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CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

CBER SCIENCE SYMPOSIUM

DAY 1

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P R O C E E D I N G S

(9:30 a.m.)

DR. ELKINS: Welcome to the 2024 CBER Science Symposium where we are finally back to an in-person format, but also hybrid. Thank you all for being here. Our first speaker really needs no introduction, particularly the CBER folks.

But just briefly, Dr. Peter Marks, received his degrees, both MD and PhD from NYU, did further training at Brigham and Women's, and then assumed a series of more progressive and responsible positions both in academia and in industry, but came to CBER as the Deputy Director in 2012.

And is now, of course as we all know, the Director of the Center for Biologics. Peter is going to talk to us this morning about some of the history of CBER. So, welcome and thank you for starting us off.

DR. MARKS: Thanks so much. So, first of all, let me just welcome everyone to today's Science Symposium, both here in the room and those

online. This really gives us an opportunity to hear both about the impactful science done by people outside the center that relates to the products that we regulate, as well as both to hear about the science done in the center, and also to celebrate that science, for instance, with the posters done at the center.

So we really appreciate the people to take the time to attend today. I know a lot of work went into getting this together. Before I get started, I want to take a moment to thank Monica Young, Emily Bronstein, and especially Karen Elkins. It takes a lot to put this together. There were a lot of -- I didn't know from seeing a lot of the emails. So thank you so much.

So today I'll start off with what will be a historical perspective. There are people in the room who probably have a better historical perspective than I do, having lived through it. But I will tell you the historical perspective that I have.

And some of it has been helped because although he retired a few years ago, we had a person who was in charge, our records receiver named Joel Misner, who kept me very well informed of CBER's history. So he had only been working here -- I think when he retired, he had only been working for CBER for about 50 years.

But also then after that, we'll have a session this morning on cell tissue gene therapies. And this afternoon, on advanced manufacturing.

So let's see, what I will do here is talk a little bit about our origins, talk about where we were physically located, something about the science that we've done knowing I can only touch on the select amount just to give people the flavor, and a little bit about what we've done on regulation over the past two years.

So like most regulatory agencies in the United States, it's very unusual that a regulatory agency comes into being because someone thinks it's a good idea to regulate something. In

general, regulatory agencies come into being because something bad happens. And that's exactly how CBER came into being before FDA came into being.

So in 1901, the two biologic products that were relatively commonly made around the turn of the century, that is the 19th to the 20th century, were diphtheria antitoxin and smallpox vaccine. And unfortunately, in two separate instances in that year, children died from contaminated product.

In one case, it was really a tragedy because a horse had been euthanized that had tetanus, and had been -- the terminal bleeds had been packaged up. And unfortunately, they were just released, even though someone could have made the connection. So, it really kind of shows you what can happen in this situation.

What ended up happening is in 1902, Congress reacted, and the Biologics Control Act in 1902 was passed which required the licensing of manufacturers of vaccines, serums, and antitoxins,

and it also authorized the inspection of manufacturing facilities.

And then, by 1903, there was a regulatory authority, which was initially located in the Public Health and Marine Hospital Service. It was renamed at that point, the Public Health and Marine Hospital Service.

And then, as you'll see, it eventually became part of the National Institute of Health, and since 1972, FDA. So we predate -- the early portion of CBER's history predates FDA by about three years.

The Biologic Control Act of 1902, it's worthwhile reading because it shows you -- in what is only about four pages of printed text, contains the essence of what we do still for biologics.

And it actually was quite a powerful Act, in that it gave us things like recall authority, something that other medical products still don't have in some cases. And you can see that it was Theodore Roosevelt who signed this into law.

Now where we have been over time is kind of an interesting story. This is the hygienic laboratory of the Public Health and Marine Hospital Service. As a trivia question we can ask, where is this located? It happens to be on Staten Island of all places, and it was built actually before the turn of the 20th century.

This is a picture, which is about 1915. It could be a little earlier, but the hygienic laboratory was where dysfunction on biologics regulation sat with. It sat there until about 1930, at which point it became -- the hygienic laboratory became the National Institute for Health, which then subsequently or around the second World War, became the National Institute of Health.

Here is the National Institute of Health campus around 1947. You can kind of see -- let's see. I'll even try to use the laser. You can kind of see that's still Building 1 there, although the rest of these buildings are -- I really can't. I think the clinical center is

somewhere over here now. That's kind of an interesting look.

And now, this is a building that some people may -- send shutters down people's spines. That is Building 21 -- sorry, Building 29, around 1960 when it was dedicated. Actually an interesting piece of this, there are lovely pictures if somebody wants to look at them down in the documents, the control room.

The King of Thailand dedicated this building when it was opened in 1960. It was, at the time, supposed to be a state of the art laboratory. Some know that it probably became not so much the state of art relatively quickly, but we grew enough during that time that additional space actually had to be built.

First, Building 29A, which was built in 1965 through 1967. And then, Building 29B, which was built in the early 90's to house the expansion of biologics regulation.

As an interesting note, biologics were regulated by the National Institute of Health

right up through 1972, at which point, they were transferred by the then Secretary of Health Education Welfare to the FDA.

If anyone wants to know the reason why, it probably had a little bit to do with some trouble with influenza vaccines around that time. And so, that was decided to move biologics regulation over to FDA.

That said, these buildings stayed on the NIH campus, along with their occupants. And the laboratories stayed on the NIH campus right up through 2014 when we moved over to White Oak. And here at White Oak, here's a picture of Buildings 71 to the left, and 52 which is a lab building. So we were all located on one campus.

In between this period, we were located -- the labs on the NIH campus, and the various review offices in about six different buildings along Rockville Pike. I can even remember back to that time because when I came, we were located all up and down Rockville Pike, and on Nicholson Lane.

So we've been around a little bit, and

now we're consolidated on this campus. Interestingly it's -- you can see this in the NIH heritage here though is very real, and is a part of why our research and regulation are so -- those go hand in hand in the center.

So let's talk a little bit about the science that's been done at the center over time. I couldn't do justice to it all without putting everyone to sleep, or without going through a lot of information. But just to give people flavor of some of the really groundbreaking research that was done.

Margaret Pittman, who was at the center from 1936 to 1971, was the one who discovered various different varieties of H Flu, and found that Type B caused serious disease in children, such as meningitis and pneumonia. And her work formed the basis for development of an effective antiserum and a vaccine to protect against serious disease from H Flu Type B.

This is the kind of work that actually has been on multiple times since the center.

Another example of this is Paul Parkman, who became Center Director ultimately, and Harry Meyer Jr., who were working in the 1960s, developed the first effective experimental vaccine for Rubella by passaging the virus extensively in culture over two years.

And they prepared a live attenuated viral vaccine for testing in children that ultimately was further developed, and that resulted in the first licensed Rubella vaccine in 1969. So quite another accomplishment.

And then, another -- again another along the lines of things that were done at the center, one of the real challenges has been the cost effective manufacturing of vaccines.

And it turns out that the polysaccharide protein vaccine conjugation technique that was used in the vaccines was quite expensive. But Robert Lee and Carl Frasch, developed a new vaccine conjugation technique that they patented through the center.

And that technique was much less

expensive, and that ultimately led to a development of Meningococcal A Vaccine, which could be produced at a fraction of the cost of the one being used in the United States.

Ultimately that technology was essentially given away -- or donated, and resulted MenAfriVac, which has been used and deployed widely in Africa. This vaccine is credited now with saving over 100,000 lives. So quite an accomplishment stemming out of the CBER labs.

And then, more recently just -- and this is a smattering, so this is -- I hope I don't offend anyone. This is just -- everyone should know that I want to recognize everyone's research. But I'm just going to recognize a smattering of research, and some of it's a little older from recent research, but it's worked that's proved its importance over time.

You know, people may be aware that the current acellular pertussis vaccine, seems to not be quite as effective as the old whole-cell vaccine that was used, the killed whole-cell

vaccine. And Todd Merkle and colleagues wanted to understand why that seemed to be the case.

They did something that -- again the center has come through pretty well, developed a model. This is actually down here, a green baboon, I think. And that green baboon model was used to try to look at what the cause of failure was, and just to get to the bottom of this.

Basically it was found that although acellular vaccine protected against disease related to pertussis, it failed to prevent the infection and transmission. So if you had kids around who were around kids who were not vaccinated, unfortunately you could still pass on the pertussis.

So again, really interesting finding that may lead to the development of better vaccines. I can tell that their parents didn't really like the old whole-cell vaccine causes fevers of 101, 103 degrees, which unfortunately then becomes associated with febrile seizures.

So people like the newer acellular

vaccine better, but as -- when you have problems like we do in the United States today where vaccine coverage rates often dip below 60 or 70 percent in certain, very local regions of the country, it's a problem. So hopefully, a better vaccine coming in the future, and hopefully people will take it.

In the area of blood research, you know, when I first came to the center there were a fair number of reports of new lobby on products being associated with blood clots, and there were a variety of serious blood clots. Everything from clots in veins and the legs, to heart attacks and strokes, and really was not clear on what was causing them.

And I remember being in the clinic in the early 2000s and through the 2010s, where we were wondering what was going on here. And during a cluster of these associated with specific globs of immune globulins, it was the insight of some of the researchers at CBER that maybe this was a contaminant.

Potentially even a Factor XIa was identified as contaminating a certain immune globulin perhaps, and being associated likely with the causation of these clotting events.

And Mikhail, Ovanesov, and colleagues developed a nice assay for this, which is now routinely used to make sure that there is not a contaminating Factor XIa above a certain level that can cause it. So again, how our regulatory research makes a difference on the lives of people by helping to make better products.

In the cell therapy research area, and this is really an evolving area where stem cell therapies are used all over the place, we really don't understand very much about them often.

And several years ago, a group of scientists from different disciplines at CBER tried to better understand what was going on with these products. Steve Bauer and colleagues looked at the ability of mesenchymal stromal cells to differentiate, and found that basically different cell lines made in a similar manner and then

passage, could look very different.

And it became clear that passaging cells in a culture really did affect them very dramatically. Now you might say, well does that really matter a whole lot that people should have realized that?

It turns out that this turns out to be very important for us from a regulatory perspective because these data help form the basis of how we can say that cells that are put in an incubator, and let's divide one or two times, are not the same as the cells that are harvested. Therefore, they are more than minimally manipulated. It also gives you insight as to the variability that we're dealing with xylotherapy products.

And then, finally, not to forget our biostatistics and epidemiology colleagues, we've had a lot of work done at the center and in this area, and real world evidence has been one of the areas of focus.

And it's been used for several years at

the center for the evaluation of product safety. But now also, we've been exploring it for the evaluation of effectiveness, and our staff have used -- our scientists have used a rigorous methodology. There are protocols that are written up front with hypotheses that are documented. These are not fishing expeditions.

And an example of what has been done, which I think is pretty impressive, is Hector Izurieta and colleagues used a Medicare database to look at high dose versus standard dose Influenza vaccines. And this was done shortly after a large randomized clinical trial was done in 36,000 people looking at that comparison.

And you can see, they were able to use something like 2.5 million people comparing, you know, 900--some aught thousand high doses, versus 1.5 million and a half plus standard does recipients, and come up with improvement and effectiveness to 22 percent, which was quite close to the 24 percent that was shown for prevention of improvement -- in prevention of Influenza-like

illness in the randomized trial.

Except there was something a little better with their data because as opposed to a role of efficacy of 24 percent reduction in Influenza-like illness, it actually was a 22 percent reduction in hospitalization, a hard outcome that really, very easily in pharmacal economics, translates into cost reduction. So it just shows that sometimes that real world evidence can actually be as powerful as clinical trials conducted in a more standard format if the setting is right.

So just to finish up, I want to spend a couple minutes talking about some of the regulatory things we've done over the past years, and just the past couple of years. I don't have time to go through as many of the different things as I probably should. But I figured I'd pick, like, from the greater tips.

So we had to address the COVID-19 pandemic, a little thing that's only supposed to happen once in a hundred years, we hope. But we

have to always be ready for these events, so addressing this was a very important thing, and learning from it was very important.

We talked a little bit about modernizing the blood donor criteria, and advancing development of novel gene therapy. And again, the most important operator here is this is just to mention a few of the things. Again, I don't want to take up too much time.

But during the pandemic, it became clear that FDA could play a major role here in helping to move things forward in part because our knowledge at CBER regarding vaccine development stems from everything from selecting strains -- that's what we do for Influenza.

We help to select strains, up through looking at manufacturing technologies with what some of the things we do in our labs, up through helping to make sure these vaccines are studied to be safe and effective.

And then, making sure the lots are released, are safe and meet criteria. And then,

we follow post-market surveillance. So we have kind of stem to stern, soup to nuts, whatever analogy you'd like, the whole gamut of vaccine development.

So it really put is in a unique place when the pandemic came along to think about what we could do to accelerate the pace of vaccine development. Why does it need to be accelerated?

So at the beginning of the pandemic, various manufacturers, it was actually two or three of them, came in with timelines for a vaccine that took about two years or more.

So that two, two and a half year timeline was very jarring because that could be juxtaposed next to data from the Centers for Disease Control and Prevention which showed that during the extra year wait from -- if you can do it in one year, you would lose X number of patients or people. And if it took two years, you were going to lose Y number.

That Y number was somewhere around the order between and 3 million larger. So that's a

large number of lives to have at risk that you could potentially do something about. So we looked back at, you know, what was done for traditional vaccine development. Traditionally, vaccines are a very low margin product. People try to derisk the development incredibly, and so it proceeds just like, you know, from one stage to another.

Phase one studies get completed. You'll look at them, and then you'll start phase two studies if that looks good. And then, if phase two looks good, you do phase three studies. And for a number of vaccines, there's not commercial scale up till after the phase three data come in. And so, this can actually really delay availability.

So the piece -- the insight during operation warp speed, one of the insights was, could you essentially do things in parallel rather than in series? And that included everything from doing phase one, two, three trials -- and by the way, the Pfizer trials was the one, two, three

trial.

The large vaccine trials that were done were phase one, two, three studies, you might say, what does that -- how does that help? Well every time you start the study, you have to go through contracting and IRBs. So by eliminating that, that shrunk time.

Additionally, it was decided that once the vaccines were shown to be immunogenic, even if they had not yet to be shown effective, we would just start scaling up production, and that was done.

Now that could have been a wasted couple billion dollars, but considering that the one-month shutdown starting in March of 2020 cost over a trillion dollars, a couple billion dollars? What the heck? Boy I'm sure I'll get flack for that from someone in Congress, but it was a reasonable risk to take.

And then, there were other things we realized we could do. We issued very clear guidance, and we also threw -- and I think this

was an incredible thing that the staff in our center did, is they made themselves in the Office of Vaccines, and the Office of Biologics Quality Biostatistics Epidemiology and even the people in our Office of Communications, made themselves available, essentially almost 24/7 so that we progressed through and answered questions in real time, rather than having the usual formal meetings. And that, I think, make a big difference.

And this is the mRNA COVID-19 vaccine timeline. People are aware that on January 11, 2020, the first sequence data were obtained. By March, they were first in human studies with two manufacturers for the mRNA vaccine. July, the randomized trials were initiated. And by October, we had the first randomized trial date available.

By November, we were reviewing. And in December 2020, by the 11th and 19th of December, we had issued two different emergency authorizations for these vaccines, and people were getting vaccinated days later after the emergency

authorization. And so, really a tour de force of what CBER was capable of doing working together.

In terms of other areas, people may be aware of the HIV epidemic, which dramatically affected our products. In the late '70s, early '80s, CBER regulated clotting factor concentrates for hemophiliacs. Many of those at that time, in fact, were made from pools of human plasma.

And unfortunately, at the time when HIV came along, most of those pools became contaminated with HIV. We didn't know it at the time that it was HIV. But in retrospect, that led to about 95 percent of hemophiliacs who were treated with factor concentrate, those who had severe hemophilia in 1980 to contracting HIV, and many of them ultimately died of AIDS.

But in order to deal with this, deferral criteria were put in place in the mid-'80s that were relatively strict. They basically tried to prevent people from, who had risk factors from donating, even though we started to have tests which could pick up the virus. And that was

because the tests were not perfect.

And so, this overlapping set of protections was what was necessary to keep the blood supply safe because this was -- as people who are old enough to remember, it was considered really a tragic failure of safety of the blood supply.

So these measures were put in place. The issue was that the test got better and better and moved towards nucleic acid testing, we did not necessarily keep pace with updating our deferral criteria.

Eventually by 2010/2015 period, it became clear that other countries were starting to move away from indefinite deferrals of men who had sex with men, and were starting to think about shorter deferrals like a year or so.

And ultimately, FDA moved to, based on the science available, moved to a time-based deferral. Ultimately that was not felt by the community to be an acceptable and fair way of doing deferrals because there was the questions

of, well people who had one partner were at risk of having a new infection, it was low if they donated.

And so, ultimately we looked at the date, and had modernized to an individual risk assessment for blood donors based on everyone's risk for the potential HIV transmission. So again, we use science to kind of modulate our regulatory approach.

And then, finally I'm just going to move onto where we are in cell tissue and gene therapies. There's a lot we can say in the cell therapy area, but I'm going to talk about gene therapies in part because I think as we look forward, one of the most exciting areas that we are regulating here -- and some of these are cell-based gene therapies too.

So we currently have 20 approved gene therapies in the United States. If you count -- just so you know, you can count these differently and say they're either 19 or 21. So I'm going to argue with you, but we use the average of 20. And

if someone wants to see me later, I can tell them why.

But the deep blue ones on the slide are the modified T Cell therapies, which include six chimeric antigen receptor T cell therapies, and Tecelra which is for synovial sarcoma. And so, finally, a non-hematologic based T cell product.

The ones in green are genetically modified stem cell therapies, notably for sickle cell disease and beta thalassemia, including Casgevy which is the first CRISPR/Cas9 genome edited product that has been approved.

Relevance since -- at 3:30, we'll be hearing Jennifer Doudna on that. And she can -- you can ever ask her when she first reported that and realized there's not a lot of years between when she described it, you know, a decade plus a little bit, between when it was -- that system was first well described, and when -- for genome editing and when Casgevy was approved.

So one of the issues here is that this is a field that has been very exciting, has been

slower than we might like to grow an application in part because many of the challenges that exist.

These are generally small populations that are being affected for many of these gene therapies, at least for this first wave of gene therapies. And by definition, we have to deal with small populations because Adeno-associated viral vectors cannot be made at scale that could treat tens of thousands of people. That's not currently possible.

So we're dealing with, by necessity, treating small numbers of individuals. But that unfortunately brings a problem with commercial viability. So we're taking action at the center to try to deal with that.

And our office of therapeutic products has been working on advancing the manufacturing for gene therapies, applying the platform technology provisions that were passed by Congress, looking to define the use of accelerator approval more clearly for gene therapy, looking to see if we can work with our European colleagues,

and our other global regulatory colleagues, to accelerate convergence of gene therapy regulations and review.

And then, trying to use some of what we've used from the pandemic in terms of communication tools to see if that could help rare disease therapy. When you think about it, CRISPR is an incredible poster child of platform therapies.

When I think about this, this is to me, you know as peering over for science that we're doing at the center, a very exciting place to be. Because when you look at the prime editor taking a reverse transcriptase and linking it to CRISPR, you suddenly have a molecule that has incredible power in terms of editing DNA. Almost like word processing, with limited length across the genome.

And because it can potentially put in sites to do directed insertion of larger stretches of DNA, again potentially very important tool. So this is obviously something that we look over towards the future I think will be very exciting

for us, and it will keep us occupied and in business regulating things, as new technologies come up that will be passed prime editing.

So I think just to summarize, we have a rich history deep in regulatory work based on scientific foundations. This combination of doing science while we're doing regulation is really well suited to the brisk pace of scientific progress that's going on in this field.

As somebody who, when I worked in industry worked on small molecules, it's true there are developments in small molecule synthesis, how to make sure a large Grignard reaction doesn't really blow up too badly when you're adding a catalyst, MEL catalyst.

But all kidding aside, it's not the same as the pace of change we're seeing in this area of biologics. And I think we can see that in all the areas we regulate, not just vaccines and blood products, but also cell tissue and gene therapy.

So thanks so much. I really welcome you today, and look forward to a very exciting three

days ahead. Thank you. And what? Let's see, we can try to do a couple of things here, which is first -- let's see here.

DR. ELKINS: Questions in the room are available by way of the two microphones.

DR. MARKS: I think we have, like, a couple of minutes of questions. And maybe at the same time we can --

DR. ELKINS: And then, we have someone monitoring the chat on the Q&A section online for questions online.

DR. MARKS: One last thing just so that I just show this while we're getting questions, and really just so the people realize here. Our staff is our most important asset. This is back in the day when we had people in the building in large numbers, which we occasionally do still. This is one of our group gatherings. Okay.

DR. ELKINS: Yes. You may need to turn on that mic. There's a little slide bar on the top, right above -- yes there you go. You got it. Thank you.

SPEAKER 1: Hello? So, thank you very much, Dr. Marks. It's given various insight into this, and so is very helpful and supportive. So I have two questions for the vaccine.

So with Mpox vaccine for in the future, would you still be able to set forth viable accelerator process phase 1, phase 2, phase 3 altogether?

DR. MARKS: So I think the question was about an Mpox vaccine.

SPEAKER 1: Yes.

DR. MARKS: Whether you could move ahead a vaccine. I think that would be one that you'd have to come in and have a conversation for. But I think there's no reason, in principle, why one couldn't design that type of a protocol in advance with the office's input.

With pre one, you would want to make sure that you had input with the office just to make sure they agree with all of the statistical considerations before you start out.

SPEAKER 1: Thank you. And my second

question was in gene therapy. So we see that there are a lot of moving supports from everyone, and we would need a lot of approval nowadays. And my question is that -- because I see that you have put a lot of effort on the pilot program global regulatory as well.

So for the (INAUDIBLE) either one is (INAUDIBLE) for FDA, and EMA, and Canada, and UK somewhere, would you be able to be responsible to coordinate with the FDA directly to put a global inspection, particular inspection, instead of working with an agency?

DR. MARKS: Right. So the question has been, I think, could we do -- are you saying reliance on individual and other country's inspectors rather than use our --

SPEAKER 1: Yes.

DR. MARKS: So in this area of biologics, we are working towards getting towards mutual reliance. But it is a slow process because we want to make sure that our inspectors, and the inspectors in whatever country are looking at

things in a similar manner.

So we'll hopefully get somewhere. Right now, we're not quite there yet in vaccines. We're going to get there hopefully in not too distant future in cell and gene therapy.

SPEAKER 1: Thank you.

DR. MARKS: You're welcome. Other questions? Okay.

DR. ELKINS: I'm told we don't have anything online.

DR. MARKS: All right.

DR. ELKINS: So thank you again, Dr. Marks.

DR. MARKS: Okay, well thank you. And I will take this opportunity -- well are you going to introduce Dr. Kohn?

DR. ELKINS: (No verbal response.)

DR. MARKS: Excellent, okay.

DR. MAZOR: Okay. Good morning everyone. My name is Ronit Mazor. I'm a researcher in the Office of Gene Therapy, and on behalf of my co-chair, Dr. Alexander Zhovmer, and

myself, I'm happy to welcome you to the cell tissue and gene therapy session.

I'll start by introducing our keynote and first speaker for the day, Dr. Donald Kohn. He is a distinguished professor at the University of California in LA in the department of Microbiology, Immunology, and Molecular Genetics and Pediatrics. He performs labs and clinical studies on gene therapy for blood diseases, especially in primarily immune deficiencies and hemoglobinopathies.

His research is focused on developing and improved methods for adding and editing genes for human hematopoietic stem cells, and validating these approaches in early phase clinical trials. And after that, we'll follow up with Q&A. Thank you and welcome.

DR. KOHN: Thank you very much. It's a real honor to be asked to speak to all of you. I really appreciate the work you're doing keeping us all safe here in the timeline of a vaccine development. Again it's just amazing that within

a year of a new disease described, that we were getting vaccinated and partying six months later. So it's really fantastic.

So I decided today that I'm going to talk about just a single topic. We do work on several diseases and several modalities. But sort of my career I just realized sitting here, next year will be 40 years I've been working on ADA-SCID. Still haven't gotten quite where we'd like to be, but I'll talk about our work over the last three, four decades of my lab work on this.

This is my conflict of interest. I'm going to talk about a lentiviral vector that we developed, and I'm an inventor. It's not my retirement plan. I would be happy if it ever got commercialized. And we've spun off a little company, Rarity, which I have equity in that will try and license it when I do consulting.

So as I said, I've been in this field a very long time, just quoting Paul McCartney, and some of you who are older can remember that version of him. So the hypothesis for the work we

do of using gene therapy with hematopoietic stem cells is that gene therapy is autologous stem cells that are corrected with the normal gene, who have sustained beneficial effects from blood cell production or function without being in complications of allogeneic stem cell transplants.

And I'm a pediatric bone marrow transplanter, and besides recurrence of primary disease, graft-versus-host disease is a major problem that we deal with. It's still quite vexing.

And so, if you do autologous transplants, that should completely eliminate GVHD risk and also significantly the need for pretransplant immunosuppression. So a lot of our conditioning drugs, Cyclophosphamide through therapy, ATG steroids, are immunosuppressive. And same thing, post-transplant with Cyclosporine, Tacrolimus steroids, et cetera. All those are not needed in auto gene therapy.

But as we've learned, we still need to make some space in the marrow to get engraftments.

And so, the concept is quite simple. The long term hematopoietic cells need to make the gene normal in those. They will then develop into a lifelong source of genetically corrected blood cells at whatever lineages are needed.

And then, just many of you, those of you who aren't in the blood cell area, C34 is a marker that sort of marks about one percent of cells in marrow. It contains the stem cells that are mainly -- actually progenitors we often call the HSPC population.

And so, the two approaches are adding the gene or editing the gene. And this is sort of the cartoon of it. So we collect and isolate stem cells from the patient in the laboratory, so ex-vivo. We either add a gene with the viral vector that we'll integrate into the chromosome, therefore be passed along to all the progeny cells.

Or if we edit the chromosomes with CRISPR and other methods now, that will also be a permanent change of the stem cells and the

billions of blood cells it makes. And so, that's -- the ex-vivo patient then receives conditioning to make space so the cells are infused back into the patient.

And so, part of the field development was learning what's the best way to handle these cells ex-vivo to keep them happy, to get them activated but not differentiating. And so, over the years different cytokines as they were identified after early stem cells were developed, it's sort of a combination of FLT3 Ligand, SCF or C-Kit Ligand and Thrombopoietin, kind of has been around now for about two decades. There are variations on it, but that still seems to be the best combination of what we know currently.

The sort of -- the conditions that we have the cells under, so we usually just have them in bare plastic because if you have a stromal marrow layer, or another type of stem cell, that makes them happier.

Recombinant fibronectin was identified as sort of serving that same function. And now,

more recently there's some transduction enhancers that actually significantly increase gene transfer.

We started off with fetal calf serum. Everyone's moved to doing things serum-free. And then, the viral vectors that have been used has sort of changed over time. We started out with mirroring gammaretroviral vectors in amphitropic envelopes.

They would get into human cells. Given a leukemia virus, the enveloped seemed to be a little more efficient. And then, about the early 2000s, most people switched to using lentiviral vectors which are more efficient.

And one of the big things about lentiviral vectors is you can transduce the cells just in overnight cultures. So we put them into culture overnight to activate them, partly turning on the LDL receptor which is the receptor for VSV-G envelope, add the virus the second day, and freeze the cells.

So they're only in culture two days

because we still can't really, to a large extent, expand hematopoietic stem cells. Every day they're in culture, we tend to lose them. So the shorter culture period with lentiviral vectors over retros, they require the cells to undergo mitosis. It seems to give a better cell product.

And of course, now we're in the age of editing, as Dr. Marks referred to. And so, we're still using the same conditioning, and we're looking at types of modulated that will improve the type of edit we want, homology-directed repair for example.

And so, the procedure is relatively straightforward and, you know, when we do it in our academic lab or three or four post docs doing it, obviously accompanies the burden of quality and documentation is much higher.

So we collect the cells from the patient at a clinical site. They come to a GMP lab. We isolate the C24 fraction to enrich our targets. And then, they just culture typically for a day of pre-simulation, a day of transduction, and then

typically the cells are then cryopreserved. All the release testing is done when the drug product meets release criteria. The patient then comes in for conditioning and cell infusion.

So the disease I'm going to talk about is Severe Combined Immune Deficiency. This is just a little background. I think everyone here knows this. So bone marrow makes -- is where the hematopoietic stem cells are, makes all the blood cells except the one special one that has to go to college first, and that's the T cell.

So it goes from the marrow into the thymus, the university of T cells, where it takes of course V(D)J 101, rearranges the T cell receptors, and then comes out as mature T cells that are hopefully not autoreactive, but are reactive as sworn antigens.

And in SCID, that doesn't occur. So for a variety of genetic defects, T cells don't develop. Neither B cells that don't develop or don't get the T cell help they need to function.

And so, SCID is the most severe primary

human immune deficiency where there's absentee in B cell function, and K function is present or not at depending on the genetic cause of SCID.

And this disease was uniformly failing infants throughout mankind's history before treatments were developed from severe progressive infections acquired, you know, shortly after birth after they start getting out in contact with the world, and also, we typically -- failure to try chronic diarrhea.

And so, we now know that SCID can result from at least 20 different genes can get some of the same clinical phenotype, and it can be broken down into several categories. So one way to look at it is those involving T cell signaling, most common is the IL-2 receptor gamma, the common cytokine chain, which is about a third of SCID patients, JAK-3 which is downstream, IL-7 receptors, et cetera.

Then there's combinations needed for V(D)J recombination. So lymphocyte-specific RAG-1 and RAG-2, but also other genes that are

involved in any EJ non-homologous end joining is part of V(D)J recombination.

If you're missing those, you have both SCID and also severe radiation sensitivity. So conditioning them for the transplant needs to be done much milder. And then, there's one that I'll talk about, a purine metabolic disorder, which wouldn't have been thought of as a cause of SCID ADA deficiency.

And we know that an allogenic transplant would be the cure to this as the first successful human allotransplant was done in 1968 for a young boy with -- or baby boy with SCID.

He got stem cells from his sister. This was before there was even age-linked typing. But they had done a mixed leukocyte culture. They didn't react, and he's alive and an adult now with his sister's immune system still working for him. And then, Robbie Park, when my mentor did the first transplant for ADA SCID.

And in general, there's a very high success rate for SCID patients if they have a

matched sibling donor, limited only if they acquired a severe infection before the transplant.

The problem has been most patients don't have a matched sibling donor. So we either use T cells that we have though typically from a parent, or a matched unrelated donor. In the past, those success rates have been lower.

And so, this is just a pie graph looking from a recent study from the primary immune deficiency treatment consortium looking at 250 recent patients with SCID, what their genotypes were.

So this again shows about 30 percent with IL-2 RG, percent with ADA, and 20 percent with RAG-1 and RAG-2, and still a small fraction with unknown genotypes. There's still some new causes that need to be discovered.

And so, ADA SCID, and I'll talk about it the cause of about 12, 15 percent of human SCID from among those 20 genes. And that means there's about 8 to 12 patients born a year in the U.S. and Canada. So this is not the public health risk of

our time, but it's still, you know, an important disease that we can do something about.

And so, it was the first genetic form of human SCID with a biochemical and genetic bases were determined. In ADA SCID patients they had profound lymphopenias T-B-NK+SCID typically from lymphotoxin as needed tablets. I'll show a figure of that.

And so, there are therapeutic options. There's allotransplant like I said with sibling, matched unrelated donor, or Haplo. There is an enzyme replacement therapy. So in 1990, an orphan drug polyethylene glycol conjugated both on ADA was approved.

And kids can get injections of that once or twice a week, and that will lower the pools of the toxin metabolite enough for it to be partially immune reconstitution.

It's very expensive, about $300,000 a year or more. As they get older, it only leads to partial immunity, but can be definitely lifesaving as a bridged transplant. And there's auto

stem-cell transplant with gene therapy.

So this is -- I'm sure you all remember this from your biochemistry class. So the enzyme deoxyadenosine that deaminates to deoxyadenosine which can either be broken down to uric acid or salvaged.

And in 1972, when Eloise Giblett at the Puget Sound Blood Center was looking for a donor for a patient with SCID, she looked at number of different proteins that came in different isozyme forms to see which siblings shared the most with the patient, and discovered the patient lacked ADA, and the parent had half normal levels. And she made the deduction and absence of ADA could cause SKID.

And so, we now know the reason is in lymphocytes, there's very high levels of the kinases that will phosphorylate and trap it. And dATP is exhibited for arrived nucleoside reductase, and it acts as a lymphotoxin.

And so, in the earlier days this is paper from 2012 looking at an outcome of 106

patients with ADA SCID at five centers who had allotransplants. So you can see again, those with matched sibling or matched family donors had fairly good outcomes, 80, 85 percent, which those with the low matched donors, their survivals were much worse because of the immune differences were more likely to have graft-versus-host disease.

Fortunately, this is improving. So this is a paper again from the Primary Immune Deficiency Treatment Consortium looking at the outcomes for ADA SCID patients. And again, looking to an earlier era, the outcomes with transplants getting the enzymes or not were about 80 percent.

In the more recent era, it was not significantly worse that gene therapy where gene therapy looks a little better. And so, we're definitely getting better with our allotransplant outcomes, and that's obviously good news.

So this is my lifetime history of treating ADA SCID patients. I'll briefly walk you through this. So back in 1993, we had version 1.0

of our gene transfer methods as I showed you in the previous slide.

So I offer out the C-Kit Ligand in bare plastic plates. We treated three newborns who had been diagnosed in utero with ADA SCID, collecting their cord blood as a source of CD34 cells. And we transplanted them without any conditioning. We got really minimal in grafting. There were a little bit of T cell productions, but very little in graft in those conditions.

So we spent about a decade in my lab to try and make the vectors better, our crossings better. Those cytokines I showed you were being discovered and produced in recombinant form.

And so, we opened up a new IND in 2000/2001 using sort of these second generation conditions with C-Kit Ligand, FLT3 Ligand, TPO serum-free medium combinant fibronectin, and a lentiviral -- a retroviral vector that we made in the lab using -- promoted seemed to stay on better in mouse stem cells than below the vectors that had been used before.

So we treated four patients with that in 2001/2002 in a phase one trial. Then we were on hold for three years when patients in -- studies in Europe, for X-SCID developed leukemia. The FDA put all the trials on hold while it was trying to figure out what was the risk factor.

During that time in fact, there was a very important paper published from the TIGET group in Milan describing treating two ADA SCID patients, same kind of approach, except they gave them reduced intensity conditioning Busulfan.

So low dose conditioning, kind of the equivalent of 200 centigrade if you were transplanting in a mouse as opposed to a 1,000 being full conditioning. And they weren't -- these were children that couldn't get enzyme therapy, which was always a concern that lumping would select the effect. And they both developed immune reconstitution.

So after we got off hold, we had modified our protocol to start giving Busulfan to future patients. And I actually moved across

town, and then from CH Lady UCLA, we opened up a phase two trial and we treated ten patients under that. And it was supported actually by hour one from Orphan Products Division of the FDA.

But it was clear that lentiviral vectors were emerging as a better virus. They were more efficient into getting in human cells. They seemed to have less genotype toxicity potential.

So with colleagues in London, we developed a lentiviral vector, EFS-ADA. We treated a total of 33 patients between 2013 and 2018. Then we licensed to a company. I'll tell you all about that story. It came back to us just two years ago. We've now treated -- we'll treat our seventh patient from this new go-around this Friday actually.

So I'll tell you a little about these more recent trials, just a little bit about the retro to make a couple points, and then a little more about the lenti. So first of all, conditioning. So this is the Los Angeles college sports version of it.

So if your bone marrow is full of these USC stem cells, footballs, and you want to transplant them, if you don't give any conditioning, so no conditioning, there's very little space and you get very little engraftment of the donor cells, even in an antilogous setting. Certainly the allogeneic is a big risk of rejection.

If you give full conditioning and really empty the marrow space you're giving the cell, you'll get large donor engraftment. But it turns out, especially with auto again where there aren't the alloreactivities, reducing density conditioning can lead to engraftment of some stem cells. So that's what's being used now for gene therapy in many conditions.

So this is the retroviral vector that we developed in my lab in the mid-90's that we did in trials through the 2000's. So a typical gammaretroviral vector with a long term on repeat with a very strong promoter drives expression of the trans gene, and we used our favorite

conditions.

And this is just a graph I made a number of years ago of one of the patients, one of the best out of that series of patients. So up in the top left. So she was number 8 in the series.

She was born in 2011. She was three months old at the time of treatment. She got a good cell dose of 6 million per kilo. The vector copy number of the product, 2.7, was good. And at this point, we're giving a single dose of Busulfan, so the levels varied and we're currently shooting of 4,900. So she had a very good level of Busulfan.

So everything worked right, and this was the outcome. So you can see that over time after the transplant, the lymphocyte count, the T cells, the 4s, the 8s, the B cells and Ks all came up.

And in the lower corner, the 24 months, she had 2 to naïve to memory cells. She was making new T cells. And on the right, the vector copy number of her blood cells, granulocytes were about 1.4.

We know the few times we've done marrow, the vector copy and the granulocytes reflects very much the stem cell level because there isn't really any advances to granulocytes having ADA or not, whereas the PBMC had gone up because that's where the T cells, B cells, and K cells that need ADA. So pretty much you select for those that have the gene. I'll show you a little bit more of that.

So this was actually all the patients from that series of ten patients. So on the upper left is their vector copy number, and their granulocytes. Again sort of their marked stem cell or their gene chimerism level.

And you see there's a wide range of engraftment. The worst one, 401, was our oldest patient, 50 years old, who had a very low cell dose. And in fact, we put him back on enzymes after six months because he met our failure criteria.

But on the top right are the level of marked PBMCs. So you can see that despite the

level engraftment, they almost all went up to that higher level of almost one copy per cell because that's the selective advantage. The one that didn't, in blue, is the one who was on enzyme therapy that blunted that selective advantage.

And the lower graphs are just the same data instead of sort of graphed on the lines. So you can see the levels of the patients. The bars represent their medians, and I'll use those as a variable in the next figure. And so, again the granulocyte vector copy number is a circuit for engraftment of gene corrected stem cells.

And so, if we use that along the x-axis, what's the engraftment level? And this is averaging all the points for each patient after the two years of the initial study. You can see that the level of engraftment varied as we saw before, but everything correlated with that.

So ADA activity went up the more the engraftment there was. The level of the bad nucleotides lowered, the higher the copy number was. Lymphocytes, CD3s, CD19, IGM, all went up

with better engraftment.

And these four patients in the boxes are the four that had sufficient B cell function to get off immunoglobular placement. So they kind of suggested with this vector at least if you have a copy number of about 05, which is about five percent of corrected stem cells, that's enough to restore B cell function.

But again, that was with sort of the first generation kind of vector shown at the top where the long term and repeat enhancer promoters are what drives strong transient expression, and also when you're making the vector tight. The tighter the vector, those enhancers can turn on cellular genes near where it lands.

And because they land relatively randomly throughout the genome, those that happen to land next to an Onko gene can turn on that gene and lead to clonal outgrowth, and that's what happened in the X-SCID patients. It later happened in CGD, and we've got all these inpatients treated, fortunately all in Europe.

And so, the whole field really moved the second generation of vectors shown at the lower part where the enhancers are deleted, and you actually delete it in the plasma. And when it's reverse transcribed, the deletes just get copied over, so it's called self-inactivating, or SIN vectors.

So you use an internal promoter to drive expression of the trans gene. If you use a promoter, that's reasonably strong to make a good amount of product but doesn't have enhancer activity. So, PGK, elongation factor alpha, are ones that meet the criteria, or it can be a lineage specific in order to direct expression to a specific blood cell type for example.

So this is a vector now that we've been using for the last decade or so. So it's the lentiviral vector with the SIN configuration. And it has the elongation factor short promotor driving expression by code and optimizing the cDNA, and adding this viral element, WPRE, that stabilizes the message, which you get nine times

more ADA per copy than without that. So single copy per cell is enough to really correct the metabolic function. And then, there's the other specifications of the vector there.

And so, this was made jointly. And in fact, the vector was made in a lab of Adrian Thrasher and Bobby Gaspar at the University of College London. We both made a number of vectors, and this was our favorite.

And so, we jointly did the pre-clinical work. We looked at the efficacy, and we found that it had expressed more ADA per copy than the retroviral vector we used before. And then, we did extensive safety studies.

So in ADA deficient mice, we didn't see leukemia or clonal expansion, nor in human CD34 seen in immunodeficient mice with either vector. So those models were not very informative in a sense, or the risk was low.

When we looked at integration site patterns, the gammaretroviral vector as commonly seen, was more often near transcriptional start

sites in cancer-related genes than the lenti.

And in an invitro mirroring lineage negative bone marrow model, the gammaretroviral vector showed -- caused clonal expansion, whereas the lentiviral vector did not. And these are some of the data from that study.

So the mock transduce cells form no colonies. With a very strong retroviral vector RSF-91, many colonies were formed. The retroviral vector that I showed you we used in that phase two trial scored in that, and the lentiviral never formed any colonies. So based on this myeloid skewed proliferation asset, it made the lentiviral vector look safer.

And so, we went out to do a series of trials in parallel in the U.S., and then in London at the University College of London Great Ormand Street Hospital. So the MHRA approved it in 2012, FDA in 2013.

We've used vector -- made it at the Indiana Vector Production Facility, an academic facility. The same labs were divided between

London and Los Angeles. We used the same vector lots.

And so they, up to about 2018, treated 20 patients in their trials, 10 on trial, 10 by sort of hospital exception. We treated 21 patients initially using fresh cells. And then, we did a trial where we cryopreserved the cells and treated 12 more patients with that.

And so, this is sort of the scheme up for the patients. And so, with the fresh trial, you know, we would consent the patients typically remotely, and a screening test done to make sure they were eligible.

And when they were good to go, they would come to UCLA. We would then get them on Monday. Tuesday, take them to the OR and do a bone marrow harvest, put in a pick line. If by Tuesday night we knew we had enough stem cells to get a single dose of Busulfan, and then Thursday the cells would be washed and brought to the hospital and infused, and if everything went well at Day 30, we'd stop their enzyme therapy and then

follow their immune reconstitution. And we treated 21 patients under that approach. And then, we moved to the frozen -- or the cryopreserved trial, where we dissociated. So we made the cells, and then froze them. They did full GMP QA release before the patient came back for the second admission.

And now, we've had time to split, and PK adjust the Busulfan. The Busulfan is a drug that is a very large, person to person variation, in pharmacal kinetics. And so, we give three quarters of the intended dose on Monday. Measure that patient's clearance and adjust the dose on Wednesday.

And we were giving two to three full variations, and, you know, someone would get a half per kilo, some others would get three per kilo on that second dose. And so, we'd get much more precise targets. I'll show you that. And Friday, the cells are brought to the bedside, thawed, and infused immediately.

And so, this is just a map of where the

patients -- we got very good Canadian geography. About a third of our patients came from Canada, and we arranged for them to come to UCLA, stay overnight, get their transplant, et cetera.

And so, these are data from that -- those trials comparing the fresh and the cryo. So the ages were not different. The CD34 doses, the vector copy number of ADA activity, and the ADA per vector copy, and sort of the potency measure of the vector were not different. We're trying to show comparability.

But one thing that was different was the Busulfan level that I mentioned. So six single six dose, with a wide range of areas under the curve by PK adjusting with a much smaller coefficient variation of the dosing.

And so, these are some of the outcome data. This is a comparison of the fresh to the cryo, and all -- and you can see, the lines are all superimposed. And so, the outcomes -- so this validated that the cryo preserved formulation worked as well as the fresh cells had.

And then, this is just one more graph on this. These are, again, the granulocyte vector copy numbers. So I show you in the gammaretroviral vector, the wide range of engraftment levels we got. Now you can see with the lentiviral vector being much more consistent levels with gene marked stem cell and engraftment.

And so, we published results of the two-year U.S., and the three-year U.K. trials a few years ago. And we reported 50 patients treated with this approach in parallel trials, and with the approach I talked about.

And what we reported was 100 percent overall survival, 96 percent event-free survival. So 48 out of 50 had engraftment and sustained a mirrored constitution. Two of the patients did not work.

One of our patients just did not engraft, and we couldn't figure out a cause except she took two harvests to get enough cells. We did trio sequencing, and couldn't figure out a genetic basis for it. And the one in London was very sick

at his harvest time, so he had a low cell dose.

And so, this is -- I like showing this. This was in an oncology meeting. This is the Kaplan-Meier curve for overall survival. This is the event-free survival, so it shows the event -- one event in each trial. And this is just one graph in the figure showing T cell reconstitution.

So the patients came in on ADA enzyme, and they only had T cell counts of about 2 to 300. Those actually dropped a little bit after we stopped the enzyme, but they came up over time, over -- it took about two years for them to reach their maximum T cell counts.

And we did extensive vector integration analysis to make sure there weren't any clonal expansions. This is just one figure chosen at random. So the figure on the left represents the top ten most frequent integrands, or the colored bars. So they're all at, like, one percent or less than the gray area, the other ones.

In this patient, there were 8,624 other sites maps. So highly clonal engraftment with no

clonal dominance. And we saw this in all the patients at all the tide points we looked at.

And in fact, sort of -- part way through this study, we internally did vector site analysis. So on the bottom is from the gamma retroviral vector, and you recognize the names of many of the genes there. MECOM and LOM-2 are two of the genes that have been involved in clonal proliferation of other studies. And there's other stem cell active genes in that list.

Whereas, the lenti is really a very benign pattern. And the genes that are prominent, we think are just ones that happen to be near the nuclear core, you know, just by chance more likely to get hit, but really no oncogenes are common integration sites.

And so, right now we're doing the long term follow up on this cohort of patients. And so, the subjects are now 6 to 11 years out from gene therapy. And the immunity has been sustained, and there have been no product-related adverse events.

There have been no subsequent events, so all remain well without needing to go back on enzymes or have an allotransplant, other than the two early events. And the vector integration study analysis, we looked at our most recent PBM samples recently with this paper that we're writing, and we didn't see any clonal expansions, now out to six to eleven years.

And then, we've continued the Kaplan-Meier curves. And again, there's no events and all are still alive. And the vector copy numbers, and the granulocytes, and the PBMCs are stable. So it really looks at least for a decade now, we can see the graph it looks quite stable. We hope this will be, you know, lifelong.

So that's the good news. But there are challenges taking things that worked so well from an academic lab to commercialization. So along the way, we applied for Orphan Drug Disease designation breakthrough therapy rare pediatric disease designation, all of which we received.

And so, in fact in 2016, we licensed it

from our two universities, University College of London and UCLA, to Orchard Therapeutics. We transferred the IND to them. They became the sponsor.

And after working on it for about four years trying to develop the CMC to a level required for BLA, they decided to give up on the project and return the license to the universities in 2021.

So no patient received this treatment after 2018. And during that time, we accumulated a waiting list of 30 kids who were are on enzymes sort of being temporized. We didn't have matched sibling donors.

So in 2022, the IND and the remaining serum, California to do for general medicine funding, came back to us with a Type C meeting with FDA in April to propose a new clinical trial. And that opened up in early 2023 to treat patients. And we made a couple of changes, the changes which aren't always good, but we think this was a good one.

So we switched from using bone marrow to using immobilized peripheral blood. We had gained much more experience with leuko freezing five kilo babies from other trials, and we seemed like we got more cells. And we added a transduction enhancer to the small molecule Poloxamer, which was marketed as lenti boost, which really increases gene transfer.

And so, this is just a cartoon of this. This whole series of molecules that have -- that are sort of the antipathic, and there's this whole family of them based on the length of the sides. And the one in the upper left corner, F-108, is the one that's used for this process.

And so since we reopened we've now treated, as I've said, our seventh patient who's being treated. So this shows, yellow is when we manufactured the product, and green is when we treated the patients. And we have one more to do after that, and then the CIRM grant will be depleted.

And so, just a little bit of data from

that trial. So on the left is showing the cell doses the patients got. The 12 who got the cryopreserved bone marrow in 2016 to '18. And now the eight we've made products for using immobilized peripheral blood.

So you can see, we're getting twice as many cells per kilo per patient with immobilized peripheral blood that's probably easier to undergo leuko freeze within a bone marrow harvest. And then, on the right is just showing the T cell counts, and those that we have data on, and so they're all reconstituting as we saw before.

And so, you know, I started out thinking I was a scientist and learned some along the way. I'm a drug developer. And so, we're trying to move this to BLA to make this widely available.

And so, another rock. While I may not have the answer, but I believe I've have a plan, I'm not sure how good the plan is, but this is what it is. So our plan was to open up the trial in January of 2023, which we did, treat three patients. We actually treated eight.

We've engaged with a CDMO to plan a pathway to commercial manufacturing. We had a meeting with FDA in November of 2023 to review -- well we requested a November -- we had the meeting in January of this year to review our plans and get input on it.

We then submitted a CIRM grant to fund moving forward. The CIRM then put their grants on hold for six months, so the grant was delayed six months. It went in July. We don't know yet if we're funded.

If we're not, that may bring this all to a halt. If we are, the plan is to work with the CDMO to develop commercial manufacturing of the vector of the drug product, the analytics. We've already developed a historic control data set from the PITC patients who've had allotransplants for ADA SCID.

And then, we would treat a small number of patients with commercial product. And with biomarkers, use that data, plus all the historical data, for a BLA submission. And so, we're working

on a path to market authorization.

And one of the challenges is the quotes that we've gotten from three or four different CDMOs of what it would cost to take our academic-based process that my four post-docs do in a UCLA building on a shoestring to establish commercial grade manufacturing for the vector in the drug product of 25 to $40 million dollars.

And so, we hope the CIRM grant will cover about two-thirds of that. And so, we've established a public benefit corporation to license the IP contract with the CDMO, and hopefully develop this into and market the therapy.

And so, I'll just close by pointing out, and this is sort of complement to the slide that Dr. Marks showed, so these are 18 blood cell diseases that have shown good evidence of clinical efficacy in at least in academic trials, and five are these are approved. The ADA is a gammaretroviral vector stream zealous, approved in the EU. The other four are licensed products in

the U.S.

And so, we have many products that we have good clinical evidence for. And how we go from proof of principle in academic trials to a licensed product is a real challenge as I talked about.

And so far the safety record has been quite good for these. The one exception is myelodysplastic syndrome developed late in the trial, while although it's a lentiviral vector, the promoter driving the gene was a gammaretroviral LTR that probably caused genotoxicity. And we're working on alpha fallacy notes which we think will be another very good indication.

So then, just to summarize the novel cell in gene therapy has been developed to treat severe pediatric disorders, such as the genetic diseases, as I've talked about, are cancer and leukemia. Every pediatric cancer is a rare disease.

There therapies have led to major

improvements in patient well-being, notably for otherwise fatal or severe pediatric disorders. These complex cell and gene therapies have been provided safely and effectively at academic medical centers under researched clinical trials.

However only a small number have reached licensure in the U.S., E.U, and U.K, due to more financial considerations than impeccable feasibility, and converting academic-based proof of principal manufacturing approaches to commercial-grade manufacturing is very expensive. And so, we need new models to produce and provide these therapies for rare diseases to make them available to patients who will benefit.

And then, last comment is there's a lot of work now in in vivo delivery where you wouldn't have to take the cells out to condition of the patients, but just deliver the genes to the stem cells and see too.

It's not variant, but when it is that will disrupt all of what I do, and we won't need bone marrow transplant doctors to -- ideally it'll

just be the injection to hit the stem cells.

And so, then I'll just thank my group that does all the work that I've shown you. This is our clinical team. These are our many collaborators, and these are our source of funding. Thank you.

DR. MAZOR: Thank you very much. Now the audience can feel free to stand behind the microphone to ask questions. And while they're coming, I can ask the first question.

So you've shown really an amazing path from the initial design of a molecule to clinical trials and possibly a BLA. Other than the funding that you've mentioned, what would be the other major pain point that you would point out, or the ones that took the longest?

DR. KOHN: Well I think, you know, it's just what each step requires -- so I have a slide that I didn't include that shows, you know, to get to the initial trial, we had three or four grant applications that we went through. And I tracked, and I went through a pre-ID meeting.

So it takes a long time. And again, on an academic, we are running on RO-1 kind of budgets. It is very hard to, A, to do that work, and it's hard to get funded for the things you need to extrapolate to a clinical trial. You know, do three large scale replications that's not a hypothesis. It's going to make a study section very convinced that, you know, they should fund it.

And so, I think -- I'm fortunate to be in California, to have CIRM. But I think that's a unique source. And to get funding to move these, to click, even phase one trials it is very challenging.

MS. LUCAS: Thank you for the excellent talk. Tiffany Lucas from CBER Gene Therapy. I'd like to hear your thoughts on long-term follow up for patients based on your experience.

Obviously we don't have the same data for the lentiviral vectors as we do for the gammaretroviral vectors. So as a clinician, you know, you showed some wonderful data with various

cell populations and BCN tracking over time, insertional site analysis.

But what advice would you like to share with us in terms of long-term follow up for safety and efficacy of these lentiviral vector generated C-34 products?

DR. KOHN: Yes excellent question. You know, it's -- if it was an unfunded mandate. So you can't get a grant to follow long term. We have enough programmatic money that we can do it, you know.

Even again for an academic program, we're now following 60, 70 patients from our different trials on long-term follow up. So a researcher who probably spends about half her time coordinating with the -- you know, so we -- so most of our long-term follow up, it's done at their home doctors.

And so, we send them a form to fill out, so we capture the information we need. We send them Fed-Exed to mailers with the tubes in them, so they can fill them, send them to where they

need to go, to a lab for ADA, out-phased to us to VCN.

So it's a moderate amount of work to do. I think it is worthwhile. I think, you know, we don't, as you said, we don't have a lot of long-term data. That's where we hope a journal will be interested in our long-term follow up paper.

Because, you know, we need to know what the results are, and are our engraftment levels persisting or are they falling off? Are there late effects? And so, I guess finding a way to fund it would be, I think, very helpful to many groups.

But in terms of the amount of information it's raised, it seems reasonable. So basically we will have a clinical note from the home physician who saw the patient in exam. We have a method of what meds there are. I need a count of the meds and adverse events. And so, I think we're able to keep a reasonably good track of them.

The other thing though is as we get further out, more and more patients are being lost to follow up. They just -- they're fine, and they don't want to go see the doctor.

MS. LUCAS: And along the lines of the burden of peripheral blood versus bone marrow sampling, any thoughts there on patient burden, and the initial value of the data?

DR. KOHN: Yes so we almost never do bone marrow samples. You know, they are pediatric patients, so it's -- you have sedation typically. It's a big deal. So I think you can follow everything from blood.

All of our protocols, if there's anything abnormal in the blood at the vector integration site, or vector copy numbers going up or something, that then we would do a bone marrow. But we almost never do bone marrow. So it's all done in peripheral blood.

DR. MAZOR: Okay. Last question.

DR. KOHN: Yes?

SPEAKER 1: Thank you very much. This

is a very inspiring work that you've done. So I have a little bit -- tiny bit of a question for the content of lentiviral vector. I'm asking you about the work with the lentiviral vector.

On a CMV, and on a promoter and EFS, I think in my experience, EFS tend to have more aggregation, and a higher viral vector to either in the production. If it's okay, do you see any aggregation on the quality of lentiviral vector?

DR. KOHN: Well so the vector plasmas that we use for lenti are the original ones from Naldini, et cetera, from the saw. So they have a CMV promoter driving vector transcription during packaging.

But the internal promoter that we use various ones, CMV it turns out is not a very good promoter for hematopoietic cells. So we rarely use that as our internal promoter to drive the trans gene.

But we spent a lot of time studying lentis. We can talk about afterwards. You know, the main issue for lentiviral vectors is after

they get longer, their tightness goes down, and their transduction efficiency goes down.

SPEAKER 1: Thank you, but I just have one comment about that. I think in the future in vivo, I think there's a very good charity and we would have work in country with the CDC we are getting. And I think we can design to be 34 for research, I think it would be part of the cost saving.

DR. KOHN: And I think transportable around the world hopefully.

SPEAKER 1: Thank you.

DR. KOHN: Yes.

SPEAKER: I'm from Pennsylvania Department of Health. Thank you so much for very much working and the information presented. I'm wondering like when you have to do a second therapy or if some patient is a lady with a lentiviral therapy, do you subject any issue with the preexisting immunity?

DR. KOHN: That's a good question. You know, I think there -- first of all, I don't know

how much this has been studied, and what kind of immune response the patients get to the cells. So they're transduced ex-vivo, and washed, and given back.

And so -- and the vectors don't express any viral proteins. So it's just whatever was in the viral particle that might still be associated with the cell to induce an immune response.

But we've never really looked at re-administration, and I don't know if people even measured antibodies to dsVg or HAV-GAD in recipients of gene therapy to know if there's a problem.

But I don't know if any indications if I repealed and looked at regiving them, you know -- looked for -- liked like it's stable so you wouldn't need to retreat for the primary indication.

SPEAKER 1: Yes. My other question is not related to the SCID, but in your slides you showed that it is -- it will lead to department deoxy noting and part of the uric acid. And as we

all know, it can release enough uric acid that leads to the disease gout.

DR. KOHN: Right.

SPEAKER 1: So do you see such symptoms also? And when you replace the adenoids by gene therapy, do you see any, like, indicative of the development of uric acid too?

DR. KOHN: No we haven't. You see, I think, it's sort of putting the pathway back in balance.

SPEAKER 1: Yes.

DR. KOHN: So we haven't seen imbalance and overproduction of uric acid, and none of the patients exalt gout of the follow up time.

SPEAKER 1: Thank you.

DR. KOHN: Okay.

DR. ZHOVMER: Our next two speakers come from FDA, and our first talk will be given by Dr. Ronit Mazor. She's a Principal Investigator in the Office of Gene Therapy and Gene Therapy Products from FDA. She completed her PhD in immunology in University of Tel-Aviv in Israel and

doctorate training in National Health Institute in Bethesda.

Before joining the FDA, she worked as a senior scientist in MedImmune AstraZeneca in their antibody discovery and project engineering. She was focusing on prediction and mitigation of immunogenicity of interpretive proteins. And at the FDA, all her laboratory studies went to interaction between an immune system and viral vectors used for gene therapy.

Our second speaker, and I'm going to list the second speaker at the same time. It's Dr. Zuben Sauna, and he's a Principal Investigator and Director in the Division of Hemostasis and Office of Therapeutic Products at FDA.

He received his PhD from Kumaun University in India and training also in the National Health Institute. Doctorate of studies of pharmacogenetic phases of immune response to proteins used in therapeutic interventions, including new modalities such as gene editing.

He loves to use the combination of

computational in vivo and ex-vivo approaches to investigate why some individuals or some populations have response to therapy, and why others do not.

And after this to talk through at the session, though you can ask your questions. Thank you.

DR. MAZOR: Okay so thank you, Alex, for the introduction. And thank you everyone for coming in person, and for those calling in online. I'm very excited to share the work we've been doing in the past few years in my lab in the Office of Gene Therapy.

So here on the left is our sixth year group, or at least most of the members of our group, and our group really focuses on investigating immunogenicity aspects of gene therapy.

We use methods in silico, in vitro, also ex-vivo where we collect TBMC samples in vivo to look at different models and different AAV serotypes with different trans genes, which are

either models or actual relevant clinical models.

So the definition by the FDA for gene therapy are all products that mediate their effect by transcription or translation of transfer genetic materials, or by specifically authoring the host genetic sequence.

On the left, we have an example of an ex-vivo where we extract stem cells from the progenitor cells, alter them ex-vivo, and then introduce the modified cells back to the patient.

In vivo gene therapy, which is what we focus on, we do direct delivery of patients to the patients using viral vectors. And one of the most common viral vectors that we see in many of the submissions is AAV, adeno-associated virus.

On the right is the development through the years where the first AAV submission was submitted to the FDA in 1995. And since then, it's been a very interesting and bumpy road. We now have six approved AAV products from various serotypes, and hopefully many more to go.

So based on this advanced regulatory

load, you can imagine that the FDA had good interest and better understanding the efficacy and safety of this novel modality.

So a little bit about AAV. Novartis AAV is single strand DNA power virus. It's non-prosomeric in nature, and it requires a helper virus for replication, such as adenovirus or herpes simplex virus.

The capsid is complete with 60-sub units that come together to form the capsid. And the AAV genome shown on the right includes a rip in the cap gene that are captured by ITRs. For gene therapy vectors, the rip in the cap gene are replaced with a gene of choice. So for AAV vectors in gene therapy, they are very popular and sought by investigators because they are relatively simple to manipulate.

They have a pessimal expression in the host cell nucleus which prevents or reduces the risk for insertion with the genesis. And they have the potential to persist in non-dividing cells for a long time, which can allow for

systemic expression.

Due to the viral origin on the AAV, it has a lot of immunological challenges that we encounter. So I broke them here to pre, during, and post-treatment. Before therapy, we see pre-existing antibodies that usually occurs due to natural exposure to a natural virus.

Between 30 to 85 percent of the population has pre-existing antibodies to one serotype or another. And that varies a lot depending on the test you use, or the serotype.

The zero positivity is highly impacted by geographic impacts, also by age and sex. And these antibodies cause neutralization. So essentially at this high titer, they can completely omit the therapeutic effect.

It can also cause accelerated clearance. And in many clinical trials, it results in patient exclusion. In clinical trial where they do not exclude patients that have pre-existing antibodies, that can cause a dose increase which then patients that did not have neutralizing

antibodies can experience toxicities.

Considering during the day of treatment, we have concerns for immediacy immuno-related responses, such as the innate immune activation, risk for infusion-related reactions, and complement activation.

After therapy, we have activation of the adaptive immune system. That includes formation of neutralizing antibodies. 100 percent of systemic delivered AAV will result in formation of high titers of AAV. When we delivery it directly to an immune -- to some immune privileged organs, we may see lower levels of immunogenicity.

We have formation of cytotoxic T cells, and those T cells target either the AAV or the trans gene. And that results in decreased or loss of efficacy, and can also result in liver toxicities. It also has ganglia toxicity and immunogenicity for the trans gene itself.

And the factors that contribute to immunogenicity can be broken through the patient, the product, and the treatment mode. We have

prior exposure to other gene therapy or even a patient that was treated.

The immune state, the patients that are immunocompromised would have a lower probability to have immunogenicity. The genetic background is the patient's HLA, and if they've seen the HEL4 and the trans gene is, the CRE negative status, the sex and the age, and other medications that they're receiving at the same time in therapy.

For product, we have the ethical content. So in the AAV, we look at either B cell or T cell epitopes on the capsid. The aggregates that have a very strong effect on activation of the innate immune system, post translation modifications such as deamidation. We've had some studies that showed that deamidation can increase the immunogenicity in some patients, and decrease it in others.

The CpG content -- because remember this is actually inside. You have a DNA strand, and the content of MC capsid which can increase the antigenic clone. Treatment mode can also have an

effect on immunogenicity, the frequency, the dose, the duration, and the route of administration.

If we can deliver it, the AAV, to -- with catheters directly to the target, sometimes that can prevent the initial neutralization, tropism of the AAV. And if we do combination therapy, that can suppress the immuno response.

So now, I want to share a recent project that we did in the lab, led by a staff fellow, So Jin Bing. She's a staff fellow and a reviewer for CMC for AAV products where we actually designed a next-generation AAV that has its T cell epitope -- one of its major T cell epitopes removed.

So the first step would be to identify where the T cell epitopes are in the AAV? To do that, we developed a simultaneous epitope mapping for both helper and cytotoxic T cells. We simulated PBMCs from 52 donors, with a heated AAV that is empty. It does not have any trans genes, so we can focus on the immuno response to the AAV.

We expand the cells in vitro with cytokines that are meant to help expand both

helper and cytotoxic T cells. So the CD IL-2 is meant for CD IL-4, and the IL-7 and IL-15 are meant for the cytotoxic T cells. And then, we -- after 14 days of expansion, we restimulated the cells with peptide libraries, spanning the entire sequence of AAV.

Now in a perfect world, we would test each one of those peptides separately, but it's extremely laborious. So we pool them into pools of 12. And when we get a positive pool, we then deconvolute it into the individual peptides.

To look at the immune response, we use ELISpot with interfering gamma in IL-2, as well as intercell with cytokine staining, so we can also characterize the T cells that are responding.

This is a representative response. This donor had a very strong response in Pool 9. And Pool 9 is composed of Peptides 97 through 108, and you can see that it was Peptide 103 and 104 that contributed to the response of this donor.

So using this method, we expanded cells from 52 donors, and we got the map that you see on

the left. The epitope in Pool 9 was the top-ranked one. And the deconvolution shown on the right shows that it was really Peptide 103 through 105 that were responsive in all of the donors that responded. And this epitope was present in 23 percent of the donors that we looked at. We further characterized the epitope looking at which T cells they activate. You can see here on the flow that it is, once again on CD4. This is a CD4 helper T cell response.

And we also depleted the CD4 or the CD8. And you can see on the bottom right that the response is abrogated once we deplete the CD4. So this is an MAC2 CD4 epitope.

Further characterization asked which HLA molecule is presenting. So we have three major presentation molecules for Class 2, DR, DP, and DQ. So we use antibody inhibition assays that would interfere with the presentation with either the DR, the DP, and DQ, and we found that this epitope is almost exclusively presented by the DP presentation molecule.

Then we went back and looked at all the positive donors that had a response to this epitope, and looked at their DP alleles, and we found that it was diverse. There was no one allele that responded, which indicates this is a promiscuous epitope.

Identification of a promiscuous epitope is advantageous in the fact. First it means if we eliminate this epitope, we'll be able to sell it to a diverse population, not just people with this specific HLA.

But also in the literature, there's a correlation between the strength and the importance of an epitope, and the promiscuity. The more immuno dominant the epitope is, the more promiscuous it is. So this sits with confirming that this is a strong and important epitope.

So now, there's one slide that I would like you guys to walk home with. It's this slide, which includes the engineering that we've done. So the next step once we've identified the epitope, we wanted to look, is this epitope highly

conserving other AAV?

So there are several, 13 natural AAVs, and many ones that are introduced, that are developed in the lab. And we looked at this region, and looked at homology to the other AAVs on the left.

We found that this epitope is highly conserved across the 13 natural AAVs, which means that if we can solve this epitope for AAV-9, we will probably be able to solve it to a lot of other AAVs.

Interestingly, AAV-5 which we can see because this pointer doesn't work very well for me. But if you look at -- if you focus on AAV-5, AAV-5 was not conserved. So there are five amino acids that are different between AAV-9 and AAV-5. And we thought that if AAV-5 does not include this epitope, maybe this is the way to solve this epitope and reduce the immunogenicity.

So in the middle, we have in silico prediction where we compared the HLA binding possibility of either the wall type epitope in

AAV-9, or on the right AAV-5. And indeed, we found that AAV-5 is predicted to have a much weaker HLA presentation for this epitope.

So then, we made -- synthesized the peptides that include the peptides from AAV-5, and compared them to the ones from AAV-9 shown on the right. And as you can see here on the bottom, AAV-5 does not have this epitope. So these five amino acids solved the epitope.

So we were very excited about that, and we went back to the molecular biology side of the lab and started making new AAVs that have either the five-point mutations that would essentially introduce the chimera from the AAV-5, or just two-point mutations that were predicted in the algorithm to eliminate the epitope by themselves. We designed both AAV with GFP fluorescent, GSB fluorescent so we can characterize it in different methods.

On the right, we have what we like to call the CMC characterization. We wanted to confirm that the introduction of the two or the

five-point mutations did not increase -- did not change or compromise the yield of the production of the AAV, the size of the particle, the thermal stability, and the percent of MC capsid. And indeed, we found that mutation resulted in highly comparable AAV vectors.

Then we went onto to characterize this activity in vivo. Here we have three different cell lines. And in black is the wild-type AAV, and the gold is for the pink and the blue to not be different or better activity.

And indeed, we found, and as you can also see it in the GSP illustration microscope images, that the mutations did not compromise the in vitro activity of the AAV.

Okay this is the second model where we used the nano luciferase. And again, the activity, the in vitro activity was highly comparable. But you see those big things in gray in these preps AAV-5 was -- had very good performance. But if we still compare the wild-type AAV-9 with the two mutant activities, it

is very comparable.

And then, we went on to characterize the biodistribution and transduction activity in mice. So the mice were treated with either the wild-type or the two mutants. And we also had AAV-5 as the control.

And we found that looking at the NanoLuc admission, we have very similar transduction activity because the entire mouse is shining. We can't really see the biodistribution here, so that will fall in the next slide.

But if you look at the quantification of the signal, we have very similar transduction activity. So these two-point mutations or five-point mutations do not compromise the activity with in vivo.

Now in order to validate the biodistribution, the mice -- before we sacrificed them, we injected them with a nano -- with a substrate, the luciferase. And then, isolated the organ, the brain deliverer along the muscles.

And on the right, you can see the

quantification of those organs with very similar biodistribution. We further harvested these organs, isolated the DNA, and looked at the AAV-derived DNA and to further characterize the biodistribution.

And we found that at least for AAV-B1, so the two-point mutation we had extremely similar biodistribution. So those five-point mutations -- those two-point mutations did not change the biodistribution.

So the goal of the mutations was to affect the immunogenicity. So we did a few immunogenicity confirmations. The first was just to make sure that those two -- those few amino acid changes did not create a new B cell epitope.

So this is an antigenicity acid where we take human serum from I think it's 50 different donors where it's pooled, and we validate the antigenicity, the ability of that serum to neutralize our mutant AAV.

So while that's in the slide, you can see that we did not see any major changes in

antigenicity. If anything, it was a little bit reduced, which is not surprising because we did change it a little bit. But we did not create a new B cell epitope.

So when the final confirmation that the epitope is indeed gone once we make it in the whole constellation of the AAV, these are the specific peptides -- this is the -- these are the peptides that contain the epitope.

You can see on the left that for wild-type ones, we expected the cells from wild-type AAV, we get an immune response, but we do not get that immune response as a result of the activation with AAV-5, or the two mutants.

And we also characterize -- we expanded the cells with the entire -- with the AAV, and then restimulated with an entire peptide library spanning the sequence of AAV. And we did not see any raising up of cryptic epitopes or new epitopes that may come up as a result of the mutation, so we were very excited about that.

And to summarize what I've shown you,

we've identified a novel and promiscuous immuno-dominant T cell epitope in a viral capsid protein AAV-9, that can be eliminated through a rational-designed chimerism without compromising the function or potency.

Such designs can result in safer and more efficacious gene therapy by reducing the T cell mediated toxicities, and by preventing T cell mediated deaths of transduced cells. Potentially this can result in longer persistence of transgenic expression.

And similar rationale, immuno silencing could be applied to other AAV vectors, and also other therapeutic proteins. And I just want to say -- to acknowledge my lab members and our collaborators. None of this work can happen without.

And So Jin Bing is bolded because this is the staff fellow that did most of the work. And thank you for your attention. And at the end of Zuben's talk, I'll be happy to take questions.

DR. ZHOVMER: Thank you, Dr. Mazor. Dr.

Sauna?

DR. SAUNA: Good morning everyone, and thank you Ronit for giving the introduction sort of to my talk as well. So my fundamental interest in my lab for over a decade has been to understand the immune responses to therapeutic proteins.

And what I'm going to talk to you today is in the context of novel modalities, and try and make a distinction between what we find in the therapeutic proteins that are purified proteins that we inject into individuals, and when we get the same proteins by gene therapy or gene editing.

So this audience doesn't really need to get the importance of novel modalities. It's just being gene therapies, cell therapies, and increasingly CRISPR cast-based genes, which hold immense promise in treating, you know, previously almost untrackable diseases.

Now understanding immune response to these modalities may be pivotal for improving the safety and efficacy of these therapeutic proteins of these therapies. Pre-existing and induced

immuno responses are a key concern during the Deblar Pentar (phonetic) regulation in almost any biologic that the FDA regulates.

So before I get into novel modalities, such as gene therapies, let me give you a little bit of background and context in terms of this unwanted immunogenicity that we have in proteins, that we use them in therapeutic applications.

And here I have tried to summarize sort of the techniques and technologies that I used for trying to predict or determine if a particular modality is going to have an immune response. And I very loosely ranked them in terms of decreasing through birth, and increasing cost and complexity.

So the simplest thing you can do with the therapeutic modalities is do it in silico analysis. And there are very good algorithms available now. But what most of them do is essentially they don't really tell you about, you know, little immunogenicity.

What they do predict and predict very effectively is whether a particular peptide will

bind a particular image, and with what affinity. You can do the same kind of thing in an actual assay, and increasingly use these in silico tools that are so powerful that, you know, that there's very little difference between the results that you will get between measuring these in vivo, in vitro.

You can also use human blood derived cell-based assays such as dandarid cell-based assays or T cell effector assays to look at cytokines that are produced by T cells and get a sense of whether your protein or peptide is actually illicit -- is likely to illicit an immune response in the sense, do they activate some T cells?

You can do more advanced assays like MHC tetramer guided epitope mapping for -- to map T cell epitopes. And there's an assay called the MHC associated peptide proteomics assay, which is a very powerful assay that we are increasingly using which actually gives you a sense of about antigen processing and presentation, which can

actually allow you to identify naturally presented and processed peptides on the MHCs of cells obtained from donors.

And you can do other, you know, more advanced assays like protein-specific T cell application. And if you want to get an idea about the in vivo effect of these proteins or peptides, you can use HLA transgenic mice, which are you know, very expensive and very complex to do.

So before I -- about a decade ago, many of these assays were not that routinely used during drug development. And the reason for that was that the clinical utility of these assays was poorly understood.

And then, there came this particular story that we worked with the company, Novo Nordisk, that you know, got into this problem with the particular protein. So Factor 7A has been used as a bypass therapy for people who have antibodies to Factor 8, and cannot be treated with Factor 8 for hemophilia patients.

And for over two decades, there were no

reports of anti-factor 7A antibodies in hemophilia patients. Novo Nordisk made three mutations in Factor 7A, and they went into clinical trials without green clinical studies such as using the assays, such as the ones I've described previously.

And with just three mutations, they ended up with an incident of immunogenicity of 11 percent. The drug was removed from development, and we worked with Novo Nordisk to try and understand whether in the real world these assays that we have been talking about would have some utility.

And you know, this poses a series of questions. Do mutant peptides that they generated bind and actually attach to molecules with high affinity in both in silico and in vitro. We showed that they could.

Mutant peptides presented in the super MAPPs assay, and the answer was yes. Do mutant peptides that bind with high affinity illicit a T cell response? And the answer was also yes.

Most importantly, is there a clinical importance to this? And the answer is a resounding yes because antidrug antibody positive patients do carry HLA Class 2 molecules that bind to mutant peptides with high affinity.

And with this introduction and the learnings that we've got with therapeutic proteins, let me switch to key differences that you observe when you're using therapeutic proteins versus novel modalities. And let me start off with, you know, talking about therapeutic proteins where we got most of our learnings from.

So here we're talking about CD4 T cells, which are MHC Class II restricted, and preprogrammed for helper functions such as activation of B cells to secrete antibodies.

Antigens ingested in, like I said, the protein of the antigen into endocytic compartments of macrophages dendritic cells or B cells are presented to CD4 positive T cells as peptides are drawn to MHC Class 2 molecules. And therapeutic proteins are almost always extra cellular and

immune responses are driven by the MHC Class II CD4 responses.

In terms of bio analytics where we have, you know, decades and decades of experience here, me as well as products in CBER, bio analytics for assessing the immune responses to protein therapy is largely focused on accurate determination of antidrug antibodies, and determining whether these antibodies are neutralizing.

Now let's move to another kind of immune response you can get which is CD8 T cells, which are MHC Class I restricted, and preprogrammed for cytotoxic function directly killing target cells.

Now endogenous synthesized antigens and the cytosol of all cells are presented to deviate T cells as peptides bond to MCH Class 1 molecules. Novel modalities illicit diverse immune responses based on the root of administration, delivery systems, et cetera.

So there is a much more complicated scenario in terms of these novel modalities compared to, you know, a simple -- a type, but

it's not really simple. But what we've been using to make it simple, the therapeutic protein.

And bio analytics was assessing responses to novel modalities cannot rely solely on the identification and characterization of antidrug antibodies, and these assays must be fit for purpose and carefully designed for every application.

So let's look at, you know, the Cas protein used in CRISPR CAS as a model system. So for in vivo clinical applications of CRISPR-Cas, immunogenicity would be a key concern. Cas proteins are of material origin. Many of them are human pathogens, so high emergency risk is expected for these particular proteins, even for the FDA guidance.

Now pre-existing antibodies to Cas-9 and pre-existing T and B cell responses to Cas-9 have been reported, but also in others. Also genome emitting in mouse livers was accompanied by an increase in CD8 plus T cells, cytotoxic T cell response hepatocyte in both doses, and complete

elimination of genodermatotic cells.

A patient in a CRISPR-mediated disrupt and restoration was demonstrated in a canine DMD model. However Cas-9 specific immune responses put a critical barrier of a successful AAV CRISPR therapy. Serum Cas-9 antibody and PBMC at least spot-confirmed Cas-9 specific responses in two dogs were treated in this manner.

So as far as Cas-9 is concerned, you could deliver it as an mRNA, and the Cas-9 is made increasingly presented to Class 1, and you get a CD8 immuno response. You could also give Cas-9 as an RMP particle, but it would be presented in an extraneous protein, and engage with an MHC Class II, and illicit a CD4 based response. So either scenario is possible given the current state of the art and how we, you know, use Cas proteins.

So now to try and understand, you know, as much as an erroneous explanation for AAV, what are the epitopes and how do you use sort of phase out the immune response of Cas proteins?

And the first question is the

non-trivial task of selecting a cohort of donors for ex vivo assays. So presentation of peptides direct from the protein by the major histocompatibility complex, MHC, is a necessary but not sufficient condition for eliciting an immune response.

Now the MHC is the big elephant in the room. The MHC is polygenic. Every individual contains several MHC genes. It is also polymorphic. The population has variance of each gene, and the MHC genes are the most polymorphic in the human genome.

So getting a cohort of donors that is representative of your population of interests is itself a challenging task. And here we -- you know, I describe a tool that we've developed, and I'm just showing the illustration of this tool, you know, where this orange bar is 10 million randomly picked cohorts of 50 donors each.

And on the x axis, we show a statistical measure of the Jensen-Shannon distance score. And this is an arbitrary measure, and the lower the

score, the closer it is to your -- whatever entity you're comparing it to.

And here we are comparing our pool to the distribution of HLA alleles in our pool versus that in the general population that you're interested in. And the green lines show that you -- very powerfully decrease the Shannon distance score when you use this algorithm to basically select your donors of interest.

Then we wanted to use the score to donor, and look within this diversity of HLA alleles. What is the presentation of Cas proteins using this MAPPs assay? That is which you directly measure the peptides that were presented when you, you know, give the protein.

And on the chart that shows you the flow chart is basically a MAPPs assay for Class II where you feed the protein of interest and antigen-presenting cells, and then pull out your MHC associated with the peptide, and see whether the peptides are presented or not.

And this graph -- this picture basically

shows you the basic power of the MAPPs assay. So you have, you know, the dotted line essentially showing you the percentile rank in terms of binding affinity of every possible peptide in the human proteome, a million randomly generated peptides or peptides in very large protein-like factored into one milligram factors.

And the gray area shows you the binding affinity of the ones that -- the peptides that were identified in the assay. And you see that it's skewed very strongly to the left showing that you always pick up tight binding modalities.

However the opposite is not true. In the bottom graph, you show -- you see in gray all the tight binding peptides that you find in a protein factor. Whereas, the colored bar shows the ones that are actually identified in a MAPPs assay.

So this is, you know, one of the reasons you want to use a more sophisticated assay rather than just a in silico binding assay. And here is, you know, in our donor, the Y axis has each donor.

And each of these little squiggles shows a peptide that was identified using this MAPPs assay.

And now, we -- if you want to get a sense of what are the biological meaningful epitopes, what we have here are, you know, overlapping peptides covering the entire MAPPs peptide, the entire Cas protein. And then, these were used in a T cell assay which this is using flowcytometry using three different cytokines.

And this is the graph. This was the graph that is of interest because here what we defined as biologically meaningful peptides are peptides identified by the MAPPs assay, and are also capable of eliciting a T cell response in a T cell-based assay.

So you -- you know, from this very large protein, this is the peptides that are relevant in accord of donors which is relative -- which is comparable to our population of interest in this case because when we regulate drugs in the U.S., this is based on an non-nematic population.

Now to -- this is all about Class II,

which is again based on our learning from, like I said, protein therapies. But Class II is not the only thing of interest. Like I mentioned previously, efficient genome editing can occur even in the presence of assay Cas-9 immunity.

However genome editing can be accompanied by an increase CD8 T cells, and a cytotoxic T cell response. So how do you handle this kind of a problem if your root of administration is likely to illicit a Class I based response?

And this is more recent work where we have tried to -- so there are very few official assays that you can use to identify MHC Class I proteins. And here we have actually used cell lines which model allelic cell lines in the sense that each cell line has only one MHC Class I allele because these were a gift from Derin Keskin at Harvard, and the citation is given here.

So we take these cells. We do a lentiviral transduction of our protein of interest in this plate, Cas-9. And we essentially sort

these cells repeatedly until we see expression of the protein of interest intracellularly. And then, we go and do the same kind of, you know, pull down and MAPPs analysis of these peptides.

And here you see, you know, the ten donors that we've used, and each of these different peptides derived from Cas-9 that bind to each one of these Class I proteins.

And so much for Cas-9, we know that Cas-9 is from a human pathogen. You expect it to be immunogenic. But what about the new Cas proteins that are being generated? And here I'm giving you three examples. One is a Cas-9, and then there is Cas-12A which is from human -- from a common cell. And then, Cas-5, which is not even from bacteria, but from a bacterial phase.

And the assumption is that these Cas proteins would probably be safer immunologically because most humans have not been exposed to them. And we -- and as we see, that is indeed true.

And so, here is a couple of graphs showing B and T cell responses to alternatives of

Cas-9. And this is measuring antibodies, and again, in large pool of donors. And you see over here quite clearly that Cas-9, Cas-5, and Cas-12 all have pre-existing immunity in the human population.

On the right hand side -- the left hand side, you see antibodies. On the right hand side, you see an allele spot-based assay looking at T cell responses. So you can see that both pre-existing in T and B cell immunity exists in these alternatives to Cas-9 as well.

This is another graph looking at Cas-9, Cas-12A, and Cas-5 on -- using a MAPPs assay. And again, you know, though there are relatively fewer Cas-5 peptides that we've found, essentially it shows you that all of these proteins seem to have some kind -- some level of pre-existing immunity.

And with that, I want to end with some, you know, some unanswered questions that we have in terms of our regulatory responses with regard to these novel modalities. So what -- we do not even know at this point what is the clinical

relevance of the adaptive immune response to novel modalities.

What assays, reagents, or statistical measures, such as cap point determinations, do we need to evaluate immunogenicity in the clinic for these novel modalities? And do we need more standard -- metric standardization? My guess would be, yes.

There are very few, if any, reference standards. And if you need these, who will build the cap? Would it be a community effort, an individual effort in the lab? Developing in silico tools is the cheapest and highest throughput. We are doing it.

And you know, we really need tools that are more specific to these modalities. How do we design the assays the reflect the influence of the mode of delivery on immunogenicity, which is much more diverse than other modalities and for therapeutic proteins?

And what in silico, in vitro, ex vivo including allele assessments that we need? Like

in the case of Factor 7 that I've described that gives, you know, manufacturers and other individuals confidence that if they invest in these kinds of clinical studies, that is going to be clinically meaningful.

And developing in silico tools, we need to go beyond peptide MHC binding predictions and developing complex mathematical models. And you know, right now there is -- the FDA just ended a pilot program that's become a regular program for more model-informed drug development approaches that the FDA encourages. And there is a space here for the development of more complex tools.

And with that, let me acknowledge individuals in my lab. My collaborations within -- the collaborators within the FDA, and also a research co-op agreement with Editas Medicine which, you know, is involved in the development of Cas -- of gene editing therapies based on Cas proteins. And with that, I'm happy, along with Ronit to take questions.

DR. ZHOMER: So now we are going to open

our Q&A session. And please, you can take the microphone.

SPEAKER 2: I'm Cherise and I'm from Stanford University. So the question is for either one of you. So I wonder if adaptive immunities -- I'm sorry, trained immunities is being considered in this when you both stated adaptive immune response.

But there is an emerging field of leukemia innate immune cells and memory-like responses among those, particularly myeloid cells, and K cells. And I wondered whether there's any intention to look at those as well in this context.

DR. MAZOR: Okay. Thank you for the question. It's a great question. Indeed for AAV, we're seeing a lot of involvement of innate activation. Most of the studies have seen -- really focus on, you know, the DNA gene inside the AAV because it has this serum pump signals.

And yes, there is -- there are evidence of activation of monocytes and extracellular cells

in many of those myeloid responses. So absolutely, there's a lot of interest. And we have on person in the lab who keeps looking at that as well.

DR. SAUNA: Okay. So I would just, you know, add one thing briefly to that in that in these -- definitely there is, you know, value and understanding from a scientific point of view.

But in a -- from a practical point of view from -- with limited resources, what is it that -- you know, from a point of view for a manufacturer, what do you do what will give you some kind of meaningful information before going into the study?

And that becomes a very different kind of question and a proposition. And it is not easy to find examples and tools where you can show on a one-by-one basis that this is -- this assay, or this group of assays, is actually going to give you something that is clinically meaningful.

And that is, you know, just beginning after decades and decades beginning to emerge for

therapeutic proteins. We have much less understanding of immune responses and the complexity is much higher for novel modalities.

And you know, and again, it has to be much more thoughtful because you have to really understand that particular product, that particular situation, and figure out what are the most useful assays that might be for your, you know, your particular situation.

DR. ZHOMER: Thank you. Your question please?

DR. GOLDING: Hana Golding, Office of Vaccines. Really two excellent talks, and very thought provoking. And I think the common thing is that you're both using very important both an in silico and in vitro way to identify epitopes that are recognized by combined many HLA and can activate to the four cells.

And then, that can either raise the question of whether removing some of these epitopes can then prevent the immunogenicity of other AAV vectors or Cas-9. So the question to

you is maybe take the next step, and ask.

I would like to better understand whether removing or changing CD for epitopes, how is it actually going to affect to effective mechanisms of adaptive -- either antibodies that are very problematic, especially against AAV and other viral vectors, as well as cytotoxic cells?

Do you think there will be a way to really modify the overall immune response to this important treatment that's usually given multiple times?

DR. MAZOR: Okay. Thank you for the question. The short answer is yes. I do believe it can modify and reduce the immunogenicity. For AAV, it was not known yet of course, but in my previous slide from the NCI, we worked on therapeutic proteins that were highly immunogenic.

And we found that if we can eliminate some of the T cells, the major one, the dominant one based in mice models, it was very effective in preventing completely, or I think it diminished it by 80 percent, the ADA, the antibody response.

Furthermore we then translated that into a clinical anticancer therapeutic. And while it did not prevent the immunogenicity, the PK allowed patients to receive, I think it was additional two cycles before they had those very high neutralizing antibodies. And the PK still had some antibodies. So they did natural neutralization once you -- we removed some of those CD4 T cell epitopes.

Having said that, for AAV it's a double challenge because we both have CD4 immunogenicity that we received from many therapeutic proteins, but we also have the cytotoxic T cells, the ones that are causing the liver toxicity.

So right now, So Jin Bing, she's in my lab working on trying to eliminate both the CD4 and the CD8, and it's challenging. It's a lot of work, but we hope -- we believe once it's done, we will try to show in mouse models that it works first.

DR. SAUNA: Yes so, I mean I completely, you know, concur with Ronit is that it is

desirable, and it is what she's been trying. You know, we try it in different models as well.

With proteins, you know, again like Ronit said, you're removing the engagements. So essentially, we are talking about immuno silencing. All we are doing is trying to reduce the engagement with MHC Class I or II.

And again, given the diversity of HLA alleles, you know, it's -- you do the -- you kind of come up with a workflow where you, you know, either -- your choice is between trying to disengage as many as possible with whatever degree of disengagement as possible. Or engaging a few and it becomes much more personalized.

Then you would have, you know, molecules which are applicable for this subset of HLA alleles for example. And for normal modalities, you know, again this is -- again do you know -- I mean, let's just take Cas-9 for example because I've just chosen to use it as a model.

Do you try and to make a protein that is both Class I and Class II immuno silenced? Or

generally to -- because you're not going to simultaneously, at least for this protein, simultaneously use it in a manner where it is, you know -- where it's risk equivalent for both of them.

For example, if you're giving it an mRNA base, I mean you just might want to, you know, stick to the MHC Class I, which have the added benefit of -- you tend to get far fewer MHC Class I epitopes. Then you'll end up with MHC Class II, which becomes, you know, much more challenging.

So again, the answer is the same. Yes we have ideas, but --

DR. MAZOR: Yes I think all of these are very good things, as long as of course they don't kill the function of it.

DR. SAUNA: And that's constantly there in every -- even in the algorithms. I mean, you build them, and you start with, you know, something of the other to -- I mean, at the end of the day you test it.

But we, for example, avoid making

mutations and conserve residues. We, you know, use the tools that identifies deleterious mutations and avoid those sites. So I mean, there are ways to get around that, but it's still challenging.

DR. MAZOR: Thank you.

DR. ZHOVMER: Very nice, thank you. Last question.

DR. ELKINS: Actually we have two questions online, but we'll try to be kind of succinct about them.

AAV is still a popular strategy for delivery of gene editors. Presumably they could be persistently expressed in transduced tissues for days, weeks, or months. Do you have kinetic data, time course data, for any known immunogenicity assessments over time? And if so, how does the immunogenicity change over time? Maybe that's a yes, no, and maybe.

DR. MAZOR: I think it's nice because it's a question that marry Zuben's lab and my lab together. It's delivering the editor through AAV.

I think the short answer is, we don't know.

I have not done kinetics for immunogenicity. We're looking into more relevant clinical transients, but we have not -- it's not going to be an easy experiment to do.

DR. SAUNA: I have nothing to add.

DR. ELKINS: And this one can be tough too, and it might go for discussion later. Do we know why sometimes AAV doesn't cause antidrug response, even in situations, where logically, you would think it should?

DR. MAZOR: So as I said and tried to be cautious in my introduction, as far as I know, when we deliver AAV systemically to patients with a normal immune system, we expect 100 percent immunogenicity. And not just 100 percent, high tide or very neutralizing, staying for a long time.

There are ways to deliver the AAV. Again if you deliver it to an immune privileged organ, like directly into the eye, that at those cases you don't get 100 percent immunogenicity.

Sometimes it's even lower. But for just in general, it's a viral vector and the immune system knows what to do with it.

DR. SAUNA: So I don't know about AAV, but this is the call of what -- I mean, our concern has always been whether you use a protein, whether you use AAV, or whatever. There are -- it boils down an individual response, like I said.

In every instance, there are some people who will get a response, and there will be some people who will not get a response. And for proteins, it's much easier to do. And people can refer to our papers and other papers as well that look at all the different personalized risk factors and, you know, what the importance is.

One powerful tool that is now emerging, and we have just, you know, started to actually get a tip of the iceberg is that you can -- for therapies which have been approved and have been used in the clinic for a long time, there are large, very large data sets about that that include genetic data sets for AAV diseases and

others.

And machine learning tools are a hypothesis way of addressing this question when you have, you know, when you have -- and it's in a kind of different context. This is what Dr. Mazor touched on in real world data.

So you use the data which we get from registries from patient advocacy groups, et cetera. And interrogating those data with, you know, machine learning and AI tools is one excellent way of trying to identify genetic rick factor that can help you segregate responders from non-responders.

DR. ELKINS: So that's the last word. Please thank all of our speakers and moderators for a wonderful opening session. We have a short lunch break.

DR. RANDOLPH: I talked to you today about some of the -- some new technologies that we've been working on for vaccines. Trying to address some questions and limitations of current vaccine technologies that currently make it

difficult for us to reach, in particular, all parts of the world with vaccines, where we could easily be doing a much better job.

So vaccines are tremendous, and clearly they're the winner, right? Any time you can have something that prevents you from getting a disease, I'd rather have that than having something that fixes having a disease.

Beyond that, the overall health benefits in terms of lives saved when you list them out for vaccines are just so huge. They dominate everything. And still we frequently find that we don't have adequate vaccination programs, even for vaccines that exist and are good vaccines.

And so, there some reasons why vaccines don't make it into people's arms. Some of them obviously we've heard about with COVID, and there's all kinds of political things and vaccine resistance.

But there's also reasons that prevent people who would really like to have vaccines from getting vaccines. And some of those are things

like the cold chain requirements with the need to maintain vaccines under rather strict cold chain conditions that really prevent them from working.

Especially in underdeveloped countries, but also in this country in places. We're really having trouble getting vaccines through the cold chain to where they need to be reliably.

The logistics of vaccination campaigns are really complicated, but the instability as antigens and adjuvants in vaccines. There's another factor is that many vaccines, most vaccines now, are requiring multiple doses.

And so, there's just this pretty steep drop off in patient compliance for getting a multiple dose series into your arm. That of course is even more difficult if we're talking about trying to deliver to underdeveloped countries or places that don't have really good healthcare logistic systems. It's hard enough to get one dose of vaccine to people, let alone three or four or five sometimes.

So I'll talk a little bit about how we

can get around some of these problems. And to get around at least the cold chain problem, we need to be able to store complicated assemblies. Viruses, proteins, they need to be stored for long enough at a high enough temperature so that we can get them out to where they need to go.

Nature has a really good strategy for this actually, and that is that nature tends to put things into glasses to stabilize them. There's an interesting example that just reappeared this year, which is that you may have seen the beautiful pictures of the lake in Death Valley what refilled slightly during the massive rains.

That lake had been sitting there storing at 120 degrees Fahrenheit for years and years. When the lake filled up with its six inches of water, brine shrimp all of a sudden appeared. And so, those brine shrimp eggs had been stabilized against Death Valley temperatures for year.

The way that they did that, the way the brine shrimp achieved that is by forming glasses,

organic glasses within their eggs, that enabled them basically to lock down motion and prevent the -- prevent any damage to proteins, DNA needed for them to sort of pop out of their glassy stasis when they get water on them.

Same things that happened for things like Lotus seeds found in Chinese tombs that have been 2,000 years old, add water, and they grow a plant. And those are stabilized as well by sugar glasses that are formed inside these seeds.

So vaccines have used this approach successfully before, and actually rather frequently. So by freeze drying a vaccine, you form a glass, and that glass typically gives you better long term storage facility for things like proteins. And so, this sounds like a way we could avoid some of the cold chain requirements.

There's a downside though in practical application of this, which is lyophilization may cause acute damage to vaccine formulations. Much of that damage seems to come from the adjuvants rather than the antigens involved.

The adjuvants that have been traditionally used in vaccines include aluminum salt, and those tend to aggregate during lyophilization. They tend to aggregate especially during the freezing portion of lyophilization.

But lyophilization also destabilizes lipid-based antigens, which is sort of the -- part of the new generation of adjuvants that are appearing in vaccines. It also destabilizes the lipid components of lipid nanoparticle vaccines for mRNA.

So why does this happen? And then, what can we do to get around it? So one of the things that happens during freeze drying is that you have freeze concentration, correct? So as you form ice, the ice crystalizes out, and that results in the concentration of everything else to higher and higher levels.

And so, if you freeze in vaccine preparation, all of the antigens and adjuvants that are there become more and more concentrated as that water leaves as pure ice. And they can

become 15 to 20 times more concentrated with everything being that concentrated, right?

If you had a protein that maybe had good stability at 150 milli molar salt, does it have that same stability at 1.5 molar salt? Probably not, right? So as these things get more and more concentrated, things can be -- can fall apart.

That high ionic strength also destabilizes aluminum hydroxide calwood (phonetic). Those are used as alum in adjuvants, and that can cause aggregation. If you have a lipid adjuvant such as those used in mRNA nanoparticles, that freeze concentration can destabilize emulsions that leads to coalescence, making giant aggregates of the preparation.

There's another thing that happens during freeze-drying that's dexterous, and that is there's an ice water interface that's formed. Proteins and adjuvants as well can absorb to ice water interfaces, and those cause instabilities.

Ice, when it's forming, it expands as we all know. And during that expansion, things that

are absorbed on that surface become mechanically damaged from mechanical stresses.

And then, when you're done with the freeze-drying process, you have -- you quite typically have your antigens that used to be on the surface on an ice water interface are not on an air solid interface, and that also can cause damage to these delicate molecules.

The way that glasses form during freezing is related to this process, that is as the temperature decreases, ice crystals grow, and things become more and more concentrated. And as they get more and more concentrated, they get gooier and gooier. We start making solutions that become more and more syrupy, thick, and concentrated.

As that viscosity goes up, motion slows down. And eventually, things become so concentrated and so viscous, that basically no more motion occurs. And in fact, so little motion can occur that ice stops freezing, or the remaining water stops freezing to make ice because

this can't move around to do that anymore.

And that point is called the glass transition temperature at maximum freeze concentration, or Tg prime. Once we have concentrated and cooled things so that they are at that temperature or below, things are locked down.

You can think of a piece of candy as that final state. So a jolly rancher candy is glass, it's an organic glass, sugar glass. It feels like a solid. It's really a super-cooled liquid, right? We formed those kinds of glasses around our vaccine particles, things that are inside that can't move around, okay?

And lyophilization takes roughly in the order of thousands of seconds to get from the initiation of freezing where that freeze concentration starts to the point where everything gets locked down in that glass.

And during that time as things become more and more concentrated, damage may occur, right? So there's a kind of danger zone that I call that is the time between initiation of

freezing, when solutes begin to cryo concentrate, and the point where we kind of lock things down into this jolly rancher candy that prevents damage, right?

So one approach that we've been using is to try to shorten the time over which this danger zone, and embed vaccines in glassy matrices that are formed by spray-drying. So spray-drying is a process where basically you just make a spray of liquid into warm air or warm gas, and if you make the droplets that are being sprayed small enough and the air is dry enough, you can form glassy microparticles within about 100 microseconds.

So we go from having a spray of liquid to this tiny jolly rancher candy glassy preparation in a tenth of a second, two-tenths of a second or so. You avoid ice water interfaces being formed, and importantly, you minimize the time that you spend in this cryo-concentrated region where high concentrations of everything else might damage your product.

So when you do that, you can embed lots

of things in it, and it can become much more stable because they're essentially locked down in this highly viscous environment. It's basically, you know, if you duct tape the kids together, they can't misbehave as much, right?

We're just going to slow that motion down incredibly. By incredibly, the viscosity of the sugar solutions that we're spraying goes from being roughly the same viscosity as that of water to ten to the fifteenth times more viscous. So they act basically like solids.

So as a starting example here, if we put a virus into these glassy particles, in this case it's a bacteriophage, those viruses can be stored almost indefinitely at even very high temperatures.

In this particular case, we stored bacteriophage at degrees in these glassy powders for a year without losing any its infectious activity. And if you had stored -- tried to store the same bacteriophage in an aqueous liquid environment at 37 degrees, we lost I guess what

seven orders of magnitude of activity were essentially completely killed.

So we can put vaccines into glassy states. We can put complicated assemblies into glassy states. It could be a vaccine. And that will stabilize them, but there some other vaccine challenges that are important here as well. And one of them is this requirement for multiple doses.

So by way in which we are going to combine some technologies to both things at once is using a technique called an atomic layer of deposition. And this is very foreign to the vaccine world.

It's a method that puts deposits, ultra-thin, nanometer thick layers of metal oxides on surfaces. It's used in the semiconductor industry. It's used to make the powders that coat the inside of fluorescent lightbulbs. And it seems probably remote from vaccines. It was certainly seen that way from the beginning, I guess.

It's a technique where you can put absolutely precise layers. By precising it, we can count the number of molecules, deep of these layers that we can coat things with.

So the example is aluminum chemistry. If we start with a surface that has hydroxides on it, which could be hydroxyl groups on it which could be something like sugar, a sugar particle. We expose that to trimethylaluminum. That has a reaction that proceeds essentially instantaneously, and coats the layer with methyl alumina.

When we switch from that to water vapor, methane is kicked off and we regenerate the surface where the surface now has one layer of aluminum oxide on it. We can repeat this cycle as often as we want. Every time we do that, we add one layer of aluminum oxide to these surfaces. So each of the cycles that we run deposits 2.33 angstroms thick layer of alumina.

For convenience, if you want to think about that and avoid some of the chemistry,

alumina is sapphire, or sapphire is alumina. So we're basically putting a sapphire coating that's a few nanometers thick on whatever we want to put it on. In this case, it's going to be a stabilized protein class.

We do that in a very chemical engineering environment. These are fluidized beds. Fluidized beds, basically if you take powders and blow air through them, they start to bubble and they look almost like mud. Although what is spending the particles is the air and not a liquid.

And so, we can conduct this reaction inside this atomic layer deposition, inside a fluidized bed reactor, so it's very efficient. It's what they used to make tons per day of this material in the semiconductor industry and the lightbulb coating industry.

So with really low cost, it turns out you can add these nanometer-thick layers of alumina on the surface of these particles. So this is what it looks like. We start out with

spray-dried particles. These are five macron-sized particles.

And then, we can apply these nanometer-thick coatings to those. So you can see in this, the right hand SCM image, an image of those shells, we've blasted them open by using the electron beam to sort of show the thickness of them. They form these really thick -- really thin sapphire coatings on top of sugar microbeads.

So what could that have to do with vaccines? Well first of all, if we take things like a virus that we could be using as a vaccine, right? We can put those in these glassy powders. They're already pre-stable. When we add the extra coating to that and -- these extra coatings of thin sapphire layers are further protected.

In one case here, we looked at three different bacteriophages. These have actual potential therapeutic applications. They're good for addressing multiple drug-resistant bacteria. But you can keep these phages active in this case for nine months at 37 degrees.

Why is the 37 degrees important? Obviously body temperature. But that means we can actually inject these powders into an animal or a human. And those powders, the content of those powders, inside of them would be at 37 degrees. The contents would remain stable as long as the coating remaining on the surface.

So the next stage of this. I have never particularly worried about taking a shower while wearing my sapphire jewelry. Sapphire doesn't dissolve. We don't need to worry about that, but nothing is completely insoluble at the molecular level.

And so, when we coat these powders with 20 molecules deep sapphire, and inject that into an animal, that sapphire, those ten molecules deep sapphire do dissolve. It just takes weeks to months to do that.

And so, when that happens, eventually we release the contents and essentially, we can release a dose of vaccine if we'd like. This release is very pulsatile. So because we can put

extraordinarily uniform coatings on the surface of these particles, they also dissolve at a very well-controlled rate. When they finally dissolve, everything is released all at once.

And so, we have -- we can speed that up by putting them into some media that causes them to go a little bit faster. You can see that we deposited some release versus the number of coats.

So we can put a different number of cycles on, different thicknesses. Again each of these coats is 2.33 angstroms thick. And those a very good correlation, so we can dial in exactly what we want each of our particles to release.

So now, what we can think about doing is saying, well if we have a vaccine that is a two or a three-dose vaccine, we can give that as a dose that it releases immediately, one that releases in two months, one that releases in four months. And that's exactly what happened.

So in this case, we've taken some labels, fluorescently labeled PHV virus vaccine. And if you have it uncoated and administered just

all on the adjuvant, what you see is that it can remain at the injection site in the mice for about a week.

If we put on 250 layers of this, we can move that release out to about ten weeks. And so, you can time then when you want a vaccine to be released from these particles simply by adding in different numbers of coatings.

And you can have all kinds of flexibility at that point. You can make mixtures of powders to release a various -- different times. You can make everything release much later as you'd like, right?

So in vivo -- I mean, it shows an in vitro solution data before. In vivo, we can dial in the immune response timing by changing the coating levels as well. So these, on the right, we have a graph of the number of molecules, each sapphire we put on these microbeads. And then, plotted that against the weak to peak titer for a vaccine that we've stabilized inside these preparations.

So these work really well in terms of reducing the number of doses that we might need to have. So this is some data for an HPV Type 16 vaccine. We're plotting HPV at antibody titers, conventional two-shot liquid formulation. And you can see the usual sort of initial antibody response. If you boost, it pops back up again, and then slowly decays after that. A single shot though of the coated -- 250 coats formulation not only requires only a single dose, but it actually gives about an eight to ten-fold higher antibody response.

We had some interesting ideas about why that might be happening. But we have succeeded here in removing and reducing the number of required doses from two to one while still giving us a superior response.

We haven't lost the thermal stability part of this at the same time though. It's really remarkable here. We've taken these HPV virus vaccines, encapsulated them in these sapphire coated DVs, and stored them for three months of

temperatures up to 70 degrees.

Degrees is too hot to hold onto, right? And yet, these vaccines are stable for that long. So completely out of the cold chain, and producing a good response that's better than the conventional vaccine in terms of antibody levels. And in this case, we've looked at several different serotypes, so you can do multiple serotypes at the same time as well.

So some of the examples that I showed with the HPV were for a protein-based antigen, and if there's bigger challenges, it might things like stabilizing a viral-based vaccine, in particular, envelope viruses such as rabies.

So if you look at what rabies looks like, it's complicated. It's got a lot of structure to it. So lots of structures means there's potentially lots of things that could go wrong.

However when we take rabies vaccine which is administered currently as a five or seven-dose series, and it has to be stored under

cold chain conditions, we can first of all take this rabies vaccine and convert it by spray-drying it into a glassy state into something where we can go up to 50 degrees in this case without seeing any loss of antibody responses.

And we can also do this kind of coating and delayed response, things that spreads out, things like can have multiple doses in one. Here we sort of demonstrated how we can delay antibody responses by putting different levels of alumina coating on these, one 60 nanometers thick, and one 35 nanometers thick. You can see we just shift that immune response as things basically release at a time that is later and later as we put on more and more coats of aluminum.

And once again, the interesting thing about the overall effect of this is not only that we have a stable vaccine, but it's also more potent. So if we look at -- if you'd taken that spray-dried vaccine without the coating, reconstituting, and injecting it, we get a good response. But when we put those in these

sapphire-coated microbeads, that response goes up by about an order of magnitude again in terms of the antibody response.

And this was unaffected when it was stored at degrees for a month. So all of sudden, now we have a rabies vaccine where we can replace multiple doses with single doses, and they don't need to have cold chain anymore, okay?

I'll also note that not only did we get overall antibody titers higher, the neutralizing titers are also in order magnitude higher when things are coated with this atomic layer deposition coatings.

So some of the directions we are going in the future with this are that not only can we -- by controlling, not only is the stability and the -- but also the time of release, and the amount that gets released, is a new set of flexibilities we can have in vaccine design and vaccine delivery.

So Shane Crotty and Darrel Irvine have published recently some very interesting results

suggesting that you can get much better immune response in terms of avoiding B cell immuno dominance in germinal center guanosine and quality improvements by going to sort of a sustained flow release of antigen.

And they demonstrated that using osmotic pumps that were implanted, or multiple escalating doses. And that's nice, but multiple escalating doses are what we're trying to avoid. And the stability challenge of implanting an osmotic pump and having the stuff come out in an undamaged fashion is pretty daunting.

No one's also going to use implanted osmotic pumps as a vaccine delivery system. So we wanted to make a single-shot formulation that recapitulates some of the benefits of what Crotty et al. had shown with osmotic pumps, but using our system.

And so, we're doing that using the HIV envelope protein trial, this N332-G2 molecule. And basically, the idea is again we'll put our particles -- construct our particles containing

those antigens. We'll coat them with different levels so that they release at different program times, and see if we can increase the immune response as a result of that.

So we put this protein into our particles and coated it with different levels. And you can see the distinct effect of the different levels. Each level releases at a different time. And so, as -- we can program that in. In this case, we went from 30 to 200 coats, each of those giving us a different release time of the antigen.

And we put that together with a dose format where we gave about 10 percent of the dose -- initially 10 percent of the dose comes out of day seven as the coating dissolves. And another 70 percent comes out in two weeks.

And compare that to what happened when you just give a single bolus dose of the same quantity of this particular protein, and that's in black. In red, you can see where we put in the single dose, but with controlled release of these

three spots for the ALD system.

And once again, we get this boost roughly -- in this case, these are AUC values, but a significant boost in immune response over what happens with a single bolus dose. So we've been able to essentially recapitulate what Crotty et al. did with this -- with an implanted pump using a controlled release microparticle system.

So with that, let me stop but just say that we're really excited about how you can use some technology that's been used at large scale in the semiconductor industry for making spray-dried glassy particles that we can coat with alumina and other metal oxides.

We can stabilize things as complicated as envelope viruses, stabilize proteins, stabilize protein antigens, and that lets us get to single-shot bolus release formulations that can now be used to design really complicated and interesting multiple-dose regiments for vaccines that may really expand our ability to do things like really target and address broadly

neutralizing antibodies, for example, in vaccine development. So thanks.

DR. VILLA: Thank you very much. Really fascinating talk. We have a question right here, Doctor.

SPEAKER 1: Yes. This is a very interesting talk. The concept is very good. I'm from Office of (inaudible), and I have one concern that you're giving a single dose shot of multiple doses.

So if the patient reacts to the vaccine, you have no recourse of taking contents out, right? So how will you treat that?

DR. RANDOLPH: Yes that's true. You can't take it back out. The conventional vaccines as antigens absorbed onto alum release over the course of a week, and you can't take those out either.

SPEAKER 1: So that's it? You have one shot, and one does.

DR. RANDOLPH: Yes.

SPEAKER 1: And then, you can control

the adverse reaction beyond that.

DR. RANDOLPH: Well in actually all of these examples, we're delivering exactly the same total dose, it's just kinetics of biodistribution are spread out. But you're right, you know, you can't get it back out.

SPEAKER 1: Yes. So you may have to think of that in the future.

DR. VILLA: I think there's another question on this side.

SPEAKER 2: Yes I actually have two questions. The first is more practical. Well they're both practical. In terms of polysaccharide conjugate vaccines, do you have a way to measure if the interactions between your glasses and alum or aluminum phosphate are similar, or how are they different?

Because when I think of what you're doing, for some reason I'm thinking more of a solid. And when I think of the others, it's more of a gel, and maybe that's a very fine line. But one seems more amorphous and malleable to me.

DR. RANDOLPH: Maybe you can repeat that.

SPEAKER 2: Yes. So are these -- the basic question is, are the interactions between polysaccharide conjugate vaccines and your -- have you -- first of all, have you looked at them? I'm guessing you have.

DR. RANDOLPH: Yes.

SPEAKER 2: And are they different in your materials, in your glasses, your sprays, than they would have been in say the alum, the classical --

DR. RANDOLPH: They are different. So there's essentially almost no interaction between our aluminum coatings and what's on the inside.

SPEAKER 2: That's very interesting. How do you measure that? How do you determine that there's no interaction?

DR. RANDOLPH: So the way we've done that is by doing the experiment of putting the antigen on the outside of the particle where -- or on the inside.

SPEAKER 2: Yes.

DR. RANDOLPH: And we only get these immune bursts -- enhanced immune response when things are in the glassy state inside the particle.

SPEAKER 2: Okay.

DR. RANDOLPH: All of our coding is done on glassy particles where things can't move around. So anything that's on the inside of the particle never even sees the coating level.

SPEAKER 2: Sure.

DR. RANDOLPH: SO there's a lot less interaction. So in terms of, you know, antigen absorbing to alum, we have far less surface are available for that to occur. And most of it is protected on the inside of these glassy stasis.

SPEAKER 2: Okay. And the second question was, you talked about dissolution, and breakdown came to mind, chemical breakdown. So what -- are the breakdown products of your glasses the same as alum, or are they different?

DR. RANDOLPH: Yes the same.

SPEAKER 2: Okay.

DR. RANDOLPH: It's just an oxide, yes.

SPEAKER 2: So it just takes a little bit longer for this to break down?

DR. RANDOLPH: It takes that long for the alum to break down too.

SPEAKER 2: So it's the same timeline basically?

DR. RANDOLPH: Yes the same timeline, except we're just -- we're not looking at -- I mean, in our case, by the time it breaks down, that's going to be pretty coincident with complete clearance, right?

If you inject alum, that alum is going to hang around for months and months, and slowly it does fully dissolve, but it takes an awful long time. Our layers are so much thinner than even the primary particles in alum.

SPEAKER 2: Okay.

DR. RANDOLPH: But they disappear in much faster (inaudible).

SPEAKER 2: Thanks.

SPEAKER 3: Just a quick question. Have you looked at what can -- or I'm sorry. Have you thought about naturally stable viruses like vaccinia virus or variola virus?

And I'm sure Shane would have thought of that anyway, but do they have any special characteristics that contribute to that stabilization, so smallpox 100 years, maybe more for example.

DR. RANDOLPH: Yes. I think if you think about some of the cases where smallpox may have been stabilized in, you know, rodent dens and other things, it is entirely possible that it was just partially stabilized by -- in sort of dry glassy matrices as well.

And there certainly are viruses that are inherently more stable than others. And so, you know -- but looking at lenti virus, a large -- the different bacteriophage each have their own intrinsic stability.

And yet, basically when they're put in the glass, everything stops, and they all look

kind of the same. So I think it's -- I mean, it's hard. It's obviously impossible to say that it's going to be the same for every single virus.

We were little surprised actually that, like in the case of the envelope viruses which have this sort of watery layer in between the outer surface and the membrane, that those would be stabilized by drying. They work.

DR. ELKINS: We have a feisty group of online questions. The first one is partially with CDS, but is about the safety of the ADL-coded particles from the release of the material itself. What do you know about physiological responses to that?

DR. RANDOLPH: So physiological responses, we haven't seen -- so this is all in mice, but we haven't seen anything in terms of the difficulties with any kinds of adverse reactions, or things like that.

I mean, basically what happens is we're going to be releasing aluminum oxide, which is again the same thing that's being released when

you have an alum-based vaccine. So the track record on that is 100 years of alum-base vaccines with pretty good safety profiles.

DR. ELKINS: I've got it. Next, can either of the techniques be used in drug deliveries, particularly oncology treatments?

DR. RANDOLPH: I would love to say yes, but I'm not sure how strongly I'd want to say yes. The reason for that is that in these glasses, the concentration of actual antigen is pretty low. And so, the amount of powder you'd have to give somebody for many treatments would be just too excessive.

So it'd have to be something that's pretty potent. But for some things that are really potent, yes. And the advantage of vaccines, of course, you're giving microgram doses.

DR. ELKINS: Thank you. Next, is WHO looking at the technique to address the cold chain problems, particularly in Africa?

DR. RANDOLPH: WHO is not directly.

We're doing this with Bill and Melinda.

DR. ELKINS: They have more money, I hear. Okay this is an interesting one. The ADL process itself usually takes place in a harsh environment such as high temperature and pressure. How do you deal with that, and can the biologics withstand the environment that's needed?

DR. RANDOLPH: Yes. So that was maybe the surprise, again, that something that's used in a semiconductor industry would work this way. It's actually low pressure, and the temperatures that we use are not as high as would be use in the semiconductor industry.

And when you do that, it takes a little bit longer and the yield is lower. And so, my colleagues were very worried about making tons per day. We're very worried about how this would add another cent per dose, and we weren't worried.

So you can adjust those conditions so that the temperatures are such that we don't lose activity of proteins and protein antigens, you know. If we're talking about exposures, each of

those cycles takes minutes. And when we're talking about exposure, it's 60 degrees for a minute. And stuff has been stabilized so it can maintain 60 degrees for months.

DR. ELKINS: Very good. Can you use the technology for sequential release of different antigens, different immunogens?

DR. RANDOLPH: Sure and that's where the next round of this flexibility goes. If we're doing sort of polishing for steps for a vaccine where we might want to direct the immune response by suddenly altering the antigen over time of release, we can certainly do that.

DR. ELKINS: Okay and last but not least, do you see potential for the use of technology for cell and gene therapies?

DR. RANDOLPH: Yes.

DR. ELKINS: Good answer.

DR. RANDOLPH: No I mean, anytime if you want to think about delivering any viral vector that might be used in some of those techniques, you can -- we can deliver those. We can stabilize

and deliver those vaccines. You'd have to figure out the reason why you want to do that, and the temporal characteristics to be looking for. But yes, I think you can do it.

DR. KHOSHI: Great. Thank you very much. Good afternoon everyone. I'm Amir Khoshi, and I am Head Staff Fellow at Laboratory of Rutgers Hematology, Division of Blood Component and Devices.

It is my pleasure to introduce the second speaker, Dr. Kaitlyn Sadtler. She is a Chief of the Section on Immunoengineering at the NIH. Dr. Sadtler was selected for the Forbes 30 Under 30 list in science, the MIT Technology Review, 35 Innovators Under 35, the World Economic Forum Young Global Leaders, and the National Academies of Science, Engineering, and Medicine New Voices Program.

At NIH, Dr. Sadtler has, in her labs, expertise to the fight against COVID-19, leading a study that detected 16.8 million undiagnosed SARS Coronavirus 2 infections in the U.S. After the

first wave of the pandemic. Please welcome Dr. Sadtler.

DR. SADTLER: Okay. Thanks so much for that introduction. I'm going to pivot a little bit today to chat about some of our lab work specifically in the realm of traumatic tissue injury and reconstruction. So we're looking a little bit earlier in the timeframe, kind of pre-clinical development/kind of that initial discovery phase.

And in terms of our biologics, we're looking at -- we're working with biomaterials medical device implants. But then, also us as a biologic anytime you have any sort of intervention.

First and foremost, I want to say a huge thank you to my lab. I'm about five years into leading a group, and have really enjoyed my time there. I'm not the one doing the pipe heading anymore. So huge thanks to them. It's been a wonderful environment to work in.

So first off, in terms of traumatic

injury, what are the problems we're dealing with, and what are we facing here? The stats that always have kind of surprised me was that traumatic injuries are actually the fourth-leading cause of death overall in the United States, and it's the leading cause of death for people under the age of 45.

Of course, a lot of people notice this. And back in 2016, the National Academies down in D.C. generated a consensus report, and set forth this goal of minimizing those deaths and bringing them -- bringing preventable deaths down to zero. So they called this, Mission Zero.

If we look at progress on that, unfortunately preventable deaths have only risen since 2016. So this was and remains a problem. After that -- traumatic injury after that event, patients on the immune side of things, which is what we look at, suffer from both activation and immune suppression conditions making them a very interesting and complex patient population.

And if you talk with any clinicians that

work in the emergency department, they will see that they meet patients on their worst day of their life. And bottom line is, even if they get through those first few golden hours and through that survival window, there's a lot -- there's a long road of recovery that they have to deal with.

So when it comes to recovery from soft tissue injuries, which is what we work on, current standard of care is skin grafts, muscles flaps, and all of those come with donor site morbidities.

If you have other organs that are damaged, there's limited availability of donor organs. And then, in addition to these accidental injuries, surgery also causes problems. And so, we have accidents, but then also just day to day procedures cause tissue damage.

And so, our lab at the NIH focuses in on this problem both from a fundamental level looking at basic immunological discoveries, understanding how the immune system interacts with both injuries, and the materials that are implanted to treat those injuries.

Those mostly we use mouse modeling. But we also go ahead and apply those fundamental discoveries with a goal of developing new materials to help with wound healing and tissue regeneration. And this is all in this platform of clinical discovery and translation because ultimately speaking, we want to make therapeutics for humans, not just mice.

So we had a mission statement. Our goal is to understand these fundamental mechanisms of how our immune system responds to injury and medical device implantation, and how we can engineer those immune results to promote tissue regeneration and recovery from traumatic injury. I forgot I added a little pizzazz on that slide.

So I got the keys to my lab in late January of 2020, which is really great time to start a lab. And I go back to this slightly modified quote of, smooth seas never made a good captain.

So if you do any Googling, you might find some COVID work from our group because like

with everybody with a lot of -- you hear at the FDA, we pivoted. We tossed our hat in. We used that engineering approach to solve an immunology problem, and we worked on those SARS-CoV-2 serosurveys. However, that wasn't our goal coming in.

Our goal coming in was immunology in the context of traumatic injury. And luckily working at the NIH, we'd be able to pivot back. And one really exciting and kind of a lucky collaboration that we had was inside the government.

We were actually approached by the Department of Transportation that was doing a drug study in trauma patients during the pandemic. And they were curious about SARS-CoV-2 incidents. And so, we had this wonderful intersection of our SARS-CoV-2 work, along with the trauma work.

And so, ultimately we did do some work on SARS-CoV-2 seroprevalence, and in this population we did find a difference in comparison to the regional studies from that same time period.

But we were sitting on a pile of samples. We were getting SARS-CoV-2 antibody data. But there was so much more we could learn from those samples. And so, we had this very large collaborative effort from multiple trauma centers that were external to the NIH.

We worked with a contracting company. Ultimately those samples were routed to the NIH where we collaborated with other institutes, including NIAID. And also our friends in DoD across the street to understand the immune response to human trauma to help inform some of our biomaterials design work.

And so, we were looking at a 1,00-patient cohort study on human immune response to traumatic injury. A whole lot of clinical data in this data set, but ultimately speaking, we have a variety of different injury sources. So things like motor vehicle crashes, falls, gunshot wounds. And then, we also have a variety of injury types. Lacerations, fractures, and the like.

When we evaluated 59 different proteins,

we could actually find some new trauma response proteins. And some of them were really interesting. So overall, we found some of the major players.

So if you look down at this graph, IL-10 is up in trauma patients. That's a very standard thing that's noted that IL-10 kind of dampens the immune system. However there were a couple that had been previously reported, and one of those that was really cool for us was IL-29.

Mostly we had a hunch that it might be interesting, and we couldn't find anything in the literature with IL-29 and trauma. There was one paper on IL-29 of substance.

This was an interferon lambda. It is a cousin of interferon gamma. It has some structural similarities to IL-10. But it was really interesting because it was one of the highest upregulated. So IL-29 here is third, just past IL-10 in this, and it hadn't been described before.

What was even more interesting is it

wound up being a predictor of survival. And so, people with higher levels of IL-29 actually had a higher likelihood that they would survive their traumatic injury.

And when we wound up combining a little bit of machine learning, and then ultimately deciding that fractions were easier, we could develop this algorithm which we call, VIPER, which had five different proteins.

And when we combined those in this equation here and we evaluated that score in trauma patients, we found that it was quite predictive of patient survival. And so, we called it VIPER to get it across an editor's desk.

And then, we're looking into some of these proteins and the potential functional roles of these in terms of trauma recovery and therapeutic application. And so, we're really excited about that.

And this is a huge, you know, systemic study. We're looking at blood plasma. And if we think about those systemic responses, of course,

they can alter bodily function. There's this massive surgical corticoid, so there's this huge systemic immune response when you have trauma.

But ultimately speaking, the injury context can really change that local immune response. And so, I'm a big believer in the tissue-specific immunity. And so, a response in the skin is not going to appropriate for a response in say the nerves or the eye.

And so, ultimately those local immune responses really matter in wound healing tissue regeneration. And this is something where we moved into our mouse model to start digging a little bit deeper.

And so, we work primarily on skeletal muscle. And I really love this figure because it shows the complexity of the immune response in skeletal muscle repair. And so, early on we have skeletal muscle damage and signals, such as interferon gamma which is a more Type 1 inflammatory response can stimulate myoblast proliferations.

So stem cell proliferation, you need stem cells in order to regenerate tissue. However later on, that's got to shift to a more Type 2, or regulatory immune response, in order to get fusion of these myoblasts into myotubes to form new muscle fibers.

And so, what's really cool here is we kind of have this pattern. We have an immune pattern that needs to be solid to help heal and regenerate tissue. So it's not just sighting off of those infections, but it's actually promoting the reconstruction and regeneration of the tissue itself.

And this is kind of the general dogma of wound healing, which is an early Type 1 inflammation, followed by a Type 2, or regulatory inflammation to resolve. And one thing that really bothered us.

Do you see this huge mass? And you think about trauma as massive injury, a massive insult, and it's pretty instantaneous. Whereas, a viral infection will take some time to build up.

A car crash injury is usually less than a second.

And so, if we look at this and think all of that inflammation is going on, how do we not get autoimmunity if you have this massive disturbance in homeostasis? There's a massive disruption in your tissue. What mechanisms by which has our body made to go in and handle these traumatic injuries and make sure that we don't start attacking ourselves?

So just as a bit of refresher on immunologic cell tolerance, or kind of the way that our body knows what's us and what's not us, this happens -- tolerance largely happens in the thymus system. It's an organ that is above the heart and happens before birth.

This transcription factor called AIRE is activated in these cells called MTEX. And basically, it presents a lot of cell proteins to T cells. If they react to it, they can either be deleted or turned into Tregs. Furthermore, there are patients that have a defect in AIRE, and those are associated with autoimmune diseases.

And then, peripherally, so outside of the thymus and the periphery, there's a variety of different cell types that are at play in maintaining this peripheral tolerance, and those include regulatory T cells or Tregs, conventional Type 1 dendritic cells or cDC1s, and plasmacytoid, DCs or pDCs.

So there's been a lot of prior work on immunology and Tregs in muscle damage. We certainly aren't the first. A lot of these detailed mechanistic studies involving these two models.

The cardiotoxin, or CTX-based injury model, or freeze injury cardiotoxin is an injection of the snake venom. It causes innervation and degeneration. And freeze injury is exactly how it sounds.

And then, in terms of what's been discovered, Diane Mathis's group has been a real pioneer in this area. And they've shown that Tregs respond in an antigen-specific fashion. So they were responding to cell proteins to go ahead

and help repair this muscle tissue. And these are canonical Tregs characterized by Tox CD3 and CD4 expression.

Others have shown that exercise induces Tregs. And if we think about exercise, exercise has muscle damage. So there's multiple types of muscle damage that have been associated with Tregs. And you can get even more details, and go in and find different proteins that's basically associated with the Tregs that are important in these processes.

But the great thing about these bio models and why we lean on them so much is that they're predictable. They result in complete regeneration. So when something goes wrong, you can tell it's gone wrong. They're easy to evaluate in a world of complex immunology, having something that's a little bit more straightforward as far as an outcome is wonderful.

However how applicable are these models to the clinic? So when we're thinking of biomaterials, and we're thinking of that clinical

translation element, let's take a look back that 1,000-patient cohort that we had, and look at the ICD-10 codes.

What about toxic cachectic snake venom? How many of those patients have snake bites? Zero. And we're looking at about 7,000 to 8,000 per year in the United States. What about freeze-based injuries? We also had zero. 2,000 to 3,000 in the United States per year.

What about physical traumas? Anything that induces physical tissue damage. 992. 99 percent of those patients came into the ER with physical tissue damage ranging from motor vehicle crashes, crush injury. We also had some questionable decisions with fireworks in the mix. And so, the number per year in the U.S., 24.8 million.

Now if you remember, I said that surgery also causes trauma. We have 64 million surgeries in the U.S. each year. And materials are often implanted to help heal wounds and fix these damaged tissues.

So we need a complementary approach. Not one or the other, but we need a complementary model to go along with those basic biologic models to really understand what might be happening in the clinic and in patients.

And as I was mentioning, surgeons put a lot of stuff into people. And so, when we look at repair wounds, we have biologic products for wound repair. So this is an example of decellularized extracellular matrix, or collagen-based scaffolds. This is being put into a diabetic foot ulcer.

Those materials have also been used in larger traumatic injuries, such as volumetric muscle loss. This work is being pioneered Steve Badylak at the University of Pittsburgh.

There's also synthetic materials that are implanted for reconstruction, not necessarily designed to integrate with the tissue, such as breast implants and gender-affirming care.

Here you can actually see a negative immune response against breast implants. So this is what's called a foreign body response. And so,

you can see the fibrotic encapsulation of these materials. So the immune system matters both for the integrating and healing, but also the ones that don't.

And as mentioned, anytime you have a surgery or an implantation, it causes trauma. If you've ever been in the OR and watched a surgery, it's especially if you've been in an orthopedic OR, it's not a gentle procedure per se. So you're generating that tissue damage, even though it's not an injury per se.

And so, what we used is we use a volumetric muscle loss, or a VML model. It is a surgical excision model, so we go in and we physically remove muscle tissue. And then, we go ahead and we -- and it's a permanent defective muscle function.

So this is about 30 percent defective muscle function here, and we go in and we implant two different materials to hit the immune system pretty much with a sledgehammer. This is our version of generating that binary in a non-binary

world.

And so, we have a material that's been associated with positive outcomes in wound care and regeneration, which is the extracellular matrix, or this ECM-based biomaterial here used in hernias, diabetic foot ulcers, things like that.

And then, we have polyethylene, which is a hydrophobic polymer. Great when that stays together, but when you have where particles are breaking off, you get inflammation and fibrosis. And so, if we compare these two and figure out what makes the good, what makes the bad, we can identify targets for rationally designed therapeutics.

And so, what happens to wounds without cell tolerance? Let's go back to that gene, AIRE, the one that's responsible for central tolerance. And so, we'll go ahead and take their approach of breaking it and seeing what happens. So let's look at a mouse that does not have AIRE.

So down here where you've got the pink, which is your muscle tissue, and here is your

inflammatory front, and this is the polyethylene material. Here's your wild-type mouse and here's your AIRE in knockout count. You see this massive expansion in that inflammatory front, which was confirmed with CD45 DNA. So we have a massive immune infiltration.

Furthermore it's made worse with materials. So we can see that the inflammation here, just in the injury itself, in the AIRE in knockout mice. However the second you add that polymer, you have a massive induction of inflammation that's much worse that your wild-type mouse.

It's probably one of the worse genotypes I've ever seen in a knockout mouse. And so, I jokingly said, here I am. Don't put plastic in people without cell tolerance. Generally the body doesn't like it. So a big takeaway.

Here is cell tolerance, kind of duh takeaway, but cell tolerance seems critical for wound healing, and especially with material implantation. So what are some mechanisms of this

tolerance in normal mice?

So I'm not going to dig into this story because it is published. I mean, I would love to chat with you guys about a little bit of our unpublished work. But one of those cell pics that I had mentioned previously, we wound up investigating a bit more in this model because polymerogenic CDC1s are these classical dendritic cells.

And what we found was when we treated it with a material that really promoted the regeneration, we had enrichment of these cells where it's that fibrotic inflammatory material. We had enrichment of other dendritic cells, not so much these CDC ones.

Furthermore, when we looked online at cells that had previously been implicated in this repair process, we saw that this affected T cell activation, and they also affected MP, macrophage polarization.

Now both of these have been implicated in the repair process. And ultimately, if we

knock out the CDC1s, we get defects in this tissue repair, defects in the biogenesis. We get fat appearing where it's not supposed to be. We get calcifications appearing where they're not supposed to be.

If we look the other way and we look at what might be recruiting them, we wouldn't know how to take inspiration from tumor literature and said hey, if NK cells can bring in CDC1s in a tumor, why won't it be the same in wounds? And it was.

So we found a recruitment of NK cells that produce this chemokine XCL1 that correlates with this XCR1 expression on these eugenic cells. And furthermore, if we stimulated NK cells with damages associated with molecular patterns in vitro, we could go ahead and get up regulation of this gene expression suggesting you've got tissue damage which stimulates NK cells to create XCL1 to bring in your CDC1s that ultimately affect the immune activation in the environment.

So basically, we've kind of found a new

pathway for cell tolerance in immunoregulation after traumatic injury using this very intense injury model. And one thing that we had seen in this study, which was kind of like, huh, interesting moment that we decided to dig in a little bit deeper were these weird regulatory CD8 positive T cells.

And why we were interested in those is CDC1s are capable of this process known as cross-presentation. Basically they take antigens from outside. Instead of talking to CD4 T cells, they actually talk to CD8 T cells.

And so, when we look at these CD8 Tregs, we can think about some of the past and history. They are controversial. Some people think they exist. Some people don't. So I'm going to present you everything we've got.

Basically they were first described in the 1970s. They're characterized by the expression of CD8 Helios and Ly49 inhibitory receptors in mice, or KIRs in humans. They have been associated most prominently.

Mark Davis's group at Stanford has chatted about them in autoimmune compensation after CD4 reg depletion, and the mouse models multiple sclerosis, also in severe COVID-19 where you have lung damage. And then, by another group in transplant immunology.

So in our group, we have both RNA sequencing and flowcytometry. We do see them. They express Helios. They express these inhibitory Ly49 receptors, and low levels of CDA.

I will say that there are CD3/TCR beta positives. CDA is negative, and Ly49 inhibitory positive cells, and there are two. So something else is in there that kind of looks like them. And we found these in muscle, blood, and lymph nodes of mice.

I will say they are very sensitive to sample processing stuff. I'm pretty sure if you look at the tube wrong when you're prepping them for flowcytometry, these antigens fall off. So that is a possible explanation of not seeing some of these things, but we do see them with RNA C10

flowcytometry.

So ultimately, we don't really care what you call them, we just care what they do. So I'm going to refer to them CD8 regs, but this is the cell population that I'm talking about today.

So what have we found out? Or specifically, a post-doctorate fellow in the lab of this year found out about these CD8 regs. So when he went ahead and looked at the gene signature, he found out that they shared gene expression patterns with canonical Tregs to the ones that Diane Mathis's group had implicated in muscle regeneration. And then, also natural killer cells. So again the killer in the regulatory phenotypes.

Furthermore for those interested in two-cell receptor sequencing, they were very diverse, and they were correlated with self-reactive motifs. So previously, work published in immunology found that hydrophobic residues at P6 and P7 of the CDR3 were associated with self-reactivity, and we see that really

highly enriched within the CD8 reg class.

When you look at the different types of T cells, and you look at the clonal expansion, you can see that your standard helper Ts, your standard Tregs, your standard CD8s, they all are clonally expanded. But then, down here this cluster Number 6, those are CD8 regs. So they're not clonally expanded. They've got a very diverse T cell receptor chart.

And then, of course we want to know, are they existing in human muscle? And so, when we dug into a published single-cell earning sequencing data by Ben Cosgrove's group, we took a look at the CD3 positive population.

And within the CD3 positive population, we looked at those. They were positive for these KIRs, which are equivalent to those Ly49 receptors in humans -- in mice. We did find them pop up within this subgroup. So they are also present within human muscles.

Unfortunately not too many people want to give up their muscle for single-cell

sequencing. A little bit harder than tumors, so the data availability is something that's lacking on the human side. But we are working with some trauma centers trying to build that up a bit.

And so, ultimately our theory was potentially these CDC1s and these CD8 regs were communicating. So what evidence did the CD8 find that this might be true? First off, they expressed the proper protein machineries. So they have the tools to talk to each other.

So again, we were using single-cell RNA sequencing, and we were using flowcytometry. We found that these CD8s expressed things like the CDC1s, expressed Qa-1, which is the MHC-I that CD8 regs recognize.

They expressed them cytokine and chemokine receptors that overlapped. And ultimately, they had the costimulatory molecules, and the adhesion molecules necessary to form an immune synapse.

Furthermore because an immune synapse is a cell communication event, they have to be in the

same spot in order to talk. And so, when we look at spatial RNA sequencing, we could see that there was spatial colocalization of genes associated with CDC1s and CD8 regs within mouse muscle tissue.

We are working on protein level with immunofluorescent staining. The available antibodies are not the best for this, but at least on the gene expression level we were able to see this colocalization. And it is more so than other cell pairs in that environment.

And then, functionally if we go ahead and talk things into a dish, and I promise those all have stars, and we look at T cell proliferations with APC T cell culture, look what proliferates, and we look at CDC1s with damage associated molecular patterns, and no PMPs, no LPS in this, this is just muscle homogeneous. This is just injury gamish (phonetic).

We see that these light red bars which are CD8 regs, they are the ones that preferentially proliferate in comparison to things

like these CD4 T cells which are way down here. And so, they actually preferentially proliferate even over the canonical CD8s.

So we wanted to then figure out how close this phenotype was to any sort of autoimmune response. So Aditya Joysyula, who is a post-baccalaureate fellow in the lab, ran some bulk RNA sequencing on the AIRE knockout mice, chose these autoimmune mice, as well as BADIS3 knockout mice, which lack the CDC1s.

And we saw that the BADIS3 phenotype was almost completely recapitulated within that autoimmune phenotype. If we went and looked at gene set enrichment of this -- so this is kind of correlative thing, what about gene function? We saw that in the immunoregulatory interactions. So that kind of immunoregulatory phenotype was also decreased in the BADIS3 knockout, so in the ones that don't have CDC1s in comparison to our wild-type mice.

So what this tells us is that the mice that lack CDC1s, which we previously showed can

prime CD8 regs, have an autoimmune-like phenotype and decreased immunoregulatory interactions.

So how are these doing what they're doing? We had an idea, which is basically regulating pathogenic B cells after trauma. This would line up with some of the work that was done in the infectious disease world on these cells. And so, we're digging into this.

When we look at the lymphocyte compartments within the different treatment groups, we can see that the B cell, especially these B2 B cells, so antibody producing B cells, greatly expands. And the more pathogenic environment, this is the pro-wound healing. We hardly have any of them.

And then, when we look at the B cell receptor sequencing, we can find some shared sequences. Interestingly, the most prevalent sequence was the most prevalent in each treatment group.

So there appeared to be some possible non-pathogenic sequences there. But there were

also clones that were specific to the polyethylene treatment group. And mind you, this is just a plastic going in. This is not any proteins or anything.

And so, these clones must be against cells. I will put an asterisk there, or microbiome. Of course, other stuff are in there. But this is not against the material we point in.

And so, again we were wondering if these CD8 regs could potentially be eliminating pathogenic B cells either by directly eliminating them or eliminating the antigen-presenting cells without T cell help.

Now this is working on in vitro killing assays for these, but technically a nightmare because everything just wants to die before we even get to the assays. So we're working on it, and our cellular isn't the best from mouse clubs.

But what we could do is go ahead and look back in vivo and try and see if we were getting in the right direction. And so, when he went ahead and looked at B cell prevalence in CD8

knockout mice, he could see that there was actually a significant increase in B cells in the injury site with that pro-regenerative material treatment, the one that didn't have any B cells, in the absence of CD8. So this suggests that the CD8 compartment is regulating B cell presence in wounds.

So what about other wounds outside of the muscle? All we've been talking about so far has been skeletal muscle. So Aditya went in, and he looked at different single-cell RNA-seq databases.

He took our data and this different -- and this gene signature, this blue cluster here for the CD8 regs, and could find that gene signature elsewhere in things that are wounds, but not really thought of as wounds.

So for example, the mouse myometrium in a preterm birth model. And so, this starts to overlap with pretty much tissue damage. And so, we have these CD8 regs that are present here, and we found them in other data sets. And as I

mentioned, we are in the process of collecting local tissue from traumatic injury to go ahead and get some human sample data as well.

So ultimately, what I hope I've convinced you of is in this giant network of cells that we've found, we are seeing either direct killing of these B cells, or at least a regulation of these pathogenic B cells by these regulatory CD8s that are primed by CDC1s, and we hope to kind of flesh out the pathway a little bit more and fill in those pieces as we get some more data.

And so, big picture, CD8 regs are primed by CDC1s that respond in a polyclonal fashion that they represent a rapid cell tolerance pathway that evolves from traumatic injury.

Particulate hydro polymers exhibit exaggerated responses in mice without cell tolerance. And going back to the comment of, don't put plastic in people without cell tolerance, we think of things outside of medical device implants, and I'm sure you all have heard of microplastics.

And so, we can start to think about and expand this beyond the traumatic injury sense, and think about those smaller tissue damages, and other potential triggers of autoimmunity that might relate to these more extreme tissue damage scenarios that we generate in the lab.

And so, with that I want to give a massive thank you to everyone in the lab. But most of the work done today was completed Dr. Aditya Josyula and Daphna Fertil. Daphna is applying to PhD programs this fall, so snag her. She's fantastic.

Also a massive thanks to the alumni from the group, collaborators in NIBIB, others at the NIH as well as their extramural collaborators, especially from the trauma centers.

I want to thank the patients from the trauma centers. Without them, of course, the work could not be done. As well as my tenured advisory committee, and my mentors that have never stopped helping me out after my training.

So I'd be more than happy to take

questions now, or you are welcome to email me with questions later. Thank you.

SPEAKER 1: So I come from Pennsylvania. And thank you so much for a really exciting and informative presentation. I was just asking like you said, don't put plastics. But we do see that for knee replacement and always put metals. So do you have any comment on that?

DR. SADTLER: Yes. So I said, don't put plastic in people without cell tolerance. So I will flag that. So what I think happens is probably there is either some sort of genetic predisposition or environmental trigger that happens before that event that is what kind of gives that two-hit or multi-hit to lead to autoimmunity.

You know, I've got pierced things in my ears, right? So I've got medical devices in me. Most of us have something. So most of the time, it's fine. We're really interested in kind of when it's not, or when it's flipped to not, or when those materials kind of get more fibrotic.

So one example is women with breast implants. Some women have complaints of autoimmune-like syndromes when they have breast implants. However, when those implants are excised, those symptoms resolve.

And so, that's just being kind of investigated. So it does suggest that it might be a reversible phenomenon. And so, it's certainly much more needs to be evaluated, and I think there's going to be a genetic component.

There's going to be multiple environmental components. But if we can figure out the underlying biology, we might have a better chance at treating those people that are dealing with autoimmune-lie conditions.

SPEAKER 1: So do you think even the external -- again also like for any artificial legs and artificial hands, they would also be having some of the same things?

DR. SADTLER: So it depends on the application. And I will say again, all these medical devices have gone through lovely folks

like your offices to make sure that they're safe, so I definitely don't want to suggest that they're not safe.

With external implants like protheses, some have integrating -- so for example, bone integrating materials to help mount that prosthetic. And so, this would be applicable to anything that is in contact inside the human body.

And again, a lot of us have medical device implants that are fine. It's just a question of those folks that say something goes wrong. And it might be learning from those folks when something goes wrong, which helps improve implants for everybody too.

SPEAKER 1: Thank you.

DR. VILLA: Thank you. I have a question for you. So when -- I come from the office of blood. When I think about trauma, I think about hemorrhagic shock and transfusion. And there's increasing emphasis on pre-hospital transfusions, even of whole blood.

And so, do you think that if you're

getting allogeneic blood product in the setting of a traumatic injury that that could modulate some of the immunology that you're seeing?

DR. SADTLER: Certainly. So I think anything that modifies that -- and I will say we are working with folks. So while most of our work has been a little bit later on in that kind of regenerative reconstruction phase, we are working with some folks over at Department of Defense on some of those initial kind of traumatic and shock instances to understand a bit more about what's going on there.

And so, I'd say yes. Any time you're kind of modulating that a bit, you're going to have some sort of change. I think that there's probably going to be a very overwhelming hemorrhagic response from the host that receives that kind of blood volume, and that hypovolemic response which is going to possibly just take over those early stages.

But if there's any sort of persistence with those transfusions and things like that,

that's something where it could come into play. But I am not aware of data on that, but that's also outside my field.

DR. VILLA: Is there any shock or hemorrhage in the model that you used?

DR. SADTLER: We do not, but we're working with some folks that do use shock and hemorrhage models. So our goal is to start integrating some of those variables with it. We avoid hemorrhage right now, so this is just kind of a physical tissue damage.

The other thing that we're trying to integrate with this is ischemia. So a lot of times if you've got a large volume metric injury and you've got the blood loss, things are torniqueted and you have a massive ischemia profusion of that.

So we also have -- kind of other projects, including an MD PhD student that's working on some of the ischemia re-profusion damage because not only do you have the physical tissue damage, but you've also got downstream

after that tourniquet comes off.

DR. VILLA: Thank you very much.

DR. SADTLER: Thank you.

DR. KHOSHI: Any more questions? All right, thank you so much Dr. Sadtler.

(Recess)

DR. VILLA: All right, now we have three speakers from CBER, Dr. Joseph Jackson, Dr. Kyung Sung, and Dr. Alex Zhovmer. I will give a brief introduction of each, and they will speak one after another. And then, we will have a Q&A at the end.

Dr. Jackson is a staff fellow in the Laboratory of Cellular Hematology, the Region of Blood Component Devices, Office of Blood Research and Review at CBER. Dr. Sung is the Chief of the Cellular and Tissue Therapy Branch in the Office of Cellular Therapy and Human Tissues at the Center for -- at CBER. And Dr. Zhovmer serves as a Principal Investigator in the Laboratory of Immune Biochemistry at CBER.

Please welcome Dr. Jackson.

DR. JACKSON: Okay thank you. So I would like to thank the Center management, and also the Center moderators for inviting me to speak today. I am a staff fellow in the Laboratory of CBER in the Laboratory of Cellular Hematology.

And I would like to talk today to you about our evaluation of 405 nanometer visible blue light as a novel pathogen reduction technology for plasma and platelets. And please note my disclaimer here.

So in the U.S., there are more than 14 million units of blood transfused on estimate every year. And available blood products include ex vivo-stored plasma, platelet concentrate, and packed red blood cells.

Standard donor-screening questionnaires exist prior to donating blood to assess the risk of transfusion transmitted infections, and each unit of blood donated in the U.S. is routinely screened for various infectious disease pathogens using FDA-approved assays.

The U.S. blood product supply is safer than ever before due to the use of standard safety measures, and although residual risks do exist due to new and emerging pathogens and unexpected bacterial contamination.

And so, a proactive approach continues to enhance protection through broad spectrum and activation of pathogens in blood products with an additional layer of safety. And this was termed as pathogen reduction technology.

So some of the currently approved or developing technologies that utilize UV light exposure are shown on this slide. The first is the intercept system which uses Amotosalen as a photosensitizer in conjunction with UVA light exposure, which is in this technology, is approved in the United States.

The Merisol System, which is approved in several European countries, uses a similar methodology of exposing products to UVA/UVB light in conjunction with chemical treatment of riboflavin.

And the third developing technology is the Theraflex System which exposes product TVC. And the concept here is simple, that bacteria viruses in parasites have genetic material, and the replication can be stopped by interfering with their DNA/RNA replication through chemical and UV treatments.

Although this does have some unintended consequences -- so for example, one report here demonstrates that in plasma, the use of pathogen reduction technology is associated with a significant decrease in coagulation factor activity. And in platelets, it's been demonstrated that UVA exposure can harm platelet membrane integrity, signaling pathways and the function of micro RNAs.

Thus leads us to our rationale of using 405 nanometer visible blue light as a novel PRT developed here at CBER. So visible light treatment of blood components could potentially offer a safer alternative to UV light methods while preserving product functions, and see to an

activation of pathogens without external photosensitizers are possible, which is in contrast to that of UVA and UVB methodologies.

405 nanometer light also has success in bacterial wound healing, and as well as surgical instrument sterilization, and has been shown to activate bacterial endospores.

And 405 nanometer light can been used at levels that are lethal to microorganisms without harming exposed mammalian cells. And therefore, based on this rationale, we initiated our evaluation of 405 nanometer light as a potential PRT.

So now I want to move onto show some proof of concept data on 405's light -- 405 nanometer light inactivation of bacteria, viruses, and parasites. So in collaboration with our group -- a group in the U.K., we demonstrate here that 405 nanometer light has antibacterial activity in plasma.

So in this experiment, a variety of different bacteria where you see were spiked into

plasma at low, medium, or high titer concentrations, and then exposed to blue light, or not exposed as a control.

And you can see that for the majority of the bacteria tested, there is a significant reduction, nearly 100 percent inactivation of all bacteria when exposed at a light dose of 360 joules per centimeter squared.

405 nanometer light has also been demonstrated to be antibacterial in -- when bacteria is spiked into platelets. So this example data here, we spiked 200 milliliters of human platelet concentrate with a low density of staphylococcus aures, and then treated the samples with light. And you can see we have a dose-dependent reduction in surviving bacterial load.

I want to move on now to demonstrate 405 nanometer light's inactivation of viruses. So here, we show that 405 nanometer light is effective against HIV-1. So in this experiment, 10 nanograms per mill of p24 of HIV-1 was spiked

into plasma, followed by treatment with light or not as a control.

And you can see here within five donors, our control levels we see high levels of HIV-1 p24, which is -- we don't see any differences with just 30 minutes of light treatment. But by five hours of light treatment, we have a significant reduction in p24 levels. And this equates to a dosage of 270 joules per centimeter squared.

On a similar note, we also demonstrate that 405 light is effective against HCV. So an HCV sub-culture strain was spiked into both plasma and platelets, followed by treatment with light, and then co-cultured onto Huh-7.5 cells to allow for the subsequent focus from the units to be assessed.

And you can see, compare it to the unexposed samples, which are shown in blue. Exposed samples have a significant reduction of HCV levels, even within a dosage of 162 joules per centimeter squared.

Now I'd like to move onto discuss how

405 nanometer light can inactivate two different parasites. So the first here is -- it's an activation against T Cruzi, which is the causative agent of Chagas Disease.

So here on the left, you can see that if we inoculate T Cruzi onto MK2 cells for six days, either after untreated or with light treatment, you can see that there's a significant reduction in the number of T Cruzi parasites in our light-treated group as compared to that of our control.

And this has been -- this is also quantified. We demonstrate here that T Cruzi levels are decreased a significant log reduction after five hours of light treatment in both platelets and plasma. And we also have demonstrated that in a mouse model of Chagas Disease, there was no infection 180 days post-inoculation in our light-treated group.

And finally, I want to demonstrate 405 nanometer light effect -- an activation effect on the Leishmania Donovani. So in this particular

case, RAG2 mice were inoculated with human plasma that was spiked with Leishmania, and either treated or left untreated, and allowed to infect the mouse for seven to ten weeks. Upon which the spleen and cervical lymph nodes were harvested, and Leishmania was quantified.

And you can see for the both the spleen and the lymph node organs, the presence of Leishmania is much higher in our untreated control group, and significantly reduced in our light-treated groups.

So overall, I've demonstrated that 405 light has an activation effect on bacteria, viruses, and parasites. And the mechanism is thought to be due to the -- it's thought to be due to the excitation of photosensitizers within the bacteria or parasite cells, such as porphyrins or flavins. And these allow for the production of reactivate oxygen species, which subsequently damage the cellular integrity.

But what about cases acellular pathogen cells such as viruses? Well research suggests

that flavins or other photosensitizers are associated with viruses from cell culture -- from the actual cell or cell culture media.

So here, you can see that when we use a fluorescent indicator on reactive oxygen species, we have very low levels of fluorescence within out PBS negative control, as compared to when we add our hydrogen peroxide which it serves as a positive control.

And then, if we look at HCV which was stored in DMEM media completed with fetal bovine serum, we can see that especially in our exposed samples, we have much higher Ross levels.

So what about the effect of 405 light on host cells and plasma? So we looked at a variety of different in vitro metabolic parameters for platelets, including platelet counts, but also PH, lactate, glucose, et cetera. But we find that compared to our control, there's no significant difference after exposure to 405 nanometer light.

We also looked at levels of platelet activation in platelet apoptosis by looking at

molecules called P-selectin and phosphatidylserine expression. And you can, again, appreciate here that compared to our control samples, light-treated samples did not induce significant differences in either P-selectin or apoptosis markers, suggesting that light does not cause platelet cell death nor activation.

Another marker that we looked at is platelet function was the ability for platelets to aggregate. So collagen mediated platelet aggregation, again, was not changed between test and control samples.

And we've also recently published that 405 nanometer light treatment of platelets subsequently causes platelet mitochondria to undergo metabolic reprogramming to endure this light treatment.

405 nanometer light also preserves platelets in vivo, survival, and recovery in a SCID mouse model. So in this case, platelet concentrates were spiked with staphylococcus aureus, and were treated with 405 light for eight

hours before transfusion into a SCID mouse. And platelet recovery was followed over a period of 24 hours. And you can see no significant difference between that of our control and our light-treated groups.

And finally, I want to demonstrate that 405 light does not cause untoward harm towards plasma coagulation factors. So in this case, we used APTT or PT-based potency assays to essentially measure the potency of individual coagulation factors labeled here.

And when we compare it to a characterized reference plasma, you can see that for the majority of the coagulation factors that are studied, there were no major differences in each factor.

So in summary, I would like to say that 405 light is an effective microbial cyto tool for the tested organisms in ex vivo-stored plasma and platelets, and that they are not harmful to platelets or plasma with regards to the tested in vitro parameters and in vivo SCID mouse model.

This light source has the potential to be a pathogen in activation technology for ex vivo platelet component safety, and therefore comprehensive evaluation of this technology is further warranted.

There are my acknowledgments. So I would like to thank everyone here. And yes, thank you very much. Thank you.

DR. VILLA: Next we have Dr. Sung.

DR. SUNG: Hi everyone. Thank you so much for joining today's seminar. So I'm Kyung Sung, and I'm from Office of Cell Therapy and Human Tissue. So my lab is really developing different engineered tools for the manufacturer and characterization of cell therapy products.

For today, I'm really going to focus on -- to talk about some of the advancements in in vitro systems that we've developed, with the goal of enhancing the functional assessment of the cell therapy process which are really complex and heterogenous.

So let's see if I can figure this. Okay

so this slide shows the product. They are regulated by the Office of Therapeutic Product. And as you can see, these OTP really regulates the diverse of the product, that includes gene therapy products as well as the cell therapy product.

So when I say the cell therapy products, I typically refer to those highlighted in the red box, the stem cells and stem cell drive the product. And the functionally mature and differentiated cells, such as retinal pigment epithelium cells or chondrocytes and keratinocytes, and also the combination products such as tissue engineered product.

So let's look at a typical cell therapy product manufacturing process. So typically, the cells are first isolated from donors, and when donor and patients are the same, the product is autologous and the donor -- and when the donor and patients are different, then they're an allogenic product.

And of course, for the allogenic product, the donor eligibility screening and

testing is really important to minimize those unwanted cyto trials. So when -- after cells are isolated, they are typically expanded at the manufacturing site. And sometimes they are stored in cell banks until they are needed.

And then, it goes through the final formulation. And for tissue engineered products, they are many times seated in certain biomaterials or scaffolds. And then, it's administered into patients.

So it looks pretty straightforward and simple process for the cell therapy of manufacturing. But when you actually look at the manufacturing process, people do a lot of testing from the source material, cell bank, and in process testing, and also the testing for the final product to release.

So it's a lot of work. And the in process testing and final product release resting is really important to make sure that the manufacturing process is well controlled. And then, people can produce the therapeutically

effective product consistently.

And the point I want to make from this slide is that FDA is really flexible in terms of the assays that people choose to use, and the type, and the level of testing are really product dependent. And these testing and strategies can be really improved with defenses in the regulatory science.

So what are some challenges in cell therapy product characterization? And in my opinion, really one of the main challenges is cell therapy characterization is the lack of the standardized and relevant testing for both in process and release testing.

For many cell therapy products, the cell populations are really heterogenous. And you know, there is one image that I just put on this slide that only captures about ten cells. But you can tell that they all look different.

So the cell populations are really heterogeneous, and this cell heterogeneity is typically influenced by the donor and tissue

sources, and also the, you know, many things that happen during the manufacturing process, and also the recipient condition.

And this heterogeneity really complicates the development and the qualification of the characterization assays. And for both in process and release testing, many people still rely on very traditional assays, such as viability measurements and cell proliferation assays, and certain surface marker expressions.

And these assays are very meaningful, and they provide really good information on the cell product. But many times, I think that these are not sensitive enough to capture the heterogeneity, and the complexity of this cell product.

And because of that, I think they often fail to capture the therapeutic relevance and the functional potency of the manufactured cells. And so, many cell therapy products typically involve multimodal mechanism over action, or many times the mechanism is unclear.

So because of this uncertainty, it is really difficult and challenging to develop a quantitative assay that are sensitive enough to detect really small functional differences between the cell batches.

So let's say if we are -- if some person is using a testing that is not sensitive, or that's not really relevant, then it is highly possible that, you know, a batch is incorrectly characterized as potent or not potent which really impacts the reliability and consistency of the manufacturers of products, which is a big challenge in cell therapy manufacturing.

So these are the challenges that my lab is aiming to address for cell therapy manufacturing and characterization. And as I mentioned on here, there are inadequate markers that are predictive of the cell phase and cell fate.

And then, there is typically a poor understanding of how cells interact with their microenvironments. And also there is poor

understanding of the cell fate and survival post the transplantation.

So through a regulatory science project, our lab is really aiming to develop and improve assessments for the cell therapy products, the characterization, and also potency assessment. And we also aim to identify the product attributes that are more predictive of fate gene effectiveness.

And on the next slide, I'm going to talk about an example of the assay that we developed in the lab using micro physiological system to predict the vascular gene and engineering a potential of the mesenchymal stromal cells, MSCs.

And this study was led by Dr. Johnny Lam who used to be a scientist in my lab. And that these two studies were published in the two papers that I listed on this slide.

So the MSC is a really popular cell therapy cell source for a cell therapy manufacturer because it can be isolated from different tissue sources, and can be directly

differentiated into an adipocyte, or a chondrocyte, and also the osteoblast.

But it also produced a lot of paracrine factors for immunomodulation and the vessel regeneration, angiogenesis, and vasculogenesis. But then, the challenge is really it uses a multimodal mechanism of action, and it's one of the cell lines that's really poorly characterized. So it's really difficult to have a controlled manufacturing process, and also the well characterized release assays.

So for the vessel regeneration, what I have seen so far is people typically use ELISA to quantify the regular secretion from MSCs, or other pro angiogenic factors to confirm the functional activity of these cells for vasculogenesis.

And like I said earlier, I think these assays lack sensitivity to detect the small changes in the product release which could lead to the inconsistent outcomes. And also, there is concern about the inconsistent release of the angiogenic markers because of the heterogeneity of

the MSCs, which could really complicate the standardization of the quantitative assays.

So in this project, we wanted to develop quantifiable but sensitive assays that can really predict this vasculogenic functional activity of MSCs. And we used this really simple micropaedic system to develop these assays.

And I have more explanation in the next slide. And the image on the bottom right corner is the vasculatures that are formed within our devices. And we collaborated with a curator to develop this micropaedic system.

So we're using this one system for post vasculogenesis and angiogenesis, which are similar but they're not the same. So the vasculogenesis are more of the novel formation of the blood vessel from endothelial progenitor cells. And this mainly all occurs during angiogenesis.

And angiogenesis is the SPROUT formation from the existing vessel. And this commonly happens during wound healing and the treatment of ischemic tissue. So to study this vasculogenic

and angiogenic bioactivity, we use the same system. But then, we change the loading configuration.

For the vasculogenesis, we basically suspend it, endothelial cells in fibro and hydrogel. And then, we injected them into the central channel, which is shown in pink color. And then, we added MSCs on both sides of the micropaedic shown in blue and green. And then, after cold culture, we basically fixed the stain and images of this culture.

For angiogenesis, we injected a blank hydrogel into the central channel, and then we coat the one sidewall of the hydrogel with the endothelial cells to create a pre-existing vessel.

And then, we added MSCs into the top channel shown in the purple. And then, we looked at the SPROUT generation from the bottom of the hydrogel to the top of the hydrogel. And you can see some representative images of the vasculogenesis and also angiogenesis.

And then, we used our lone fibroblast as

our positive control mostly because it is well known that the lone fibroblast can induce a new vessel generation when they are cold cultured with endothelial cells. And then, our negative control was endothelial cell only condition without any stroma cells.

So for both vasculogenesis and angiogenesis, we did some hydro screenings. So we get the MSCs from different donors at two different passages, so they are manufactured differently. And then, after cold culture we image them, and then we did some automated -- the image analysis.

So basically, we quantified 21 different sub parameters that's related to the vasculogenesis and angiogenesis to more quality. And to reduce the dimension, we did the principle components analysis. And then, we found that the principle component 14 captured more than 70 percent of variances.

So we used PC14 as our vasculogenesis and angiogenesis score. So the higher score means

it's more vasculogenic and angiogenic, and with the green is more vasculogenic and angiogenic.

So you can see that we were able to identify some MSC preparations that are more vasculogenic and angiogenic compared to other MSC lines. And you can also see the score for the lone fibroblast and also our endothelial cell negative control.

But what was interesting from this data was that the cells that were vasculogenic were not angiogenic. So you know, even though we are using the same assays, same cells, the assay really needs to be fine-tuned for the context of use, and the end point that we're analyzing.

So for example, the Rb9 cells were very vasculogenic in this analysis. But then, the Rb9 shown in the red box, they are not angiogenic. So that was really interesting. And so, then we looked at a little bit more details about the angiogenic SPROUT generation.

And then, what we noticed is that there are two distinct phenotypes when you look at the

SPROUT quality. There is a T cell dominant morphology that to me looks like more like a cell migration instead of like a real aluminized vessel formation. And there's also the soft cells in the SPROUT generation which is thicker, and it's a more aluminized vessel.

So when we see that these SPROUTS are more polygenetic, it's interesting to see that the Rb9 in our -- the heatmap in that analysis, they are not very angiogenic. But then, when you do the SPROUT for further analysis, they actually generate more soft cells on the SPROUT formation, which could mean that these cells actually form like a more meaningful structured angiogenic vessel, the formation.

So it's really -- we really have to look at the different -- the endpoint to really understand what's going on in our systems. And then, we also need the base angiogenic cell analysis, and tried to correlate that with vasculogenic and angiogenic bioactivity.

And that what we found is that the MSC

group with a higher vasculogenic activity maintained the higher baseline expression of fibronectin and that are coupled with a suppressed expression of angiopoietin and IGFBP family, which was really interesting.

And when we did the correlation, the fibronectin and angiopoietin are two things that were significantly correlated with the vasculogenic activity. And then, when we looked at angiogenesis, like the cells that we looked at didn't really show good correlation.

But then, we detected that there was a statistical significant correlation between HGF expression and then the degree of the soft cell dominant angiogenic expression. And so, the angiogenesis is a more quality system which is quite important. And then, we didn't notice any significant correlation with the T cell dominant angiogenesis.

So in conclusion, we think that we have developed a high throughput of vasculogenesis and angiogenesis bioassay for measuring the MSC

bioactivity. And both vasculogenesis and angiogenesis subbase consistently demonstrated patterns of the heterogeneity of different MSC preparations.

And we noticed that in general, the early passage MSCs exhibited greater bioactivity and produced more robust and urbanized vessel formation. And HGF emerged as maybe a potential T regulator for MSC vascular formation.

And the difference that we observed between Rb9 high vasculogenic for lower angiogenic activity underscored the importance of selecting the right assay. It sounds like the right cells for the intended use.

And really the purpose of developing these assays was to enable the development of more reliable and functionally relevant assays for ensuring the quality and back to back consistency of MSCs or cell therapy in clinical trials.

So with that, I'd like to acknowledge my lab members. Even though I only talked about Dr. Lam's work today -- but we have many different

projects going on in the lab. But hopefully next time I can introduce those as well. And also acknowledge our collaborators. Thank you so much.

DR. VILLA: Thank you, Dr. Sung. Next we have Dr. Zhovmer.

DR. ZHOVMER: Today I'm going to talk about use of advanced cell count tissue systems (phonetic) for immunotherapy testing. And we're working on a food allergen, but that bond cell count tissue systems as you might think may be used not only for food allergen.

Here we have an example of the system we use to analyze the material of cancer cells. And in the blue, you see there is a part that is made of a coagent. And it helps to delineate. We're optimizing base material, and then delineate the material of cancer cells. I don't have to pursue that cancer's important by why food allergens are important, and why we think the system also is important to study the food allergens.

So the food allergen is -- and in general, an allergen is one of the most common

global pathologic conditions, and it affects up to 50 percent of the population in Europe.

And in people, more than half will experience anaphylaxis at least once in their life. Yes anaphylaxis is not very fatal, and only 0.2 or 2 percent of people will die because of anaphylaxis. But what it tells you is that you're almost guaranteed, if you have an allergic experience, a near death state.

So let's narrow down and go back to food allergies in the United States. In the United States, there are approximately 15 to 30 million of Americans, or ten percent of the population, who have a food allergy. And food allergy is recently in the top three causes of anaphylaxis, along with drug and venom-mediated anaphylaxis. And it's disproportionate in kids because kids are responsible for 80 percent on anaphylaxis. And in part because it's very hard to control yourself at this age.

And you can see the blue line, the most common causes of food allergy and anaphylaxis is

milk, eggs, soy, sesame seeds, wheat, nuts, peanuts, fish, and shellfish. And for kids, it's really hard to avoid this allergenic -- these allergens. And this blue line puts the cause up to 90 percent of anaphylaxis.

So for this reason, there is a push to develop a therapy for allergies. And recently, we had only the avoidance, Epinephrine, as a way to treat this state. And additionally, we can think about the use of antihistamines.

But recently, we go to more options, and I can mention the products that can be used for desensitization of monoclonal antibodies. And also, some promising approaches, and a few in cell and gene therapy.

And these approaches, they can be used as monotherapy, and they also can be used in a combination which is going to complicate the regulatory review of this product.

And it's also too hard because I am a CMC reviewer. From a CMC reviewer, it's hard to help to review the development stage of a product,

though we are provided with evidence that support the safety and effectiveness of a therapy.

So the major problem for development process is that it's very long, it's risky, and it is expensive. So ideally, we'd like to make it as safe and risk-proof as possible because it might take you five to ten years, and then it's, okay it doesn't work. I've got to move.

And how it usually starts. It usually starts in vitro. On the left, you can see there is a picture of a mouse, but it's often seen in clinical studies. Whereas the picture of the human is the symbol of clinical studies, but it all starts not in mice or people. It all starts in a cell -- in the lab in a cell culture dish.

And we assume that this is going to work. That hours from the dish is going to show exactly the same outcome as a result from the mice and as a result in vivo. And this is a big assumption.

What we have to do because these cell culture experiments are so cost expensive is so

simple. So this is the development of where therapy starts. And I'm going to show you an example because it's always good to show examples from your personal experience as well.

We came up with -- we have a great idea. Let's develop a therapy. And let's do this exercise, and let's try to make a therapy for food allergies. So how do food allergies start?

This is immunology 101, and it starts with the presentation of an antigen, or allergen in this case. Two of the immune cells and the adrenergic cells. The adrenergic cells are going to present this allergen in the form of epitopes to T cells.

And T cells and B cells are going to interact with whichever source under a specific T cell or under a specific B cell are going to give a license to B cells to convert into the plasma cells and start making the antibodies.

For vaccines, it's very good if you can stimulate an immune response, and particularly if an individual one responds. But sometimes, it can

also stimulate an IgG response, and that's also good if you're talking about, like, protection.

But sometimes, this response is raised against a particularly harmful antigen. So I'm talking about food antigens, which we'll call allergens. And in this case, the plasma cells start to produce the IgG antibody against milk, eggs, shrimp, whatever.

And these antibodies are going to be secreted from plasma cells by going to bind the muscles, and other stimulations. Basically you are going to your favorite restaurant, or you're going to give lunch to the kids, and they will have a consumption of an allergen.

The muscles are going to trigger the allergic reaction. But an allergic reaction, it can be mild, or it can be severe in the form of anaphylaxis. It all depends on the time or the dose, and other factors.

But this is the cause of the problem. We have cells that are making the antibodies. And let's think about very recent advances in cell

therapy. There we can deplete the pathogenic cells.

And the cell therapy is usually used for cancer where we can use the CAR cells to get rid of the cancer cells. So let's think, can we get rid of pathologic B cells, but then make them into antigen antibodies?

And here, we're giving an example where we designed the NK-92 cells outside the toxic cells. So we gave them a receptor, the CAR receptor. So a genetic-modified cell line that expresses ovalbumin in a context of a CAR receptor.

And we simulated a target. And the target is a human monocyte which has a receptor for antibodies. In this case, we used Anti-OVA IgG antibodies because Anti-OVA IgG antibodies is less characterized. But the idea is, if our approach works, then in that case it's going to find and kill biogenetic specific cells.

Okay. So the green cells are CAR cells, and the red cells are target. And the work -- and

here it goes. It's there. So this approach potentially works very nice in vitro. And it's very good to learn a number of mistakes about them. We say, okay it's works in vitro. What about in vivo?

So here you can see that there is a spleen that we extracted from a mouse, and there are some OVA-specific IgG-1 cells in the spleen. I can seem them in the white. In the intestines, there are some OVA-specific IgG cells, but they're making pathogenic antibodies.

And then, we inject our CAR cells in the mice, and we got this much OVA-sac that lasts this little. So we got a very modest effect from the therapy, but this is very effective in vitro. But in vivo, it doesn't work.

And it's not too surprising because this is a common oculus cue of the CAR approach because it's very good for blood counts, and it's not so good against the cancers in the folate tissues.

In the case of an allergen, most of the targets are embedded in solid tissues. So we've

got to go for a way to allow ourselves to invade the folate tissues. And CAR cells are usually not very good in this.

So how do you approach this? And here we did a study that was not specifically designed for allergies, but was designed to answer how we can stimulate invasive CAR cells.

And if you don't know how, a good study for them is a screening. You have a library of compounds, and you can screen dozens, and hundreds, and thousands of them. And you're using a system which can show you using primary joined hue. We use the parameter of the cell speed. So we use the T cells, and we use the cell speed as a readout, and we tried the different compounds.

So on the left top, you see the cell speed. It is tested with the control cells in a control dish, so in the regular cell culture experiment. And in different conditions, the regular speed is about ten microns in a minute.

So then, with this, this is Compound 1, and this is just the result. And you see that the

speed is about 5. When we tested Compound 2 at another speed, it was about 15 greater when increasing the speed of the cells.

So then, we're going back to the mouse, and we do see the results are exactly the opposite. The green compound is actually going to decrease the speed of CAR cells in tissue.

In the red compound -- but we initially think, okay it's a negative. Actually it does increase the speed of the cells in tissue. And this result shows us how misleading it can be in vivo experiments when they're trying to translate results to in vivo.

And we could avoid this if we use the system that has not just a flat surface, but has a texture that can mimic the structure of a tissue. And here on the right, you can see the use of dishes, none are textured.

And in this case, Compound 1 is truly positive, and it does increase with speed of CD8 cells. And Compound 2 is going to be a negative where you can see the decrease of migrations. So

this is just like one aspect where the use of cell count tissue system got a little bit more which we can use to help us to get better results.

And again, why don't we just use the mice? We can't just use the mice because mice a very expensive, and you cannot start the screening with 10,000 using mice. And the second point is ethical issues.

We cannot do 10,000 experiments with a group of mice who have to reduce. So there is a need, an alternative approach to animal testing. And some of the systems can be used as substitutes.

So I already showed you this, and this is a system of texture. In this case, this is a very similar of what I drew because it's extra, and it sits on the surface of a regular plastic dish. But it does give cells the extra, like in tissue.

On top of this, we also do the embolization of mechanics of the system. And mechanics is important. I already showed you why

the texture is important. But mechanics is also important.

And it's especially -- it's known for people who work with stem cells because if you use a soft gel for culturing stem cells, you will get adipocytes. If we use something stiff like plastic, you will get chondrocytes and osteoblasts.

So the mechanic properties of the environment are going to effect the expression of genes in a different way, and it will get a different outcome of an experiment with cultured cells.

Another aspect we are trying to study is the effect of a confinement on the behavioral cells. And in this case, this is a step from the system tissue-like. We'll use the granular gel.

And in addition to -- we can study interaction of cells, we have come across different cell types. I can see in this case, this gel was also -- it also contains the fibroblasts. But somehow, we're going to mimic

the extracellular matrix with a cellular component.

And the last piece that we were trying to work on, this is the DNA augmentation of gels. And why it's important, it is important because this technology allows us to change experiments as we go. So this is the basic idea.

But instead of just putting the antigen or problem that presents like a color gel, the fibronectin, whatever is used to activate the immune cells in the experiment, instead you are making an intermediate part that is made of DNA.

And using this DNA, what can you do? You can change the experiment as it goes. It's probably not as important of cultured cell lines, but certainly important with primary cells. The amount of samples is limited.

Although a human-like sample -- this is a sample, V-1. You cannot go back and collect the sample again. But in this experiment, you can do it in a what if fashion. So you can test Ligand 1. You can test Ligand 2 and Ligand 3 and Ligand

4. And then, based on the exchange by -- a DNA-based exchange of a Ligand that is achievable in and of its system.

And as a summary, while we are working on the alternatives to animal testing. With this is relatively simple. They can be inexpensive as they are. As a matter of fact, they are skeletal so you can think of this. It doesn't require you to do the large animal experiments for all the screening.

And they also can mimic certain aspects of in vivo-like behavior. And our lab develops this advanced cell culture system. And if you want to develop a new therapy or improve testing of an existing product, we will help, and we will be happy to help you and to talk about this. So think about this as a Lego. They are from a simple block. You can build a whole city of Lego, like anyone. And this is what we're trying to do in our lab.

And this is my acknowledgments. So to Dr. Mar (phonetic), who is the scientist in our

lab. So Ashley, she's our student and also the two collaborators. To Dr. Dandema (phonetic) from Penn State University, Dr. Sheheil (phonetic), also from Penn State, and Dr. Afonin (phonetic) from University of North Carolina. Thank you very much for your attention.

DR. VILLA: So thank you, all three of you, for a wonderful talk. I'd like to invite all three of you up for some questions and answers. Thank you. So if anyone has any questions in the room, please feel free to use the microphone. Dr. Elkins has some questions from online. Do you want to go ahead and kick us off?

DR. ELKINS: Sure. The first one is for Joe. When applied, is the light pulsed or constant?

DR. JACKSON: Hello. Yes the light is a constant source of the duration of the light treatment.

DR. ELKINS: Thank you, and the next one is for Kyung. I may have missed this, but are you using a totally defined medium in your vessel chip

cultures? And if not, could serum or other biological medium components cause variability? That's the first question. And then, there's another one.

DR. SUNG: No. We just use the commercially available media, and then they're our priority, the supplements that they recommend to use. And we haven't used the -- we're really well controlled in media. But I agree that that could -- that's another factor that could really -- the variability, yes.

DR. ELKINS: Thank you. And then, have you compared the performance of the chip using cuvettes versus ECs from another tissue source? It may be irrelevant, but it would be interesting to know.

DR. SUNG: So for those vasculogenesis and angiogenesis -- so we only use the UVAS because we wanted to keep the endothelial cells consistent -- constant over our experiment.

And we -- I think we used, you know, iPSC drives for endothelial cells once, and we got

very different results. So we decided to speak to UVAS for our initial study.

DR. ELKINS: And that's all I have online.

DR. VILLA: I have more questions here in the room.

SPEAKER 3: Okay. So this is for Kyung also. So the chips that you presented were very complex and the outcomes from your study was, you know, very elegant with a lot of outcomes.

So do you envision tests like this to be used as a, you know, a release or test? Or are you looking for more biomarkers that can kind of streamline the process?

DR. SUNG: Yes. So it could go to -- either way. This is really a simple assay. And if there's the time, then you know, if the assays are all characterized, then it can be used for release testing if there's the time to do three days of cold-culture, and then a few extra days for this high-content imaging analysis.

But you know, I presented that we are

also trying to identify the gene markers that correlate with vasculogenesis and angiogenesis. And that's the other way. We're actually focusing more on that direction, that we really want to identify some markers that, through these assays, that we could use potentially use for the release testing.

DR. VILLA: Next.

SPEAKER 1: Yes. In the 405 nanometer light for the passage density removed -- so do you think that if the pathogen has the porphyrins or other photosensitizers, it will be more effective than those which do not have them, like viruses? So do you prefer it for more to those pathogens which are having photosensitizers?

DR. JACKSON: Thank you for the question. Yes it's actually interesting because we -- in the case of our HCV study, we see inactivation occur in much less time than in other scenarios.

So to say that 405 light may be more effective due to the presence of porphyrins versus

that of viruses that may not have it, it suggests that viruses do have some type of photosensitizer present there, and it still elicits an effect. And at least in the case of that particular virus, a faster effect.

SPEAKER 1: But did you see any porphyrins of any photo products? Like with you, we see (inaudible) or something like that.

DR. JACKSON: I don't. Other than the production of reactive oxygen species, I don't think our lab has overlooked that deeply into that.

SPEAKER 1: Thank you.

DR. VILLA: I also have some questions for the speakers. So Dr. Jackson, so in blood components, when you treat them, they may have different amounts of pigment in the plasma from an individual. How much donor to donor variability do you see in the susceptibility to blue light?

DR. JACKSON: In terms of the platelets?

DR. VILLA: In either platelets or plasma, if there's pigment that could interfere

with the process. Has there been much difference depending on the source of the plasma?

DR. JACKSON: That's a good question. There is definitely donor to donor variability, which is I think, pretty common for many platelet or blood-product samples. But when compiled together, or overall, there doesn't seem to be major differences between the donor.

DR. VILLA: Thank you. And a question for Dr. Zhovmer. So it is very interesting how the mechanics of the culture system can affect it. Have you looked at cold culture systems with multiple cell types, and does that accentuate those differences between regular in vitro cultures and just some more specific models?

DR. ZHOVMER: The answer is yes. We're investigating that cold culture and different cell systems. And usually, it's a migration of T cells within the layer of the endothelial cells or epithelial cells because we're interested in the -- I know just basically for interaction to which extent they interact.

And yes, there is a difference depending on how you culture your cells. And I think it's enormous for both endothelial cells and for epithelial cells like the integrity of beta AIRE.

DR. VILLA: Thank you very much, and a question for Dr. Sung. So are there plans to compare this to other release criteria directly, clinically, for the cellular products?

DR. SUNG: Yes that's a really good question. Yes I mean, we really want to compare these to other traditional assays, such as you know, bariatric expression or even in vivo assays. People use the mouse model to measure the MSC vasculogenesis assay, so we plan to do that if the resource is available in the future, yes.

DR. VILLA: Thank you very much. Any other questions in the room? All right, Dr. Elkins. We're okay online?

DR. ELKINS: We are okay online.

DR. VILLA: All right. So with that, I think we have a short break now. At 3:30, Dr. Jennifer Doudna will be giving our symposium

keynote address. This will be a virtual presentation. It will be broadcast here in the room and online to all of our online participants.

We're all really looking forward to it hearing that talk from Dr. Doudna. So I'm going to close this session, and say thank you to our keynote speakers, our three speakers on the stage here. It's a really interesting look at advanced manufacturing and analytics, and we really appreciate everyone's time and attention today. Thank you.

DR. ELKINS: Yes. And for those of you in the room, we'll be broadcasting here. We have some light refreshments, and we will just switch over to the virtual presentation. Come back and join.

(Recess)

DR. ELKINS: So our final speaker for the day truly needs no introduction. Dr. Jennifer Doudna is at the University of California Berkeley where she's been for quite some time. Her original undergraduate degree was from Pomona

College, followed by time at Harvard both as a graduate and as post-doctoral fellowships.

But her career has been devoted to studying nucleic acid biology, particularly RNA biology, and that led her to one of the more remarkable pieces of biology uncovered in recent years, that of repeat palindromic syndromes that the bacteria use to defend themselves against viruses.

The CRISPR-Cas technology went from being an astounding piece of basic biology to being an incredible tool for medical treatments in work time, and that is her subject for today. So without further ado, please take it away, CRISPR therapies. How can genome editing become a standard of care? Thank you.

DR. DOUDNA: Hello everyone. Thank you so much for that kind introduction. I am thrilled to have a chance to talk with you all today about CRISPR therapies, and something that I care deeply about, along with my colleagues. How can genome editing become a standard of care? It's something

that I know you all are deeply committed to as well.

I want to start with a few disclosures. I am a founder of a few companies that are working in this space. I also work as an advisor to a number of companies that are excited about genome editing in different areas. And I'm a director at three of these companies.

So our story really begins with one of the most ancient aspects of biology, namely how organisms defend themselves against viruses. Probably ever since there was life on our planet, there were viruses trying to take it over.

And this is a picture of bacteria getting infected by phage, bacteriophage, and they face the same challenge we face as humans or as does any other organism that has to fight off foreign DNA that's getting injected in a form of a virus, or coming in through other means.

And in bacteria, there is an adaptive immune system known as CRISPR. We got started studying this back in around 2007 after

conversations with Jill Banfield at Berkeley alerted me to the presence of a likely RNA-guided adaptive immune system that was found in many different kinds of microbes.

And through fundamental science, we investigated how this works, and that actually led to an understanding that there are enzymes that are at the heart of these adaptive immune systems that function as RNA-guided DNA cutting proteins.

And I'm showing you two examples here. Cas-9 on the left, which is the enzyme that Emmanuel Charpentier and our students began investigating as part of a collaboration. And then, on the right, a protein called Cas-12 that is representative of another type of CRISPR-Cas RNA-guided endonuclease.

These are enzymes that have the ability to recognize sequences of DNA, typically 20 base pair stretches through RNA base pairing that recognizes that sequence, and then they trigger a double stranded DNA break.

And it was by investigating that

fundamental biology that we realized that this system which operates so effectively in bacteria to target and cut foreign DNA could be repurposed as a programmable system to introduce targeted changes in the genomes of organisms, like our own and like plants, that are -- have a much more sophisticated system of recognition of damaged DNA, and can thereby introduce targeted changes to DNA sequences after a double stranded break is introduced.

And one of the great things about CRIPSR is that these proteins turn out to be very robust platforms for all kinds of associated technologies. So you may be aware that nowadays, you know, this is -- we're now at about 12 years out from the original publication about how CRISPR-Cas9 works as an RNA-guided endonuclease.

And now, it's possible to use this same protein for making targeted changes to individual nucleotides in DNA, changing the transcriptional profile of cells by targeting genes for up or down regulation of gene expression, and by inserting

new sequences of DNA after a double stranded break or even after a single stranded break.

And so, these have become work horses for researchers around the world. And as we'll talk about today, increasingly they are being used as actual therapeutic modalities.

If you're curious about all of the different flavors of CRISPR-Cas9 proteins that are out there, and all of the different ways that they're being utilized, I refer you to CasPEDIA.

This is a website that we put together at the Innovative Genomics Institute that captures all of the enzymes that are currently being employed for research and for various kinds of application in genome editing.

These proteins, as you can imagine, are under very active development. So this, we work hard to try to keep this website and the database behind it up to date. And I credit many graduate students and professors at different organizations around the world that have been part of this CasPEDIA team, and worked actively to keep it up

to date and useful.

So I want to turn now to thinking about how we actually use CRISPR as a therapy. And one of the things that's very exciting in this field is that along with all of the fundamental science that's been conducted, starting very early in the field, right after the publication of the work that Emmanual Charpentier and I conducted on CRISPR-Cas9 and published in 2012, scientists were already thinking about how to use this tool to cure genetic disease.

And I want to turn to sickle cell disease now as a great example of a disease that had been very well defined in the field. We had a very good understanding of the biology of sickle cell disease. And that meant that there was an opportunity to use a genome editor, like CRISPR, to have a meaningful effect on patients.

So just as a quick summary of sickle cell disease, this is a disease that results when a patient inherits two copies of what's called the sickle cell gene. This is a gene encoding the

beta-globin protein.

It's a protein critical for oxygen-carrying in the red blood cells of our bodies. And a single base pair mutation in this gene creates a so called sickle form of the protein that gives rise to classic sickled red blood cells that can clog arteries and cause organ failure, and certainly great distress in patients.

So this is an example where having the ability to manipulate DNA sequences in a precise fashion can have a meaningful impact on patients by literally correcting the disease-causing mutation. Or as I'll show you, making a different change in DNA that can override the effects of this mutation.

And so, you know, the interest in sickle cell disease goes back many decades to a time when there were clearly patients around the world that had a defect in their ability to carry oxygen in their blood.

And so, scientists investigated the biology of this system. And in research that was

done by Stewart Yorkin at Harvard, and many other laboratories, it was discovered that normally during human development there is expression of proteins Alpha and Gamma, gamma globin genes, that form a fetal form of hemoglobin that leads to a change.

There's a switchover in gene expression that happens right around birth in which the gamma globin gene, the fetal form of hemoglobin, is repressed. And in turn, the beta-globin, or adult form of hemoglobin, is activated. And so, you can see that change occurring here.

In patients that have two copies of the sickle cell form of betta globin, however, they begin to experience sickle cell disease symptoms right around here, right around three months after birth.

And so, in investigating the biology of this process and how this gene, this switch in gene expression occurs -- kind of a fascinating example of gene expression that gets altered over time in a very controlled fashion normally, it was

discovered that a transcription factor called BCL11A is responsible for repressing fetal hemoglobin expression in normal cells.

And by determining how this works, and where this transcription factor and the regulatory elements that control its production in cells is actually located, when CRISPR came along, it was possible to interfere with the expression of that BCL11A transcription factor by making a targeted change to the genome in a region that's normally responsible for enhancing its expression.

And I'm showing you here just, you know, a cartoon of the design of an RNA-guide molecule. This purple molecule here that can recognize a sequence in the BCL11A enhancer region of the genome, make a targeted interruption to this sequence, that in turn leads to repression of BCL11A. And as a consequence, a continuation of fetal hemoglobin expression in people even after birth.

And so, this is a change that can suppress the effect of the sickle cell mutation by

producing fetal hemoglobin that can effectively serve as an oxygen carrier in red blood cells, and override the effect of the mutation.

And on the right is a close up of the details of this. And I'm showing you this in part because it's really a great example of how having a fundamental understanding of how CRISPR-Cas9 works, in which it recognizes a 20-nucleotide sequence in DNA.

So this is the site where the guide RNA comes in in base pairs, but importantly must be right next to a small motif in the DNA known as the PAM sequence that allows the DNA to open up and enable RNA base pairing.

This is all essential to the function of Cas9 as a genome editing molecule. And this is part of the fundamental work that was done in your laboratories with Emmanuel back in the beginning of our collaboration.

So that understanding was then used to initially treat patient-derived cells in a laboratory, then in animal models, sickle cell

disease, and eventually in patients. And today, this is how the therapy is actually implemented.

It's possible to remove blood stem cells from patients, add the CRISPR-Cas9 ribonucleoprotein, or RNP, to the cells in a dish in a lab, and to induce gene editing along the lines of what showed you where the BCL11A transcription factor can be disrupted, and thereby restoring the production of hemoglobin in these cells.

And so, once the cells are edited, they can be validated in the lab, and then reinfused into patients using a process that is effectively a bone marrow transplant. And this is not a fantasy. This is in fact a therapy that is -- has been shown to be highly effective in patients in clinical trials.

And in December of this past year, the FDA approved this therapy for treating sickle cell disease in a moment that I think was -- felt like a real triumph for everybody. All of us in the field, from fundamental researchers to clinicians,

to of course patients that could benefit from this.

And so, this has been incredibly exciting for me personally, and for all of our students, all of our lab members that have worked so hard to figure out how these enzymes work, and to ensure that they can be used in a way that will be safe and effective at treating disease.

And I have to point out that this therapeutic enzyme that is approved by the FDA is in fact the identical enzyme that Emmanuel Charpentier and I began researching more than ten years ago. And so it's, you know, a real sort of poster child for the value of fundamental science and where it can lead to real world applications.

So along with the celebration is also kind of reality check, a realization that there's a lot more work to do, and that's because right now the cost of this therapy is high. It's about $2 million a patient. And a lot of that comes from the fact that the molecules that are being used in these therapies are expensive to make.

So the manufacturing costs are high. And importantly, technologically the delivery of these enzymes into the cells where they can do their work and have a clinical effect is challenging. And so, this is really the kind of classic delivery challenge.

And I want to turn to our attention to now is that I wanted to tell you a little bit about some of the work that we're doing to try to address this delivery challenge. So I'll talk about research that is currently unpublished, so it's new work in the lab that we're doing with a variety of collaborators.

And then, I want to tell you just at the end a little bit about what the Innovative Genomic Institute is doing to work closely with folks at the FDA that will ultimately be able to, we think, change the pipeline for drug approvals, ensuring that we have drugs that are safe and effective, but can also be brought hopefully to patients in a faster timeline that will make it easier to treat people, especially those suffering from a rare

disease.

But let's start with how we deliver CRISPR-Cas9 as a genome editing technology. We think that there's a lot of value in delivering assembled ribonucleoproteins for in vivo genome editing.

And so, this is a little bit different than, you know, using say a virus to deliver genome editors or any other kind of molecules where you have to have the viral genetic material encoding the molecules that you're delivering. And it's also distinct from using lipid nanoparticle where we're delivering messenger RNA. For example, the COVID vaccine is an example of that.

We're really talking about something different from either of these types of modalities where we're thinking about how to deliver a preassembled protein with this guide RNA that will be ready to go when it gets into the cell, and has to go to the nucleus, and make changes to DNA.

And the advantages of this are that,

first of all, the editor is preassembled. We're not waiting for protein expression, or RNA transcription, and then assembly of the editor. We add it to the cells in a preassembled state so editing can happen fast.

And secondly, it's transient. So that means that instead of being expressed over the long term through a virus that persists over time in cells, or messenger RNA that might stick around for a while in cells, we're in a situation where these entities, these preassembled protein RNA complexes get turned over typically within about 24 hours. That's about the half-life of these particles in cells.

And that means that there's a limit to the editing capability of these particles. And as a result, we can limit any kind of off target editing that might happen over time.

And thirdly, the components are recombinant. And so, in principle, they could be produced in large quantities in a cell-free setting, again analogous to what was done for the

COVID vaccine. And that could, in principle, reduce the cost and make it a lot easier to produce these in facilities that are maybe less specialized over time.

So in thinking about approaches to RNP delivery, there are several ways that various groups have explored to do this. Electroporation is a method that's currently being used. So when we talk about the FDA approved CRISPR therapy for sickle cell disease, you might wonder, well how are those cells actually being edited?

And the way that works right now is the cells removed from patients are being electroporated with preassembled protein RNA complexes that can do the editing. So it's type of an entity here.

We also know that it's possible to add peptides onto the end of the protein that's being used for this in this kind of a capacity that give the protein cell-penetrating properties. And so, that's the CPP, cell-penetrating peptides, that can allow these proteins to access cells, and to

get into the nucleus more efficiently.

The challenge there is at least for now, these are not programmable in the sense that we don't have ways to control which types of cells they access. They simply give them general cell-penetrating properties.

And then, over here on the right are different ways that currently are being explored to do a more targeted delivery of these types of molecules into cells. So there are virus-like particles that take advantage of properties of viruses that can have particular cell tropism, allowing them to get into only certain types of cells.

And then, on the far right, lipid nanoparticles that can assemble around entities as I'll show you, including assembled protein RNA complexes, and lead to cellular delivery. And there are various ways that are being tested right now for making these types of particles also programmable or at least favored different cell types for delivery.

And so, what I'm going to propose to you today is that I think we could imagine another modality that really would sit right here, right in between a viral-like particle and a lipid nanoparticle.

Imagine that you had a particle that was comprised of a very minimal set of components, but still retain some of the things that we like about these viral-like particles, and that they could be programmed to enter just particle cell types.

And to deliver there preassembled protein RNA complexes efficiently into particular types of cells without messing with any other cell types, especially if we were using these in vivo, and they had some of the properties of the lipid nanoparticles that are so attractive, namely easily manufacturable without requiring cells to make them.

It sounds kind of like a fantasy. But we're working on this hard right now, and just really imagine that there's a space here to explore that could be really very exciting for the

next generation of genome editors that will be able to do targeted delivery in vivo.

And I think that eventuality will really transform the field and make it possible to deliver this, you know -- deliver, both in the direct sense and in the more figurative sense, this technology to many more people around the world that can benefit from it.

So just to say a little bit about how we're approaching this idea. So we call these envelope delivery vehicles. They're EDVs, and this is work that was started by Jennifer Hamilton when she was a post-doctoral scientist in my lab. Now she started more than seven years ago.

She came from a virology background. And her idea was to take what we know about lentiviruses, so these are viruses like the HIV virus that are very effective at infecting immune cells, T cells in humans, and include on their surface fusogens, like this molecule represented here called VSVG, that we're capable of cell to cell -- particle to cell fusion but didn't have

any particle cell tropism.

And then, provide the tropism by expressing on the particle surface a single chain antibody fragment that would provide cell surface recognition and could allow penetration of particular cell types by these particles.

And to make sure that we could incorporate just the cargo that we were interested in delivering in the Cas9 protein and it's guide RNA, Jenny Hamilton made fusions of the gag protein, which is part of the structure of these lentiviral particles made with fusions of gag with Cas9 that could then be -- allow automatic packaging of Cas9 and its RNA guide inside the particle.

And we've played around with different ways of displaying single chain Fvs. One example was shown here, but we have other types of connections that we've explored between the particle surface, and the single chain antibody. And there's probably a lot more work to be done there.

But even in our original designs, we found that these particles could be used very efficiently for targeted cell type specific delivery of Cas9 that would lead to very high efficiency genome editing in cells.

And if you're curious about what those data would look like, I'd point you to these papers here on the left are two that talk about this fusogen that I'm referring to, this VSVGmut.

And then, on the right is a recent article from our lab, and there are others as well by Jenny Hamilton previously that show the efficacy of using EDVs for targeted cell type specific delivery in vitro. And then, of course, what about in vivo?

And I'll just show you one data point for this. This is an experiment that Jenny conducted using humanized mice. So these are mice that have their own immune system ablated, and they've been transplanted with T and B cells from a human donor.

And then, those mice can be treated with

EDVs that carry CRISPR-Cas9. We're using Cas9 guide RNA that can target the T cell receptor in these cells, and can also try to generate a chimeric antigen receptor T cell.

And we can also use a particle, and EDV, that's going to be programmed to recognize just human T cells. And we can even be more specific than that, and try to target CD4 positive T cells in these animals.

And so, I'm just showing you two different examples, two different experiments done with different number mice. And I want to draw your attention to two things.

First of all, we were excited to see that we could use these Cas9 EDVs to generate CAR T cells in the animals. And this is comparing to our positive control using lentivirus, and then our negative control using buffer. And so, we saw that in both cases, we generated CAR T cells in vivo using Cas9 EDVs, which was exciting.

And what was even more exciting was that we found that only in the T cell -- CAR T cells

that we generated in these animals using Cas9 EDVs, did we observe genome editing in the cells.

And we don't see that with lentivirus. We wouldn't expect to because that virus is not carrying an editor in its genome. Here we're actually delivering Cas9 and getting targeted integration in the cells.

And so, this was the first example to our knowledge of getting true targeted cell type specific genome editing in vivo. And since then, we've had a very exciting, very productive collaboration with Justin Eyquem, a professor at UCSF.

And I will point you to his laboratory, and to a forthcoming publication from his lab that is in collaboration with us, showing that we can now use this approach to truly generate therapeutically beneficial levels of CAR T cells in vivo that are sufficient to ablate cancer cells, and this is again in a mouse model.

And so, this -- we're very excited about the potential of this technology because we know

that it can work in T cells. And we think in the future, it will be possible to program these particles to enter other kinds of cells as well.

And so, one of the things that we're working on currently in our academic group is to understand the structure of EDVs. So this is a cartoon that shows that maturation of lentivirus that occurs.

So there's an assembly of these particles that involved proteolytic cleavage that produces the proteins that can assemble to form a capsid inside of the particle. This normally will encapsulate the viral genetic material of say HIV viruses with the RNA, the RNA genome with HIV, and this forms the mature virus.

And you can see this very clearly if you use a technique like cryogenic electron tomography for example. You can view the structure of the capsid, and you can often even see details about the molecular structure of the protein forming the capsid and the contents inside.

But with EDVs, we really had no idea

whether this capsid would still form, and if it did, where our cargo would end up. Would it be inside the capsid, outside the capsid?

And so, we've embarked on a series of experiments to explore this, and this has been really led by two Waynes, Wayne Ngo, and Zehan Zhou, as well as a wonderful collaboration with Randy Schekman's laboratory to look at the trafficking of these particles in cells.

And that has led to an understanding that we can jettison a lot of the components of the original lentiviral particle to produce a particle that's a lot simpler than this one up here.

And most importantly -- I don't have time to show you the data for this right now, but I point you to a preprint that we posted on bioRxiv that shows the data, we know that capsid is not required in our particles. And in fact, it's better not to have it.

And so, we've been able to clear out a lot of space, we think, inside the particle by

removing components that are really viral centric and create a structure that really just involves the encapsulation components of the virus.

And importantly the cell surface molecules that give it targeting tropism, and the ability to fuse efficiently with cells, but remove anything on the interior that frankly just takes up space and isn't contributing meaningfully to the delivery of Cas9 RNPs.

And as I mentioned before, we think that really the simplest form of an EDV could actually be quite similar to a lipid nanoparticle. Today EDVs are made in a cell, you know, a host cell that's a producer cell that can generate particles very much the way lentiviruses are made.

But imagine that we could produce these particles in vitro, you know, without cells, and we could do it with purified components that would give us a lot of control over the particle and its composition, its size, its cargo, and its downstream functional efficiency. This is would be, I think -- give us a lot of control over the

way that these particles could be utilized.

And so, to get there in a second collaboration with another colleague of ours at the Innovative Genomics Institute, Niren Murthy, we've been working with a lipid nanoparticle. And this is project started again a few years ago by two very enterprising post-doctoral scholars, Kai Chen in my lab, and Hesong Han in Niren Murthy's lab.

And the question they asked was, would it be possible to use lipid nanoparticles not for nucleic acid delivery, not mRNA delivery or DNA delivery, but could we actually use it to deliver a protein, and namely a protein with an RNA guide like CRISPR-Cas9?

And so, in a series of experiments that some of which have been published, and some are in a preprint that's posted bioRxiv and will soon be published in a peer review journal, we know that this -- the answer to this is, yes. And furthermore, this is possible to do in vivo.

And so, what these scientists have been

doing is assembling CRISPR-Cas9 RNPs into lipid nanoparticles. They've tried a large range of different formulations of particles that would give efficient assembly with the largest number of Cas9 RNPs that are functional. And importantly, also favoring different types of tropism.

And then, using these particles to generate genome editing cells and tissues in mice. And then, looking to see the efficiency, the accuracy, as well as the tissue tropism, namely ensuring that if we're targeting one tissue type, we're not getting a lot of editing in other tissue types along the way.

And I'll show you just a couple of data points for this, and point you to preprints if you'd like to see more information. But you know, using what we call a fairly standard lipid nanoparticle formulation, we can induce quite efficient genome editing in the liver.

And this is analogous to the work that's been announced by the company Intellia, that I'm a founder of, although I had nothing to do with

their science. And they're already in a phase three clinical trial with this type of approach for editing cells in the liver to treat liver disease.

They're not using Cas9 RNPs in their lipid nanoparticle formulation. They're using mRNA for delivery, but we think that ultimately this delivery strategy could also work for assembled protein RNA complexes like Cas9.

So here is some data that Kai Chen and Hesong Han generated in animals where they can inject this lipid nanoparticle formulation with CRISPR-Cas9 RNPs, and show that they get very nice, very efficient editing in the liver.

Here we are using a mouse model in which cell editing triggers production of the tdTomato reporter protein. So the cells turn red, and it's a very nice visual to test genome editing, and they can verify this with a deep sequencing.

By changing the formulation, it was possible to redirect these particles to the lung. So using a cationic lipid nanoparticle formulation

favored cells in the lung, and this again is using the same animal model that has -- where editing generates red cells.

And what was exciting in this case was showing that we got very little editing in the liver in this formulation or in other tissue types that were investigated. And so, we think that this strategy also has real potential, and other labs have reported the same thing that, you know, that it really looks like we can make strives in tissue specific editing using particular formulations of lipid nanoparticles.

And who knows? Maybe there's a way to combine this with the strategy of using single chain antibody fragments to get even more targeted editing of this type of particle in the future.

So I'd like to think of sort of a continuum between a cell produced particle like this, it looks a lot like a lentivirus, and then a completely cell particle over here on the right that is, you know, formulated as a lipid nanoparticle.

And maybe there's, you know, there's an intermediate type of particle here that has properties of both that you would find attractive from both a manufacturing and genome editing efficacy perspective for editing in vivo, and we'll keep working towards that goal.

And why are we doing all of this? Well you know, right now in 2024, we're in a situation where maybe a few hundred people at most have been treated with CRISPR therapies in various kinds of clinical trials. But it's very difficult for most people that could benefit from this, even with the FDA approved therapy. To get access, it's expensive, and these molecules are hard to make.

And you know, we've had this experience in a very visceral way at the Innovative Genomics Institute with the challenges of patient access because we have -- for now several years, had an approved open IND for treating sickle cell patients with a therapeutic strategy that was developed originally by Jacob Corn who was a scientist working at Innovative Genomics

Institute.

And that was developed further by partnership with David Martin and with Mark Walters at USCF Benioff Children's Hospital. And also, with folks in Don Collins' group at UCLA.

And so, it's very exciting to have this clinical trial approved. However we ran into big challenges trying to get molecules made for this trial. And it turns out that only now are we starting to enroll patients due to the difficulty of actually producing the GMP-quality molecules that are necessary for the trial to proceed.

And if we look at the timeline and the cost, it was a four-year timeline, and it cost over $8 million to get to this initial IND. And that's not sustainable if we want to have a pathway with this type of therapeutic strategy that will be effective for lots of other kinds of rare genetic diseases in humans, which is something that I think we would all agree is a great goal.

And so, we ask ourselves as scientists,

as researchers, and as you know, just human beings. What's the right thing to do here? How do we reduce this timeline and reduce the cost?

So that when we have patients that come in with a rare disease that's clearly treatable in principle with a gene editing cure, how do we get them to a point where they can receive a therapy that's safe, effective, and cost effective in a reasonable amount of time? It's a tall order, but I think it's something really worth doing.

And so, at the IGI, we've been thinking about this as -- in terms of platforms. And this is just, you know, taken from recent guidelines from the Department of Health and Human Services and the FDA, talking about platform technology designation.

We think that CRISPR really should be able to qualify for this because if you think about going all the way back to how this system actually functions in bacteria, it's a naturally programmable system.

And so, imagine that we had a way to

swap out an RNA guide that was in place for, let's say, sickle cell disease with a guide RNA that could make this effective for a different rare disorder.

Everything else could stay the same, and it might be possible, especially as we continue to understand better and better what controls the accuracy of targeting? What controls the DNA repair pathways that happen if we're using CRISPR in its classic form? Or what happens if we use this in its base editing or other forms that allow control of gene expression?

And to get there, we're thinking about a process that would, of course, start and end with the patients. Start with a patient in need. Figuring out the variant, genetic variant that is causative of their disease.

Coming up with the right editing approach or strategy, the delivery approach that's going to be effective. Testing the safety and effectiveness. Doing some kind of a clinical test of this. And then, being able to introduce it

into that patient as a cure.

If you are curious about some of the ideas that the IGI has around this, I refer you to a recent publication on this topic that was led by Melinda Kliegman who's our Director of Public Outreach at the IGI.

And I just want to show you the team involved. So this is a team that really focuses on immunodeficiency because that's where we have a lot of expertise at UCSF, particularly in the labs of Jennifer Puck and Morton Cowan. And then, all of these folks shown here are contributing in central ways to making this pipeline possible.

We can't do this alone. We need to have corporate partners. And we've been able to partner with a number of the folks in these companies here. And all of this is encapsulated in the program we call the IGI Beacon.

Which was announced recently as a partnership with the conglomerate company, Danaher, that has a lot of manufacturing capabilities, and are very keenly interested in

making sure that they can contribute to this important goal of making genome editing much more widely available in patients.

So I just want to conclude with three points. First of all, that by continuing to investigate how genome editing works, and what editing -- what delivery methods are going to be most effective, we think this type of fundamental research will actually reduce the therapeutic doses that'll be necessary, and bring down manufacturing costs.

I also think that, you know, by focusing on particular delivery strategies, and today I mentioned this idea of using envelope delivery vehicles for Cas9 protein delivery, that we can enable cell type specific genome editing in vivo where it will have, I think, a really transformative effect on the field when this becomes much more widely possible for different tissue types.

And finally, we think that academic and industry partnerships, like the IGI Beacon, will

expand affordability and access to genomic therapies. And this must be a very close partnership with the FDA.

So we're excited that you're excited. We want to work with you. We want to understand your thoughts about how to appropriately regulate this and make sure that it's handled in a fashion that will produce, at the other end, a pipeline with safe effective and affordable therapies for people.

And I just want to close with a couple of acknowledgements. This is my own team with all of our -- I think we had 12 undergraduate students in the lab this summer working in the laboratory. So I want to thank a lot of these scientists who have contributed to some of the research that I told you about today.

Of course, I'm incredibly grateful to all of our sponsoring organizations, and to the folks that pay the bills over here. And finally, I want to give a big thanks and a big shoutout to the team of IGI Beacon.

Fyodor Urnov has led all of this. Brad Ringeisen is our Executive Director at the IGI. Eric Geigridge (phonetic) controls the campus intellectual property decisions that affect the way that we can work with companies in this space.

And then, all of the scientists and clinicians that are contributing to this work. Don Khon, of course, spoke at the beginning of the day today. And he continues to be a very important contributor to this work, and is leading the clinical trial that I mentioned.

I'll stop there and, of course, am delighted to take any questions that you might have. Thank you.

DR. ELKINS: Thank you so much. And we have a round of virtual applause online, as well as in the room. There are questions in the Q&A pod, which I think you can see. If you would like to take a look at those. And if not, let me know, and I can relay them.

DR. DOUDNA: Yes great. Let me -- I'll start at the top here. So what's the key

difference between VLPs that might have two markers to increase selectivity and EDVs? Is it just the cost of manufacturing?

It's not. It's actually the components. And I didn't have time to show you the details with this today. But if you're curious, I would refer you to our recent preprint about this.

But you know, we were able to trim away a lot of the internal composition, the components of these particles so that they really look much more just like little containers. They don't have most of the viral proteins.

I think we've gotten rid of about 80 percent of the viral residues that, you know -- amino acids that normally comprise that package. And so, that means that they are just simpler to produce. There are fewer components. And in the long run, we hope we can actually make them in cell-free setting.

The next question is, how does a VLP differ from a viral vector? So I just mentioned that briefly. And importantly, we don't like to

using the word virus or virus-like particle because we think that that implies that we're using an infectious agent of some kind, and we're not, right?

We're literally using it as a container that is packaging a protein with an RNA guide. So these are particles that, you know, simply deliver their cargo, their contents, and then they go away.

Have you compared Cas9 delivered via RNP with Cas9 delivered from EDV to check differences in editing efficacy? Great question. Thank you for asking. The answer is yes, and we have some very interesting data on this. It's not published yet.

This is work by Hannah Carp, a current graduate student in the lab, who's been to show that EDV delivery is 50 to 100-fold better, more efficient, than delivering the RNP, you know, sort of on its own into cells.

And why is that? We're trying to figure that out. We think that there's something about

the trafficking pathway is different when we use the EDV. And it could be that, you know, we're getting -- you mentioned turnover rate here. It could be that we're getting more protection of the cargo by the package so that we don't have as much maybe destruction or early turnover of these particles.

I'm not sure what the reason is yet, but it's a very interesting difference. And it suggests that for ex vivo therapies that are going on right now, there may be a real benefit to using EDVs rather than, you know, naked ribonucleoproteins in -- for electroporation into these cells.

Okay the next question. Great to see tissue specific editing. This person is wondering about the biodistribution of EDVs after IV injection. Did EDVs actually arrive at off-targeting tissues, but just did not edit the cells? And the second question is, what cell types in the liver and the lungs respectively?

Yes. So with the EDVs, we're not seeing

evidence of editing in these other tissue types. But I don't know the answer to your more detailed question, which is that, what's the reason for that?

Do the particles go there, but then they do not actually getting into the cells, or did they just not even end up there? My guess is they get there, but they don't transduce the cells. That's my guess, but we don't have actual data for that right now.

And then, the question about cell types in the liver and lungs, I don't really know, you know. We have not, or at least I don't have the data handy right now showing you specifically what kinds of cells are being transduced. And by the way, that was with the lipid nanoparticle, the data that I showed you for that. So it's a little, you know, different delivery modality.

And then, another question. Let's see. Is it important that a gene therapy be shown to be effective before licensure? Of course. I mean, absolutely. It would have to be effective, yes.

And it would have to be safe. So there's absolutely no argument there.

I think the question is, how do we get to those points, but in the most efficient way possible, especially when we're thinking about very rare disease where, you know, it's probably really just isn't realistically affordable or even really practical to do a full blown clinical trial for those patients.

And in many cases, it might just be a few patients affected in total. So we have to think about how we're going to, you know, benefit them in a way that's safe and effective.

And then, secondly -- sorry, another question here. Can you use the EDV approach to selectively target antigen specific T cells in vivo, similar to a tetramer type approach?

I don't know the answer to that right now. But I think in principle the answer is yes. One of the things that we've found, and some of this is in some of the preprints that I referenced, is that these particles and their

efficacy is highly dependent on the specific antibody fragment that we're displaying on the surface.

And it probably, frankly, also has to do with the density of those antibody fragments on the surface. And even things like the connecting molecule and how long it is, you know, that connects the single chain Fv to the surface of the particles seems to also matter a lot.

So it's a lot of variables. So you know, we're trying to sort of sort through this and figure out if there's some rules or kind of rhyme or reason to it. But at the moment, we're sort of, you know -- we're still at the stage where we're just trying to figure out which variables are the most critical for efficacy, and then how to control them appropriately.

And by the way, please let me know. I can keep answering questions.

DR. ELKINS: Yes.

DR. DOUDNA: But you should let me know when you need me to stop.

DR. ELKINS: Please.

DR. DOUDNA: Okay. I'll just keep going. Okay. So can you please comment on advantages of using EDV over antibody modified LMP for cell targeting? Yes. The short answer, I don't know because we haven't tried the latter, right?

We haven't tried, you know, antibody modified LNPs. I know various groups are playing around with these and testing them. I would be very curious to try them side by side with the EDVs, and hopefully we'll get a chance to do that at some point. If anybody here has ideas about how to do that, or who should do that, please reach out because I think that would be an important thing to test.

And then, there's another question. In order to deliver EDVs to the target, do you always require prior knowledge of the receptor identity, or can they be made tropic to cells agnostic over sectors?

No. We really need to know the receptor

identity. So at the moment, at least, that's how we're doing this research. Now you could imagine potential ways to do, I don't know you know, tropism selection with cells where you maybe don't know the identity of the surface markers, but we haven't tried that yet.

Right now, we've been really focused on identifying antibodies that we know recognize a cell surface marker and a cell type of interest, and then you know, trying to work it out in the format of the EDV.

What is the release mechanism of the EDVs? Yes. It's a really good question. We're just in the beginning stages of exploring that. We're looking at using cryo electron tomography to actually follow the trafficking pathway of these particles from when they actually engage on the cell surface.

And there's membrane fusion to then how they're actually taken up into the cell, and then how they make -- their cargo ultimately makes its way to the nucleus. But you know, we haven't --

we're just at the beginning of doing those experiments.

Do you see differences in off target occurrence between gene editing in vitro and in vivo by Cas9? I would say we don't really see differences there right now. I mean if we -- this is us -- this is like the royal we. We've done, but so have many other groups, so have companies and folks doing it, running clinical trials.

You know, especially in T cells for example, you can look at primary human T cells that you're manipulating in vitro, but you can also look at primary T cells that are being edited in vivo.

And when you look at those cells, you really don't see a difference in off target occurrence. And frankly, if anything, the situation is even better in vivo believe it or not.

This is data from Carl June's lab showing that -- and this is in a paper that we published. Connor Tsuchida is the first author,

if you're curious to look it up, with Carl June as a coauthor, where we looked at, you know, Carl's clinical data.

And then, we looked at in vitro data that we had for editing human T cells in vitro. And it turned out that in vivo over time, cells that are edited accurately have proliferative advantage actually. And then, they tend to predominate over time.

Let's see here. Do you have a back of the envelope estimate for RNP delivery compared to mRNA delivery on a cost per dose basis? I don't have that. I think that's a very important thing to try to do.

It's tricky because we don't currently make mRNAs for delivery. I guess I could maybe get ahold of some of those numbers from some folks that are doing this. But I haven't done that yet. But it's a great question. I think it's a really important question, especially as we get farther down the road with these various technologies.

Is there anyone looking at osteogenesis

imperfecta using any of the CRISPR-Cas methods? I don't know the answer to that. Yes I do not know. I think that's a great question. That's maybe an NIH type of question. I don't know the answer to that.

Does EDV deliver guide RNA Cas9 have a different off target profile compared to RNP delivered method? Not that we've seen. Yes not that we've seen. We haven't done a very deep dive on that yet, and we have not done that comparison in vivo.

But when we look in vitro at cells that are treated with one or the other of these modalities, we don't see a difference in off target editing. We do see a difference in editing efficacy though, and that of course could affect off targets in the sense that, you know, if you have a more active editor, typically you also have more off targets. But in terms of, like, on that sort of -- you normalize by efficacy, you really don't see a difference in off targets.

And then, the last question here in the

Q&A is, I was wondering if you have observed the effect of the gene editing therapy decrease over time, and if you think patients might need additional gene editing interventions in the future?

Yes. It's a great question. So this is something that the company Intellia that I mentioned has looked at a little bit. And they made a public announcement last summer, a few months back, about trying this type of strategy for liver disease where they were able to re-dose patients that had received an initial injection of their editor, and dosing them a second time.

And the good news there was that at least with their formulation, they're using an LNP with, you know, mRNA delivery there for the editor. They did not see, you know, a toxicity there or an immune reaction. So that's a good sign.

But you know, clearly more will need to be done. And I think you raise a very interesting point here that, you know, what's going to happen

with gene editing as we begin to see its impact on different types of diseases, and larger number of patients?

Are we going to find that the genome editor edited cells persist in these patients? I mean, sickle cell is kind of an interesting example because the edited cells have a proliferative advantage. And so they, maybe you know, will naturally tend to persist over time, and have an advantage over the unedited cells.

But that might not always be the case. And so, I think it's a really interesting biological question. It's an interesting clinical question, and something that we'll really have to pay attention to as clinical trials progress.

Now that's the last question that I see there.

DR. ELKINS: I think that's it.

DR. DOUDNA: That's it?

DR. ELKINS: And I think we have pressed our luck with being over time. We thank you so much for a very informative, stimulating, and

exciting presentation. Obviously, it generated a ton of discussion. And we know what we have to look forward to both as scientists of regulators a little bit more. So thank you again from all of us for joining us today.

DR. DOUDNA: Thank you for hosting me. And enjoy the rest of your meeting.

DR. ELKINS: Thank you again. And folks, we are adjourned for the day. We look forward to seeing everybody tomorrow morning.

(Whereupon, at 3:30 p.m., the PROCEEDINGS were adjourned.)

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I, Michelle Begley, notary public in and for the District of Columbia, do hereby certify that the forgoing PROCEEDING was duly recorded and thereafter reduced to print under my direction; that the witnesses were sworn to tell the truth under penalty of perjury; that said transcript is a true record of the testimony given by witnesses; that I am neither counsel for, related to, nor employed by any of the parties to the action in which this proceeding was called; and, furthermore, that I am not a relative or employee of any attorney or counsel employed by the parties hereto, nor financially or otherwise interested in the outcome of this action.

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