Transcript

Christine Bevc:

Hello and welcome. I'm Christine Bevc, task lead for RECOVER at the Administrative Coordinating Center and moderator for today's webinar. I'd like to welcome everybody to today's RECOVER Research Review or R3 webinar. The overarching goal of the R3 webinar series is to catalyze the formation of a scientific stakeholder community within and beyond the RECOVER consortium. Fostering a shared understanding of the state of science and providing an educational resource for both RECOVER investigators and the broader scientific community of clinicians, patients, and stakeholders.

Hello and welcome. I'm Christine Bevic, task lead for RECOVER at the Administrative Coordinating Center, and moderator for today's webinar. I'd like to welcome everybody to today's RECOVER Research Review or R3 webinar. The overarching goal of the R3 webinar series is to catalyze the formation of a scientific stakeholder community within and beyond the RECOVER consortium. This helps fostering a shared understanding of the state of science, and providing an educational resource for both RECOVER investigators, and the broader scientific community of clinicians, patients, and public stakeholders. I want to start by thanking everyone who submitted questions in advance. During today's webinar, as Shane mentioned, please use the Q&A feature in the Zoom window to submit your questions. After the presentation, our presenters will answer as many questions as possible, and they'll also provide responses in real time within the Q&A. Please note that we will not be answering questions about clinical care.

We'll also have an FAQ document for this webinar that will be posted along with the recording of the webinar on recovercovid.org. Today's webinar is the third in our series on mechanistic pathways of PASC. Today's session focuses on organ damage and reprogramming of host tissues and organs. Our presenters will be addressing the question, how does the virus that causes Covid lead to long-term effects? If you haven't already, remember to sign up for on our website to receive future announcements and updates on the series. As RECOVER continues to grow, we want to remind our audiences that the information presented in this seminar is intended to stimulate collaborative dialogue amongst the RECOVER scientific community, as well as study participants and other interested parties. In addition, none of this information should be interpreted as medical advice.

We're excited to have three RECOVER investigators joining us today. It's my pleasure to introduce Dr. Benjamin tenOever, Dr. Douglas Fraser, and Dr. James Heath. They're joined by our Discussant Jim Stone, who will kick off our discussion and response portion of our webinar. Our first presenter today is going to be Dr. Benjamin tenOever, who holds a dual appointment as professor in NYU's Department of Microbiology and Department of Medicine. He's currently Director of the NYU Langone Virology Institute. And Dr. tenOever will help us understand

the molecular basis of long Covid in his hamster model, and the connections to olfactory impairment. Next, we'll hear from Dr. Douglas Fraser, who will take us into the realm of plasma proteomics and the use of AI and bioinformatics to investigate organ changes. Dr. Fraser is a professor and clinician scientist in Critical Care Trauma Medicine at Western University and serves as director of the Translational Research Center in London, Ontario, Canada.

Our final presenter today is Dr. James Heath. Dr. Heath is President and Professor at the Institute for Systems Biology in Seattle. Dr. Heath also holds the position of Professor of Molecular and Medical Pharmacology at UCLA. Dr. Heath will be sharing his emerging insights on the connections between autoimmunity protection, and T-cell chronotypes in his work using single cell multiomics. Our investigators are joined by Dr. Jim Stone, who will help us connect the dots between these three presentations and lead off our discussion with a short series of questions to our panelists. Dr. Stone joins us today as a member of the RECOVER Steering Committee. He serves as director of the Autopsy Services and head of the Cardiovascular Pathology Service at Massachusetts General Hospital, and Associate Professor of Pathology at Harvard Medical School. Following the questions from our discussant, we're going to open the floor to questions from the audience. And as mentioned, we're going to try and answer as many questions as possible related to today's topic. And with that, please welcome all of our speakers as I turn it over to Dr. tenOever.

Dr. tenOever

Well, thanks for having me. I certainly appreciate the invitation and it's an honor to be here. Again, my name is Ben tenOever, and I've been working on SARS-CoV-2 biology since the beginning of the pandemic really. What I'm going to talk to you today about is the use of the Hamster Model in helping kind of determine what might be the molecular drivers of long Covid, which is the theme of this entire symposium in this large RECOVER contract that the NIH has executed. So next slide. And so really the main question I'm going to really talk about today, is how is it that a virus that infects really largely is confined to the airways, has all of these non-A airway manifestations from a clinical point of view? Both from in its acute form and of course the persistent form that we call long Covid or PASC.

Next slide. And so just as a 30,000-foot overview, I think you're aware that the acute response to virus infection is largely the virus replicating in both your upper airways and in your lower airways, really the affiliated epithelium of your lungs. And while some virus does tend to get to the heart and cause arrhythmias and a response in the heart as well as complications with regards to clotting in the blood, overall, the virus and the host response is largely contained in these systems from at least for the first three to five days post-infection. Next slide. However, what we know about SARS-CoV-2 is that despite that fact and despite the fact that virus can successfully get cleared by, in the case of most infections, there are many other complications associated with having contracted SARS-CoV-2, whether it be directly after that initial acute phase to infection or even long-term

manifestations of these different clinical presentations as it relates to SARS-CoV-2. The question here is really does the hamster, which is such a fitting model of SARS-CoV-2 biology and Covid disease, can it also help predict how this phenomenon exactly happens?

Next slide. And so that's really this idea we're going to leverage an animal model to be able to know exactly the time of infection, when the infection starts, and be able to address different aspects of how all these different organs are responding to virus in real time and with the ability to control all the variables that one can control. Next slide. And so as I was saying, the hamster actually dearly did turn out to be the most superior animal model for SARS-CoV-2. Not

only do you not have to change the animal at all for it to be susceptible to virus, but we also don't have to change the virus in order to adapt to replication in hamsters. We see the same kind of ground glass opacity in the lungs that you're viewing at here that we see in human individuals. We see higher disease in males over females. We see that as hamsters get older, the disease gets more severe.

And so really in every parameter that we've been able to study Covid disease from cadaver tissue and those that get hospitalized, we see it phenocopied in the Hamster Model. And therefore, we believe the Hamster Model is probably the most superior small animal model to study this biology. The next slide. And so also very much like what we observe in clinical samples from people who've contracted SARS-CoV-2, if you take a group of say 12 hamsters and we infect them all by just introducing a relatively low amount of virus on their snout, on their nose, what you can find is that all the animals in the upper airways, you can look at the trachea, the lungs, in all of these animals are testing positive for virus replication, which is not surprising. But really with the exception of a few other areas like the olfactory epithelium, which is kind of right around the upper airways, as well as the heart itself, which is of course enveloped by the lungs.

We really don't see productive virus infection in these other organs, suggesting that it really is a virus of the airways that's contained there, despite the fact that it's causing these complications in these distal organs. And that's really what we're going to talk about today. Next slide. And so if you look at the hamster, the hamster really is modeling what a healthy individual might look like in response to SARS-CoV-2. I don't know if you can see my pointer, but if you look at kinetics at one day post-infection, three days post-infection, five, seven, 14, and so on, I hope what you can appreciate is that these animals shed a lot of virus. So they're releasing 10 to the eighth platforming units. It's a lot of virus that's coming out of their nose, and that really stays consistent from days one, two, three, four, and five post-infection. They're capable of spreading it to their cage mates. They can spread this through the air, by contact.

But by day seven, you really see an effective clearing of this virus, which corresponds really nicely as you can see the graph on the right, to the production of spike antibodies. So basically the virus comes in, the virus begins to replicate in the lungs, the animal begins to build an antibody response to this spike protein that's outside

of the virus. And as those antibodies begin to rise, the virus's capacity to maintain this infectious production drops, plummets actually by day seven, and then the virus is cleared thereafter. And so this is really how a healthy individual might respond to virus. Next slide. What's interesting about them, so you can also follow this and it looks identical. It's what we see in clinical symptoms. So there on the top there, the brown is staining of the virus. In this case it's the nucleocapsid protein.

But you can see the large airways, those ciliated epitheliums become very, very brown on day four, really hitting the peak of infection before it starts to fadeaway. And by day 14, you can really see that most of that virus is gone, although you can definitely appreciate that there are still brown spots in there, speaking to maybe a stable leftover viral debris that's left behind after infection, which we'll touch on at the very end of this talk. And so again, as a healthy individual, we do see this rise in fall virus production, which on the bottom, this so-called H&E staining, what you're looking at there is infiltration. So you're watching your immune system be called in kind of as a product of reinforcement to that infection, and that just generally delays the peak of virus infection. So you see more infiltration as peak virus production goes by. Next slide.

And so like us, in addition to falling sick and having our lungs heavily infected by virus before having all this immune infiltration come in. Hamsters also experience anosmia, that is the loss of smell. And we know this because we can do tests on these animals between days two, three, four, five, right where that peak replication is happening. And we can actually bury Cocoa Puffs underneath the bedding of the cage, and look at how long it takes for these animals to find those Cocoa Puffs, which obviously requires a sense of smell. And what you'll find is if you're testing SARS-CoV-2 infected animals between days three and five days post-infection, they take a much longer time to find that cereal if they find it at all, really demonstrating that they do in fact, like humans, suffer from anosmia. And this also in most human cases, dissipates over time. And so by 15 days post-infection, when the virus is cleared from this model, their sense of smell also returns. Next slide.

In addition to anosmia, which is kind of an acute response, we also find that there are behavioral changes in these animals. And so this is probably the most consistent and statistically-driven data that we can demonstrate. And so this is a test where you introduce marbles into the cages of rodents. And behavioral scientists have found that rodents will often bury them because they're foreign objects that create a level of anxiety. And so the rate and the pace at which these animals bury marbles, is often looked as a cognitive measurement of anxiety or stress or energy. And what we find is that animals that have been treated with SARS-CoV-2 that have recovered, even 31 days out or five, six weeks out, what we're seeing is that they tend to bury less marbles suggesting that they are undergoing some kind of cognitive dysfunction, although you know exactly what that is and how it's materializing is not for me to say beyond the fact that we do see behavioral changes that consistently occur that might serve as a proxy for something like long Covid. Next slide.

And so that is all to say that we have this animal model that has all these features, both the acute and the long-term implications that can result from a SARS-CoV-2 infection. And can we use that model to then understand what's driving that biology in general? And that's what we're going to do now. Dive into that a little bit. Next slide. And so to do that, I think we felt it was really important to first off to benchmark this. Obviously if we only worked with animals that were infected with SARS-CoV-2, anything we saw from a biological point of view could have been implicated in either the production of anosmia, or this change in behavior, and it might not be at all specific to SARS-CoV-2. So to be able to distinguish that, what we did was we did a parallel study where we took one cohort of hamsters and we just treated them with saline, they served as our mock controls. We took one cohort of hamsters and we treated them with SARS-CoV-2, the virus that causes Covid of course.

And then a third cohort of group we treated them with influenza A virus, which of course doesn't cause anosmia and is not associated with long-term complications. And what we did is we simply took these animals from peak infection all the way through clearance, and we compared really day three, which is the peak infection for both flu and SARS in this case, as well as a time point after 31 days when in both cases the virus has long been cleared by the system. And we asked, is there anything that might draw out a distinction between what SARS-CoV-2 is doing in the hamster model that would speak to long Covid in the underlying drivers thereof? Next slide.

And so this is something we call RNA sequencing. So basically it's a snapshot of all the software that's running in these various tissues at a given time in the infection. And so this is day three. So in both cases this is peak virus titers, whether you're flu infected or your SARS-CoV-2 infected. And here we're looking at the heart, the lung, the kidney, and really the olfaction system. So both the olfaction bulb and some of the olfactory epithelial cells. And what you're looking at here, red just means that a particular gene is found to be heavily enriched as it's compared to the controlled cohort. And these are statistically showing up here as genes that across the entire cohort of animals, we see these genes go up in all of those animals. And what you're looking at here, each box represents a suite of genes that all belong to really the host response to virus infection.

And all I want you to appreciate is that even though both flu and SARS-CoV-2 are viruses of the respiratory tract and are largely confined to the lungs, there really is a systemic response, meaning that this antiviral software that we see running here, is running in all of your tissues. And I'm just showing you four here, but you could really check anything from the fat of the animals, to the liver, to the pancreas, to the kidney. And in all these cases, the entire body as acting as though it's infected by virus infection. And what's interesting about this is that the fact that flu, we see this with both flu and with SARS, would suggest that whatever this response is in whichever driving this biology is not distinct to SARS-CoV-2 and therefore probably not a driver of long Covid.

However, if you go to the next slide, this is the exact same experiment, but now we're looking at 31 days post-infection long after the virus in both cases has been cleared. I hope you can appreciate here is that on that very bottom condition in the olfactory bulb, those genes are staying red, which means that despite the fact that

there has been no infectious virus for three weeks in these animals that can be detected, the olfactory bulb is still behaving as though it's infected and is still eliciting this antiviral response. And this is unique to SARS-

CoV-2 and not seen in the case of our influenza infected cohort, suggesting that this might actually be something of significance that's driving our biology. Next slide.

And so the olfactory bulb, an olfactory epithelium turned out to be a really a hotpot for virus replication. So this is called iDISCO. What you're looking at is the snout of the hamster where the aquamarine color is SARS-CoV-2. You'll get a zoom in, in a minute. But what you'll see is that the olfactory epithelium is this cavernous area where there is a lot of affiliated cells that SARS-CoV-2 replication can occur. And this is of course running all adjacent to the olfactory bulb, which is the tissue we just saw. And to the right of this, what you can see the graph is in teal, what you're looking at is the fact that SARS-CoV-2 not only replicates more robustly in this olfactory epithelial area, but it's also driving a heavy innate immune response of driving in T-cells and B-cells of the immune response to essentially come to the olfaction system to deal with this replication problem. Next slide.

And so if you take this and we do single cell, we can do those single cells at the exact time for example, where we know our hamsters had anosmia. So we know they're not getting the Cocoa Puffs they want to eat. And if we simply take their snouts and we look at all the cells at an individual level, but I hope you can find is that there are those cells that you just saw where aqua in the iDISCO movie there, those are called sustentacular cells and they get infected by SARS-CoV-2. You can see at one day post-infection here, all of these sustentacular cells light up. And so those are the cells that are being productively infected. They start dying and as a result of their death, they get picked up by macrophages and microglia that are around the olfactory zone, and they elicit this interferon response, this warning flag to tell all the cells around that a virus infection is coming and it's important to fortify. And we know that from a marker like the single cell sequencing Disney plots you see on the bottom here, this is called ISG15.

This is just a marker that basically can denote where the antiviral system is turning on. And you can see that even though virus infection is really restricted to certain cell types, that antiviral response is just turned on blanket wide. And this has consequences in the fact that, for example, your olfactory receptors that are responsible for smell, are now spending all of their bandwidth dealing with amplifying this antiviral response. And that's in fact why you lose your sense of smell, because an olfactory neuron only has so much bandwidth to give. And so if it starts bending its energy and time building up these defenses that it doesn't actually need, it loses the capacity to smell for a brief period of time until the virus is cleared, at which point it can go back to doing what it was doing and your sense of smell returns. The next slide.

And so this is not just unique to hamsters. So in fact, we did get some cadaver tissue. So these are from two long Covid patients who identified as having long Covid, but died incidentally in a car accident that had nothing to do with their

long Covid presentations. But even in these two individuals where we could actually capture the olfactory bulb tissue and check for this, they actually have the same high chronic interferon antiviral singling signatures in their olfactory bulb that we saw in the hamster. So again, every time we try to verify what we see in hamsters, it turns out to phenocopy beautifully with what we see in a clinical setting. Next slide. And so we didn't just stop there, we also looked at these different other areas. So we have here the prefrontal cortex, the cerebellum, the thalamus, the olfactory bulb, the trigeminal ganglia, and the striatum.

And in all these cases what we're looking at is 31 days post-infection long after viruses cleared, and we're comparing our flu cohort of animals to our SARS-CoV-2 infected cohort of animals. And while you see a lot of things happening in meth thalamus for both virus infections, which is a topic for another day, the two areas where SARS-CoV-2 is noteworthy is in the striatum and the olfactory bulb, which we just went over. And so there are these two areas of the brain that are showing prolonged and persistent signaling in response to virus that we're not seeing with flu. So it does seem to be something specific to this particular virus. Next slide.

And so I went to all of that to say that we have this activity happening in the striatum and the olfactory bulb that certainly explains anosmia. It might explain some of the cognitive aspects of long Covid, but still there's clearly all these other non-airway organs that are associated with long Covid. And of course this is not being addressed at this point by this particular model. It could be that the brain drives all of those manifestations, but that doesn't seem particularly likely. And so to that end, there's one additional finding that I wanted to share with you today. This is not published. Next slide. And that is if we take our hamsters and we in fact treat them with dexamethasone. So dexamethasone is a steroid. It's actually ironically a steroid we use to treat and diminish some of the many complications that come after having had SARS-CoV-2, because it induces such an inflammatory response that really after the virus has cleared, the problem really becomes inflammation more than virus itself.

However, in the case of hamsters, if we give dexamethasone in the context of a virus infection, it means that we turn down their ability to be inflammatory while the infection is going on. And so this is actually a really nice way to model what someone who's immunocompromised might look like, even somebody who is of advanced age, because we know that advanced age also diminishes your initial capacity to launch these antiviral defenses in a timely manner. As opposed to children who launched it in record time and of course show very little symptoms associated with SARS-CoV-2 biology. And so all of that to be said is if we take our two animals and we going to compare regular intranasal infection to an intranasal infection in animals that have been in which their immune system has been dampened by the administration of dexamethasone, what you can find is that in the addition of dexamethasone, we now can see virus production and tissues that are outside of the lung.

So we can see it in this particular graph in the liver and the spleen and in the GI tract. And the reason I think this data is important to mention here is because obviously because our hamsters are healthy young individuals, their immune system is always going to be able to act in a very rapid manner that is indicative of young

healthy people. But in people that have a comorbidity where their immune system might be compromised in some way, the very same dynamic that we just went through with anosmia, how you get this prolonged antiviral singling persist in the olfactory ball that then has implications in the brain. This could certainly happen in the liver or the spleen, the GI tract, even the kidney where we see it at other times, as long as the virus had a chance to actually break free from the respiratory tract and find a home in one of these other organs.

So I think that in a lot of cases this could be the underlying driver of whatever is causing that prolonged inflammatory signal to induce this signaling, is in fact what's causing all the various complications in different organs including the brain of course. And so with that, I'll go to my last conclusion slide. Next slide. And so I hope what I covered in my 20 minutes today was that in order for us to really understand what long Covid is and what SARS-CoV-2 biology is, it was imperative that we have an animal model, and that animal model is probably best served as the golden hamster. What's nice about that is that we can compare the golden hamster's response to both SARS-CoV-2, which is an RNA respiratory virus, to influenza A, which is also an RNA respiratory virus, but of a completely different category. And that allows us to benchmark things that are truly unique in response to SARS-CoV-2. .

And in doing that, we think we feel we can explain anosmia and what drives anosmia, as well as cognitive abnormalities, and perhaps even many of these other areas of other organ complications associated with SARS-CoV-2 infection. And so really to me, the real question that remains in all of this to work is what really is the driver of that sustained immune singling? And I think we'll probably end up talking about that in the questions later on, but I list a few options here, which would be, in fact, there is a reservoir of virus or at some low quantity or maybe a broken version of virus that can replicate its own RNA, but can't spread in, therefore test negative in plaque assay. It could be like I mentioned in the lung staining, that the debris left over by the virus, whether it be cell debris or viral debris, could be so stable that it's inducing the singling long after the virus is cleared. And the third, which these are not even mutually exclusive with each other, could be that virus infection actually damages a lot of the mucosal barriers we have.

And so what's happening is the initial damage was caused by virus, but thereafter it might be an interaction between our natural microbiome which is now able to reach tissues that it couldn't reach before because of these broken barriers. And it really could be a combination of these three, or any one, but these would be examples that you could achieve this phenotype. And with that, I'll stop. My last slide is just a thank you to the people in my lab and the funding sources that have enabled a lot of this work. I'd like to actually specially thank Jonathan Overdevest and Stavros Lomvardas, who did all the help with the anosmia work and single cell sequencing. And with that, I'm happy to take questions and I will pass it on to my other great group of panelists. Thank you.

Christine Bevic:

Great. Thank you, Dr. tenOever. We'll be holding your questions until the discussion portion later. And next, we'll move to our second presentation by Dr. Douglas Fraser. Again, drop your questions into the Q&A. If they're specific to a presenter, they may address them live by typing an answer or they may also hold them until the discussion portion. All right, Dr. Fraser?

Dr. Fraser:

Thank you, Christine, and thank you to Dr. Stone for the great invitation. So I'll be speaking about the vascular proliferative process going on in long Covid and how it is associated with organ dysfunction. Just a quick disclosure statement, I do have multiple positions and provisional patents related to Covid-19 and long Covid. Just to start off with a little preamble. The patients that I look at are all recruited from a multidisciplinary long Covid clinic. They're confirmed with Covid 19 infection, and I only study patients that are PCR positive. They have to have at least 12 weeks of symptoms and they have to have a partial workup done. Based on our public health surveillance, the SARS-CoV-2 infections would've been wild-type alpha or delta. So I will not present data on omicron or beta gamma. Typical study approach that we use is proteomics with machine learning and bioinformatics.

And I just want to be clear that we are looking at plasma, and plasma was used as a surrogate for cellular and tissue activities. So it's still a little unclear how proteins are released from tissues and how they turn over, but it's a great way to start and the data should be considered exploratory, not definitive. So hopefully this will help drive more hypothesis-driven research. So when we started our experiments, we were interested in the vascular system. It made sense because it joined together all the organs that seemed to be diffusely involved in long Covid. So working with Thermo Fisher, we created a vascular transformation kit that uses multiplex technology. We started there, we looked about 16 molecules or proteins that are involved with vascular transformation or angiogenesis. And we found that 14 of the 16 were elevated in the long Covid patients, and they were quite significantly elevated.

We then used artificial intelligence techniques and we started to rank their importance. And you can see that Angiopoietin 1, P-Selectin, and MMP1 are the three leaders. And when we started to use further machine learning techniques in this case TSNE plots to look at how all the molecules can define the cohorts, the cohorts are pretty well identified by 14 biomarkers. However, some noise is probably injected from some of the markers lower down. So then we only went to the top two or the top three, and you can see in the sea that the cohorts are identified very well from healthy subjects to acutely ill Covid-19 patients to the long Covid patients. We went on just to characterize some of these molecules or proteins. And again, the cohorts are healthy subjects versus mild Covid 19, severe Covid 19, acutely ill versus the long Covid patients. And they're all age and sex matched, and you can see that the proteins are significantly elevated. And when we use rock curve analysis, we find that they seem

to have some pretty good discriminatory ability with regards to determining who has long Covid versus the other cohorts.

We do use a lot of machine learning techniques. This is a technique based on Pearson correlation. And essentially what it's showing is the cohorts that we look at are on the X and the Y axis and you can see where the long Covid patients are. They're clearly different by the color coding relative to the other cohorts. We have a timeframe. So when the patients presented, the patients are typically around 60 years of age and on average they present on average at about a hundred days after post-infection. In this case, you can see how the proteins seem to be climbing still, 100, 120 days out, but they're all above the cutoff values that we were able to establish.

We did validation cohorts, so this is a completely separate cohort. We don't use any selection techniques. We just take the patients as they come in and they meet the criteria for the clinic. And these two proteins in particular, angiopoietin 1 and P-selectin discriminated the patients very, very well. And you can in fact see there's kind of a clustering around 100 days post-acute infection. So we went on from using the straight protein measurements to using proximity extension assays for over 3000 proteins in human plasma. And when we looked at over 3000 proteins and then we used machine learning techniques, brew to feature reductions and so forth, we came down to the most important proteins for determining who had long Covid relative to the other cohorts. And you can see we came down to about 119 proteins, which we've reported and you can see that the cohorts are nicely separated.

119 proteins if you're going to use this as biomarker work is clearly too many, that we then brought it down to nine protein, still had great classification accuracy. Brought it down to five, still great classification accuracy and we can bring it down much lower. And again, whether you use a nine protein combination or a five protein combination, you can see in the bottom graphs again that the long Covid patients are quite different from the other cohorts we looked at. The top nine proteins are listed here. You could see the eight went up. Well, FRZB went down. The green shows where the normal levels are, so those are healthy subjects. That's where the levels are. And the blue dots of course, are the long Covid patients. You can see a variety of molecules here. You've got chemokines involved with cell migration and sustained inflammation.

You've got proteins like AP3S2 that are involved with type two diabetes. You've got proteins involved with neurological impairment, max, FRZP, and others. So a broad group of proteins that seem to be very important for characterizing long Covid. We used natural language processing, another form of machine learning, and we went to the UniProt system to try and determine all the proteins that we were able to look at out of the 119 where they were expressed. So what organ systems were involved in, what cell types were they expressed? I was a little surprised. We did this probably about six months ago. I was a little surprised to see the digestive system came up first. I'm not now because there's been enough literature to suggest that the digestive system's in fact, the most

affected organ in long Covid. And that could be anything from GERD reflux, to vomiting, diarrhea, poor peristaltic movement in your intestines and so forth, liver impairment and others.

So not too surprising. Then we get into the lymphatic system, nervous and so forth, and they're all really well represented. When it comes to the cell types involved, lymphocytes, not surprising came up first. Leukocytes NYD next, followed by platelets. Again, not surprising, distribution. What did we do with this data? Well, one thing we've done, we have licensed much of our patented data out and we're working with a company QMC Health. And we are in the process of developing a point of care device, which is a lateral flow device, where you would use capillary blood and a dropper to pick up a set amount. You would put it into a container that contains all of the chemicals and conjugates that are necessary. And then those drops would go down on a plastic cartridge, which has your nitro cellulose paper and it would run along.

On the left is the IGG, that's IGG to for a nucleocapsid protein, to show that you've had a recent infection. And then we've got three biomarkers. You'll notice a flashlight. We're using quantum dots for a reporter. Quantum dots are about 20 times more sensitive than colloidal gold or your traditional pathways that they fluoresce, they're very stable. They offer sensitivity to the test while the specificity is offered in being able to look at multiple biomarkers at once. We have some work to do once the prototypes are finished in terms of understanding how these biomarkers will interact with other diseases, but I think it's a great start and we're not too far off. I'm going to get now into more of the plasma proteome and what we're doing with bioinformatics, which tells us a little bit more of the pathology that's going on. Again, I want to say that that we're looking at the plasma proteome as a surrogate for tissues. So this is not definitive work, it's exploratory, but it does offer some great ideas of where to go next.

Our study model already is presented. We look at healthy controls, acutely ill patients, long Covid patients, at least on average about 100 days out. We use plasma and then we're looking at over 3000 human proteins. We're quickly going up to over 4,000 proteins now. We do de-convolution for cell types and functional activities, and then we can also curate the markers into organs to determine what's happening from that perspective. And in BUC using PCA techniques that grouping the patients together, that they're clearly different based on their proteome of over 3000 proteins. The mild, severe, long Covid seem to almost rely on an access with the healthy controls elsewhere. If we look at all the patients individually, you can also see from the PCA and the plots that long Covid is very different from accutely ill Covid and from the healthy controls.

We then went on and did cellular silerdeconvolution, looking at immune cell types using the cybersort tool. And we made a few findings or had a few findings that were quite interesting. The memory cells aren't to be unexpected, but you'll notice in the upper middle NK cells took an interesting turn. The phenotype of the NK cells seem to go from an activated to a resting state in the long Covid patients. And then we also had an elevation in neutrophil related proteins as well. Here we've got a pathway showing the NK cell media toxicity. Whenever I show

you these pathways, if you see red, it means the pathway's gone up. If you see blue, it means the pathway's gone down. And in the resting state NK cells will constituently release TNF alpha GMCSF and interfering gamma.

Just some heat maps to drive home how different the patient cohorts are. And this is NK cell phenotype. You see healthy control, mild Covid-19 severe Covid-19, the long Covids. And I think just by eye you could see the difference with the long Covid patients and with a Pearson correlation next to it, you could see similarity in the proteins. We went on to do some pathway analyses and we found that TNF was our top hit, as you can see in the top left. Again, constituently released from resting NK cells. TNF activates angiopoietin system as well as the VEGF system. And you can see some of the markers on the right-hand side, with TNF highly elevated, MMP9 involved in the remodeling of the basement membrane, blood vessels, VEGF and angiopoietin1. What's intriguing is as opposed to the acutely ill Covid patients where angiopoietin two tends to be higher than angiopoietin1, there's been an angiopoietin1 to two conversion which is occurring and likely related to angiogenesis and sustained vascular proliferative response.

We also wanted to look at the neutrophils which were elevated as well, and look for evidence of something else going on in the blood vessels. In this particular case, looking for extracellular trap formation or nets. And in this particular pathway you see many of the markers are up-regulated right down to the end where you see MPO is elevated and which is released by neutrophils. Here are some of the markers and you can see they're elevated. The second one in from the top is P-selectin, which I've already shown to you and is highly elevated using this assay as well. So our angiopoietin1 and P-selectin are elevated. No matter what assay we use. They seem to be very, very consistent.

We wanted to validate this because not all of the markers for nets were available in our proximity extension assays. So we took a separate population and we looked at R 17, which is citrinated H3, and you could see it's highly elevated long Covid. In fact, you might even say it's almost specific for long Covid. And then we have elastase, which is sineprotease release from neutrophils, which is also elevated. It seems to go up in a graded way. We reported previously in Covid 19 acute illness that it's elevated and that it's associated with the neutrophil lymphocyte ratio, which can predict mortality with some success. Again, some heat maps just to drive home the neutrophil changes. The neutrophil phenotype has changed quite dramatically in long Covid. And again, we find patches of similarities and proteins on our Pearson correlations as well.

So I'm just going to get into the TGF beta pathway for a moment. And in natural fact that TGF beta pathway is actually quite quiescent, with the exception of one protein that is of interest. And that's EP 300 you can see in blue and it's down regulated quite heavily in long Covid. EP 300 down regulation would actually enhance the effects of TNF alpha on the vascular system. And we're just showing these in plots down on the bottom left with some protein protein interaction. Where TNF and where EP 300 and other markers like IL6 would be important is activation of the Hif pathway. And we've investigated the Hif pathway quite extensively and we have some

interesting results there as well. So the VEN analysis shows overlap in about 80 signaling pathways between mild Covid, severe Covid, and long Covid. But one of the top ones is the hypoxia inducible factor signaling. And you'll see three pathway graphs.

One at the top is mild Covid, the middle one's severe Covid, the bottom is long Covid, they seem to gain strength as you go down. In the top one IL6 tends to be the top instigator, but it works its way down and you get angiogenesis and vascular and changes in vascular tone as one of your results. Once you get into the severe stage, now IL6 interfering gamma and growth factors including VEGF, would all come down and they would also activate the system maybe a little bit more powerfully. And then in long Covid, the system seems to be more optimally activated, perhaps being driven almost specifically by growth factors. But EP 300 as you can see in blue is down regulated and that's enhancing the downstream effects with angiogenesis and vascular tone. So all of the pathways we're looking at, all the things we're were we're investigating all seemed to be coming down to the vascular system.

I will say hypoxia, so the patients who were admitted to hospital had some degree of hypoxia, so that's not surprising. But the long Covid, many of them were not actually admitted to hospital. So we're not sure if they were happy hypoxic patients wandering around with slightly low oxygen saturations. But there are other drivers of this pathway, and that could be CX CL5, other growth factors, but also the renin, angiotensin aldosterone pathways well could drive this particular system and those are all implicated in long Covid. I'm going to stop for a moment and I'm going to go back and I'm going to talk about drug repurposing. So one thing we can do with a proteome and the data we have, is we can go to the drug databases and we can screen them for potential drugs that have already been approved, that are safe to use in humans and that might be helpful in disease.

And we already published this one last year. So fes tyrosine kinases came up very strong in our hits when we went to the drug databases and there's an antibody against it which has been approved for ITP or immune thrombocytopenia. We thought this was quite interesting. And soon afterwards we found out that the company had actually been doing trials. And when the trials came out, we found out that this antibody against fes tyrosine kinase actually showed really good results, with improved clinical outcomes compared to placebo. I believe one phase two trial might have even had close to a 50% reduction in mortality in ICU patients. So this is a great example of how we can use the data to go back to the drug databases and look at drug repurposing to move a lot faster. What have we done for long Covid?

For long Covid, we've come up with a whole series of drugs. Some would directly modify the HIF pathway, which I've already talked about. Where you see the drug target is PhD, which is one of the enzymes. But you'll notice as you go down the drug target column, you'll see a lot of VEGF and angiopoietin. So again, everything we're doing is kind of coming down to those same mechanisms and the same vascular involvement in long Covid patients. So here's some data that hopefully will be useful going to clinical trials. With regards to angiogenesis and

the proliferative mechanisms, more pathways, the HIF involved EP 300, you work your way down, you look at the molecules that are up-regulated or should be to sustain angiogenesis and in fact that those are our findings. So that all fits really nicely. On top of that, we can look at growth factors, meeting proliferation, and these are growth factors associated with tyrosine kinases, and in particular the IGF system and the IGF binding protein system.

And again, we see changes in long Covid that would result in sustained angiogenesis and our piercing correlation show nice protein interactions. I'm going to get in just for my last couple slides, get into organ dysfunction. Now, when we look at brain dysfunction, these are markers that are curated by the company that does the proximity extension assays. And I think right away you can see from our heat maps that something very different is going on in long Covid relative to mild and severe Covid. And this is suggestive of brain dysfunction that can occur in some patients with long Covid. When we get into the actual pathways, we find that cellular migration, neurogenesis, glial cell involvement and so forth tend to be dominating the pathways. And when we look at the protein interactions, it really is about cell survival, cell death, but we were surprised to see amyloid accumulation came up very strongly. And in fact, amyloid precursor protein is highly elevated in the plasma of patients with long Covid.

Here are some graphs to show you, each of them have a different function. S 100 A is a calcium binding protein involved with neuronal development. KIA is a very interesting protein. It's called dyslexia associated protein, and it's a marker of language impairment and it's actually quite high in the long Covid patients. Amyloid precursor protein, as I've already stated, is high. GEM two, which is a blood vessel tight junction protein is high. Potassium channel IRO, which is involved in neurite outgrowth, and then snappin, which is involved in chemical transmission and recycling of synapses. We wanted to take us a little bit further. So we've been curating our markers based on all of the available literature, to come up with specific markers of particular cell types within tissues. Now, anyone of these proteins can be expressed anywhere. We fully admit that. But it's about the combination where things start to strengthen and then again, it can become hypothesis driving work.

We've looked at astrocytes and microglia two LIA cell types in the brain, and we do find a pattern shift in the proteins that we've been able to curate. And you can see from the severe to the long Covid patients, there has been a shift. So I think we are starting to see some evidence for brain dysfunction even if we are measuring them in the peripheral blood. But again, this provides hypothesis driven research. We also looked at the cardiovascular system. And again, we have changes in long Covid relative to the other patient cohorts, and they really fall across three spectrums. And if I look at the actual cell types, they involve integrins. Fibronectin is highly down-regulated, MMP7 is up, CCL5 is up quite dramatically. And that can result in sustained a sustained inflammation.

And then when we get into the pathways, a lot of what we're seeing in heart or cardiovascular tissue relates to extracellular matrix reorganization, and then angiogenesis. So we have that link between angiogenesis and that vascular proliferative process going on, as well as what's going on with organ dysfunction. And when we

get into the protein protein interactions, again, we see a lot of integrin, fibronectin, and calcium binding protein interactions, as well as that activated CCL five. So in a nutshell, where's our data going? Here is kind of what we think is happening based on our data in long Covid, is that earlier hypoxia may be activating the HIF system along with other factors, whether it be growth factors, persistent IL6, the renin angiotensin, aldosterone system, and CXCL five. All of these things can contribute. But ultimately the HIF pathways are being activated, the EP 300 is being down-regulated, that's really allowing angiogenesis and vascular tone to be affected.

We've got activated NK cells going to resting NK cells with constitutive TNF release, also activating the VEGF and angiopoietin system. At the same time in those blood vessels, we have lots of neutrophilic started traps happening involving neutrophils and activated platelets. So there's a lot going on, but at the end of the day, I think it really comes down to a vascular proliferation as being one of the major mechanisms. And I hope that what I've been able to show you today will drive some more research, we'll be able to move a little bit quicker. And I hope that the drug repurposing will also prove to be useful. Just some acknowledgements, I'm at Western University in London, Ontario, Canada. I have a small lab of great people and wonderful research and hospital collaborators. Thank you very much for your time. Much appreciated.

Christine Bevic:

Thank you, Dr. Fraser. Well, we've got some questions that have been dropped in there. Our panelists are answering those questions live, so if you submitted those, please be sure to check back. Next, we'll move to our final presentation by Dr. James Heath. Dr. Heath,

Dr. Heath:

Thank you. Is my screen showing?

Christine Bevic:

Yes, you are. Great. You're all set.

Dr. Heath:

Very good. Okay. Okay. So today I'm going to talk about, as Dr. Fraser gave a deep dive into the plasma potiome and how it relates to long Covid. I'm going to talk about a deep dive into cytotoxic T-cells and how those relate to long Covid, in particular the disease journey that patients experience that either laid them back to health or to a chronic state. I'm going to just point out Jingyi Xie and Daniel Chen as primary folks involved in this work. I'm going to talk about two independent patient cohorts, one recruited by Helen Chu, but then the primary one that we studied recruited by Jason Goldman here in Seattle. A couple of disclosures. So about a year ago, we published this paper on a number of factors that we identified at diagnosis that could anticipate long Covid. And

most of these factors actually are gone by something like six months. In fact, basically all of them except for of course the preexisting comorbidities.

But one thing that we found when we looked at cytotoxic T-cells in these patients, is that we found that if you looked at the kinetics of the T-cells over time, that what one expects is that you would have, for example, for SARS-CoV-2 cells, they would begin as a more or less a naive pool that would expand into a cytotoxic pool. And then over time, most would disappear, some might form memory. But we found that there was continuing expansion of certain cytotoxic T-cell clones, both specific to SARS-CoV-2 and specific to cytomegalovirus, which in these patients was not activated. This is what's called bystander activation, meaning these cells don't see antigen, but they are probably activated through other pathways. And cytotoxic chronotypes of these T-cells expand over time in patients that uniquely develop gastrointestinal PASC.

And this prompted us to do a much deeper dive into these T-cells. And that's what I'll talk about. The work I'm going to talk about is not published. So the way that we did this is there's a technology we've been developing over the past several years, which allows us to make the common reagent that's used to capture T-cells, which is a peptide MAC complex, generally assembled into a attachment or some sort of multi format. But to make very, very large libraries of these, and thousand element libraries or so as this type of construct here called a single chain trimer. And there's a lot of engineering and work that's been gone on. And if you look in two recent nature papers and a bio paper that we've all that we've published over the past six months or so, you can see more on this technology.

In any case, we use this technology to convert the entire SARS-CoV-2 proteome for HLAA 2.1 allele, into a library of peptide MHC type reagents, multimores. And then we used that whole library to pull cytotoxic T-cells down from on the order of 65, 70 patients that were HLA 2.1 haplotype. And then we see these particular chronotypes that get pulled down. And so I'm going to talk about these T-cells. And when we did this experiment, we actually pooled all of these patient bloods together and pulled down the T-cells, kept them on ice, did it very quickly so that we could immediately follow that with a multiomic single cell analysis of those viral specific T cells.

And so we end up with a traditional UMAP type representation. And this is just the single cell transcriptome data, but it's a little bit richer than most such presentations that one normally sees. Because for every cell, we not just know it's phenotype. We also have it's site seq, so we have a hundred and some proteins. But we also know the antigen specificity, because we barcoded our multimores to know which antigen the T-cell recognize. We get the T-cell receptor alpha and beta genes, which you need both of those to have a full barcode on the T-cell, so you can follow them over time. And then we had the whole genome on our patients. And so we used SNIP analysis between the genome and the transcriptome to assign an individual cell to an individual patient. So a very rich data set. And just to give you a flavor of what this sees for a couple of antigens, are also we looked at both C M V and SARS-CoV-2 specific T-cells.

Here is basically one particular antigen that's immunogenic, it's seen by many patients. Every color is a unique patient that has T-cells clonototypes against this antigen. And these are the different phenotypes. By and large, SARS-CoV-2 specific T-cells are sitting here. And if you go back, these are sort of naive central memory effective memory cells. And CMV unique cells are basically, like I say, they never see antigen. You don't really see an antigen exposed signature on these cells and they're really cytotoxic and more terminal phenotypes. So we take this dataset and we project it over time, and so that now we have that same dataset I told you, but we're able to follow which cells expand over time and which cells contract. And by and large, you would expect the vast majority of the cells to contract. And they do, they basically disappear. But what I was shown you here is a map of the chronotypes that contract, they're the orange ones here. And then add it two or three month,. These are the chronotypes that persist and even mildly expand.

And each of these chronotypes represents multiple cells, 10, 20 cells. And so there's a lot of statistics in this types of a plot. So then we ask how different are these cells? And it turns out they're actually extremely different. The ones that expand or the ones that contract, you can identify right at diagnosis which cells will contract and which cells will expand. They have very different proteins on their surface. They have a very different transcript. They they're cytotoxic, but they have a subset of toxic phenotype that's different if they expand or contract. And in fact, if you look at the ones that expand over time, their phenotype, and this is a UMAP made with just the proteins, is remarkably constant. And so the fact that these cells are persistent and of an unchanging phenotype, makes us call these remembered cells. Because they're there early on, they're there later on, and they don't change. Here's the ones that we call forgotten. And these are the cytotoxic cells that basically contract over time. And you can see these different trajectories of expansion or contracting for a few selective these of these chronotypes.

When you take that proteome and you analyze it to distinguish the differences between what cells contract and what cells persist, remembered cells and the forgotten cells. Here's the forgotten cells. And in fact, they look like they're just extremely cytotoxic, and the phenotype here literature publications on what are called short-lived effectors. If you look at the nature of the cells that actually are remembered, they have a host of different regulatory markers. They have NK markers, they have memory markers, they have exhaustion markers, but they're a very different and distinct phenotype. And you can see these markers are according to literature definitions, we just classify them here and what you see for the forgotten cells. So you can take two of the best markers from here, any two. In fact, you can take multiple combinations of these guys and develop a predictor, meaning that at diagnosis, how well can I predict what cells will expand and persist over time and what cells will basically be cyto cytotoxic and then disappear over time.

And that predictor is very accurate. This is the ROC curve of that, so it's like 90% accurate. So now we ask do these cells... We've done this on now because we took this initial dataset here where we had 65 patients. But now that we have these markers that we identify here, we can apply it to many patients. And now we've gone to

something like 300 and some patients. And if we look at these best of these markers, we're now able to say, are there anything about these T-cells that associate with long Covid or not? And what we find is that a lack of remembered cells strongly associates with lung Covid. And these two independent cohort, this is a mildly infected cohort. This is basically a cohort that I think more than half of the patients were hospitalized.

The implication here is that the presence of these remembered T-cell seems to offer some level of protection. And it looks like there's some sort of a regulatory capacity in the CD8 T-cell compartment that is protective. And we actually call these cells not regulatory T-cells, because it's not like they're influencing other cells. We think of them as regulated T-cells. They're actually regulating their own behaviors. And I'll elaborate on that in the next couple slides. So we took these same patient PBM cell sees and we did an analysis where one measures the single cell transcriptome as well as ATACC, meaning the chromatin accessibility in these patients and these T-cells. And so what I'm showing here are these are the patients that have these regulated T-cells that are normally protected from long Covid, and those are the patients in blue. And then the patients that have low numbers of these T-cells are in orange.

And in the ATAC seq, if you look at what genes are exposed in these regulated T-cells, some things pop up with great frequency and in particular a bunch of genes that are associated with basically the black oscillation. And there's a number of literature papers suggesting that when these genes are expressed, that the receptors on these T-cells are just black oscillated and that tends to protect them from activation, it makes them less active. It's basically just steric issues in terms of ligands that come in and bind. Whereas when you look at the T-cells that are in the patients that don't have these regulated T-cells, the things that are differentiate open are basically interferon type signaling. And so this just provides another picture that these offer protection against long Covid are regulated. Their activity is just toned down a little bit, even though they're effectors there is diminished.

When we look in particular at the chromatin in these two sets of patients, we find that there's what we call an enhancer region right next to TGF beta, which you would expect to be associated with this regulated like behavior, that's wide open in the patients that have these regulated T-cells, not in the patients that don't have it. And it turns out that this particular enhancer region basically strongly correlates or strongly associates with all the properties we see in these T-cells. And so here's associations with markers on the same chromosome. Here's association with markers on a different chromosome. And I can sum summarize that up here, where I take that one promoter enhancer region here, which is this guy here. And it has strong negative associations with these interferon and cytotoxic markers, and it has strong positive associations with the regulatory markers. So this suggests to us that there may be some sort of a genetic underpinnings of this.

And so what we did is that we look at a number of genetic variants that have been associated through a large GWA study with systemic lupus orthosis. And in these patients, we associated the accessibility and the presence of these genes in these patients. And what we see is that this is the alternate risk allele and this is the

reference allele. And the patients with without these regulated T-cells are shown in orange, the ones with the regulated T-cells are shown in blue. And what you see is that in almost all cases, the risk allele or even the openness of the non-risk allele, the reference allele, which also convert the risk for the autoimmune syndrome, is elevated in these patients that lack these regulated T-cells. And here's just a map of the particular genetic mutations. These are patients that lack these regulated T-cells here, and you can see these mutations are much higher here. And so this suggests that at least in this view, that the genome is going to be causal.

And so that there are causal relationships at the genome level that not only are associated with these regulated capacity in the CDA T-cell compartment of these patients, but also associated with the pensity of patients that don't have that capacity of regulated T-cells in their CDA compartment to develop long Covid. And it's a direct connection genetic to a systemic lupus orthosis as well. And so my conclusions are that tools for pairing antigens with CD8, I didn't talk about CD4 T-cells, but that technology is working as well. Allow us to really look across thousands of T-cell antigens and large patient populations in a single experiment, and give us a set of new glasses on T-cell immunology that we simply didn't have before. And the CD8 T-cell kinetic responses specific to the infected virus, separate patients into patients that have this high CD8 plus T-cell, I would say regulated capacity, and then patients without such capacity. And this is about the top third and the bottom third, there's an in-determinant group in the middle.

And those classifications can connect PASC to systemic lupus orthosis, and they can also differentiate SLE patients from healthy. And with that, I'll just thank my funding institutes. I'm always looking for talented postdocs and I'll join in the question and answer session. Thank you.

Christine Bevic:

Thank you Dr. Heath. And thank you to the rest of today's presenters, and they'll be turning on their videos momentarily. And thank you to all of you who have submitted your questions. We have a number of questions to get to in our Q&A and some of them have already been answered. But first I'd like to welcome back Dr. Stone to provide us with a quick synthesis of these three presentations and help lead off our discussion. Dr. Stone?

Dr. Stone:

So those were just three wonderful talks, very detailed, very informative. So one of the themes running through these three talks are the changes that we are detecting, that you are detecting in patients and your models of PASC. Particularly these enhanced antiviral gene expression, enhanced inflammation in general, especially with CD8 subtypes and even vascular proliferation. The question I have that certainly is important, and I'm trying to understand why this is happening with Covid when it hasn't happened with SARS-CoV-2, when it hasn't happened with other viruses in the past as far as we know to this extent at least, is to what role is viral persistence. And certainly viral persistence has been observed in numerous studies now in patients with PASC, to

what role is that potentially playing in the models and in the systems that you're looking at? Is it irrelevant or are the changes happening early? Or is viral persistence working along with and causing some of the changes that you're seeing? So to all the speakers.

Dr. Heath:

I can take an initial shot at that, but I'd be interested to hear what my colleagues have to say as well. What we're seeing in our data is that some patients basically have a robust ability to form cytotoxic T-cell memory and some don't. And if you have a virus that goes into some quiescent stage or becomes hidden and then reemerges by who knows what, you're likely to regenerate, you're likely to have flares. I think that's actually the kind of thing that one sees in a lot of autoimmune conditions, which is one reason why we were trying to look for this comparison with lupus, is this periodic flaring may just have to do with the capacity for immunological memory in the T-cell compartment. Now B-cells are a whole different story. I can't comment on that. But that would at least be my hypothesis for why viral persistence may be important here.

Dr. Fraser:

I don't have a great answer. What I would say though is whether it's cumulative or an amplification effect, and I use the HIF pathway as one example where you have a hypoxia induced pathway, but then it could be regulated and amplified by so many other factors. So whether it's persistent infection, whether it's reinfection, whether it's a different variant of concern, I certainly don't have the answer, but it does seem like there does seem to be amplification processes that are going on.

Dr. tenOever:

Yeah. I don't have so much to add to that. I would agree that if we do trust the Hamster Model as a proxy for what's going on, there really is no strong data suggesting that the virus can persist for longer than seven to 10 days. The examples that we've seen in the clinic are usually the exception to the rule. And so one easy way to test this would be to simply ask the question of those suffering from long Covid, does something like Paxlovid offer relief from the symptoms? And I feel like the answer to that is has been clearly no.

So it would suggest that if it is persistent viral material that's constantly ping the innate immune system, it's not actually contagious virus per se, but maybe remnants of the polymerase that allows it to self replicate or maybe really long pieces of genome that are now double-stranded that are just really stable. So that they can induce a response by microphages, microgliam T-cells. Things in the immune neighborhood will respond to that antigen or that pathogen associated molecular pattern, but in itself is not an infectious particle. That's kind of where I would fall, that it's possible, but not likely.

Dr. Heath:

I think we would agree with that 100%. You just need fragments or non-competent virus or some other signatures to emerge.

Dr. Stone:

Excellent. And another question that's always on the mind of our viewers for these R3 seminars, is the impact of vaccination on the things that you're measuring and observing. Obviously, vaccination does impact the development of PASC, although to not as great an extent as maybe some would've predicted in the beginning, but it does impact. And I'm just curious if you've looked into this at all in terms of whether your patients are vaccinated or not, and is it affecting what you're observing in the systems that you're looking at? Anyone want to take a stab?

Dr. Fraser:

Sure, I'll take a stab at it. So I know our initial studies, there was only a 7% vaccination rate in the data that I showed you because again, it was wild type alpha, delta was fairly early on, and a lot of people that wound up sick were the people who were non-vaccinated. But at the end of the day, what we're doing now is trying to collect vaccinated, non-vaccinated, as well as VOCs, and trying to see how all of these different pathogens and vaccination so forth are making a difference. And just we have to start comparing those groups.

Dr. Heath:

Yeah, I would agree. Those comparisons require basically the RECOVER cohort to do those kind of studies, and the size of the RECOVER cohort to try to understand the details of that. And it just simply hasn't been parsed through yet.

Dr. tenOever:

We can vaccinate our hamsters, which we have done, but if you do that, they just don't get infected. So we don't see any cognitive decline or no development of anosmia. So in the Hamster System it's very clear cut, but it's not quite the same per se.

Dr. Stone:

Okay. Christine, do you want to take some questions?

Christine Bevic:

Thank you, Dr. Stone. Yes. So we'll go ahead. There's a couple questions that we've received. And Dr. Fraser alluded to this first question, this was submitted with the registration. About the differences that you're seeing in the analysis with the 2020 to 2021 wave of Covid, versus the more recent variants, and whether you're seeing similarities in the organ and tissue damage. And that's open to the entire panel. Does the variant matter?

Dr. Fraser:

That's a great question and that's what we're after now. That's what we're trying to answer now.

Dr. Stone:

I would say this question comes up a lot, and I would say the problem with human patients that you have is that the variants are linked with vaccinations. So it's very difficult to tease out what a variant is doing, because you're often dealing with a highly vaccinated group versus a largely unvaccinated group. And Ben's model may actually be a way to more clearly try to look at that if he starts using different SARS-CoV-2 variants in the model. I don't know if you've thought about that yet, Ben, but that might be a cleaner way to look at variants. Because it's really difficult when you have such differing vaccination rates.

Dr. tenOever:

Yeah. We haven't experimentally addressed that particular question before, but yes, we do study the different variants, which all do behave again very much in line with what we've seen in from a clinical setting. So you do see that as the variants progress along what happened during the pandemic leading all the way to omicron, you get more of an upper respiratory infection as the new variants arise in hamster just like you do in people. But we haven't done the kind of a anosmia and cognitive function tests on those studies, mostly just because we haven't got around to it yet.

Christine Bevic:

All right. Thank you. So our next question is another one that was submitted in advance. Can we distinguish between the organ damage caused by the acute infection from, versus organ damage resulting from autoimmunity?

Dr. tenOever:

If I answered that question, I would say there is no organ damage as a result of the acute response. Because we see the exact same amount of inflammation with flu, which is not associated with all of these different complications. And so I think it's a pretty strong case for that to be true. And what's really happening is it is the sustained signaling from whether it be latent virus reservoirs, or rogue immune cells, or just a constant pinging of this antiviral system from something in the body that is doing damage over time, I would argue is a better probability.

Christine Bevic:

Thank you. All right. So the next question comes from our Q&A, and it was actually posed by a couple folks. So we're going to combine this to get a response from this, and this is back to you, Dr. tenOever. So you mentioned about prescribing dexamethasone as immune suppressant there. So what are the implications of that?

What does it mean for prescribing dexamethasone in Covid, and would you expect that inhaled steroids for asthma would cause some of these same problems as what you're seeing in your model?

Dr. tenOever:

Yeah, no, it's a really great question and it's an important thing to keep straight. So when we give dexamethasone to people in a clinical setting, it is well after the seven to 10 day period where they have infectious virus. And so it would be a bad idea to give anybody systemic steroids when an active infection was happening, because it's going to prevent your immune system from acting on this virus, which is obviously an important thing for that to do. So we do it in hamsters to artificially mimic what it would look like if you were immunocompromised in some way, which then takes on a very different biology. So from an experimental model point of view, it's a very different setting. It does speak to the fact that we should not use steroids in people who are actively infected with virus, but that's also kind of an obvious thing that no clinician would ever do.

It's interesting though, in the setting though of long Covid in that if we really do feel that some of long Covid individuals do have a latent virus, then the use of steroids would in fact make it worse. You would actually provide the opportunity for that virus to flare up again because you've now suppressed the immune system. And so in a lot of ways when people have certainly reached out to me and my thoughts on people who are desperate to do anything in this long Covid space, it seems like the safest thing to do would actually start with something like Paxlovid, where if there was any latent activity of the replica's machinery, you've got five days to clear that up and knock it out of the park.

And then after that you could try steroids to see if the driving cause of it's ever manifesting itself in this clinical complication, can be reset back down to baseline values by simply using steroids. It would actually answer a lot of questions, certainly what I thought was a well modeled grant submitted long ago that was promptly denied by the NIH. But someday I'd like to give you that answer.

Christine Bevic:

All right, thank you. And we have our next question is to Dr. Heath. Is the genetic condition that you discussed during your presentation, be one that might be mimicked in those without the genetic condition over time, possibly with multiple exposures? Or is there vulnerability to get long Covid restricted to that population that has those certain gene genetic predispositions?

Dr. Heath:

Well, the genetic predisposition I showed had two components to it. One was the risk alleles that were associated with lupus, and the second was the exposure of either the risk alleles or the non-risk, the reference allele. And it's known in the literature that even the expression of those proteins can be detrimental even if you know the risk allele. And so the suggestion would be that yet you don't necessarily have to have the risk allele to

have this autoimmune condition, it's going to be whether those genes are accessible or not. But if you do have the mutation, it's a higher risk. And I would say those mutations are also associated with some of the proteins that one would expect associated with immune activation. And so it's not that surprising.

Dr. Stone:

Have you thought about expressing this as a percentage of the risk? What percentage can be explained by the genetic component? Are you able to do that?

Dr. Heath:

Not yet. And I would love to see for example, in the RECOVER study, every single person have a whole genome collected that would allow you to do that. Good luck. But that's I think what's necessary. I can tell you that, so previously we had seen that auto-antibodies early in the disease course even at diagnosis, anticipate chronic conditions developing later on, those auto-antibodies sort of disappear. But if I looked at this T-cell story I told you, it doesn't explain what those auto-antibodies explain. There's multiple etiologies associated with lung Covid, and my guess is there may be a different set of genetic associations there. So that's why gosh, you got the samples, measure the whole genome of all these patients, and let's try to through this. I think that that's actually what's required.

Christine Bevic:

All right. We have time for one more question. And because everyone's so captivated with Cocoa Puffs, we're going back to the hamsters. And this is a question that we got about the behavioral changes. Are there ways to measure the symptom equivalent of Perosmia, where the hamsters are smelling something but it's qualitatively incorrect? Are they smelling something but they're not smelling Cocoa Puffs, they're smelling something else?

Dr. tenOever:

Yeah. I don't know how to do that. That would be a tough one to do. It may be possible, but the truth is that all behavioral tests have been designed and optimized for rats or mice. So even applying the existing tests to hamsters, like I said, I'm very cautious to say what it means or doesn't mean beyond the fact that we see statistical differences. I would imagine because the biology is so similar to what we see in humans that, that probably Perinosmia almost certainly also happens in a subset of hamsters. It's probably caused by the fact that when that baseline inflammatory signaling actually is at its height, the olfactory neurons that are taking in all these responses, their biology is basically shut off and prioritized entirely to be antiviral. And so when that goes back down to baseline and those neurons can now regrow their outgrowths, for example, I can see things getting confused and misfired where when one smell triggers one receptor, you might mistake it for something different. So I would guess that would happen, I just don't know how to actually test it in a biological model system.

Christine Bevic:

All right. And we have just another moment. Are there any closing thoughts or comments from our panelists?

Dr. tenOever:

No, thanks. This has been a lot of fun.

Dr. Stone:

I have one other question. Oh, go ahead.

Dr. tenOever:

No, no, I was just going to say thanks to the group.

Dr. Stone:

For both Doug and Ben, both of you showed changes in the brain that were more extensive than the other organs. I'm just curious if you had an explanation in your systems as to why that was.

Dr. tenOever:

So for us, we don't ever see virus in the brain. We really see virus right adjacent to the brain along the these epithelial cells called cystacular cells. And so what's radiating into the brain is actually the necessary chemokines and this pro-inflammatory environment that's allowing for the more recruitment of microglia and T-cells and B-cells, and then they're driving further inflammation for reasons I don't know. My guess would be it's debris. And so I do think the brain plays an important role, but the individuals out there who are having complications outside of the brain are also have been already pre-selected to be one of those individuals where the virus maybe did escape the respiratory tract and did find its way into the vasculature to find another organ, which has caused their particular complication. And so if we had enough hamsters, I think you would see examples of that, because like humans, they're out-bred. But I think the numbers that you would require to do that would be prohibitively expensive, but I bet that also is a similar example for other organs. It's just that the brain is the most consistent because of where replication actually occurs.

Christine Bevic:

All right. Dr. Fraser, 30 seconds.

Dr. Fraser:

I wonder if it has something to do with the blood-brain barrier. The blood-brain barrier is a unique organ in itself and there's tight junction interruptions. There's a very strong leukocyte migration pattern going on, so I

wonder if that's playing a role. But outside of that, I think we need to start looking very closely though at the amyloid question.

Christine Bevic

Thank you. And with that, we are wrapping up for today. So please join me in thinking again our RECOVER investigators who have joined us today, to share this exciting work in progress. And thank you to our audience for joining us today. The FAQ for this webinar will be posted along with the recording on recovercovid.org. The FAQ will include the answers to those questions relevant to today's webinar, as well as those submitted in advance and during the session. Questions on other scientific topics will be addressed in future webinars, and we may have already addressed them in past webinars. So there are going to be broader questions about RECOVER, and those are also going to be available on the recovercovid.org. As we close, we invite you to come back and attend our future R3 webinars, as we dive deeper into some of the broad topics that were discussed today.

If you haven't already, please remember to sign up on our website to receive those future announcements and updates on the series. And if there's additional topics that you want to learn about, be sure to jot those into the survey that just popped up on your screen. And we're going to leave the webinar open for you to complete that survey. But please join me again in thanking our presenters and thank you for joining us today. This concludes today's R3 webinar. Thank you.