'm going to just tell the story limited to T cells. Other cell types showed a similar profile, but I'm just going to limit the story to T cells for simplicity.

If you repeat dose, those same donors, the same human deviancies, I'm showing you an example of one donor on the left and multiple on the right, and you just look at the CD3-positive T cells, after a single dose, about 25% are positive. After 2 doses in 2 days, you're upwards of 54%. You get to 68% at 3 doses. I hope you can appreciate that we're sort of almost linearly or asymptotically approaching expression. In some conditions, we've been able to drive this upwards of 100%.

What also should jump out at you, if you can look at the dot plots or if you're familiar with them, is that the intensity of the protein is going up. So some of these cells are seeing multiple doses. And as you'd expect, the MFI or the amount of protein per cell is increasing linearly as well. Although I'm not showing you that on my graph, I hope you can appreciate it in the dot plot density plots.

Okay, great. So we've got what we need, right? Well, let's see does it translate in vivo because our interests are not in ex vivo lymphocyte manipulation, they're in, in vivo modification. We started in the mouse and administered a standard mRNA dose IV of about 0.3 mpk, that's a relatively low dose for us, and collected splenocytes and PBMCs. And importantly, we were able to look now not just at the blood, but at the spleen, which is a much more representative population. I hope I've convinced you. We measured similarly transient transmembrane reporters and confirmed it with imaging in multiple occasions.

Now we did this in 2 strains. We did this in many strains, but I'm going to show you 2 strains because it's representative of what we've seen across. Let's start with the T cells. A single IV injection of our immune nanoparticle expressing the transmembrane reporter system, how many of the T cells in the spleen of this mouse were positive -- in these measured positive? You can see controls are 0. Background, not surprising for both strains in the case of C57BL/6 and BALB/c. Upwards of about 17%, 20% of animals were positive after 1 dose. This is 24 hours after 1 dose. It's a massive fraction of their T cell compartment after 1 dose, and it was well-tolerated obviously.

If you break that down into those subpopulations again, the story continues to reproduce what we saw in the human blood. Helper T cells, maybe a little bit higher, upwards of 20%. You can see all the individual animals and plots for both species or both strains. Cytotoxic T cells, we are looking a little bit lower, maybe 15%, which is exactly nearest what I showed you with that in PBMC. So we're able to transect huge fractions of the T cell compartment.

How about the B cells? Now here's where it's different than what I was showing you in human PBMCs. When you take the spleen, you're actually getting all of those tissue-resident B cells as well as some very specialized populations, marginal zone B cells, plasma cells, the mature cells that are really sessile. They're locked into that organ. And there, what we saw is huge transection. In fact, higher transection of B cells than we see in the -- than we see in T cells, 30% to 40%, depending upon the strain and what you're looking at in 24 hours.

And monocytes was a very similar story. I'm just going to show you the summary data here, which is that if you look at that initial experiment across all of those strains that we did as opposed to our target of 5% with a single IV infusion, we were able to get to about 18% of T cells becoming positive, multiple lineages; 30% of B cells; and 48%, almost 50% on monocytes. Now there were lower levels detected in the peripheral blood. I didn't show you that data, but that should match your impressions at this point. Remember, the peripheral blood is the Mass Pike. And if you wait a day, a lot of cars have moved around, and what you're going to get as a snapshot isn't as robust as what you're measuring in the spleen.

So that we think represents cell trafficking, and we'll show some examples of that in just a moment. And there was also minimal signals in the lymph nodes, right? So what we're seeing is the spleen essentially. And our hypothesis at that time was that, that was probably because we didn't allow enough of those cells that we had transected to re-traffic into other organs. And so we chose a different animal system to fully elucidate that in more detail.

That was a Cre-lox mouse system, a relative Cre-lox mouse system from Jackson Labs. Now just for those who aren't familiar with this mouse system, these animals are genetically engineered so that they express a reporter called tdTomato. But that tdTomato, it's a red protein, is inverted. It's essentially hidden in their genome. And so they carry it in all cells but don't express it, except if they become exposed to a single copy of mRNA making Cre recombinase, Cre recombinase being a protein, that will lead to gene editing in vivo, meaning that inverted reporter will flip the right

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way and all of a sudden start making a ton of protein. Right? So it's a form of in vivo genetic engineering because what we're able to show is those cells -- any cell that takes up one of those mRNA and makes Cre recombinase will be permanently labeled as red. Super easy to see.

I'm showing an example of what we do with that in a liver, which is another area we do a lot of obviously discovery in delivery science. Then I'll show you in a moment what that looks like [in vivo]. Now in order to avoid capturing all the daughter cells and everything that happens downstream, we actually do this experiment at 48 hours. We follow these out for a month, and generally the story is the same. But our focus, the data I'm going to show you is just that first 48 hours.

So let's start by looking in the blood of these animals, these Cre-lox mice. Across all of those cell types, negative controls are still negative. But what I hope you can appreciate is that the percent of cells that have gone through that Cre-lox, that Cre recombinase genetic recombination is representative of everything we've shown before. 25% of T cells, similar numbers across the subtypes; 26% of B cells. Again, this is now in the blood at 48 hours. And so we've allowed a little bit more recycling of those cells, and so you get more, if you will, dilution and normalization in the cell type. Monocytes are high, and then you get obviously the granulocyte population on the right.

Now what happens if you go looking at other organs? Again, the blood I've already presented to you. But if you look at the spleen, at 48 hours and beyond, what you see is there's been recirculation of those cells. And where it's recirculated to? Good news, the lymph nodes and the bone marrow. So upwards of 6%, 9% of cells in those lymph tissues and bone marrow being positive both for T cells, B cells and obviously large fractions of the myeloid compartment. So broadly across the immune system. Not just in that big lymph node we call the spleen, that's the big kahuna there, but actually broadly in lymph nodes, in this case, an inguinal lymph node. The only way that could be happening is if we were transecting the cell in the blood and then it would migrate into that tissue.

So the last translational experience for us is that's great, that's mice. I'm going to skip over a lot of intermediate species and work and bring us to the nonhuman primate because the immune system of an adult nonhuman primate is one of our best approximations for the immune system of a human. We go in vivo here, similar sort of experiment, we're expressing a transmembrane reporter at a well-tolerated relatively low dose in the primate and get a look at the different cell types.

And if you look first at the T cell in a nonhuman primate, after a single IV injection of the immune nanoparticle expression reporter, approximately 12% of all T cells in that primate that we can harvest from the spleen are positive, just like what we've seen in the preclinical species, so very much translating across as we said.

B cells. B cells is similar story. If you look at B cells in the spleen of a nonhuman primate, about 13% of all B cells, CD19 positive cells in that primate were positive. Again, a single dose, 1 day. And then the monocyte fraction, similar to what we've seen before, is always higher. 22% of all monocytes were transiently expressed in the protein. And I'll remind you, it's a transient protein. It could have been anything. We could have reprogrammed these cells with a transcription factor. But all we did was (inaudible) reporter, show us that you made it and show -- so that when we go look, we can see it there.

Summarizing where that is across the primate, you could see the numbers there, very analogous to what we've seen elsewhere. So 12% of T cells, 13% of B cells and 22% of monocytes. You estimate that as a whole body number for a human, a 70-kilo human with 5 liters of blood, that's about 9 billion T cells. 9 billion. B cells would be about 1.5 billion and monocytes would be about 2 billion.

Now let's come back to that question. This is in nonhuman primates, what were we aiming at? About 5% of your CD8 fraction is all it takes to control the most prolific virus in the blood, which is CMV. Right? Human CAR T cell to therapy, the doses of about 300 million of CD8 positive T cells, enough to have driven dramatic effects in those patients where those cells obviously can expand, but 9 billion is not a shabby number.

And then T regs. I talked about the proportion of T regs in your blood, about 5 billion to 10 billion of them or 5% to 10% of all CD4 positive cells. Clearly, from our perspective, we think the translation of this into primates in a single dose pharmacology at these orders of magnitude demonstrates the power of this technology to address a range of immune diseases.



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Okay. So how do we deploy that? That's dose-dependent pharmacology across a range of diseases. I'm going to talk very quickly about a couple of other things and get you an example and then invite Uli to come speak. So mRNA software-like features, all of that is for naught if you create a lot of off-target safety issues. And at Moderna, we work incredibly hard to control, once we get the mRNA to the cell type, do we want it to make a protein or not. And the way we do it is something called microRNAs. There are distinct microRNAs in every cell type in the body, and these microRNA signatures really define cell fate.

Now usually, the body uses them to turn up and down the amount of protein translation that's happening, as represented by this picture. But if you deliberately encode a perfect complement to a microRNA signature in your mRNA drug, which we're providing exogenously, a different process happens. When they hybridize, that leads to direct cleavage of the mRNA and degradation, and that's shown in the call-out in the lower right-hand side. What that means is you essentially have an off-logic gate. If you're in a cell I don't want you to be in, don't make any protein. And that confers a tremendous amount of ability to control, once you can get mRNA into a broad number of cells, where do you want the -- what proteins to be made? Because again, you can put multiple proteins in and have them off in different cell types or multiple cell types.

Ruchi presented an example of that last year, and we subsequently published it in Nucleic Acid Therapeutics. And in this example, it deals with the liver, and I'll talk about why. I'm talking about the second because it's in something we're -- I'm about to show you.

So in this example Ruchi presented last year, I'll quickly summarize but I'll point you to that manuscript, we encoded a lethal protein for cells called Caspase-6, it causes cells to apoptose. And if you inject an mRNA encoding that protein into the cells and look in the liver, in this case, of a mouse, what you can see in immunohistochemistry of the control with no targeting sequences, you see absolute cell death. You see many instances of cell death.

That's probably best represented on the right-hand side by liver enzymes, AST and ALT, which we look at a lot in medicine. And you can see those are through the roof, incredibly high for both of those. Real-time cell death as a result of encoding that protein. Now if you put just 3 copies of a miR-122 targeting sequence, miR-122 is specific to hepatocytes, it is actually one of the features of hepatocytes, they have high microRNA-122 in their cells, you're able to completely abrogate that, stop that, not just in terms of immunohistochemistry looking at cell death, but you actually don't see elevations in transaminases. It's almost like the mRNA never got dosed. It is a lethal protein, and it never had an effect. That's the power of these microRNA obviously because when you encode them the right way, in this case in triplicate, in the right place in 3' UTR and a perfect complement.

Now we can use the same sort of stuff in the immune system, and we're very early days here, and I'll just show some early examples. For instance, what if you had a targeting site where you said I want to express in T cells and not monocytes. Like what would that look like? I showed you the FACS plots. We have mirror targeting sites not disclosed today that have been -- that we've been able to discern that can actually protect the amount of expression, only see about 5% or 10% reduction in the amount of expression T cells, but reduce the majority of the monocyte fraction about 75%.

What if you wanted to do the opposite? Say I want to express in monocytes and not in T cells? The FACS plots in the bottom tell the picture. But the bar graph, for those who aren't familiar with looking at them, T cells, we can eliminate over 80% of that expression while still preserving the majority of the expression in monocytes.

Now what would that look like? How would you use that? A cartoon may be the best way to illustrate it. What if you could select the protein you made in different cell types? And you could illustratively make a green or a red protein? Some on T cells and some on dendritic cells, in this case, monocytes. What you'd be able to do is create novel and specific signal between cells in those immune synapses. You could actually provide new signals, you can use whatever you want in the intracellular domains for those, that are actually specific between those interactions.

So first, you use microRNA and then you create novel protein, protein interactions to create new pharmacology. So that's how we think about mRNA software inflecting cells and driving safety and particularly in the liver.

Now I want to put all this together before handing it off to Uli to talk about traffic. So what does this all look like when you put all these pieces of technology together as an example? So can we achieve in vivo dose-dependent immune cell pharmacology in multiple cell types in a way that we



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would all resonate with? And can we do it in a way that's safe? That has no off-target effects in the liver or elsewhere? Everything I will show you involves microRNAs to achieve that specificity and safety, and particularly miR-122 to protect any off-target liver expression.

All right. So what's the system in which we chose to do this just as a system? We went into C57BL/6 mice as a first approximation. And we did an IV dose of immune nanoparticles every 2 days. Every 2 days for 8 days, 4 doses. We took the blood of those animals and then we took the spleens at the end. And that contained either a non-translating mRNA, an mRNA that doesn't make a protein, an immune nanoparticle just as a negative control, or an mRNA encoding a CD19-CAR. And the reason we did that is CD19-CAR, as many folks in here know, are very effective at moving to the deletion of specific cell types, CD19 positive B cell types in peripheral blood. That allows us to monitor those PBMCs with CD19 positive cells, harvest the spleens at 8 days and do full characterization.

Now for those of you who are biotech-grizzled veterans here, I got to say this is not a CD19-CAR T, right? This is not a CD19-CAR T. In fact, expression we expect by design to be in multiple cell types. So yes, it will be in T cells, but it will be in macrophages and natural killer cells as well as a couple of other minor populations by design.

Second thing is expression with mRNA is transient and self-limiting. That's a key feature. Whereas a CAR T will expand in its host once it's been dosed, our expression of protein will be very, very high across cells very quickly, and then it will start to fade. And those cells will have some natural life span. But we did not genetically change those cells. So even if they expand or dilute, they will lose that specificity. It's a transient drug-like effect. It's important to bear in mind.

And then lastly, CD19 here is just being used as a reporter so that we can monitor activity against normal B cells. These are not diseased animals. This is not cancer, right? These are just a reporter. And the reason we use that reporter is that as we generate these cells, which we were able to demonstrate in vitro and in vivo, what we'd expect is that they would delete the natural B cell compartment of these animals. And we would hope to demonstrate that it can be dose-dependent, as I said.

So how did we do? Putting it all together, I'm going to start by talking about T cells and specifically T cells in the peripheral blood. Gray dots here are your control animals, green dots are the ones dosed with the mRNA encoding the CD19 CAR. What you see after 48 hours of 1 dose, the animal, essentially within normal variation, all have normal amounts of T cells in their blood. Again, this is not the target cell type. This is not CD19 positive. It's just a control cell type. We looked at many. At 96 hours after 2 doses, you could still see that's true. And after 8 days, after 4 doses, essentially the means are not different from normal in the number of T cells.

If you take the spleens of those animals to get a more complete picture of what's happened in the immune system and you count individual cells, I hope you can appreciate that the number of T cells in the spleens of these animals is not physically significant different -- not substantially different between treated and controlled animals. Right? So that's the controls work.

How did we do in B cells? Now again, control animals with a non-translating mRNA, you would expect nothing. If our immune nanoparticle is doing nothing, it should do nothing. And that's what you see in the gray bars here, the mean essentially not moving dramatically. After a single dose of an immune nanoparticle expressing transient in the CD19-CAR, we saw a lowering. After 2 doses, it became statistically significant; and after 3 doses, substantially significant. From a staff perspective, P value is 0.0001.

Taking the spleens of those animals at the end, and you want to quantify the amount of reduction in B cells, what you're looking at is, in the blood and in the spleen, about 75%, 80-ish percent reduction in B cells with dose-dependent pharmacology. One dose gets you a little, 2 doses gets you more, 4 doses gets you in a week to a substantial depletion of the B cells.

Okay. So those are the key -- the big 3 that I've talked about: dose-dependent in vivo pharmacology across a range of preclinical systems able to show that we're able to modify disease -- I'm sorry, modify cell types as I just showed; using software to ensure safety; and transient expression to confer completely new functions and phenotypes. I'm sure the mind starts to race how you might use this sort of technology broadly across the diseases.



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But before you go all the way to thinking about all the things we should do in cancer and neurodegeneration and autoimmune disease, I do want to pause and talk about that fourth bucket, which is driving trafficking -- cell trafficking to facilitate cell-to-cell interactions. Where things are in the immune system is as important what they're doing, and that's been some of the limitations of other approaches that have been taken. And the ability to transiently express proteins allows us to dictate where cells are.

I'm going to invite now Uli to come up and talk a little bit about that because Uli von Andrian, who is on our SAB and is also a research collaborator with us on the platform, and I'll ask Uli to provide a little bit of a background of himself and his experience with traffic in his lab, while I swap out these computers for your massive video files.

Ulrich H. von Andrian-Werburg - Moderna, Inc. - Member of Scientific Advisory Board

Good day, everyone. Thanks for still being here and for bearing with me for another 20 minutes or so. I'm Uli von Andrian. My origin is in Munich, Germany where I went to medical school. And like all medical students, I had to take course anatomy where everything is called and most importantly where everything is located in that every organ and tissue in our body is defined by its special relationship to everything else. Except the immune system.

If you think about it, and Stephen has already alluded to this, the immune system has evolved to deal with infections. And there is no way for us to predict where an infection will actually occur in the body. So there are components of the immune system, the macrophages that Stephen has introduced, that our sentinels and our peripheral tissues to monitor all of our barriers for a breach in those barriers and invasion by pathogens. But then a very important additional component of our immune system is sort of the SWAT team, cellular agents that are independently trafficking throughout the entire body to go to wherever they are needed. And the conduit by which this occurs is the bloodstream.

So this, in principle, is easy to understand, but in practice is actually really, really difficult to dissect and I believe has great potential for the introduction of drugs and new therapeutic approaches to autoimmunity, inflammatory diseases, aging, cancer and prophylactic applications such as vaccines.

So I was fascinated by this question already in medical school, decided not to go to the clinic, but started a lab at Harvard Medical School in 1994 and have been a member of the faculty there for the past 25 years. And I've been a member of the Scientific Advisory Board of Moderna for the past 10 years.

So let's start at the beginning. Stephen introduced to you T cells, which are searching incessantly for antigen. Now antigen for most T cells come in the form of proteins that can be either from our environment, a vaccine, a pathogen, food, a parasite, commensal flora that inhabits our surfaces, or it can come from the inside. It can be insulin, added cells, collagen, anything you really want. But the proteins per se are actually invisible to T cells. They need to be presented by professional antigen presenting cells, abbreviated here as APC, which takes these proteins and cuts them into small pieces, peptides, that are then presented on the surface in MHC molecules.

So from a single protein, you can get a multitude of different peptide MHC complexes, each of which will look different because the amino acid sequence in these peptides is distinct. And our T cells in our body constantly monitor these antigen presenting cells to see if their T cell receptor can recognize this peptide MHC complex with sufficient affinity for it to become activated.

Importantly, a T cell that recognizes this peptide MHC complex #1 here will be completely unresponsive to #2 and #3, even though the peptide may have come from the same protein. The reason for that is that the T cell express a unique single species of a T cell receptor. And that happens because in the thymus, each T cell makes its unique (inaudible) product that becomes a unique reassembled T cell receptor in a process called VDJ recombination.

So Stephen has told us that there are 150 billion T cells in the human body on average. And if you ask how many T cell receptor species are there in this compartment, estimates range from between 25 million to 100 million. So there are about 3 orders of magnitude more T cell receptors than there are genes that we actually inherit from our parents. This happens because each T cell individually in the signage will take snippets of DNA and reassemble them randomly to generate one unique new T cell receptor. So this means there's actually much more diversity in the immune system than we can actually fathom.



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Indeed, if you take the most conservative estimates of T cell receptor diversity, 25 million, do the math, we have 150 billion T cells, on average, at most, you will have 6,000 T cells that make the same T cell receptor so they recognize the same peptide MHC complex. 6,000 cells with a diameter of about 1/15, the width of a human hair, when you put it in a test tube and spin that down in a pivot, you can't even see that with your naked eye.

So if these 6,000 T cells happen to see the immunodominant peptide of the next flu virus that comes our way, how is it possible that this minute population of cells find something that is initially very rare, which is a peptide associated with our upper airways where the infection starts, and then it's presented by a very, very tiny fraction of professional antigen presenting cells in our tonsils or in our regional lymph nodes. The reason for that is that T cells are programmed to monitor the body by a process that we call recirculation, where they circulate in the bloodstream, but their half-life in the blood is actually only about 30 minutes. And within 30 minutes, they get recruited into tissues, but they're not into any tissues but basically only into lymph nodes and the spleen. And this happens in a microvascular segment of lymph nodes called high endothelial venules, shown here, so there is a constant flow of circulating T cells from the blood into lymph nodes. And the lymph nodes spend about a day or so in this node looking for antigen that might activate them. If they don't find this antigen, they leave the lymph node and return to the blood, go to another lymph node and look again.

So why do they have to go to so many lymph nodes? Well, because these are local libraries of biological or immunological information that is represented in just one discrete organ or in one discrete region of our body. And this is because in all of our peripheral tissues, we have capillaries that are physiologically leaky. So we constantly lose water out of our blood circulation into the tissue. And this water has to be transported away, otherwise we swell up like balloons. And that drainage system, that's our lymphatics. So any water that seeps out of capillaries in my hand gets transported through lymphatics to a lymph node that sits in the -- in my elbow here, in the cubital area, and then from there to another set of lymph nodes in my armpits.

So any information that is soluble or that is represented in migratory cells can be transported via the lymphatics and is then collected in lymph nodes and represented there. So if you want to know, as a T cell, what's going on in my left hand, you have to go to my cubital lymph node here in my elbow. But if your investment for a node on the other side, you will have no information about this. And so this is true for all these lymphoid tissues, all the lymph nodes that drain various parts of our body, the oral cavity, the skin, the intestines, the CNS and so forth. And in each of these regions, you get information that is regionally defined based on what's going on in the system or in the organs that (inaudible) into that region.

Now under normal circumstances, there is a healthy tissue. And so the material that gets drained into lymph nodes is presented by so-called immature dendritic cells, which actually promote and sustain tolerance. So T cells that happen to be self-reactive are actually maintained in a state of tolerance, and this can assure us an immunologic piece and present autoimmunity. But when there is inflammation, dendritic cells undergo a dramatic change in their programming, and now they become immunostimulatory or mature. So these [mature] dendritic cells then enter into the lymph node where they can interact with these homing T cells. And when the T cell recognizes, say, an antigen on the dendritic cell, the T cell can respond to that.

And -- so remember, there are only 6,000 to begin with. You can't fight a war with 6,000 soldiers. So what happens is T cells start to make copies of itself and starts to proliferate very, very rapidly until there are millions of T cells that are now in the lymph node. But if this is an infection that goes on in our periphery, now the T cell is in the wrong place. So these T cells leave through the draining lymphatics, return to the blood and now they have changed their trafficking profiles in ways that they are no longer interested in going to lymph nodes. They have now already received the education they require, and they have become inflammation-seeking to access the challenged tissue and then get rid of the infection at the site where it actually occurred. And once that has happened, these T cells are no longer needed, so most of them die.

But the population or the substance of them remains, and these are longest memory cells, and that's in fact what we would like to accomplish by vaccinating somebody.

Now memory cells come in different flavors, and those are actually defined largely by the way how they migrate. Some memory cells continue to recirculate through lymph nodes like naive T cells, others prefer to go peripheral tissues and go to the skin or the gut and some go to peripheral tissues and then, like macrophages, just sit there as tissue-resistant memory cells.



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So how do these T cells actually become activated by dendritic cells? As my laboratory has developed in vivo imaging strategies to understand this, and this involves the injection of dendritic cells that we can supply with an antigen of our choice, we can take these dendritic cells in the footpad of a mouse, and then they traffic through the lymphatics, through the lymph nodes that sits behind the knee of this mouse. We can then anesthetize this animal and microsurgically expose the lymph node in this mouse. The animal is alive and the lymph node is entirely intact, and use what's called multiphoton intravital microscopy to image individual cells, immune cells interacting with each other and responding to this antigen.

Here is an example of this. So this is a low-magnification fluorescent cross-section through this lymph node, but this is in a living animal. You have to envision this lymph node is like an apple and the optic, they section through it, right through the center. Down here, this is the lymph sets of the drain from the foot of the mouse, and you see a little red dot here, that's actually a dendritic cell that's migrating into this lymph node. And there are many in the depths of this lymph node where they are also green fluorescent T cells.

So if you look here on the right side, this is actually a time-lapse video of these T cells in green and dendritic cells in red in the T cell area of this lymph nodes talking to each other. So you can see this as an extremely busy place. What is shown here, the time-lapse video, so the events that you see on the video about 225-fold faster than in real life. Nonetheless, you can see that these T cells are highly motile, and in fact they are outside of the vascular -- if you can -- as you can see here. So we have retaken a higher magnification view here. We have labeled the microcirculation by filling it with a fluorescent dye, and you see these branch capillaries here, and you see a lot of these green fluorescent T cells bothering around. Now this looks like goldfish in a pond, but all of what's black here is actually packed with immune cells, right? Only about 0.1% of our lymphocytes in this particular presentation are actually fluorescent. So this is more like sardines in a can. Everyone buzzing around like crazy, looking for information that they can process. And if this information comes on a dendritic cell in the form of an activating antigen, as shown in this example where the red dendritic cells now present a viral antigen and green cells are actually cytotoxic CD8 T cells specific for that virus, you can see this educational process occurring where these T cells are now being stimulated by this antigen by forming synapse-like interactions with this dendritic cell in the living animal.

Okay. So now we have our T cells educated. Now they need to go and find the infectious site where the antigen -- the virus is actually located. So how does this happen? Some viruses may infect us systemically like cytomegalovirus, but the flu virus will be in our airways, herpes virus will be associated with our lymphoid tissues and skin and so forth. So there is a need for regional or anatomic information as well. And it turns out that the information provided by T cells in mucosa-associated lymphoid tissues is distinct from that, that we see in skin-associated lymph node and that differs from other mucosal sites such as in the gut. And indeed, when these cells are being asked to leave the lymph node and return to the circulation, they actually have unique repertoires of traffic molecules that allows them to preferentially migrate to the anatomic region that is connected to the lymphoid tissue in which they were stimulated. So when they then enter a peripheral tissue, and I'll come back to that in a moment, now they are seeping through the micro circulation, right, where we have arterials where these cells are coming in initially. And then they pass through capillaries, and then they access draining vessels that we call post-capillary venules.

Intravital microscopists have observed over 150 years ago that regardless of where you look in the body and in what species you look, it is only the post-capillary venules where these circulating T cells can actually leave the circulation. And that is because there is a division of labor in the microcirculation where arterials regulate the blood flow through a tissue, capillaries regulate the gas exchange and nutrient exchange and the endothelial cells that coat the inside of these microvessels are basically like Teflon, always. It is only the post-capillary venules that have about 10% to 15% of endothelial cells in a given tissue that are the gatekeepers to the immune response. So there are segmentally restricted environmental signals that tell endothelial cells in the microcirculation where they are and whether they can be permissive to leukocyte at an egress or not. But superimposed from that, there are also anatomically restricted signals that tell venules in the skin to make traffic molecules displayed in the lumen that are different from venules in the brain and different from those in the gut and so forth. You can envision that like a house with many rooms, where each room is a different organ, and in each room you have a wallpaper that has a unique pattern. So T cells that are being stimulated in one particular lymphoid organ are programmed to preferentially recognize a pattern that is just found in one of those particular anatomic compartments, and they will have a strong bias to accumulate and search that location.

So here is a real-life example. This is the brain of a mouse infected with a lethal pathogen, *Naegleria* an amoeba where you see a lot of immune cells in green here that have accumulated in this tissue and you can see all the cells in the extravascular space combating this infection.



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On the left here is an arterial. With blood flowing through this arterial, it's exactly the same as what is moving through these capillaries here in the lower left and exactly the same as what's flowing through the venule. However, in the arterial and the capillary, the blood flow is so fast that you actually cannot see the fluorescent leukocytes passing through here.

Endothelial cells in the venules are uniquely capable of capturing these fast-flowing cells, slowing them down and permitting them to emigrate. So if this was an inflamed joint, you would like to have a way to either tell your T cells or your endothelial cells to act like they were in an arterial. If this was a tumor, you'd like your endothelial cells to act like they were in a capillary -- in a venule. Now this has been a dream so far, but I think now there is technology on the horizon that actually will allow us to do exactly this kind of manipulation.

So if you look in different organs of the body, we find that endothelial cells have unique patterns of these traffic molecules, shown here, that allow this differential regional bias in terms of T cell-mediated immunities. But one of our main interests is actually in the intestines. The intestine is obviously an important site of infectious diseases, causing diarrhea and one of the greatest reasons for childhood mortality in -- worldwide. It's of course also a frequent site of malignant transformation, particularly in colon cancer. So there's many reasons to want to manipulate how lymphocytes traffic through the intestines.

Now usually when you vaccinate someone these days, you inject a vaccine through the skin. If you do that, your dendritic cells will take the antigen to the skin-draining lymph node and you get skin-homing memory cells. That's great when your disease is a skin disease. But if you want to protect against an infectious disease of the intestine, it doesn't really work so well because if you give the same antigen in -- to an oral route, it's presented in the Peyer's patches of mesenteric lymph nodes associated with the gut. The memory cells you get then, you may see exactly the same antigen, but they actually express a different pattern of traffic molecules that now binds them to actually go to the intestines where they need to be. Unfortunately, vaccination through an oral route is very difficult because imagine we take all of these food items and we have all these bacteria in our guts in which we need to be tolerant. So to convince an immune response to occur by oral vaccination to things that are not replicating pathogens is actually very, very difficult. And so the world has been trying for a long time to make parenteral, so intramuscular or subcutaneous vaccines, work to protect against mucosal infections.

Now the reason why this hasn't been working very well is because dendritic cells in the intestine but not in the skin convert vitamin A that we take in with our diet into All-trans Retinoic Acid, and it's the action of All-trans Retinoic Acid on the TMV cells that imprints them with this gut-homing ability. So one way to think about this would be to say, okay, why don't we just make a vaccine and add retinoic acid to that vaccine and inject that through the skin? So it doesn't matter if the dendritic cells don't make ATRA, which has provided exogenously. And you can use either retinoic acid itself, which is very difficult and unstable -- difficult to work with, or you can take synthetic analogs like this AM80-type molecule. So we did that in mice as an experimental vaccine. And indeed, if you look in the -- if guts of an animal that is vaccinated just with antigen and adjuvants, the green dots are memory cells. You see a few of them in the gut. But if you do the same experiment and add this retinoic acid analog, you can see massive increase in these memory cells accumulating in the intestine.

So is that relevant? Well, we can vaccinate mice with an antigen through *Listeria monocytogenes*, an oral pathogen, and then do that either by adding retinoic acid or not. And then 2 weeks later, we can challenge the mice orally with *Listeria*. And so if you look at the survival curves here, naive mice that have not been vaccinated, that's the red curve here, basically all died from this intestinal infection. If you vaccinate conventionally, so you give the antigen but you don't give retinoic acid and you just subcutaneously vaccinate, you get memory cells, lots of memory cells in the blood, but they don't go to the gut, and there's basically no protection. But if instead you add retinoic acid to that, basically all of the mice survive. So that really illustrates the importance of this regional imprint. But unfortunately, there's a catch, and that is retinoic acid is actually quite toxic when given at the doses needed to get this imprinting effect.

So here, we have done this in support from the Gates Foundations with many animals over a number of years now, and you can see there's actually substantially strategic chance associated with treating these animals with free retinoic acid. And if you look at the injection site, you can see evidence for massive inflammation that occurs here. So this is a great lab exercise, but it's unfortunately not really translational. You will never put this stuff into healthy babies.

Now talking to Stephen and his colleagues with (inaudible), why don't we just teach dendritic cells and other immune cells in the skin-draining lymph node how to make retinoic acid? So couldn't we just take what makes dendritic cells in gut-associated lymphoid tissues unique and transplant



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that transiently into lymph node? Because we know that the retinoic acid is produced by an enzyme called RALDH. And RALDH is made by the dendritic cells in the gut, but not in the skin or in the spleen. And once the retinoic acid is made, it acts on a nuclear receptor to cause all kinds of [transitional] changes in this cell.

So can we express RALDH in peripheral lymph nodes? The idea would be we take these immune nanoparticles that Stephen has just described, inject them through the skin, wait 24 hours and then see what we get. So if we look in the skin-draining lymph node injected with these particles and with the RNA that's included in this particle as a fluorescent protein like in this case, mCitrine, you can see that there is massive expression of this protein now transiently in this lymph node. And mostly in the outer cortex here, this is the so-called [sub-capsule of] science. It's a very important area for allergen presentation to B cells, but also in the medulla. And what's labeled here in blue is the T cell area. You can see the T cells here or cells within the T cell area also makes this an RNA-encoded protein. And by fluorocytometry, we can see that compared to control mice, there is expression in T cells and B cells, but also in several other leukocyte substance. Okay, so that's the fluorescent protein. We can directly visualize this while (inaudible) protein, but we can ask if provision of this enzyme sufficient to now generate an immune response that is no longer biased to the skin and instead goes to the gut. And the answer is yes, we can. So here is a control situation in the intestine. You see a few memory cells. Here is the situation where we give the highly effective but toxic retinoic acid analog, and you're getting again nice trafficking to the gut. And this is in animals that we vaccinated in this case only once, RALDH mRNA, and we see expression in the intraepithelial compartment and in the lamina propria, that is basically just as good as what we get ready to give -- use this AM80. So we are quite excited about this. This is of course only one example of many that we can think of, how we can now manipulate and redirect immune responses to various regions on the body to either prevent diseases or to treat diseases potentially.

And so I would like to leave you with that. I thank you very much for your attention. I'll be happy to answer questions.

Stephen Hoge - Moderna, Inc. - President

So bear with me as I flip this back out. Some of those video files are quite massive.

So just in closing, we hopefully have shown you a number of different features that I've described. Can we see the -- let me see if I can -- right through this. Here we are. Good.

So we talked about demonstrating dose-dependent (inaudible) pharmacology across a wide range of cell types. I showed some examples of transient protein expression to [confer], in one case, towing the CD19 cells in a [authoritative] way. And we talked about how we use mRNA software for safety.

And then lastly, we provided just one example, one example using RALDH to reprogram cells. You can imagine many, many others based on the science that he just walked through to drive trafficking so we can facilitate the immune system getting to the side of interest. An example presented here was the gut, but you can imagine that for solid organs for cancer and a whole bunch of different applications.

So just closing this section before we get to Q&A very quickly. Hopefully, we've provided a little bit of insight into why we're so excited about the immune nanoparticle delivery system and its relevance to an incredibly wide range of diseases. You -- the mind does race to the things you can do in cancer, autoimmune disease, neurodegeneration generally in aging. But I want to anticipate a question that I will probably get in the Q&A, which is, what's the first program? You've shown us a CD19 car. You've shown us an immune memory in the gut and trafficking cells to the gut. You've talked about autoimmune disease, you've done even a genetic recombination experiment in mice with pre-recombinase and turning on a red fluorescent protein.

The answer to the questions is we're not talking about that today. Today is a Science Day for us. Today is the one day a year where we have committed to come and share with you just a snippet of some of the science we're advancing in the platform. In fact, even in the immune nanoparticle space, even in delivery science space, believe it or not, the immune nanoparticle is a minority of our investment in delivery science. It's sizeable. We're serious about it. But the other areas we're investing in are bigger and long term. But what we do want to do in a day like today is give you a sense of that progress, both across our messenger RNA and the things like physics as well as in our nanoparticle work.



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So with that, I do want to thank all of the presenters on the Moderna side and Uli for taking time to be here today. We're going to transition to Q&A. But just in case we are losing people on the back end of this as we do the Q&A, I just wanted to put one public service announcement, which is the next Moderna investor event. That investor event will be in New York. It's our R&D Day. That is the place we talk about our pipeline, and we'll look forward to your questions there on September 12, 2019.

So with that, I wanted to invite Melissa to come up and we'll take as much Q&A as you can bear until lunch is served. [That's for free.] The folks in the back have microphones, go ahead and start [your questions].

QUESTIONS AND ANSWERS

Geoffrey Christopher Meacham - *Barclays Bank PLC, Research Division - MD & Senior Research Analyst*

Geoff Meacham from Barclays. So thanks, first of all, for a very comprehensive day. I guess the main question is if you look at the risk profile independent of the clinical risk with all the individual programs of just the platform, others have had some speed bumps on the LNP side of things with toxicity. Maybe just help us with how you look at the platform from a derisking perspective, again, independent of any clinical data, but have -- what have you done sort of size and scope of assay to kind of mitigate toxicities and things of that nature? Just help us out with that aspect of the pipeline.

Stephen Hoge - *Moderna, Inc. - President*

Yes. Absolutely. I'll take that and (inaudible). So just first of all, others have had and we would say we've had our own along the way. Many years ago, but along the way. Melissa started by highlighting a couple of papers actually published in the last year that are in those books in front of you that talked to in great detail the science behind a couple of the improvements we've made to improve the tolerability, safety profile of our lipid nanoparticle. Whereas we started down that effort, there was no guarantee of success. But hopefully, we think that preclinical science really demonstrates it. I also think we've seen translation into the clinic of all of those key aspects to date. And we, knock on wood, we hopefully continue to see that. So we're very optimistic.

Now the science behind it, which is maybe today is the question, it boils down to really a whole bunch of features. And I almost -- [remiss in] saying 2 because of the number of people that have done so many things, but I've focused on 2 features maybe as you answer that question. The first is the actual chemical composition of the nanoparticle. At a previous Science Day a couple of years ago and in those manuscripts, we talked about how the amino lipid, which is well over half of the mass of that lipid nanoparticle, absolutely essential for safety and tolerability. The first generation immuno lipid that we've used, that others have used and actually are now in improved drugs, didn't have very good biodegradability and biocompatibility. They were fine, they were well tolerated. To be fair, they're approved drugs. But actually for our applications, what we found is when we went into primates, those immuno lipids weren't breaking down into their components, [so weren't] clearing fast. And the immune systems were getting stuck with (inaudible) they couldn't break down, and we published data in primates showing what that looks like in lymphoid tissues.

That's obviously of a concern because if the immune system finds something it doesn't know what to do with, it starts asking questions and can create problems. And so one of the key features for us was a complete rebuild of what it means to be an immuno lipid. Novel chemical matter, we've got issued [compositional] matter on that, [United States] and (inaudible). But novel chemical matter that is [not] compatible, that ultimately breaks down into food within hours of it being dust. And so that's our key feature.

The second that I'd highlight is the surface properties of nanoparticles. So we make these things, these soap bubbles, and Melissa showed some great pictures of them. And as we've described in the physics section of the lecture, we are intensive in trying to understand the surface chemistry because they will -- I know the components is great. But if you don't understand how they're organized on the surface, you don't really understand the part of the molecule that's interacting with the immune system. And it's important for safety and tolerability. We, again, in multiple presentations and even some of those manuscripts, have described how we've engineered the surface properties of those molecules, specifically so that the immune system and immunoglobulins in particular (inaudible) don't bind to them and don't need the complement activation. And that is a critical



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feature of putting a nano mass into animals and into humans, which is that you want it to be incredibly well tolerated and not have a surface handle for the immune system get a hold of and deposit complements.

The solution for that was just chemical engineering. Once you actually, through all of these great techniques, understood what was happening, you just made it not there. And then when you inject it in humans and in animals, what we've been able to see is the nanoparticles are extremely well tolerated. When we do the positive control, so we bring our legacy products, we had experience with that many, many years ago, and we see step-function change in terms of tolerability. That's been in all of our preclinical systems. That's been in our primate work, which is our key top species we're looking at. And today, as I said, it's translating in the clinic. So we're optimistic that this isn't dark magic, that ultimately it's molecular biology and surface chemistry. And if you engineer the right solutions, if you understand the problem, you can actually find the right solution relatively quickly. That's subject to ongoing data and all of the other things we have to do, but we're excited to be where we are.

Wherever you guys get to first.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

These are actually passable microphones, so you can talk there...

Stephen Hoge - Moderna, Inc. - President

I don't think we want people throwing microphones.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

(inaudible)

Unidentified Analyst

I'll try not to drop it. Yes. (inaudible), Roth Capital Partners. A number of [timed] question for you and Melissa. Great detail about codon optimization and structure. So when we think about optimization of codon from secondary structure, is that different when we think of the mRNA for the use of vaccine versus mRNA that are for systemic delivery?

Second, does the size of the mRNA matter? So you pointed out that ultimately, to optimize secondary structures, so you go through a bumpy road, the higher you can drive your expression, right? So by the same analogy, is that there will be a component of the legs of the mRNA that will ultimately influence sort of optimization? And then I have an RNA -- a micro RNA question for Stephen.

Stephen Hoge - Moderna, Inc. - President

Oh, she should be the one to answer (inaudible)

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

So to answer the first question, do we basically design our mRNA so they -- as we say, we design criteria for vaccine versus studying rare diseases and therapeutic (inaudible). And the answer is they're similar but there are differences, because we -- and inside of the platform, a lot of what the molecular biology and computational sciences departments do is to tailor the mRNAs to particular applications. And so we have a large effort underway to understand the rules by that -- what are the best features of an mRNA for a vaccine, for a prophylactic vaccine versus a therapeutic



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vaccine, for rare diseases versus an immuno-oncology target. So I can't give you a simple answer there, but there are differences. So that answer -- I think that addresses your mRNA question.

Unidentified Analyst

Yes. And then your second one, the length of the...

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

The length of the RNA. So we also have an intensive effort within the platform on understanding how the length of the RNA dictates all kinds of things, like its encapsulation efficiency, its -- how well its structure is maintained. And so in terms of the -- how that affects the overall translational output, that's hard to measure because if you're making the codon -- the codon sequence longer, you're making a different protein. And so you're -- then you're measuring apples and oranges. And so that's just not something that we normally would try to measure because it's not really comparable.

Unidentified Analyst

And then how do you determine the micro RNA target to really allow proper trafficking? How do you (inaudible)

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

So the micro RNAs -- in natural mRNA, so in endogenous mRNAs, the micro RNAs are binding, as Stephen showed in his slide, in a confirmation that doesn't digest -- that doesn't degrade the mRNA. What we do though is we -- if you make your mRNA have a sequence that's perfectly complementary to the micro RNA, then the micro RNA will act like a small interfering RNA and cause cleavage of the mRNA. So the way that we are determining what micro RNAs to target slice to use in our mRNAs, depending on what cell type we want to turn off expression in, is we're sequencing all kinds of different cell types and determining what the micro RNA profiles are in those different cell types. And then we have a big library of that. In fact, Ruchi here is involved also very much in that. And then we can -- when Stephen comes and says, "Hey, can you -- Ruchi, can you make it not be in B cell -- turned off in B cells and not in T cells?" She says, "Oh, use this micro RNA." So that's how we're going about that.

Edward Andrew Tenthoff - Piper Jaffray Companies, Research Division - MD & Senior Research Analyst

Stephen, 2 questions if I may, this is Ted Tenthoff from Piper Jaffray. Firstly, I talked briefly to Michelle -- or to Melissa about this a little bit at the break in terms of the really cool kinetics of the formations of the LNPs, but I'm wondering about sort of on the back end in terms of how you analyze sort of release of mRNA, either in the endosome or ultimately the traffic to the cytoplasm in order to translate the mRNA therapeutics. And then I have a quick other question on the lymph (inaudible)

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

Yes. So we did almost half of Science Day last year on exactly that topic, which is how do we measure how much accumulation of our LNPs or endosomes, what fraction of the RNA escapes from the endosomes and then what fraction of those RNA molecules that escape are taken up and actively translated in the cell. And in fact, as we are trying to optimize our LNPs for a different delivery goal, that -- those criteria are things that we measure and go into our pool of data that contributes to whether, are we going to use this version, are we going to use that version? And so those techniques are mostly single molecule techniques and they're imaging techniques. And if you're interested in them, some of them are in that (inaudible) paper, in one of the figures in there. But we do a lot of that. We have a tremendous amount of imaging.



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Stephen Hoge - Moderna, Inc. - President

And I would just say single-molecule imaging in cells because a lot of the activities of endosome will escape, were just being described as the base of your question. You ultimately need to reconstitute the biological systems to measure them. We do have analytical techniques to try to approximate that, and we think they're pretty good, but as valuable as x-ray guns and nuclear reactors are for generating surface properties, it's not so good for understanding your actual biological system. And So cell-based single molecule imaging has been our most cutting-edge tool.

Edward Andrew Tenthoff - Piper Jaffray Companies, Research Division - MD & Senior Research Analyst

Great. And I appreciated your immunology 101 over a year ago, so I'm wondering about sort of the gut and maybe abilities to access the immune system or train the immune system there. And could we even see a day where we have oral mRNA therapies?

Stephen Hoge - Moderna, Inc. - President

Oral mRNA therapies. So we curate the things we talk about and that wasn't on the list, so we can't -- I can't provide any guidance about the future of oral mRNA delivery. But the gut, just on the question of therapeutics for the gut or vaccines for the gut, let me present an example of how we drove, in those case, lymphocytes to the mucosa of the gut. So the profile of those cells in that case, where they were specific for the vaccine, the antigen we were providing, but you can imagine those lymphocytes having different profiles or being programmed in slightly different ways, a more tolerogenic way. And so one of the features of an immune nanoparticles approach could be the ability to transiently reprogram cells so they traffic to the gut for therapeutic benefits. And so that's -- I think that's implied by the work that was presented. But we're not -- we don't have anything to update today on oral delivery.

Matthew Thomas Holt - JP Morgan Chase & Co, Research Division - Analyst

Matthew Holt from JPMorgan. So my first question is on the first part of your talk and I know this might be difficult to do succinctly. But I'm trying to understand why you present in the context of your clinical -- your current clinical pipeline. How much of this is already in the clinic and -- versus what's next gen?

Stephen Hoge - Moderna, Inc. - President

So some of these technologies are already in the clinic. We disclosed that in the past. Micro RNA is an example. Obviously, Moderna talked about our evolution and process and (inaudible). And so you can imagine, a tremendous amount of what we talked about today actually is in our pipeline in one form or another. We do not take individual pieces of technology, and say, "Well, these things a miss." The map would blow the mind in terms of the diversity of different things that we pull together. Our best attempt to approximate that for you all is in the way we do modalities. So we talk about a modality in a specific therapeutic context, the technology that comes together there is more often than not highly common between these programs, which is the best opportunity for (inaudible) between them. But modalities have different combinations of technologies. So the other heat map would be bigger than the screen.

Matthew Thomas Holt - JP Morgan Chase & Co, Research Division - Analyst

All right. Great. And then in terms of immune cell targets, like what do you see as the low hanging fruit in the sense that works well with mRNA and could have like the biggest impact on therapeutic areas?

Stephen Hoge - Moderna, Inc. - President

So I can't answer the specific target question. I already answered it. But I will point you to the 4 things that I have. So where will we think about applying this first, or already are? Dose-dependent pharmacology. Where do you need to be able to titrate up and down your response in an



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vivo context in a way that's relevant therapeutically? Transient expression driving phenotypic changes that ultimately are also dose-dependent, a key feature in -- for some things like to fuse the CAR T example, for which that's not a key feature of those approaches. And then obviously, we talked about trafficking and micro RNA and programming. So using software to either drive cells to new tissues or -- and drive new cell interactions. So those are the features, the places we're most interested. But almost always, it boils down to us -- that first one, which is where is dose-dependent pharmacology? I give a dose today, I get an effect. I give it tomorrow, I get the same effect. I give it next week, I get the same effect. I know how to predict it if I give 3x the dose, I get 3 times the effect. That feature of our platform is core to everything we pursue and I'm absolutely certain it will be core to how we pursue [our first priority] in the immune nanoparticle space.

Unidentified Analyst

Great presentation. Quick question. As you transition from ex vivo to in vivo with multi-dosing, can you characterize whether you have any impact on cell expansion and durability? And then a little bit more on innate immunity and adaptive. We've kind of seen what's happened with the CRISPR experience, just trying to understand the similar prime areas with your technology.

Stephen Hoge - Moderna, Inc. - President

Yes. So are you asking -- is that context generally or just on the immune space, or both?

Unidentified Analyst

You presented immune, so I figured...

Stephen Hoge - Moderna, Inc. - President

Okay. So in the general (inaudible) I'll just point you to both our clinical data where we've been doing repeat dosing in humans (inaudible) for the [ligand] program as well as a published (inaudible) toxicology work that we've done in primates with chronic dosing and a number of different disease molecules. There's actually a decent size of [literature] out there. We have not with our systemic therapeutic approaches seen any dose from toxicity in these preclinical systems. In fact, the highest dose tested in our [GSP] testing, this was in some of our regulatory filings during the [IPO], in primates upwards of 3 or 5 milligrams per kilogram (inaudible) in some of those programs like MMA. And so with the no adverse event level, that includes product dosing out to many months. In the case of the immune nanoparticles, is your question, so that's (inaudible) because we actually started with that. I had to whistle past that in the context of this presentation as one of the key criteria. We could not change [cell fate] is a result of the nanoparticles. The negative control of the untranslated mRNA nanoparticles needed to get into those cells, needed to make a protein, but that protein could not make that cell more angry or less angry, more likely to replicate or less likely to replicate. It needed to go in and out and not have an effect. That took a tremendous amount of work. We're satisfied at this point in the preclinical systems and the way we've looked that we're not driving big differences in that cell population. I point to the example of the mouse data at the end where we're dosing 4 doses [of medication] just in 8 days, where we saw no changes in the untreated population or in the negative control populations in cellularity. We've obviously looked at that when we've gone to primate experiments as well. But I think it's fair to say we're still in research, and we're still -- proving a negative in science is a very hard thing to do, and so we're actively continuing to investigate and look for whether those are -- they're unintended features of the -- today we haven't seen it, but that doesn't mean it's not out there, it just means we haven't found it. So we'll keep looking.

Unidentified Analyst

And innate you're okay with?



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Stephen Hoge - Moderna, Inc. - President

Innate -- so the mRNA features I think we covered in the -- to our knowledge today, where we haven't put this in a development program where we've gone and you have an obligation in preclinical development to push in 2 species to maximally tolerated doses to define it. Sometimes, even that situation, you'll discover -- usually in that situation, your desire is to discover what the dose (inaudible) toxicity is. Because I haven't done those experiments, I can't tell you what it will be. But the doses we're using here are analogous to -- I mean, if you look at the dose numbers, they're not as strong as doses we're taking into humans and other programs. And so whether there is a change at that upper level, I can't observe yet. We do look at things like cytokines, cellularity and other sort of traditional (inaudible) markers and we have not seen anything jump out at us in that regard at these doses. Please?

Unidentified Analyst

Bob (inaudible) from Wellington. I have some more questions about the immune targeting. What happens to normal immune function while this is going on? Have you taken the -- the cells that you've [affected], have you taken them out of their normal function? And what evidence do you have that after expression is over that they actually return to the same cell type and function that they had beforehand? And then I have one other question.

Stephen Hoge - Moderna, Inc. - President

So most of the evidence that we have is in cell-based systems, because you can do more of those longitudinal experiments, you can't chase the cell around the body. But taking ex vivo human blood, we have -- you can imagine all the things we're looking at, but we have looked at the ability of activated cells to be activated, naive cells just to remain naive in the T cell complement, for regulatory cells to be able to -- still to be able to be immune suppressive when they encounter their antigen. And to the best of my knowledge now, I think all of those have not shown dramatic changes in cell fate, as I was answering the question earlier -- it's actually a design feature that we were solving for. Not everything we do has that feature, but the lead program had to in terms of its profile. Those are all still ex vivo, multi-day, maximum maybe a week sort of experiments and so you've got to go look in animals for a longer period of time and there's a lot of work ahead of us in that regard to confirm that feature, because it's pretty important that we're not actually -- other than the protein we made, we don't want to leave the signature that we were there. That's a key feature of what we're trying to develop.

Unidentified Analyst

Right. It sounds a little bit counterintuitive to me that you can do that, at least depending on the molecule you're expressing. If you're expressing a molecule that has an immune function or if you're targeting a cell to acquire an immune function, why would we expect that, after transient expression was over, it would go back to being what it was?

Stephen Hoge - Moderna, Inc. - President

I was answering the question about the delivery vehicle, not the protein that gets made. Sorry if I misunderstood the question. The delivery vehicle has the features I was describing. Obviously when we confer a function, like let's say a CAR, what you will see ex vivo in self-healing experiments is a -- is literally self-healing, right? And in an antigen-specific way. And (inaudible) you can imagine the full spectrum of antigens we've done at multiple different (inaudible). To my knowledge, I don't know that we've -- so we've monitored those cultures. We do know that when those cells become activated, they can expand. And that's a feature of a T cell activations. And when they expand, they won't naturally dilute out the protein that got made, right? So in this case if you have a CAR, they will start to dilute that out. They also aren't replacing that. We didn't genetically engineer them. They are transiently repurposed. And so as a result, it has a -- there's a dilutional effect. As soon as those cells start to expand, their actual TCR, which is encoded in their gene, starts to predominate and it's not finding its antigen. And what happens to a T cell when it's expanding and not finding its antigen? Well, eventually they apoptose. They become inert. But ultimately they're not activated if they're not seeing their antigen. The antigen specificity of those T cells is actually whatever component was transected. And as Uli said, it's quite a large range. That's in the T cell compartment. In the NK cell compartment, natural killer cell compartment, those cells are somewhat different. They will -- they obviously can

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become activated and specifically go after cells that have that matching feature. Otherwise they will still be inhibited by MHC class I, and so there's a balancing act for them, and in some of the other compartments there's sort of versions of the same. But I think the key -- maybe the answer to your question is once the cell is conferred with, let's say, an activation state that needs to [call on] expansion, that will actually self limit the pharmacology of the drug. If we dose again, those expanded cells will all have, for a period of days, the new phenotype, but then that will go away again.

Unidentified Analyst

Is that right? Would you -- the last question, would you preferentially target the expanded cells, or do you target a random fraction of all the cells? Which was going to be my other question.

Stephen Hoge - Moderna, Inc. - President

Yes. It's not random. We haven't published the mechanism, but I think the [gross,] concise answer to the question, is it is a broad fraction of all cells types. And so it represented the fraction, it's naive T cells, T regulatory cells. One thing I didn't present that we -- maybe should be obvious is that activated T cells are very avid for particles, and so expanding cells are actually relatively easy to transect. And the percentage of -- I alluded to this during the presentation, can get upwards to 90% plus. So preferentially, you can get into activated cells if that's an area of interest, but it's not an area of focus for the nanoparticle.

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

I just want to add one thing to what Stephen said. It's important to remember that not only is the mRNA transient, but proteins have their own half-lives. And so any reprogramming is dependent both on the mRNA half-life and on the protein half-life. So if you wanted to have extremely transient reprogramming, we can engineer our proteins to be very short half-life proteins just like I showed you with the GSP, where we put the little (inaudible) tag and we can make a very short half-life. And so we had lots of levers that we can pull to control the timing and how long the effects last.

Stephen Hoge - Moderna, Inc. - President

Again, we obsessively solve for dose-dependent pharmacology. That's sort of at the core, that's why I said it's the #1 objective. And that includes the ability to control the off feature of the drug. I think it's our last question before -- sorry? Oh, there's 2 questions. Last 2 questions.

Unidentified Analyst

I'm (inaudible) from Mizuho Securities New York. I understand we're not going to talk about the pipelines but we had some questions around the R&D (inaudible). I'm wondering -- one of the advantages of using RNA in vaccination is due to the adjuvant ability of RNA, right? Because it triggers a specific immune response and helps in enhancing an immune response against the agent that you are vaccinating. Now [we're -- talk here] around modifications that make these RNA immune silent, right? Not able to trigger the (inaudible) of [TLR] and everything. So my question is, do you think these modifications are going to be a problem in terms of when using these RNA as vaccines because you're avoiding the adjuvant (inaudible) RNA?

Melissa J. Moore - Moderna, Inc. - Chief Scientific Officer

So, yes. I think Stephen's going to take that.



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Stephen Hoge - Moderna, Inc. - President

I'll try to answer very quickly. So we seek to control our interactions with the immune system. So there are instances where we want more immune activation, but we always want to be in control of it. So we use the modification so that we can dictate what's the degree of (inaudible) your adjuvants of mRNA through other features that we can specifically control, including processing sequence. We also use processing sequence to drive (inaudible). So start with something clean and add back, rather than start with something dirty and only partially clean it up. And that's the philosophical approach to how we do vaccination. Last question.

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

It's Hartaj Singh with Oppenheimer. Just a couple of questions. One, just maybe take one step -- I know why you keep the clinical stuff to the R&D day, let me just take one step forward for us. I'm a clinical person, I worked in clinical drug development. So when you file an IND, there's a fairly specific process that companies have to go through with the FDA, regulators, or the EMA. I think different modalities, for example, gene therapy are changing the language a little bit and changing how FDA interacts with companies that are coming with new modalities. Obviously, mRNA is one of the newer modalities. Can you just sort of map what you talked about grossly against that process of going through target identification of understanding what it is that -- how you're going to get your therapeutic there in that sort of preclinical process? If it's possible in a gross way. And then I got a second follow-up to that.

Stephen Hoge - Moderna, Inc. - President

Okay. So I think in the context of IND filing [regulations], obviously we have a lot of experience with that. I think there is 11 or 12 INDs that we filed and opened and several Phase Is that are completed or ongoing. And even some Phase II work that's underway. And so we had a number of interactions with our regulators. Multiple continents, multiple different regulatory agencies. I think the short and punchy answer to your question is when it comes to first in human experiences with drugs, we put our pants on one leg at a time like everybody else. We have to go through the process of demonstrating all of the things, in particular that we understand the tolerability profile of our technology, its pharmacology and what we expect to see, why we predict the dose we did and what are we going to do. To date, that's largely been positive in terms of the clinical data we've disclosed, so we're pretty excited about that. But that involves -- you just -- you missed walking through the process, that involves all of the normal heavy lifting in technology, the technical development, technical operations. So preclinically, we have massive efforts in proving we can make these things, scale them up, we haven't changed their character in scaling them up, developing analytical tools, including specifications and release of it whenever we make a product, even for Phase I, that we can predict and believe that it's going to be the same as long as it's in specification and used in the clinic by those patients over time because we run multiple batches sometimes. We do normal intensive toxicology work that you're obligated to do to test something in a human, including more often than not 2 species. In the rare disease context, obviously we're doing chronic toxicology work. We've even done, in some cases, [reprotox], where you have to go show that. And all of that is to provide confidence to ourselves first, but also to our investigators, to patients and subjects who are going to use it, and the regulators that we do understand what we've made, we can predict its tolerability and therefore can advise people what to be looking for in the clinic. Last piece of that is pharmacology. You've got to pick a dose, you've got to be able to model that dose and you have to predict the response in humans. And to date we've been doing that in the traditional way. We do a bunch of preclinical translational work and very smart modeling not talked about today and then ultimately you go into the clinic. Sometimes you get a low dose or you get a higher dose -- or response than you expected but basically you need to demonstrate that in the Phase I study.

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

Great. And then just quickly, I know you -- you talked about the 4 characteristics of immune therapeutic. In all the experience you've had up until now, and that I guess adds up to kind of a risk-benefit profile, right? In a gross way again. What has your experience been in terms of prioritizing those [4]. I mean, which are the ones that are easier to get to and which have been harder to get to if that's possible to answer?



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Stephen Hoge - Moderna, Inc. - President

Well, in the delivery space, the mRNA ones are the easy ones because those came with a platform. And so some of that software like reprogramming, if we find we're in a tissue we didn't mean to be in, almost all nanoparticle delivery technologies end up in the liver and end up in the (inaudible). That's just what the liver does, it clears a lot of stuff. Being able to turn that off, which was a subject of that publication that I referenced in the presentation last year, is a key feature and that is imparted to every one of our delivery technologies because otherwise you carry the baggage of, "My gosh, I've got a CD19-CAR in a hepatocyte." Which who knows? Maybe it's a good thing, maybe it's not. But it's not the plan. So we deliberately use those mRNA features across and those have tended to be easier.

The heavy lift, the thing that takes us years and lots of time and treasure is getting to dose-dependent pharmacology, right? You can -- and particularly demonstrating that we can do that in a mouse, in a rat, in a primate and now in humans. That feature, that translational feature is the bar we set, and it's usually the highest bar. That includes, as a part of that pharmacology, tolerability. It is useless if it works in a mouse and a rat and it's toxic in a primate or it might be toxic in a human. And so that dose-dependent pharmacology, a therapeutic index and window that's wide and we understand it is the key feature that we wrestle with.

Okay. Thank you all very much for your time today.

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