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PRESENTATION

Lavina Talukdar - Moderna, Inc. - Senior VP & Head of IR

Good morning, and welcome to Moderna's Fourth Annual Science Day. Today, you will be hearing from our scientists in platform research and their work enabling the discovery and development of our mRNA medicines and vaccines.

Following the formal presentations, we will take your questions during the Q&A section. You can access the press release issued this morning as well as the presentation slides by going to the Investors section of our website. On today's call are Stephane Bancel, our Chief Executive Officer; Stephen Hoge, our President; Melissa Moore, our Chief Scientific Officer of Platform Research; and the scientists representing the broader platform research team.

Before we begin, please note that today's presentation will include forward-looking statements made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. Please see Slide 2 of the accompanying presentation and our SEC filings for important risk factors that could cause our actual performance and results to differ materially from those expressed or implied in these forward-looking statements. We undertake no obligation to update or revise the information provided on this call as a result of new information or future results or developments.

On Slide 3, please see the important indication and safety information for our COVID-19 vaccine, which has been authorized for emergency use in the United States and in many other countries around the world.

Now I will turn the call over to Stephane to kick off the event.

Stephane Bancel - Moderna, Inc. - CEO & Director

Well, good morning or good afternoon, everybody. Welcome to Moderna's Science Day. We are very delighted with the team that you're taking the time to be with us today.

As you know, we build Moderna on the belief that we could find a way to safely inject mRNA into people, to deliver a new class of medicine. This really drove our mission since the beginning. We were so excited about what this could be for patients that we set our mission, has to deliver on the promise of mRNA science to create a new category of transformative medicine for patients. And that drive to help people is what is driving Moderna.

We got very excited because we believe we could make a new class of medicine going after new targets. Not only we could do secreted proteins, but even more exciting, we could do transmembrane or intracellular protein. We believe because mRNA is an information molecule, we could go and create a much higher probability of technical success over time between the products. Because when you think about it at the end of the day, the chemistry, the formulation that we use for making a flu shot, let's say, is exactly the same as the one we use to make the COVID-19 vaccine.

We also believe that by investing in process development, manufacturing, IT, we could have a very quick time to market. And we believe that because the manufacturing process is the same regardless if you make mRNA for VEGF or COVID or CMV, that there's going to be incredible leverage of a manufacturing infrastructure or such a technology platform.

The way we think about the platform, to give you a bit of insight, is along with the following lines. We believe there is a component which is around the mRNA molecule. And there, you have sub-chapters: the chemistry that the mRNA is made of, the sequence engineering, how you decide which is the right place for the nucleotide, targeting elements so that you can turn on or off the mRNA molecule. Then if you have a great mRNA molecule, you need to bring it into the cell, and this is where delivery becomes critical. Not only the chemistry that we put the lipid around the delivery system, but the composition of each lipid. And then all the surface properties that we've learned over the years are very important to make a safe and efficacious product.

And then, of course, is how you make the products, how you make the mRNA on the one hand and as you make the lipid and get the mRNA into a lipid. So this is really what we did define as our platform.

When we started the company, we've always been very committed to science. It's really who we are. We believe you cannot do a great drug without doing great science. It doesn't happen. And so in the early days, we invested a lot to get the science ready to go to the clinic. And we are very cautious and careful because we don't want to go to the clinic too early. With a new technology, you have a lot of unknowns, and we wanted to be ready to make sure we will not hurt anybody and could have a good proof of concept.

But what is very interesting about Moderna is that as we develop the company into a clinical company and, more recently, late-stage development and commercial company, we stayed extremely committed to those investments in science. And we continue to invest across the board in science. We're not slowing down. It is not because on a quarterly call you don't hear about our science, that the scientific team is slowing down. And today, we are very happy to share with you some of the new discoveries that they have made.

The other piece that is important to understand is those investments in science in a platform enabled what we could do last year. The reason we could go so fast developing a vaccine in only 11 months, from sequence to authorization, is because of this investment in research over the years. And that's really allowed us to also learning in the clinic on over a vaccine to be ready for this moment when it came last year.

One of the most important thing that we like to talk about at the company and to think about as we think about the future is the technology S-curve, performance of mRNA technology over time. Since the very beginning, we believe that this new technology that has never had a commercial product before, is certainly poised for an S-shape technology curve. As you can see in the pictogram, we believe we are still in a very steep part of the curve and is still the best day of mRNA science are ahead of us, not behind us. And so we continue to invest aggressively in science. As you know, we have a 10x mentality at the company. We want to think big. That allows us to take bold risk and to do new things, that if you think only incrementally in front of your nose, you will never think of doing.

We also want to make sure that we optimize everything, from the quality of the data to AI, and that we build a platform that's allowing us to learn in a very strong way. The piece that excites me the most is our scale today. No mRNA in the world has the financial resources, the team and the ability to invest more in mRNA science. And it's very important for us to keep this leadership and to keep investing in science. We still believe it's day 1.

Now before turning over to Melissa, let me walk you very briefly through our agenda. We're going to start by talking about what we are doing to assess SARS-CoV-2 variants of concern in our labs. We'll then move very quickly to what we are doing in terms of mRNA engineering to make sure that we keep improving the technology of mRNA. We're going to move then to show you some new capabilities, including with homeopathic stem cells. We really believe that this is a new production modality. The teams are working on the lab with Vertex, and we think that the ability to go into stem cell will make a huge difference in the type of medicine we can do. Stephen will come back at the end to close.

So with this, let me turn over to Melissa.

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

Thank you, Stephane. I'd also like to welcome you to our Fourth Annual Science Day. I'm Melissa Moore, and I am the Chief Scientific Officer of Platform Research at Moderna. What that means is that I direct our research efforts aimed at answering 2 key questions: what are the mRNA engineering principles enabling optimal expression of different proteins; and how can we best deliver mRNA to sell types of interest?

Our goal for Science Day is to give you a look behind the scenes into everything that needs to be considered for engineering mRNA medicines, the challenges we face, how we overcome them and some of our recent technological advances that are expanding our platform capabilities.

Before I go any further though, I want to be sure and acknowledge the scores of colleagues who have contributed to and made possible all of the stories we'll be telling you about today. To sustain the rate of progress we've been making year-after-year requires much more than a village. Rather, we're more like a small city of scientists and engineers from all types of backgrounds, from mathematicians to physicists, chemists, biochemists, molecular and cell biologists, immunologists, microscopists and computer scientists. I'd particularly like to thank our in vivo pharmacology team, whose tireless efforts are enabling many advances we'll tell you about today. Together, we've built an incredibly agile and rich research culture that epitomizes our company values of bold, curious, creative and relentless.

This year, we're going to start with a topic that is of immediate concern in application, how we're identifying, assessing and ultimately predicting SARS-CoV-2 variants of concern to speed our development of second- and third-generation COVID vaccines and booster shots. After that, we'll talk about our progress on several longer-term objectives, such as understanding how modulating ribosome load can help us achieve optimal levels of protein expression, how our capacity to dissect various intracellular events is enabling us to realize our long-standing desire to rationally design lipid nanoparticles for specific applications; and finally, our demonstration that we can now efficiently deliver functional mRNA into hematopoietic stem and progenitor cells.

Okay. Let's go. So let me start this section by reminding you of Moderna's overall strategy for combating COVID-19 that we've laid out in our April Vaccines Day presentation. It's basically to stay ahead of new viral variants as they emerge. It's that simple. We're doing this by closely monitoring emerging variants of concern as well as measuring how long the immunity generated by our current vaccines lasts to understand when booster shots will be required. Regarding boosters, as soon as we identify variants of concern with breakthrough potential, we're moving rapidly to generate new vaccine versions that provide enhanced immunity to the new variants. Third, we're partnering with governments all over the world to ensure access to the most up-to-date boosters.

Now it's still early days. But as you can see from this slide, there is clear evidence in countries that began aggressive vaccination campaigns as soon as the emergency use authorizations were granted that the vaccines are working. For example, in Israel, where people over 60 were initially prioritized for vaccination, you can see the case incidents and hospitalization in this population was dropping rapidly by mid-January. A similar trend was observed in the U.S., which initially prioritized nursing home residents. In England, COVID incidence was dropping in all age groups by mid-January.

Unfortunately though, this is not going to be the end of the story. What we're finding is that as the virus spreads, it is rapidly mutating. Some of these new viral strains appear to be even more transmissible than the original strain. As we'll show you later in this talk, we already know that some of these new strains are less susceptible to neutralization by our current vaccine. And this selective pressure for the virus to evolve in new ways to increase transmission and escape the immune system is only just beginning.

As you can see from this graph, even though COVID cases are now decreasing in the U.S., India is experiencing an incredible surge in cases, completely overwhelming their health care system. Listed on the far right are the major variant families currently in circulation throughout the world. But these simple names hide the true magnitude of viral mutation. The following movie will graphically illustrate this problem. It starts in September 2020 when a new variant named B.1.617 was first detected in India. Now let's play the movie forward to today.

As you can see, in November, new branches of this phylogenetic tree start to emerge. And each new branch then spawns its own new branches and so on. At the same time, these descendants start spreading all over the world as people move from place to place. Each descendant or haplotype contains a unique set of mutations. As of today, here we go, wait until it gets to today. There we go. As of today, there are over 800 unique haplotypes just in the spike protein alone that have emerged from the B.1.617 variant in the past 8 months. And that's just 1 variant family.

Now let's look at all the known variants worldwide. In all, there are now over 23,000 spike protein haplotypes, each containing a unique combination of mutations. To put this diversity in perspective, the phylogenetic tree we just watched emerge on the last slide is this small population here. So dealing with this is a seemingly insurmountable problem.

But we at Moderna need to know -- what we at Moderna need to know from all the sequencing data can be reduced to just 2 key questions: which sets of mutations will enable the virus to break through our current vaccine? And which exact combination of boosters, i.e., individual haplotypes, should we incorporate into new booster vaccines? How can we go about assessing the risks from new viral variants? The CDC has defined 3 classes of variants based on their ability to decrease immunity from prior infection or vaccination, increased transmissibility or disease severity, decreased susceptibility of treatment or diagnostic detection. These 3 classes are variants of interest, variants of concern and variants of high consequence. At present, there are no variants of high consequence, but there are a number of variants of concern. And as you can see from this slide, there are a number of variants of concern that have been identified by the CDC. We can also see that the World Health Organization has a somewhat different list.

So how do we at Moderna decide? The answer is that first, we are building a comprehensive set of preclinical tools to assess potential variants of concern -- to the potential of variants of concern to break through our current vaccines. Secondly, we're constantly creating and testing new vaccines. Third, we're building sophisticated in-house computational tools to get out ahead of the virus.

Now I'm going to turn you over to Guillaume Stewart-Jones, Associate Director of Antigen Design and Selection in our infectious disease team to tell you about the first 2 activities. He will be followed by Wei Zheng, who heads our Computational Sciences Group, who will give you an overview of our computational approaches. Guillaume?

Guillaume Stewart-Jones

Thank you, Melissa. In order to respond effectively, we need a strategic road map. This slide shows an overview of the workflow and work packages to address the emerging variants. Starting on the left with the sequence and bioinformatics data, which flows into our computational sciences team and onwards into virology, antigen design, antigen testing and, in some circumstances, moving into clinical mRNA product development.

To understand and act as quickly as possible with our adaptable mRNA vaccine platform to the evolving pandemic, a number of sources of bioinformatics information are being constantly collected, combined and integrated. The GISAID database and protein data bank of SARS-CoV-2 antibodies bound to spike have been expanding significantly in recent months, with the deposited sequences and structures of this leading to us developing a multicomponent approach to analyzing the potential effect of mutations on antibody or serum recognition. With this information, we're now able to evaluate if any given sequence has mutations in key sites or key epitopes that may confer escape from humoral immunity generated by the mRNA-1273 vaccine. That bioinformatics information allows the virology team to construct particular stomatitis virus or VSV

pseudoviruses to understand the impact of these variant mutations on mRNA-1273-elicited serum antibodies. And the VSV platform is used in labs all over the world.

The antigen design team moves forward in parallel with preclinical mRNA production, to move forward into animal testing as boosters or primary series vaccines with neutralization and protection readouts. If we see that there is a substantial impact compared to 1273 vaccine or neutralizing capability or protection, we are able to move forward into next-generation clinical mRNA product development.

To explain VSV pseudovirus assay platform and what it enables us to measure, this schematic shows how using genetic engineering, we can take the spike protein sequence of any variant and encode that in plasmids that form a very safe, nonreplicating chimeric virus that has the SARS-CoV-2 spike protein on its envelope surface and a luciferase-reported gene encoded in the genetic information inside that virus, allowing measurement of the extent of infection of ACE2-positive cells in vitro.

If monoclonal antibodies or serum polyclonal antibodies are present in the supernatant, these block the pseudovirus from accessing the ACE2 receptor and, therefore, that no longer generates a signal. Controls are used, including those that block SARS-CoV-2 directly or the ACE2 receptor, as shown in the lower right diagram. Importantly, these pseudoviruses can be used under BSL-2 laboratory conditions and containment, whereas the live SARS-CoV-2 virus has to be used under BSL-3 containment.

Here is a further illustration to show how this works in the lab in high-throughput mode. And in fact, we have now set this up robotically. We and others have compared the authentic SARS-CoV-2 virus in the top row with the pseudovirus in the lower row. And using sera from immuno subjects for convalescent persons, we see very good agreement between the VSV pseudovirus neutralization readout and the live SARS-CoV-2 virus, indicating the pseudovirus is a very good proxy of what we would see with the live virus.

On the left, you can see how the global diversification of the virus spike protein has generated many lineages, as Melissa Moore has explained and as shown in many colors. Also, different countries have been depositing sequences at different rates, explaining the higher representation of some variants such as the B.1.17., shown in blue. On the right, we have analyzed 8 mRNA-1273 Phase I clinical trial participants with pseudoviruses of different genetic composition. As shown in the left of this table, with different variants of interest or variants of concern, and we see that the majority of these participants generate neutralizing activity against these pseudoviruses, including B.1.1.7, which has been surging in the United States in recent months and shown in blue in the dendrogram.

While we see quite broad spectrum activity to most variants in the study, some variants, including the B.1.351 and P.1, shows some reduction in neutralizing activity, yet we see some residual neutralizing activity for all variants.

If we look specifically at the B.1.351 variant, this has a number of mutations in the RBD and NTD or receptor-binding domain or N-terminal domain. And we have seen a 6- to 7-fold decrease in neutralizing activity to this strain compared to the D614G variant. Given that, we decided to move forward with the GMP manufacturing of a next-generation SARS-CoV-2 variant vaccine based on the B.1.351.

Here, we can see the immunoassay composition of the new vaccine, mRNA-1273.351 compared to mRNA-1273, where there are 12 mutations. And there are key mutations that are known to profoundly affect the neutralization capacity, including E484K in the RBD and the deletion of 242 to 244 residues in the NTD. We tested the ability of the mRNA-1273.351 or mRNA-1273 in immunized mice to evaluate the neutralizing activity against a panel of variants of interest or variants of concerns at day 36 after 2 doses of the vaccines.

And what we see is an excellent breadth of neutralizing activity across the pseudovirus panel, including to B.1.351 and the recently described Indian strain B.1.617 variant of interest, which has been shown to be expanding significantly in that region and, indeed, across the globe. The responses to the B.1.351 vaccine are even more broad across all these pseudoviruses, which contrast with the clinical data which we just saw where we can see an appreciable decrease in the neutralizing activity to that pseudovirus, illustrating that the B.1.351 is effective at filling the gap, if you like, against the pseudovirus of that same strain.

So how can we do even better than this? While the mRNA vaccine platform offers great agility and flexibility to updating the vaccine in the context of this evolving pandemic, significant resources and time is still required to perform the suite of preclinical activities. The shortest time from the

detection of a variant of concern to preclinical immunogenicity readout against a panel of pseudoviruses is approximately 2 to 3 months. And new viral variants are coming -- emerging constantly in real time. Using sophisticated computational methods, we are working to predict the characteristics of any variant spike sequence relative to the approximate impact of mRNA-1273-conferred neutralization.

And with this, I'll pass over to Wei Zheng, Lead Computational Science team member, who will detail these computational efforts.

Wei Zheng

Thank you, Guillaume. Before I jump into our computational work, I want to give a big shout-out to the unprecedented level of collaboration and data sharing from the entire scientific community. Our current knowledge on SARS-CoV-2 and recent emerging variants is built on top of viral genomic sequences deposited by scientists from across the world. The most complete online database is GISAID. As of today, there are already over 1.5 million sequences deposited, and this number is growing rapidly. Sifting through a data side of this size, however, is a truly daunting task, requiring both time and sophisticated computational expertise.

To enable our white lab colleagues to access this huge amount of sequence information and be able to perform the analysis required to design future experimental vaccines and pseudoviruses without the need for programming, our Computational Science Group collaborated with Diamond Age Data Science to develop a shiny app that we call Moderna CoView. This app allows any Moderna user to quickly sift through this huge dataset to identify sequences of interest for further investigation. With the app, our colleagues can filter for geographic region, lineage or mutations to isolate variants of interest. You can track variance prevalence both globally and at the country level; examine novel mutations as they accumulate on varying backbones; make mutation positions to spike protein structure; possess the prevalence, growth and antibody escape for mutation combinations; and finally, easily output the filtered datasets for further analysis or to initiate experimental assessment. All of these features are greatly accelerating our pace of development.

Meanwhile, our computational team also monitors spike mutation and lineage information daily to feed into antigen design decisions for our colleagues in infectious disease. Using the Indian variant, B.1.617 as an example, we first identified the most prevalent haplotype, now known as the B.1.617.1 lineage. When the surge in cases in India started about 2 months ago, we quickly learned that this haplotype contains 3 mutations unique to the .1 lineage and doesn't contain the other 6 unique mutations that are specific to the .2 lineage. As a result, our infectious disease colleagues have now designed new pseudoviruses and experimental vaccines for both the .1 and .2 sublineages.

Although there are a huge number of viral sequences being generated all over the world, it is important to note that the sampling, that varies substantially by country, as documented in this recent Nature news report. For example, the U.K. and Denmark have generated many more sequences per confirmed case than either India or Brazil. Thus, one cannot readily inform how fast a particular variant is spreading simply from the total number of the positive sequences.

To get around this problem, we have begun to track haplotype prevalence over time at both the global and country levels, normalized to total case number. As you can see, for the U.K. and South Africa, where the sample index was deep, the country-level and global-level prevalence track very closely. But for other countries, such as Brazil and India, with low sample index, the inflection point at the local country level can be 2 to 3 months earlier than the global level. Detection of new variants of interest as soon as they start to climb in an individual country should enable us together jump-start on future preclinical studies.

Moderna is also paying back to the scientific community by actively sequencing viruses from COVID cases in our clinical trials and epidemiology studies. We're working to share this data back to GISAID as well as integrate them into our internal modeling efforts.

So far, I have only been talking about virus sequence information, but we can also leverage information on protein structure. At this time, almost 400 high-resolution structures of the SARS-CoV-2 spike protein has been deposited in protein data bank or PDB. Many of these are complexes of the spike protein with either the ACE2 receptor or a monoclonal antibody. This gallery provides just a small sampling of this type of data.

Analyzing the subset of structures, wherein the spike protein is complex with a neutralizing antibody, enabled us to create a contact map showing which positions on the spike protein are most important for neutralizing antibody recognition. Haplotypes containing mutations at base positions

are thus more readily likely the haplotypes containing mutations and other positions to evade antibody recognition and potentially also vaccine protection.

One way to use this information to predict vaccine escape potential is to score different spike variant haplotypes by how many mutations occur within the neutralizing antibody contact surface. Indeed, when we use the total number of mutations falling into the contact surface to predict the neutralizing -- pseudovirus neutralization assay by sera from mRNA-1273 Phase I trial participants, as Guillaume just introduced previously. This model clearly works.

Compared to the original Wuhan strain, which is labeled by black dots, B.1.1.7 only contains 3 mutations in the contact surface and didn't cause much loss in neutralizing antibody title. On the other hand, the haplotypes we tested for B.1.351 contained either 6 or 7 contact surface mutations. These mutation sets resulted in substantially decreased neutralizing antibody titles.

Up to this point, I have been telling you about our efforts to analyze existing variants. But can we do even better? Could we proactively identify and evaluate problematic spike protein mutations before they even emerge in nature? So instead of always one step behind new viral variants, could we get out ahead of it? We are working furiously to make it possible.

This slide shows 3 examples of different modeling strategies with increasing complexity going from left to right, which may lead us to a proactive computational solution. The first model on the left is the most straightforward, the antibody footprint model I just talked about. Note that it only uses coding structure information independent of variant prevalence in the world. This is, thus, something we can leverage for proactive prediction. The model in the middle is a random forest model using just the presence or absence of single mutations as predictors. Random forest is a decision tree-based machine learning method. For this method to work, we need to train it on some reliable experimental data. For example, we can train it on part of the neutralization data, predict the rest and reiterate. It can also be trained on other data types collected on mutants that are not yet circulating. As you can see in the lower middle part, with a modest set of neutralization assay data, this machine learning method was already doing a decent job in predicting neutralization. As more and more data come in, we will be training it on this larger integrated datasets to further improve model performance and assign risk scores for individual mutations.

The model on the right is a natural language model developed by Bonnie Berger, Bryan Bryson and colleagues from MIT. This was published in Science in January this year. In network, they also build a neural network model called constrained semantic change search or CSCS, which used about 4,000 spike protein sequences from SARS-CoV-2 and related virus species. They aim to predict the viral escape by constraining for correct grammaticality, while asking for high semantic change. In the lower right part, they also overlaid their predicted semantic change and grammaticality scores against those 5 extinct mutations identified in another experimental dataset, labeled by red crosses. Ideally, if a prediction is perfect, we want to see the red crosses overlapping with the yellow region in the plot. But as of now, there are still room to improve.

At present, this model also cannot handle insertions or deletions, but we know that B.1.1.7 and B.1.351 and many other emerging virus do contain deletions. So we need to further expand the baseline of research for our application.

I talked about training these models using mutant data that are not yet circulating in the world. Where do these mutant data come from? One source is deep mutational scanning. This is a high-throughput technology to assess the biophysical properties of protein domains. Some academic labs have already made impressive progress and accumulated large amounts of data for the SARS-CoV-2 spike protein. In the example I'm showing you here, Jesse Bloom and coworkers at the Fred Hutchinson Cancer Center in Seattle started by building a comprehensive mutant library for just the receptor-binding domain or RBD of spike protein. Note that since this mutational analysis was performed on only a small portion of the spike protein far from a fully functional virus, it poses absolutely no public health threat.

This library was then incorporated into a yeast display system, which expresses up to 100,000 mutant RBD variants on the yeast surface. Using this yeast display system, these researchers were able to sort the variants into things according to their binding affinity for the ACE2 receptor. Each sorted thing was then analyzed by next-generation sequencing and be convoluted by computational license to quantify the impact of every single mutant. This is an extremely fast way to simultaneously assess the effects of many mutations in parallel.

We are now looking into applying similar methodologies to assess the effects of mutation combinations. In summary, our vision is to integrate all these types of information using AI and machine learning to accelerate our vaccine development process. From the lower left panel clockwise, the data source we just discussed and will be integrating include the spike mutations and lineage annotation from GISAID. Their prevalence over time, viral sequencing data from our clinical trials and real-world epidemiology studies, structured information from PDB, deep mutational scanning data from academic labs and our collaborators and our internal pseudovirus neutralization assay results, we aspire to build a highly efficient and accurate model system to predict variant escape rates and informed design of our next-generation vaccines.

But the work process and tools we're building are not just for our fight against the SARS-CoV-2, they will also be enabling for our work on other rapidly evolving viruses such as seasonal flu and HIV. Thus the lessons we're learning from SARS-CoV-2 will leave us even better prepared to fight future viral outbreaks. Thank you.

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

Thank you, Guillaume and Wei. Now let's turn to another topic, mRNA sequence engineering. To achieve optimal performance for our mRNA medicines, we need to fully understand the basic principles of how -- governing how much protein a given mRNA will make. In nature, some mRNA molecules are translated thousands of times, producing vast quantities of housekeeping or structural proteins. Conversely, others have evolved to make just tiny amounts of protein, for example, mRNAs encoding key regulatory proteins such as transcription factors. What are all the features that control how much protein is produced from a particular mRNA? Is our current understanding from decades and decades of academic research on translation and degradation of endogenous mRNA is complete? Are these rules the same for our therapeutic mRNAs? And are all currently established dogmas correct or do some need reevaluation? Needless to say, we've been working on this problem for much of Moderna's history, and we've made considerable progress.

In past years, we've told you about how we gain control over the tendency of the ribosome to miss the first start codon and initiate protein synthesis further downstream, a process known as leaky scanning. We've also presented our now published work on understanding the relationship between codon optimality and mRNA secondary structure within the protein coding region. As a result of these and other sequence engineering efforts, we no longer need to test multiple sequence variants to identify a fit-for-purpose mRNA. Indeed, so high is our confidence in our mRNA design abilities that when the sequence of SARS-CoV-2 became publicly available on January 11 of last year, and we had quickly agreed with our colleagues at NIAID on the exact protein sequence to express for a vaccine, it took our in-house mRNA design team just 1 hour, 1 hour to design a single mRNA sequence that we immediately put into GMP production.

In another 42 days, LNP formulations containing this mRNA were delivered to NIH to initiate the Phase I clinical trial. And now the same mRNA sequence has been administered to hundreds of millions of people worldwide as the core component of our first SARS-CoV-2 vaccine, mRNA-1273. It should go without saying that I am beyond proud of our mRNA design team for this singular accomplishment. It actually still gives me goose bumps when I talk about it.

So are we done? Of course not. As Stephane indicated in his opening remarks, at Moderna, we believe the sustained investment in basic research is critical to our long-term success. We also believe in achieving deep understanding of the mechanisms underlying unexpected observations as these unexpected results mean that we still have things to learn about the underlying biology, and they provide us with insights into new engineering principles. This type of insight sometimes requires years of focusing on a single problem until the solution is finally revealed.

In this next section, we'll tell you about our continued efforts to understand how sequence and mRNA secondary structure within the protein coding region affects protein output. Two years ago, we told you about a set of 30 mRNAs encoding the same short-lived green fluorescent protein, or EGFP, but that differed only in the synonymous codons used to encode that protein. As illustrated by the squares in the plot on the right, these mRNAs spanned a broad range of codon optimalities and mRNA secondary structure. But they also varied well over a hundredfold in the amount of protein they produced.

But why? Here to tell you the next chapter of the story is David Reid, an Associate Scientific Director in our mRNA Science department. David?

David Reid

Thanks, Melissa. Today, I'll talk about a domain that we're quickly coming to see as central to the practice of mRNA engineering. That's optimizing ribosome load. You can see the data that Melissa just described here, where each line on the right represents the amount of a green fluorescent protein generated by a different mRNA sequence. These values were derived from images like the ones you see in the upper right. We also described previously that differences in protein expression are driven almost entirely by differences in mRNA stability, where, as one might expect, those mRNAs that are the longest lived expressed the most protein. You can read all about these findings in our 2019 paper that I referenced to the lower left.

Now understanding how to modulate protein expression by changing the mRNA sequence is a powerful lever for us to control the performance of an mRNA drug. But to understand what that lever is really doing and to push the boundaries of mRNA performance even further, we needed to move beyond these simple correlations and characterize mechanistically what drives differences in protein expression. And the first place we look to understand that was that translation.

The ribosome plays a dominant role in orchestrating the life of an mRNA inside the cell. You can get a sense of this just by looking at the physical form of an mRNA in the cytoplasm, as shown in this classic electron micrograph. MRNAs are coded with many ribosomes along the whole length of the coding sequence. In this illustration, each of the dark spots is a ribosome bound to the same mRNA. And as you move from left to right, you can even see that the nascent protein chains become longer, representing the fact that the farther right ribosomes have translated more of the mRNA. This is the form that most mRNAs take on inside cells, not naked and free floating but, as said, coded with ribosomes doing the job of making protein.

These ribosomes right along mRNAs, which we call polysomes, can form a vast array of superstructures from rods to circles to something more Rosetta-like. And since translation is such a fundamental part of mRNA function, we wanted to first ask, how does ribosome density relate to protein output? If we want an mRNA that generates a lot of protein, should that mRNA have a lot of ribosomes or a few? Second, can we use these learnings to engineer mRNA drugs? And third and more specifically, by deeply understanding the mechanisms that connect translation to mRNA function, can we develop levers that precisely tune the activity of mRNAs and push the boundaries of mRNA engineering?

Now when it comes to ribosome density, a reasonable intuition might suggest a simple answer to the relationship between ribosome density on mRNA and the amount of protein that, that mRNA generates. If an mRNA is bound by few ribosomes, there are a few of the machines doing the work of making the encoded protein. And on the other hand, if an mRNA has more ribosomes, you might get correspondingly more protein. There's plenty of academic literature to support this notion that an mRNA with more ribosome should yield more protein. But even with all these data, we didn't yet have a direct assessment of the relationship between ribosome count and protein output for mRNA drugs.

Does this reasonable intuition apply? To determine the number of ribosomes for each of the synthetic mRNAs that we generated, we've turned to polysome profiling, a biophysical method developed in the 1960s, but is still considered state-of-the-art. In this method, the contents of a cell are extracted then layered over a sucrose gradient. This gradient is then processed in an ultracentrifuge, spinning at tens of thousands of rotations per minute for several hours. This centrifugation process separates non-translated mRNAs from mRNAs bound by -- with a few ribosomes from those bound by many ribosomes. Each PQC in the right-hand plot represents an mRNA bound by a different number of ribosomes. As you can see, natural mRNAs endogenously in the cell, can be bound by a wide range of number of ribosomes, from just one to dozens.

To measure ribosome density for our engineered mRNAs, we took our library of fluorescent protein encoding mRNAs that I described previously, which are identical in length in untranslated regions and differ only in their coding sequence. And we pulled them together, and we transfected them into cells and used polysome profiling to separate mRNAs based on the number of ribosomes that they have associated with them. We separated this polysome gradient into fractions then used RNA sequencing to determine the amount of each mRNA sequence variant associated with each number of ribosomes. Using this approach, we can define the number of ribosomes associated with each mRNA.

And here are the results. On the right, each line represents the distribution of an individual mRNA in our polysome gradient. In this plot, if a line peaks in a gradient fraction associated with, say, 4 ribosomes, then that mRNA typically associates with around 4 ribosomes. We're also showing the paired expression level on the left. As you can see, the different mRNA sequences have polysome gradient distributions that peak in different positions, indicating that they're associated with different number of ribosomes. And as we highlight the individual sequences, I hope you can

appreciate that, surprisingly, those mRNAs with the highest expression levels, in blue, tend to associate with fewer ribosomes; while those mRNAs with the lowest expression levels, in red, tend to have more ribosomes. That suggests that protein output may be negatively correlated with ribosome load.

Now we can calculate 2 important metrics for each mRNA sequence based on these polysome gradient distributions. First is the percentage of mRNA that's associated with any ribosomes at all. This is, of course, important because an mRNA without ribosomes isn't making any protein. As you can see on the left, this metric is largely consistent across the different mRNA sequence variance, irrespective of ribosome load or protein output. You'll be hearing more about this metric and how it helps us to understand lipid nanoparticle function later today.

Now the metric where we do see differences between our mRNA sequences is in the average number of ribosomes per mRNA. Here, reflecting what we observed qualitatively in the polysome gradients, there's a range of ribosome load from around 4 to around 7 ribosomes. And counterintuitively, we see that those mRNAs with the fewest ribosomes, around 1 per 200 -- 1 ribosome per 200 nucleotides confer the highest levels of protein expression.

Now how can we leverage this information for mRNA drugs? How can we engineer an mRNA that will carry a small number of ribosomes for high protein output? How can we engineer an mRNA with high ribosome load when a lower protein output is needed? To really understand what's driving these differences in ribosome density, we had to go one level deeper into the realm of kinetics.

Ribosome density is determined by 2 different kinetic parameters. One is the translation initiation rate. That's how frequently ribosomes are loaded onto an mRNA. This is typically expressed in ribosomes per second. The second kinetic parameter is elongation rate, how quickly a ribosome proceeds through the coding sequence of the mRNA. All else being equal, the faster a ribosome goes through an mRNA, the fewer ribosomes that we'll have. Elongation rates are typically expressed in codons per second.

So are the ribosome densities that we observed explained due to variation in initiation rates or due to variation in elongation rates? The answer to this question could determine how we engineer ribosome load and mRNA function in the future. So to measure these different rates, we developed a new kind of experimental technique, a polysome-based ribosome runoff. In this method, we use a small molecule called harringtonine, which freezes ribosomes at start codons but allows already translating ribosomes to continue. As time goes on, ribosomes are progressively released. They run off, that is, and ribosome load decreases.

As you can see, you can see this in the polysome gradients in the top right. We can then again apply RNA sequencing to measure ribosome load in cells treated with harringtonine for different amounts of time. These ribosome load values let us calculate initiation and elongation rates. Here's how. For each mRNA, ribosome load will descend from a certain initial level with a certain slope. This slope represents the initiation rate in ribosomes per second. To assess elongation rates, we can take advantage of the mathematical relationship between ribosome load initiation rate and elongation rate, shown on the left.

Here's how the runoff data look for our library of GFP sequence variance. These mRNAs all have descending ribosome load after addition of harringtonine, but they start from different initial ribosome loads and they have different slopes.

And here are the numbers that we derive from these runoffs. First, looking at elongation rates, we see that there are essentially no differences across the different mRNA sequence variance, suggesting that elongation rates can't explain the differences in ribosome load. On the other hand, when we look at initiation rates, we do see that there's meaningful differences between the different GFP sequence variance. If we compare these differences in initiation rates back to the average ribosome load, we can see that differences in initiation rates can essentially completely explain the differences in ribosome load. So this suggests that among the mRNA sequences that we've tested here, initiation rate is the determining factor for ribosome load.

This gives us a series of relationships that leads to a counterintuitive result. mRNAs with low initiation rates infer low ribosome load, which in turn conveys high mRNA stability and high overall protein expression. So through these series of relationships, it turns out that mRNAs that load ribosomes onto the mRNAs the slowest, end up driving the highest protein expression.

As I've just shown you, understanding how initiation rate influences ribosome load and protein expression gives us a lever that we can pull to precisely control the expression level of our mRNAs. Moreover, by understanding the mechanisms that drive this behavior, the gears and pulleys that the lever moves, if you will, we can take mRNA design from a guess-and-check discipline into an engineering discipline. This lever that we've talked about today takes us place among many others, some of which we've talked about in this forum in previous years.

With these tools in place and more always under development, we're continuously more able to make an mRNA that generates the right amount of the right protein or the right amount of time in the right cell type. And as we build these tools into our mRNA drugs, we will be able to target more indications with ever more precision and do so with confidence.

Thank you.

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

Thank you, David. And with that, we will now take a 10-minute coffee break, and we'll see you back to start talking again about intracellular events affecting LNP performance. Thank you.

(Break)

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

Welcome back. I hope you all had a relaxing break, and you're ready to hear some more science. Now let's turn to another of our long-term objectives, our continuing efforts to enable rational LNP design. For optimizing LNP performance, there are an incredible number of things that we need to consider. Starting in the upper left-hand corner of the slide, there are the exact chemical components. We can modulate the ultimate LNP structure by varying these components, varying their ratios with respect to one another as well as the process at which they're mixed together to form an LNP. Once we formed an LNP, key properties for investigation and optimization include chemical and physical stability of the final formulation, where in the body the LNP travels and what cells prefer to take it up, how it interacts with proteins or opsons in biological fluids and how these opsons affect cell tropism, the efficiency of uptake by desired cell types, the efficiency of endosome escape and what fraction of delivered mRNA successfully engages with the translation apparatus.

Importantly, each new route of administration and target cell type requires consideration and optimization of all of these parameters. Now we can't possibly cover all of these topics in one webinar. So today, I'd like to focus on a recent story involving our efforts to deliver a new delivery vehicle, to develop a new delivery vehicle for a particularly difficult to transfect cell type. We're not yet ready to review what that cell type is, but I want to use the data to give you some insight into our thinking process when it comes to developing new delivery vehicles optimized for particular applications and how we go about overcoming unexpected intracellular barriers.

The story starts with 2 LNPs of different composition that came out of a large diversity screen using a primary human cell culture model. We'll call them LNP-A and LNP-B. As you can see on this slide, neither was particularly efficient at transfecting these primary cells, with both yielding reported protein expression, in this case, green fluorescent protein, in only about 1% of the total cells. If instead of looking at protein expression, however, we now look at LNP accumulation using fluorescently labeled LNPs. You can see that LNP-A got into about the same percentage of cells as those expressing protein. So LNP-A was clearly suboptimal for cellular uptake. But once inside the cell, these LNPs were able to efficiently deliver functional mRNA into the cytoplasm.

Conversely, these primary cells clearly had a much higher affinity for LNP-B, with this LNP detectable in over 60% of the cells. In addition to that, LNP-B accumulates to much higher levels per positive cells than LNP-A, but only a tiny fraction of the cells that took up LNP-B expressed the desired protein. So LNP-B seemed pretty optimal for cell uptake but highly suboptimal for functional mRNA delivery.

So the question became, how could we -- could we somehow combine LNP-B's propensity to get inside these primary cells with LNP-A's ability to deliver functional RNA? To do so, we needed first to understand why LNP-B couldn't deliver functional RNA. And to investigate this, we turn to single molecule imaging.

So what I'm showing you here is our system that we use for single-molecule imaging that allows us to determine the translational status of mRNAs in the cell. What we do is we have a reporter mRNA called NPI luciferase or nascent peptide imaging luciferase. And that reporter mRNA encodes a protein that ultimately goes to the nucleus. And that protein, on its in-terminus, has a tag that we can fluorescently label with antibodies so that we can see both the protein that's made in the -- that goes into the nucleus and the nascent peptides as they're being translated. At the same time, we can detect the mRNA by using fluorescent oligonucleotides that hybridize to the mRNA. And therefore, they can detect both translating and un-translating RNA. This is what one of the single molecule images looks like.

And what you can see here is that the red molecule is a non-translating RNA because it only has signal from the FISH probes, the fluorescence in situ hybridization probes. And the yellow molecule is a translating RNA.

So now returning to our story, maybe the problem with LNP-B was an inability of the mRNA to escape the endosomes once it had gotten into the cell and gain access to the cytoplasm. These panels show that this was not the case. Indeed, even though a larger fraction of mRNA remained in the endocytic compartment when delivered via LNP-B compared to LNP-A. If we count the number of single mRNA molecules in the cytoplasm, as denoted by the white boxes, LNP B delivers more cytoplasmic mRNAs than LNP-A.

But if we now look at the translationally active mRNAs in these same cells, you can see that while a larger percentage of cytoplasmic mRNA molecules delivered via LNP-A are translationally active, the vast majority delivered via LNP-B are translationally inactive.

Why? Maybe some component of LNP-B was permanently damaging the mRNA, so it could not be translated. In this slide, I'm only showing you the cell imaging data for LNP-B. This top row here is very similar to the data I just showed you. And again, the bar graph indicates that only a small fraction of the cytoplasmic mRNA molecules delivered with LNP-B are translationally active. But if we take that formulation and now extract or de-formulate the mRNA and then deliver it by electroporation, the translation efficiency comes back. Thus, LNP-B was not permanently inactivating the mRNA molecules.

Okay. So we've rolled out endosome escape and permanent mRNA damage. What else might explain the low protein expression with LNP-B? Well, maybe one of its components is generally toxic and globally inhibits all protein translation in the cell. So to test this, we devised the method to test the impact of LNP-B on global translation. To do so, we dosed cell culture cells with LNP-B, waited several hours for them to take up the LNPs, and then electroporated in our NPI look imaging reporter, waited another 2 hours for that to be expressed and then looked at the image.

In a control experiment, we did the same thing, but we left out the LNPs. So if LNP-B was inhibiting translation, then we should see less translation in the upper panel and more translation in the lower panel. But instead, what you can see here is that LNP-B does not inhibit translation, and so we got very similar amounts of overall protein expression in the cells that were LNP loaded as opposed to the control. So that wasn't the problem.

So again, what is the problem? We've now checked off endosome escape. We've checked off that the mRNA is not permanently damaged. And we know that it's not a general translation problem. So next, we thought, well, maybe LNP-B simply too sticky. Now what do I mean by that? What do I mean by it being too sticky? I mean that when the LNP is in the endosome and then fuses with endosome membrane to release the mRNA into the cytoplasm, if components of the LNP are too tightly bound to the mRNA, they may accompany the mRNA into the cytoplasm and prevent its association with the translation apparatus.

So to test this, to see -- to test the stickiness of LNP-B, we used a ribogreen assay. And so in this assay, what we're doing is we take our lipid nanoparticles, and we disrupt them with detergent. And at the same time, we add a dye that becomes highly fluorescent upon interaction with RNA. So you can see when the LNP -- the RNA is in the LNP, it's not fluorescent, and then you get a much increased fluorescence as it comes out.

However, with LNP-B, and as you see in this -- the data here, when we add the fluorescent dye and detergent alone, we did not see any fluorescence. And what we found that in order to regain the fluorescence, we needed to titrate in a competing anionic species that would then compete with

the LNP components bound to the RNA and remove them from the RNA so that now the dye can interact with the RNA. And so it turned out that LNP-B was extremely sticky. We had to titrate in quite a bit of ionic material in order to get our fluorescence back.

So having learned this, this then gave us the clue that we needed in order to optimize LNP-B. And we did so by varying process parameters such that we could mitigate the stickiness that was the case of LNP-B. And I'm pleased to tell you that now we've developed a new LNP, LNP-C, and this is our current lead candidate for this tissue type. And you can see that we now have been able to very much increase our protein expression in these primary tissue culture cells.

So with that, I'd want to summarize and say that single-molecule imaging is an invaluable tool for dissecting intracellular events affecting mRNA function. In addition to endosome escape, we need to consider slow lipid component release that can adversely impact functional mRNA delivery. And finally, our mechanistic elucidation of intracellular events has allowed us to engineer LNPs capable of accessing difficult-to-transfect primary cells with efficient endosome escape and high functional mRNA delivery.

And with that, I'm going to hand you off now to David Alvarez, an Associate Director in our Platform Immunology department. He is going to tell you about our recent success in transfecting hematopoietic stem and progenitor cells. David?

David Alvarez

Thank you, Melissa, and hello to everyone. In this closing chapter, I will present our advancements in delivering mRNA to some of the most rare yet indispensable cell types among all blood cell lineages of the body, the hematopoietic stem and progenitor cells.

Before introducing hematopoietic stem and progenitor cells, I will first describe the mature and fully differentiated blood cells that make up the hematopoietic lineage, their functions and their origins. These include platelets and red blood cells, which despite lacking a nucleus, serve critical physiological functions from blood clotting to gas exchange. Now the remaining hematopoietic cells of the body are referred to as white cells and encompass 2 main branches, the myeloid cells and the lymphoid cells. The myeloid cells, some of which are depicted here, are central components to the innate immune system, our body's first line of defense. Lymphoid cells, on the other hand, including cells such as natural killer cells, T and B cells, primarily make up the adaptive immune system, which through cytotoxic T cells and antibody secretion by plasma B cells, provides immunity and memory against pathogens.

With respect to function, hematopoietic lineages are intimately involved in maintaining homeostasis and human health. These include several critical functions such as coagulation and gas exchange, as mentioned earlier, maintaining normal blood pH, cell turnover and clearance by phagocytic cells as well as immune surveillance of all peripheral tissues and organs by cells of both the innate and adaptive immune systems. In contrast to these homeostatic functions, there are hundreds of hematologic or immune-related disorders that are caused or exacerbated by cells of the hematopoietic lineage. In many cases, these cells interact with host tissues to drive chronic inflammation and disease, including autoimmunity, such as type 1 diabetes, MS or rheumatoid arthritis, inflammatory bowel disease, asthma and allergy, cardiovascular disease and atherosclerosis as well as blood disorders and hematologic malignancies and many more.

From a therapeutic perspective, especially for chronic indications, altering the course of an illness may require continuous targeting of specific cell lineages. Although if one can find and target the cellular origins of a given lineage, that may have a lasting impact on the course of disease.

So where do all hematopoietic cells come from? Well, all hematopoietic cells originate from a process called hematopoiesis, from the Greek word to produce blood. This process generates and replenishes all the mature, fully differentiated blood cells found in the body and does so throughout one's entire lifespan. This process originates very early during embryonic development and occurs in ways through different anatomical niches before finally transitioning after birth to the bone marrow, where hematopoiesis is constantly maintained throughout adult life by the presence and function of the hematopoietic stem and progenitor cells.

Hematopoiesis is classically organized into several tiers, which are constantly evolving. At the apex is the hematopoietic stem cell, or HSC for short, which are rare cells with long-term self-renewal and pluripotent properties, which confirm them with the ability to give rise to all hematopoietic lineages over a lifetime. In the second tier are multipotent progenitors, or MPPs. These too are pluripotent cells but with limited self-renewal

properties, which sustain hematopoiesis mainly under homeostasis or steady-state conditions for an extended period of time, often for months. Next, are the common myeloid progenitors, CMP; and common lymphoid progenitors, or known as CLPs, which can differentiate into myeloid and lymphoid lineages, respectively. The next tier contains more committed precursor cells that further specialize into lineage committed cells, which give rise to the mature hematopoietic cells of the body.

Collectively, these tiers are often bridged together and referred to as the hematopoietic stem and progenitor cell pool, or HSPC for short, an acronym used extensively throughout today's presentation.

Now to fully appreciate the breadth of hematopoiesis is to understand hematopoiesis by the numbers, and the numbers are staggering. Recent studies have estimated the total number of cells in the adult body to be approximately 30 trillion, of which 90% are of hematopoietic origin. Among these hematopoietic cells, 84% are red blood cells; nearly 5% platelets; and up to 11% are white blood cells.

To meet the demands of the body, hematopoiesis must balance the production and destruction of specialized blood cells every day. Now given the variation in life span and cell turnover dynamics, most recent estimates suggest the total turnover rate of the human body is 330 billion cells per day, with about 86% of these cells being blood cells, mostly of bone marrow origin. This equates to approximately 3 million cells per second, the majority of which are red blood cells.

Now to fill this demand in blood cell production, recent work has estimated the numbers of active HSCs that are making white blood cells at any one time to be in the range of 50,000 to 200,000. Taking into account these statistics puts the overall frequency of HSCs in bone marrow at 1 in 10,000, a very rare cell indeed.

In this setting and within our mandate of expanding the frontiers of mRNA therapeutics, we pose the question, can we deliver mRNA to hematopoietic stem and progenitor cells in vivo? Imagine for a moment the difficulty in finding such rare cells deep in bone marrow cavities at a frequency of 1 in 10,000. In fact, this illustration conveys that message clearly of what 1 in 10,000 looks like. Despite the seemingly overwhelming odds against finding such rare cells, there are, in fact, additional obstacles particularly from a biodistribution perspective. These include the scavenging and sequestration of LNPs following systemic delivery by circulating blood cells, the liver and the spleen, which may make LNP delivery to the bone marrow even more challenging.

To cope with some of these challenges and to determine whether systemic LNP delivery reaches the bone marrow, we use high-throughput multiparameter flow cytometry to interrogate cells harvested from the body. This workflow incorporates staining cells with fluorescent antibodies specific to a wide array of surface proteins characteristic of HSPCs or mature cells. And by multiplexing with up to 15 fluorescent labels, we can comprehensively phenotype and quantify rare cell types. Then by interrogating cells at a flow rate of 10,000 cells per second, we can readily accumulate data on over 10 million cells per sample and up to billions of cells across studies.

To give you a clearer picture of how this workflow is used to interrogate HSPCs, we use a panel of antibodies to stain mature cell lineages to exclude them from downstream analysis, and instead focus on the lineage-negative cells, which by default contained progenitors. Using flow cytometry, these lineage-negative cells are then further gated on and subdivided into the relevant HSPC subsets depicted in the hematopoietic tree, depending on the species being studied. We then use a number of statistical and visualization tools to analyze and interpret this high-dimensional data for evidence of LNP uptake, association and delivery of the mRNA payload.

As mentioned previously, the scavenging and sequestration of LNPs by other organs may limit distribution to the bone marrow. Therefore, we explored this biodistribution to the bone marrow at the single cell level post systemic injection of a fluorescent LNP. And by flow cytometry, we were able to detect a fluorescent signal in bone marrow cells that upon further gating expressed surface markers consistent with HSPCs, including the long-term HSC.

To further complement this approach, we used an image stream imaging flow cytometer to provide visual proof of direct LNP uptake or association. Indeed, shortly after systemic LNP delivery, we detected punctate fluorescent LNP signals in sorted lineage-negative and enriched stem cells.

Now what these data demonstrate is that despite the odds, systemic LNP administration can access cells in the bone marrow compartment. But what these results do not speak to is whether mRNA can be successfully delivered to HSPCs, leading to translation of mRNA payloads. For that, we turn to a different experimental approach. To determine whether mRNA can effectively be delivered to HSPCs, we used Ai14 reporter lights, which contain a floxed STOP cassette sequence, upstream of the gene encoding, the red fluorescent protein, tdTomato. Here, all Ai14 cells remain nonfluorescent, unless Cre recombinases introduced into a cell and its nucleus to edit the Ai14 locus and remove the STOP cassette.

Upon excision of the STOP cassette, the cell is able to transcribe tdTomato under the control of a constitutively active promoter, leading to robust and permanent reduction of, even if the cell divides. This is of particular relevance because it allows for lineage tracing in successive cell generations, a hallmark of stem cell activity. Now our approach is to deliver LNPs that encapsulate the mRNA encoding Cre. And by doing so, we can mark cells that have been successfully transfected, as highlighted by these flow cytometry plots and image stream data that clearly showed the induction of red fluorescence in monocytes, as an example, that have acquired the LNP-Cre *in vivo*.

To provide evidence for mRNA delivery to bone marrow HSPCs, we treated Ai14 mice with LNP encapsulating pre-mRNA, and harvested bone marrow cells, 48 hours later. By flow cytometry, we gated on lineage negative cells and using established stem cell markers in lights, we observed the induction of red fluorescence in several HSPC populations, including on total enriched stem cells or LSK cells, several subsets of MPPs, in addition to the long-term HSCs as is highlighted in the hematopoietic treat.

Now since true HSPCs cannot be definitively identifying using cell surface markers alone. We complemented our approach by using ex-vivo colony forming unit assays, which provide a functional HSPC readout because it demonstrates whether a population of cells exhibit any proliferative potential by giving rise to colonies of cells. Therefore, to determine this, we played a small number of bone marrow cells harvested from LNP Cre-treated Ai14 mice. In a methyl cellulose-based media enriched with world factors and cytokines that support the growth of several hematopoietic lineages.

Over a 14-day period, we observed by microscopy, the formation of clear colonies starting at Day 5, which substantially grew in number as well as in colony size, of which 30% of all colonies were, in fact, tdTomato-positive, a value similar to the transfection observed by flow cytometry. Therefore, these data show that a fraction of bone marrow cells originally transfected by LNP Cre *in vivo* do, in fact, possess hematopoietic progenitor activity. To further determine whether LNP Cre did, in fact, transfect HSPCs in the bone marrow, we made use of the permanent induction of red fluorescence in Cre exposed cells as a useful tool to conduct long-term lineage tracing. In this experiment, we treated Ai14 mice with LNP Cre and monitor blood hematopoietic cells for the induction of red fluorescence for over 8 months.

As shown here for platelets and red blood cells, we observed the appearance of red fluorescence from day 3 onwards up to 8 months post-LNP injection. This culminated in approximately 27% to 30% of all platelets and red blood cells becoming fluorescence. In addition, we also monitored myeloid cells and [lymphoid] cells where we also observed the induction of red fluorescence. In fact, the frequency of red fluorescence in myeloid cells at 29% to 32% was remarkably similar to the values obtained for platelets and red blood cells. Now in lymphocytes, we observed a slower and steady incline in red fluorescence over time that has yet to reach the levels observed in other cell types, and likely due to the longer life span in these cells.

Now to formally demonstrate LNP transfection of the long-term HSC, we performed serial primary and secondary bone marrow transplantations in lethally irradiated recipient mice, which is considered the gold standard. In this model, we transferred cells from Ai14 donor lights that were initially treated with LNP Cre into primary lethally irradiated recipients and monitor those recipients for the appearance of red fluorescent blood hematopoietic cells. After several weeks, we harvested bone marrow from some of the primary recipients and transferred them into secondary lethally irradiated recipients and again, monitor them for the appearance of red fluorescent blood hematopoietic cells.

We first examined platelets in the recipients, as shown as open circles compared to the original donors depicted as closed circles, and observe the appearance of red fluorescent platelets in primary and secondary recipients to levels similar to the original donor, suggesting that bone marrow HSCs were transfected by LNP Cre in the original Ai14 donor and serially transferred. Moreover, we observed some results with red blood cells, which like platelets, lack a nucleus, and therefore, further support the contention that stem cells upstream of those lineages must have been initially transfected.

In addition to platelets and red blood cells, we also monitored mature myeloid cells, including monocytes as is depicted here, and lymphoid cells were presented by B cells. And similarly found the induction of red fluorescence in these lineages in both primary and secondary recipients to levels similar to the original donors. Collectively, these data show the reconstitution of all hematopoietic lineages after the first and second transplant, demonstrating unequivocally that LNP Cre can target bonafide long-term HSCs.

Now with respect to overall transfection efficiency, our initial data suggested

[Audio Gap]

as a single systemic LNP mRNA injection can target approximately 25% of HSPC populations. But can we achieve higher transfection in HSPCs? Therefore, we examine whether increasing the injection frequency or type of LNP formulation administered had an effect on overall HSPC transfection. As depicted in this schematic, we injected Ai14 fluorescent reporter mice with 1, 3 or 5 injections of LNP Cre and monitored their peripheral blood for the appearance of tdTomato mature hematopoietic cells. As shown in this first plot, we are looking at the frequency of tdTomato cells, in this case, platelets in the peripheral blood. Here, the dotted line denotes the final injection and marks the start of the observation period. And it is clear, not only for platelets, but also red blood cells, monocytes, CD4 T-cell and others, that increase in the frequency of injections is able to increase transfection to approximately 60%. Now in addition to evaluating repeat dosing, we also investigated the impact of LNP design and formulation on transfection. To this end, we initiated screens of different LNP mRNA formulations and determine transfection across a variety of cell types and organs in vivo. As shown in this bar graph for just a fraction of the LNPs screen to date, we observed increases in reported expression, in this case, for mouse HSPCs, depending on the LNP formulation used. Highlighted in red, the LNP is used in the work I'm presenting today, and we are continuously making improvements in this regard.

Next, we ask, can we deliver LNP mRNA to human HSPCs? Well, to investigate this, we propose the use of an innovative animal model that recapitulates the development of human hematopoiesis in vivo. In this so-called humanized mouse model, immuno deficient mice, our first xeno-engraftment with human CD34+ progenitors obtained from human cord blood, which include human HSPCs. And in approximately 12 weeks post engraftment, the mice become reconstituted with the full human immune system with TMB cells, NK cells in myeloid subsets as well as human HSPCs that take up residents in mouse bone marrow.

In these humanized mice, we injected LNPs encapsulating mRNA and then stained bone marrow cells using antibodies specific for the reporter protein as well as antibodies directed against human cell surface markers to clearly identify HSPCs of human origin. Again, using flow cytometry, we focused on the various HSPC subsets highlighted previously and noted variable degrees of transfection in different HSPCs, including multipotent MPPs, megakaryocyte in Aristorod progenitors, or MAPs, and granulocyte macrophage progenitors, or GMPs, which averaged approximately 40% transfection efficiency. Now in common lymphoid progenitors and in the human HSC, we observed approximately 10% transfection.

In summary, these data demonstrate successful LNP mediated delivery of mRNA to human HSPC subsets, in vivo in humanized life. In addition to examining human HSPCs. We also investigated whether systemic LNP administration can deliver mRNA to HSPCs from nonhuman primates in vivo. To test this, we dosed nonhuman primates with LNPs encapsulating a reporter mRNA and then stained bone marrow cells using antibody specific for the reported protein as well as antibodies directed against nonhuman primate cell surface markers to clearly identify HSPC subsets in the species.

The bar graph shown on the right depicts the frequency of reporter expression among stem cell subsets where each data point represents a single nonhuman primate. We focused on total CD34 positive progenitors, which include various stem and progenitor cell subsets. In addition to multipotent progenitors, which were further identified by expression of 2 well-established self surface proteins that delineate HSC activity and flurry potency in the nonhuman primate. At 24 hours post LP administration, we observe transfection in both CD34-positive progenitors and an enriched CK-positive, CD90-positive multicore progenitors, which averaged greater than 10% transfection and reached up to 20% to 30% in some animals. Taken together, these data demonstrate that a single systemic LNP injection can deliver mRNA to bone marrow HSPCs in vivo.

In closing, I would like to summarize our key findings today. Firstly, that we have demonstrated in vivo mRNA delivery to the bone marrow following systemic LNP administration, leading to HSPC transfection and long-term modulation of all hematopoietic lineages. Furthermore, we have shown that different LNP formulations and repeat dosing can enhance transfection of mouse and nonhuman primate bone marrow HSPCs as well as

human HSPCs using humanized most small systems. And so that brings an end to this chapter. And before I turn over to Stephen, I would like to thank you all for listening in today.

Stephen Hoge - Moderna, Inc. - President

Thank you, David. For those who don't know, I'm Stephen Hoge, and I'm the President of Moderna. I've had the privilege of leading our research and development organization for most of the history of the company. And it has been my greatest privilege to work with scientists like Melissa and many of the folks that you saw today. As Stéphane started out in the beginning of the day, we believe we've been on a 20-year journey of bringing forward the best mRNA science and delivery technology to hopefully have a huge impact on medicine.

Over the last 10 years, we've continued to invest many billions of dollars in the basic science, technology and manufacturing platform that have allowed us to have a big impact in the last 2 years. No doubt, 2020 and 2021 have been a point of inflection in the history of our company and perhaps our industry. We've had a huge impact and helped many hundreds of millions of lives as a result of the investments in the last decade. But what hasn't changed is Moderna's commitment to continue to invest in that basic science. And as we look forward over the next 10 years, we are fully committed to continue to invest just as much, if not more, in the basic science of messenger RNA, its delivery technologies and manufacturing technologies. We believe that those investments are what will set Moderna apart competitively and really form the foundation of the future impact in terms of health and disease that we hope to have.

So with that, I'd like to invite the operator to prepare for question and answers, and I'll be joined, during that, by both Melissa Moore, our Chief Scientific Officer; and Stéphane Bancel, our CEO. Operator?

QUESTIONS AND ANSWERS

Operator

(Operator Instructions) Our first question comes from Matthew Harrison with Morgan Stanley.

Matthew Kelsey Harrison - Morgan Stanley, Research Division - Executive Director

I have 2. Maybe if I could just start with as part of the work you're doing on variants of concern for COVID, it looks like the focus here is all on antibody response. I was wondering if you could just comment maybe on some of the other immune compartments and in particular, memory B cells and what your thoughts are there in terms of durability and impact on some of these variants of concern? And then I have a follow-up.

Stephen Hoge - Moderna, Inc. - President

Sure. Well, thank you, Matthew, for the question. So it's obviously an area of extensive research. We've looked broadly at cell memory in our previous studies, particularly T-cell memory and CD4 compartments and have previously published on that. And I think there's been a large emerging scientific literature showing that the both Moderna vaccine and some others have been able to generate really strong T-cell responses across a range of populations, which is an important component of establishing that long-term memory, that CD4 help. We've also been looking and characterizing in preclinical species, the B-cell compartment. And looking at the key components of that B-cell compartment, including follicular help T cells, but also B cells. And that's actually been a big part of our preclinical literature that we published with our colleagues, particularly through the VRC and NIH. And in general, what we've seen is the establishment of long-term memory, long-term long live plasma cells in that work. And we haven't gone in characterized plasma cells in that B-cell compartment in great detail in the clinical space. We've really been focused on the efficacy of the vaccine and rolling it out, but we have started to see, as was recently published work from academic groups, showing that vaccines actually can generate, particularly mRNA- 1273, can generate long-lived plasma cells and B-cell immunity.

I think that's all good news. What I don't think we know, and we're still going to have to follow very closely as the science evolves, is how does that B-cell memory, particularly as measured by humoral responses, hold up over time? And at what point do we start to see reinfections with SARS-CoV-2 and the emergence of symptomatic disease and heaven forbid, severe disease and possibly mortality. It seems to us that the question is, if not when -- sorry, when not if. And the reason we say that is, as everybody really comes to believe that SARS-CoV-2 will become an endemic human coronavirus -- virus. If you look across the 4 endemic human coronaviruses, reinfections happen on a pretty regular cadence. So we're optimistic that we've got a really strong vaccine. It's -- obviously, the clinical data has been terrific. And the translational data suggests we're going to have the best possible long-term memory but also, I think we have to be realistic that these viruses have evolved over millions of years to successfully come back and reinfect humans and now humans. And at some point here, that will happen with SARS-CoV-2.

Matthew Kelsey Harrison - *Morgan Stanley, Research Division - Executive Director*

Great. And then on the HSPC work, I guess the sort of broad question is, what do you think is an appropriate proof-of-concept disease to maybe study this in? And I guess, secondarily, given that you've been able to demonstrate both getting it into the marrow compartment as well as the potential to have repeat dosing, I guess, would you first go after something that you think you could you just handle with one dose into the marrow compartment given potential safety risk with repeat dosing? Or do you not see potential safety with repeat dosing?

Stephen Hoge - *Moderna, Inc. - President*

Thanks for the question. Well, look, I'll maybe start, and Melissa, if I miss anything, feel free to add. I think we're -- look, we're really excited by this. It's been a lot of work to get to the stage. We're very confident in our platform's safety. At the end of the day, you're asking a question about where we will point the platform for first candidates. The traditional approach we've taken when we move into new modalities as we try to pick diseases where the biology is pretty well worked out, you don't want to take a high degree of biology risk. And so in many cases, that points you to monogenetic diseases, which have a defect in the hematopoietic lineage. We can think of anemia as a pretty classic place where you might get benefit from amplification of whatever editing or correction you can do. But there are other defects that exist in terms of the evolution of different cell lineages, which we might be able to sort of correct as well.

And so we're not going to be specific about which of those candidates we're working on. I can assure you, we are working and pretty excited by that. But it is definitely an area where we think we'll be able to move forward with both single dose activity in the case of some gene corrections, but actually repeat dose activity, because we do think that's a key feature of messenger RNA as a platform as opposed to a viral vector approach to this. And we'll be prudent in doing this in the most responsible way, obviously, going after diseases where there's an incredibly high unmet need also affords a more favorable benefit risk calculation. But we do believe, over time, the risks are going to be quite manageable for in vivo gene editing or bone marrow correction, and we're looking forward to advancing the science there in the years ahead.

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

Let me just mention -- I'd take on something at the question or ask, it's about safety of repeat dosing. So we've solved that problem a while ago. And previously, we've shown that we have delivery systems for in vivo for intravenous delivery where we can repeat dose. And so that's not really a safety issue at this point.

Operator

Our next question comes from Salveen Richter with Goldman Sachs.

Unidentified Analyst

This is Elizabeth on Salveen. We just wanted to get your thoughts on the ability of SARS-CoV-2 to mutate materially in perpetuity. And do you think there will be some point where there's limited future ability for the virus to develop new materially different novel mutations? And then what would be the resulting impact on the development dynamics and the need for a strain-specific vaccine?

Stephen Hoge - Moderna, Inc. - President

Well, maybe I'll take a first half at this and then again invite Melissa to fill in. I never underestimate the ability of evolution to continue to find new and better ways. I think it will be hard to assume that forever, there's going to be a best answer here and that evolution would stop in terms of the coronaviruses. I look to the -- perhaps the last beta coronavirus pandemic, which was caused by, we think, OC43, 130 years ago, that is an endemic human coronavirus. It re-infects people every year. And it can cause significant disease and even some mortality still. And it has continued, if you look at the basic science, to evolve its spike protein as well as other proteins to try and now what has happened over time in those coronaviruses is you end up with sort of a dominant viral family or subfamily clade, that's really a few (inaudible) in any given season, but there's constant evolution in the background.

I think what we're seeing right now with SARS-CoV-2 is much more the sort of explosive evolution where there are many different threads moving forward in parallel. And that really is probably where there's this great genetic diversity that we're seeing evolve, but there is some convergent evolution on some best features, let's say, in the spike protein for adaptation and infection in humans. And so I think it's a bit of a mixed answer, which is we're in a period of time in the pandemic, where we're going to see quite a lot of explosive evolution in many variants and a lot of -- if you really think of it, the virus is competing with itself for its ability to adapt and infect in humans. And then over time, that will likely converge on some winning modes. And then if you look at the endemic coronavirus, as an example, probably select down to a series of winning clades or viral families. But continued evolution for perhaps for as long as we -- it is an endemic coronavirus, which we would expect would be for many decades, if not centuries.

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

Well, I was just going to add to that, that the big way to slow down the evolution of the virus is to knock down the number of people that it's transfected into. And if we can just decrease the transmission, that will knock down the evolution rate, quite significantly. Yes. Go ahead, Stephen. Sorry.

Stephen Hoge - Moderna, Inc. - President

That's right. I think the second part of the question that I was going to touch on very quickly was the -- and what is our commitment on continuing to adapt our vaccine? And I think the short answer is, as we've committed ourselves to doing our part to stop this pandemic, and that means as many times as it takes that we need to update our vaccine, if we see variants of concern that we want to, as Melissa said, do everything we can to suppress its opportunities to, in fact, even asymptotically infect people and perhaps get a chance to evolve. That's something we're going to do. Now that's not going to continue in perpetuity. I think it's -- we're entering a very dynamic phase of the pandemic, where we're going to want to do that pretty regularly based on the science, but we'll follow the science. And then over time, it might converge to -- as it slows down the pace of evolution, hopefully, because we are able to suppress infections that will converge to a less frequent update.

Operator

Our next question comes from Michael Yee with Jefferies.

Michael Jonathan Yee - Jefferies LLC, Research Division - Equity Analyst

I had 2 questions. One was, given all of your work around variants, it would seem to me that a multivalent approach would make sense. I appreciate you don't have that data, I haven't reported that yet on COVID. Can you just maybe speak to the most obvious logic of that being the strategy going forward? I know competitors seem to think

maybe just a single valent would work. So maybe just talk to that because I would think that would speak to what you just said about decrease in transmission and evolution. So that's question one. And then question 2 was, I guess, about the revasum load technology. Can you maybe just speak to where or which indication or which protein that might make most obvious sense for where you're thinking about going with that first and where we could see that evolve first in the clinic, et cetera?

Stephen Hoge - Moderna, Inc. - President

Great. I'll take the first and leave the second clearly for Melissa. The -- so on the first question, we have published preclinical data on our multivalent approach. And I think in that case, you do see clear evidence again in preclinical species, that a multi billion approach generates the broadest immunity across, not just the boosted antigen. So not just against the viruses that are included in that bivalent vaccine, but actually against the forms of the spike protein that are not included. And that's not surprising because the more you diversify, you diversify your antigen that you're using in your vaccine, the more you're kind of also filling in the gaps because you're generally generating an immune response that's more broad-based. And that's what sits underneath our belief that ultimately, the clinical data, when we do talk about it, will support moving forward with a multivalent platform.

We think it's been shown to be beneficial in other cases to pursue multivalent approaches. It's certainly a feature of the flu vaccine or the annual flu vaccine that you cover several strains of flu. And we're -- we've got a lot of experience with that. Even in the clinic, our 1653 program is actually a multivalent respiratory vaccine that we've been dosing in children even. And so we're -- we believe it's a feature of the platform. We can do that. We believe it's the right thing to do, epidemiologically, and we actually think that even our current preclinical data showing mRNA-1273.211 and preclinical species supports that idea. So whether it's bivalent or trivalent or quadrivalent in the future, that really depends upon the evolution of virus. But we're able, with our platform, to add that diversity, and we're actually -- we do believe the science points that in that direction long term.

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

So with the -- with regard to the ribosome load question, I think one of the things I can tell you is that our understanding of how the sequence structure then code on optimization in the open reading frame or the coding region affects ribosome load. That's part of our current design algorithm. And so a lot of our current and soon to enter clinical trials, mRNAs have -- are designed with those features in mind. I can't specifically point at individual ones. But what we're hoping is by developing all these different levers that we can use to modulate ribosome load is that as we design even more medicines in the future, where particularly, we might want just a very tight burst of a tiny amount of protein, let's say, some regulatory protein. That's where this becomes particularly important.

Stephen Hoge - Moderna, Inc. - President

Yes. And I want to underscore something Melissa said there, which is we invest pretty substantially in these technologies. And they -- because it is -- we ultimately believe we are a digital system and a digital biotech. A lot of it gets updated into right away how we design things. And so many of the features that we present at Science Day, even a day like today, have actually been put into our vaccine candidates, like the multivalent candidate that I just spoke about, because we are constantly making sure that as we design candidates and bring them forward, that they're using the state of the art as Melissa and team have continued to advance it. And so it's a feature of the technology that allows us to do those things even at a platform level, almost like software updates.

Operator

Our next question comes from Jeff Meacham with Bank of America.

Alec Warren Stranahan - BofA Securities, Research Division - Associate

I wanted to ask a follow-up on the multivalency approaches to the platform. I guess it's related to COVID, but also broadly applicable to your pipeline. So can you speak to how you optimize the different mRNAs, i.e., are they all created equally with respect to immunogenicity based on what you have thus far? And then is there an upper limit to how many different mRNAs you can add to a single product? I'm just trying to think about perhaps 2 to 3. Or is there a limit of 5 different products that potentially you can put into a single dose?

Stephen Hoge - Moderna, Inc. - President

Great question. Thank you. I'll invite Melissa to talk about some of the design features of mRNAs and how our platforms use across in just a second. But maybe I'll start with the second part of the question, which is we haven't [hidden] an obvious upper limit yet. Our CMV vaccine includes 6 messenger RNAs, including 6 different proteins, 5 of them come together inside the cell to form a transmembrane antigen. Our flu vaccine, we are going after many different strains of flu, 4 seasonal strains of flu. Plus, we have the 1653 vaccine. I already talked about, which has 2 different antigens in it. And so we actually have quite a lot of development experience, combining 6 and more, over time, antigens.

And so preclinically, we actually have evaluated in the range of species higher numbers, closing in on double -- actually double digits, low double digits. And so far, we have found an ability to continue antigens and maintain good immunogenicity, which is a really great sign for the platform. Now we'll have to prove that over time and deliberately so. But at least for the near term, certainly, up to 6 feels, well-documented from a development systems perspective, and we think going up into the low double digits is not even impossible. So we're excited to prove that over time. The obvious benefit of a single needle, a single injection that provides broad protection across a range of viruses is there, from a patient perspective, from a health care system perspective, even just from a personal experience perspective. So we're quite enthusiastic about that. Melissa, do you want to comment about how we design?

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

Yes. So I mean, one of the things that's really fun because I'm really the technologist and thinking about the technology, is just all of the amazing things that you can think about doing with mRNA. And so the things you can think about is if you had multiple mRNAs in a lipid nanoparticle, we can put micro RNA target sites in the 3 prime UTRs of one mRNA and a different one in a different mRNA. So even if the lipid nanoparticle delivers both of those mRNAs to the same multiple cell types, we can have it so that one mRNA is only made in one cell type and another mRNA is only made in another cell type.

Another feature that maybe we'll try to put into future designs is can you design it so that you can deliver a couple of different mRNAs at the same time, but one of them is expressed first and then the other one is expressed later by putting in different design principles into the mRNA. So it's just so many levers that we can pull just by designing the mRNA sequence. And so it's hard to answer that what's the limit because we haven't really approached the limit of what we can do with this technology. But I can tell you, my scientists are having a lot of fun trying to just push those limits and see what all we can do. And then once we have discovered that, okay, here's a lever that we can a new lever we can put in, then we talk to our colleagues in the therapeutic areas and say, "Okay, what kind of -- can you use this? Can you imagine a therapy for this and a use for this?" And then it's a back and forth conversation. So there just aren't many, many possibilities.

Stephane Bancel - Moderna, Inc. - CEO & Director

Yes. And if I can maybe just add a word, which is why we have been so excited for so long to invest in this science because it's a platform. As Stephen said, and Melissa said, information platform. If you go back to traditional pharmaceutical company, which is more kind of an analog world is when you invest in science, we invest beyond one program. And as we know, 90% of the programs never make it to market and that -- most of that

learning is gone, specific to the molecule to a program. What has always given us a lot of conviction to invest aggressively in the science and now that we have even more capital, we want to invest even further, is the fact that when Melissa and our team discover one new trick on how to use technology, not only as they describe it, the embedded into the design software, the drug is on studio. So all the drugs moving forward can use that. But in terms of investment return, which is why Stephen and I have always been very aggressive to invest in science is you can use that feature forever, for all the new drugs on the road, and that's really an incredible value creation process.

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

Well, that's not even just that feature. I mean, every time we get a new tool in our toolbox, then it gives us a whole new set of combinations we can do. And so it's just -- there's a lot going on.

Operator

Our next question comes from Gena Wang with Barclays.

Huidong Wang - Barclays Bank PLC, Research Division - Research Analyst

That makes me wanted to go back to the lab to do event research instead of my equity research.

Stephen Hoge - Moderna, Inc. - President

We're hiring, Gena. We're hiring.

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

Yes. We've a lot of openings.

Huidong Wang - Barclays Bank PLC, Research Division - Research Analyst

I would take a sabbatical, I would take a sabbatical at some point and then go to visit Boston. So I have one question regarding the RNA translation. And I have 2 questions regarding the liponanoparticle. So for RNA translation for polysome, just wondering, have you tested the integrity of the translation with enhanced the polysome loading? And then my 2 questions regarding lipid nanoparticle. The lipid nanoparticle C, just wondering what kind of modification you made to the [ion lipid] to make it less sticky? Is that making more positive charge? And a second question regarding the lipid nanoparticle is for the HSPC delivery. I'm wondering if you're using the whole body as denominator, what percentage of a lipid nanoparticle delivered to the HSPC? And then, what is the most component -- the important component to increase HSPC delivery?

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

Okay. There's a lot of questions to unpack there. So I'm -- and I was trying to write them down seriously, and I didn't get them all written down. So let's start with the first one, though, and I just wanted to ask you, going back, when you were asking about the integrity of translation on the polysomes, what do you -- can you just what do you mean by that a little bit more?

Huidong Wang - Barclays Bank PLC, Research Division - Research Analyst

Yes, sure. Like a more, like the protein, did you like -- would that introduce any mutations or the -- like some mistakes for the proteins, the final product?

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

No. I mean, the number of -- most, as we pointed out, on endogenous mRNAs are translated by multiple ribosomes. And in fact, the numbers are staggering. The number of protein molecules that you can get out of one mRNA for endogenous RNAs is up to 10,000 different molecules. So in other words, the ribosomes travel that thing 10,000 times. The current estimates of just endogenous translation mistake rates, like putting in the wrong amino acid, and that happens because it's a manufacturing process, and your body is doing this all the time on your own mRNAs. It's about one in 10,000 amino acids are incorrect. We have looked at our mRNAs in terms of the ribosomes and where they are on the mRNAs and then trying to estimate a mistake rates. And we don't see anything -- we see no increase over what's there on the endogenous mRNAs. And so I don't think that the ribosome, the number of ribosomes is going to affect the integrity of translation. We haven't seen any evidence of that. And we can also look at just the length of the RNA that made -- I mean, the length of the protein its made? Does it get to be full length? And it's -- and we never see any problems with that. So we're making full-length proteins.

Now with the second question is the one I didn't get written down. Can you repeat that question, please?

Huidong Wang - Barclays Bank PLC, Research Division - Research Analyst

Sure. That's a lipid nanoparticle C that you made a modification make it. Just wondering what execution was that? Was that like...

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

If I could have told you, I would have told you, right? So that's sort of our secret sauce, and we're not ready to reveal that. I'm sorry. The third one of your questions was, what percent of the nanoparticles get into the HSPCs? And we're -- we'd like to get those numbers. We don't have those numbers yet. And so we're not really there yet. Then what we focused on more is what percentage of the cells can we get transformative.

Huidong Wang - Barclays Bank PLC, Research Division - Research Analyst

Was that in the low, say, low single-digit range?

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

Again, I don't have those data, so I can't speak to it.

Huidong Wang - Barclays Bank PLC, Research Division - Research Analyst

Okay. And then also, again, the component of the lipid nanoparticle, which is the most important? How do you do to increase HSPC delivery?

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

Okay. So again, this is one of these things that we're not quite ready to talk about yet. So we eventually, when we get into the clinic, and we will tell, but not today.

Stephane Bancel - Moderna, Inc. - CEO & Director

As you can appreciate, Gena, there's a lot of know-how that we developed over the years. And it seems that our company is interested in many. So we cannot share that sensitive -- no information.

Operator

Our next question comes from Mani Forohar with SVB Leerink.

Mani Forohar - SVB Leerink LLC, Research Division - MD of Genetic Medicines & Senior Research Analyst

I wanted to drill in a little bit on practicalities around -- thinking about the clinical stem cells. You talked about (inaudible) we have a really interesting mouse data interesting nonunlimited climate data. When you think about obviously, human being substantially longer-lived than mice, for example, how do you think about dosing for these cells that are quite replicative, proves many daughter cells or supplying functionally all the lineages of the entire metabolic system. How do you think about addressing potential dilution of your mRNA, in the case of rapidly developed -- in the case of rapidly reproducing cells? And sort of how do you think about dosing, frequency and any potential differentiation in your effectiveness of mRNA translation in different types of daughter cells? We've seen differences between the efficacy between lineages derived from the HSCs that you've treated.

Stephen Hoge - Moderna, Inc. - President

I mean, that's a great question. And I think it gets to this topic of target selection in our first diseases, which we're not ready to sort of provide specifics yet. But I think there's a couple of things that do guide us. One is that you obviously -- if you're just going to do, let's say, an epigenetic or cellular modification but not genetic modification. Then what you would want to do is pick a protein that you know is going to have a substantial effect on the downstream lineage. There are some things that can do that, that are well-described in the literature. I don't want to get the specific targets. But things where you could imagine expressing transcription factors or other modifying features that would actually dictate a whole lineage-dependent change, which can be relevant in several diseases. Then there's the idea of replacing a protein that is actually deficient in daughter cells in that lineage as it dilutes out, but where that protein gets handed down through multiple generations. And there are proteins in cells that do have that feature. And that would be one where you're not doing, let's say, a lineage dependent change, but you're making sure the daughter cells have a protein that they're missing that could be very important for the physiology of that cell later on.

And you don't need a lot of just a handful of copies, and there are arguments that have that feature. And then there's the question of all the way upstream, whether or not you use this approach to modify the original genetic defect in some way. And that's obviously something that's not lost on us and on others and that this technology can be useful for. So really, it's about using our ability to dose -- have dose-dependent pharmacology, get into the hematopoietic stem cell compartment and then direct either with very transient expression of a protein that impacts lineage or transit expression of a protein then will survive through the daughter cells or even modification of that hematopoietic lineage. And we're going to take all 3 of those approaches depending upon the disease.

Mani Forohar - SVB Leerink LLC, Research Division - MD of Genetic Medicines & Senior Research Analyst

Great. That's helpful. And hopping over to the -- some of the computational tools you discussed. There was a lot of conversation around different geographically, in terms of the pool of data you're getting about variants of COVID-19. Obviously, South Africa, the U.S., U.K., et cetera, emerging markets, very different proportion of the reporting of emerging variants. Taking that and applying that to flu, obviously, there's a tremendous historical pool of data regarding genetic influenza virus, the human experience was quite long. Are there ways for you to leverage that existing pool of data across many, many decades for the development of variance for a multivalent flu vaccine? Or is that something that starts from scratch every year and every year sort of a new race to find the right targets?

Stephen Hoge - Moderna, Inc. - President

Great question. I think our mission as a company is more to do better, right, to develop a new way of identifying that. And we're not constrained by the traditional limits around picking only 4 strains. And that will allow us to more flexibly, we think, develop solutions that might anticipate better what the flu season and circulating strains will be. Now in the short term, I think you'll find us follow in the footsteps of others because to be completely fair, there is an established process for the WHO and for governments to agree on what vaccine components need to be there for a seasonal flu booster. And so we will not yet totally disrupt that. We'll probably participate that. But then look for ways to improve that over time. And one of them that you pointed to is bioinformatic historical modeling and prediction. But then also prospectively, looking, as I just said a moment ago, to add more antigens and think about how we provide the greatest amount of information diversity in our booster so that we can achieve the greatest possible protection against the seasonal flu strength.

The last thing I'd say is the same can apply to COVID as it moves into an endemic virus and starts to circulate. We can learn a lot from the data that's been collected about the endemic coronaviruses and how that evolution might because again, these are mostly spike proteins that people develop immune responses to. So we will be doing this broadly in our respiratory platform, but definitely also in fluid.

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

Yes. I just wanted to bring up the point. I think it's a really interesting question that you're asking, that the -- how flu evolves is different from how coronavirus is evolving because coronavirus has a single RNA genome. And so it's mutating internally to that genome. Flu is a -- each flu variant has 8 different RNAs in it. And there are different versions of those 8 different RNAs that are -- and so when people are infected by multiple flu viruses, you get recombination. So it's much more like having more like chromosomes almost and so it's a very different evolutionary process than coronavirus. And so I think the -- obviously, the tools that we're developing will be very applicable to flu, but it's -- informatically, it's actually even a more complicated process.

Operator

Our next question comes from Simon Baker with Redburn.

Simon P. Baker - Redburn (Europe) Limited, Research Division - Head of Pharmaceutical Research

Two questions, if I may. Going back to the COVID deep mutational scanning that you talked about in the Jesse Bloom paper that you referenced. I just wondered how deeply you'd explore that mutation set in the paper to, in a sense, predict a vaccine escape experimentally before it happens or whether that's practical at this stage we can necessarily give you a speed with which you can advance new mRNA vaccines against SARS-Cov-2? And then a question on ... --

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

Can I answer the first question before you get to the second question? So the Jesse Bloom paper just recently came out. And what we -- we presented that because some of that is aspirational. So the one thing about the Jesse Bloom paper is they only looked at single point mutations. But what we're seeing in the virus, obviously, are multiple mutations at the same time and combinations of mutations. And so one of the things that we are working to do is set up that deep mutational scanning, but to do it using combinations of mutations, not just single mutations. And we do believe that by generating that data and combining it with our bioinformatics analysis, it will be something that we can feed into our machine learning models to help us predict breakthrough mutations. So it's really -- that's something that we're trying to give you a view of what future things we're building.

Simon P. Baker - Redburn (Europe) Limited, Research Division - Head of Pharmaceutical Research

Great. And that's very helpful. And then on ribosome load and kinetics around that. I was just wondering, is that a generalizable result irrespective of mRNA sequence lengths? Or do you see the relationship changing as the sequencing increases?

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

So that's a really good question. And we are doing internal experiments now to test that question. But what I can tell you is that this concept, that smaller polysomes make -- actually end up making more protein, has it's been very anti-dogmatic in the academic field. And so because we always -- we, me and everybody else, always thought bigger polysomes, better, more protein. And so it's causing the academic community to reevaluate the previous data on lots of data that come out from endogenous RNAs. And what we're seeing is that this is also true, that this optimal spacing around 200 nucleotides apart is something that is true for different RNAs within the cell and not just our therapeutic RNAs.

Operator

And I'm not showing any further questions at this time. I would now like to turn the call back over to Stéphane Bancel for any further remarks.

Stéphane Bancel - Moderna, Inc. - CEO & Director

Great. Thank you very much, operator. Well, to all of you, thank you so much for joining and spending time with us. We really appreciate it. I would like to thank the Moderna team and the platform team, especially for all the progress we have done in the last 12 months, and for their relentlessness and their boldness, which is very Moderna. If you think about the company now, we have one modality that's commercial for infectious disease vaccine modality. We now have with our rare genetic disease in the clinic, 5 modalities that are in clinical studies. And as you can see from the work here, we have 2 emerging modalities. As you're highly aware, we have no modality where we're working on with Vertex in the lung, and we look forward to giving you some update there. And as you saw today, with hematopoietic stem cells, there is some very interesting data and ideas the team have, in term of how do we get a new class of medicine using messenger RNA plus LMP into the clinic to help patients for already complicated diseases.

Our next rendezvous is going to be on September 9 for R&D Day, but we look forward to welcome you, not to talk about science but to talk about clinical data and clinical programs. And in between now and that time, we will have, of course, our Q2 earnings call. With that, have a great day. Thank you very much, and stay safe. Bye-bye.

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

Bye.

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