Immune Webinar October 19, 2023

ME/CFS Research Roadmap Webinar Series – Immune System
Open Session
Thursday, October 19, 2023

Vicky Whittemore: Okay. So, good morning, everyone. It's my pleasure to welcome you to the second in our series of webinars as part of the ME/CFS Research Roadmap Webinar Series. Today we're going to be focused on the immune system. And I'll just give some brief introductory comments. I should introduce myself. I'm Vicky Whittemore. I'm a program director at NINDS, where I oversee grants on ME/CFS, as well as work together with my colleagues at NIAID and across NIH, and Dr. Koroshetz, director of NINDS, to coordinate the Trans-NIH ME/CFS Working Group.

So, I'd just like to recognize all of the members of our Research Roadmap Working Group of Council. All of these individuals have been busy organizing each of the eight. We now have added one, so there'll be eight webinars in this series. And my special thanks go out to the chairs of each of the working groups, as well as all of the members of each of the working groups. And today, special thanks to Derya Unutmaz from the Jackson Laboratories and all of the members of the Immune System Webinar Planning Group who have worked to organize our webinar today. And I also would like to thank the NIH team working with me at NINDS that's really been sort of here in the background and helping to support this whole effort. My deepest appreciation to all of you for your extra work and hard work to make this happen.

So, I would just like to point you to the website where you can go to get more information about the webinar series as well as click on the link that links you over to the website where you can register for each of these upcoming webinars between -- there's one upcoming next week, two in November, one in December, and two in January. So, just some guidance for webinar participants today. So, the goal of these webinars is to identify research priorities for research in each of these topic areas.

So, as I said, today we're focused on immune system involvement in ME/CFS. So, we've asked the speakers to address what do we know, what don't we know, and what do we need to know to really accelerate the research to move forward toward clinical trials. So, this is not your typical research conference or research webinar. So, the focus really is getting into looking at those questions and research priorities for research going forward. So, the questions for each speaker will be addressed after each presentation. So, put your questions in the Q&A. It's likely we will not be able to address all of the questions we receive. We've asked the speakers to answer questions in the Q&A that we might not be able to get to. And we're not able to answer questions related to your individual health issues. So, please refrain from asking those types of questions. This is not a patient conference. And so, we really cannot answer those kinds of questions in this

forum. So, your questions should focus on clarifying things that the speaker said or focus on research priorities in this area of ME/CFS research.

So, we will have an opportunity for additional feedback. You can send an email to this mecfsresearchroadmap@ninds.nih.gov, email address, and we will respond to that and take your feedback. The best way to receive announcements and updates from NIH is to sign up for this NIH ME/CFS listserv by going to this link, https://www.nih.gov/mecfs. And so, watch for announcements coming soon to participate in discussion on a crowdsourcing platform called IdeaScale. We'll be posting the research priorities that came from the Nervous System webinar back in August very soon. And subsequently, we'll be posting the research priorities for each of these webinars for additional community input and feedback.

So, with that, I will turn it over to the chair of the Immune Webinar Planning Group, Derya Unutmaz, who's a professor at the Jackson Labs in Connecticut. So, thank you. And I'll turn it over to you, Derya.

Derya Unutmaz: Thank you very much, Vicky. I'd like to also start by thanking you and NIH and all the supporting team for initiating and organizing this really fantastic series of webinars. And I welcome all the attendees. So, today, we're going to focus on immunology. And we have a very, very fantastic group of speakers. And hopefully, we'll have some discussions around the questions. It's a very tight schedule, so I want to get started right away and introduce our first speaker, Dr. Nancy Klimas. She is a professor of medicine at the Department of Clinical Immunology, College of Osteopathic Medicine, and the chair of the Department of Clinical Immunology at Nova Southeastern University. I'm sure a lot of you know Nancy because she has been involved in ME/CFS for many, many years. She's been recognized for her outstanding clinical work and basic research work as well. She's also studied Gulf War illness, fibromyalgia, and recently Long COVID. So, without further ado, Nancy, the floor is yours.

Nancy Klimas: Now, I'm good. I was going to double down on the thank you back. Thank you very much for inviting me. And I'm really pleased to be here. So, I was asked to give sort of the clinician and clinical immunologist type of overview to get this session started using these key questions that Vicky just reviewed. So, the focus of this talk and all the talks is to review what we know, what we need to know, and where do we go from here. And the double-down focus is how do we get to effective clinical therapy in ME/CFS. That's the ultimate goal. So, what do we know? We have been working on immunology for a long, long time in this field. My group, and many, many other groups have published hundreds and hundreds of papers on the role of the immune system or the biomarkers of the immune system, or the effects of the immune system. And we know a lot, a whole lot. So, we know there's inflammation. We know there's neuroinflammation. We know there are issues with immune competence, pathogen surveillance, and pathogen control. There's newer work, which you'll hear a lot about today, on immune senescence and immune exhaustion, and very exciting work in autoimmunity. Again, the speakers today will be focusing on that. And so, we have all these areas that have a considerable knowledge base. And then we have more literature on specific cells. And this field has initially focused, as much of immunology has focused, on the role of the lymphocyte in human disease, chronic disease. It's a very important cell, and it does a lot of things. And a lot of our literature is based on lymphocyte function, lymphocyte subtypes, and so on.

But there are other cells that are engaged in this illness and matter, and perhaps matter a lot. One which deserves a lot of attention is the role of the mast cell. There's a condition called chronic mast cell activation. We think of mast cells as allergy cells, but in fact, they're very inflammatory cells that can get on a slow kind of weak thing and cause a lot of symptoms and misery. Platelets matter, and we're learning that a lot more in Long COVID. Macrophages matter, they're the first step in any memory type of immune response. And neutrophils, pretty much completely ignored neutrophils, which are virtually half of the immune system. And so, these are all potential targets for clinical intervention and for a better understanding of what's going on in this illness. And they also can reflect things that tell you important knowledge. For instance, an antibody response

to a virus might be the only thing we can find that says there was a virus or there is a virus. Sometimes we can't find the actual bug, but we can see its immune signature. And so, these things all matter.

So, to start with inflammation and neuroinflammation, it's been described many times in many ways. There's some knowledge about general immunology that matters when you're trying to interpret all these types of papers. When we think about inflammation, we often fall back on models of autoimmunity like rheumatoid arthritis where there's complement activation, immune complexing, and a whole cascade of things that happened that respond to classic autoimmune types of drugs like non-steroidal anti-inflammatories. But in ME/CFS, what we actually see is a signature that's more obviously inflammatory in the cytokine panel, in the chemical messages that the immune system's making that promote not just inflammation but a lot of immune functions. And that this inflammatory cytokine signal is accentuated when you exercise. So, you'll see many studies, and some presented today, about how we use exercise in our research to actually see the signal and actually see the cascade of events that follows such a challenge that have intervention points. I call this getting in front of the tail of the dog.

Often what we see in a patient is how they feel today and what's circulating, but when we use the exercise challenge, we can see what happens first, second, third, and fourth and hope to treat the first. So, that we're not having to treat all these other downstream events. So, it's an exciting way to approach this illness, which is, of course, made worse by exercise. And so, it gave us this real opportunity, I think, to use this way, this kind of window into understanding the illness. So, what we have then is a lot of bench work, basic science, cool modeling work to tell us that there is inflammation and that there's neuroinflammation. I'll go into that in a moment. But what we do not have is a lot of targeted clinical trials that focus on that. In fact, we have very few and practically none. And so, these targeted studies with biologics haven't happened very much. And the physicians that are treating this and know it's there are left with a bag of tricks that is basically using their clinical knowledge and things that they can actually use and have reimbursed by third-party payers or aren't so expensive the patient couldn't afford it themselves. And it's predominantly nutraceuticals.

Now, nutraceuticals are drugs, and they need study. But we are doctors seeing patients with an immune signature, and we're going to treat it any way we know how. And this is somewhere in the space where most of us are actually trying to treat this illness. In Long COVID, there's also evidence of thrombosis and micro thrombosis. So, if you think about the body's bloodstream, and you see the great big arteries, the heart, the aorta, then the next bunch of vessels that are kind of the size of your finger, and then the next bunch of vessels that are about the size of straws, they get smaller, smaller, smaller until they're threads, and this is the microvasculature. And the microvasculature's really letting one cell through at a time in this speeding kind of way. They're rushing through delivering oxygen and glucose to the distal tissues, to the fingertips and the skin

and the organs, and so on. And in Long COVID, what we've seen is that there's evidence of micro thrombotic events and endothelial inflammation, the lining of the blood vessels, and that these are also targetable areas for intervention. We don't know if this is new knowledge. And we should have been looking there along more closely, although some people have in ME/CFS. But we do know that in Long COVID, that's another targetable point for intervention.

So, this is a demonstration of a microthrombi and what's happening here. And what you see is, at least in Long COVID, the ACE2 receptor is the place where the virus is bound. So, you can imagine there was a lot of inflammation right here. Your immune system goes right to where the infection is, and it goes after it. And so, it causes this space around here to be inflamed and damaged. And even after the virus is gone, there's still going to be inflammation. So, certainly, in Long COVID, we're looking at the role of the endothelium and this damage as a persistent thing that perhaps triggers cytokine release, inflammation, adhesion, microthrombi, and so on. And there are some clinical trials that are just getting off the ground now that are really focused on this. It's a very exciting time because we think we have a targetable point. We're always looking for targetable points. And in a systemic way, you're looking at your whole body's vasculature and microvasculature, and it's sort of everywhere.

But when we're also looking at inflammation, we sometimes look at a specific compartment and perhaps even only see it in a compartment. This has been particularly true in the brain, in neurologic studies, where we really care about neuroinflammation. Why? Because the brain is Grand Central Station for everything. It manages your autonomic nervous system, your neuroendocrine system, and your thinking. It's an incredibly important space. We're all glad we have one of those. But if it's got areas of inflammation, even if it's tiny little punctate areas in different spaces, it will affect that area and how it works. And so, there's a fair amount of brain work in neuroinflammation, a lot of its imaging. Kind of cool because then you can see it. And sometimes you need to see it, to believe it, but mostly because the brain is not a place where you can grab a biopsy.

And so, we either have spinal fluid or we have images, but that's the way we can look. But when people have looked, whether it's through spinal fluid or through imaging, they have found inflammation. And so, it's a space for a targetable point for treatment. Another space, and you'll hear a lot about this today, is the GI tract. This is because the GI tract has obviously a lot of foreign antigens. I mean, you got your microbiome, right? You got a lot of bacteria and fungi down there. And they're speeding through the gut, rolling by. And the immune system in the gut is intense. Every knuckle of your hand, there is a lymph node all the way down. They're called Peyer's patches, and they roll all the way down the gut. And there's a mucosal barrier that's supposed to keep a lot of the noise that would normally make the immune system get all engaged on the gut side and not on the bloodstream side of that barrier. And so, there's the mucosa, there's mucus, there's a layer of antibody, IgA antibody, and all this is supposed to protect your

bloodstream from having to deal with a massive amount of immune activation that could be going by. And so, this is what really matters. And we see this in our patients, this disrupted mucosal barrier that allows and is a potential inciting point for a lot of the chronic immune activation that then drives inflammation, autoimmunity, and other things. So, we care a lot about this gut barrier.

And in clinical practice, we're already treating it. In the absence of clinical trials in ME/CFS, doctors don't sit on their hands. They do what they know how to do, and it's something we know how to do. And so, we, or at least, we know how to do it somewhat. So, we do with the tools that we have, we approach that problem. We hope to have trials to help us do it more accurately with guidelines. In the brain, here's the neuroinflammation. I like to put this one up because these guys should get credit. The Japanese, Dr. Watanabe's group was the first one to apply PET scanning. Before we could even use these PET scans in the United States, they were using them in Japan. And they applied it to ME/CFS, and they showed neuroinflammation. Since then there've been a series of other PET scans with increasing precision and better ligands for testing neuroinflammation and one negative study.

But all the other studies have been confirmed these original findings of inflammatory metabolites and inflammation in the brain. So, it's a fair target. Oh, I was going to say spinal fluid. I didn't get to put the literature on this. But Dr. Baraniuk was the first one and there's been several others since, including -- well, there's been a number. I think of the Scott one and Marie Scott [spelled phonetically] some in her group. But they're using proteome or metabolome studies of the spinal fluid to see what's being generated. And at least in the studies that I've seen so far, definitely, there are metabolites and inflammation. And when earlier I said, we don't really see complement in immune complexes and things in the periphery when we measure it, in the spinal fluid, they did see complement activation. So, there was evidence of more than one type of inflammatory cascade going on in the brain in these ME/CFS. For the longest time, and from the very beginning of anyone that studied, MRIs have shown these tiny little dots, like pencil dots, of what they call type 2 flares.

And the normal person has a few of these. But in ME/CFS, we're seeing 20, 30 of these, a lot more than usual. And what are those? So, well, presumably they're areas of inflammation, the tiny little spot, not a great big thumbprint like you see in multiple sclerosis, but a spot of neuroinflammation. Now, when you think about neuroinflammation, it's not something you see by itself. You see inflammation and there are all these different cells, including different types of immune cells in the brain that could be contributing. But the big ones that people talk the most about are the glial cells, contributing to neuroinflammation. But oxidative stress matters. And oxidative stress can drive inflammation, and inflammation can drive oxidative stress. It's a vicious cycle. And so, you don’t see the one without the other, and that makes these imaging

studies of oxidative stress terribly important. There was an early paper by Dr. Natelson, and Dr. Shungu, that showed oxidative stress.

And again, I love images, using MRS spectroscopy that uses MRI imaging, but measures by spectroscopy the metabolites of the oxidative pathway. And they could look at the redox state of glutathione, and they could also look at lactate in the cisterns. And they saw the build-up of lactate just like you get a charley horse in your muscle because of free radicals building up and causing a lactate build-up. And then boom, you've got a charley horse in the muscle, and it's not functioning right. Well, in the brain, you've got lactate building up. And you can see it in the cisterns in these images. But you can also see the regions by region where the oxidative stress is happening. So, you can't talk about neuroinflammation without talking about oxidative stress. It's a targetable point. And so, again, we're looking for clinical trials in ME/CFS, and we need these studies. There's one funded right now at the NIH, Dr. Shungu, that we're all waiting to see what happens. It's an exciting study. And then I talked about specific cells. So, in the brain, there are cells that are particularly inflammatory, and microglia are the ones we think the most about. But there are also cells in the periphery that are communicating with the brain and can generate these types of things. So, there's a lot of work talking about these brain-body connections across the immune system, but also cells of mast cell lineage in the brain that also have the same inflammatory cascade types of things.

And so, you'll see I can't give this talk without a shout-out to my dear friend, Dr. Theoharides, who has published so much that's clinically helpful in the field of mast cell activation. And now he's turned his attention to Long COVID and ME/CFS. We're excited he's in the field because he moves straight to interventions, which is great. So, he published some papers. But there are some others too, and they can absolutely be targetable points. Have we had any of these studies yet in ME/CFS that are powered sufficiently to tell us something we might act on? No. Do we need them? Absolutely. So, you're going to hear me harping about the need for clinical trials, both as we need treatments, but also because they're proof of principle. When you have a very targeted thing that you can target and you can fix that spot, and then you step back and see what happens, you can say, "Oh, well, that worked," or, "That didn't work," or, "That worked a little." And it begins to tell us how we might use things singly or in combination in how we design these studies. But this mast cell activation syndrome is a deal because it's not that common in the community, and it's exceedingly common in ME/CFS. It's exceedingly common in Long COVID. So, there's something there in which we need to be aware of. It can be diagnosed. There are diagnostic strategies, and it can be treated.

And so, it could be treated more effectively if we had more trials. But at least, we have some knowledge that's based on evidence on what to do. You'll hear a lot about low-dose naltrexone in ME/CFS logs and places and things. And that's because we do things in clinical care that we have borrowed from the comorbid literature. So, this is a good thing and a bad thing, okay? In

fibromyalgia, Dr. Younger did this beautiful job of doing an early Phase 1 and then a Phase 2 that really suggested LDN, low-dose naltrexone, can reduce pain in fibromyalgia patients. And the Phase 3 has yet to be done, but it's safe. It's easy to use. It's compounded. So, it's not something you can just write a prescription for, you kind of need to know what you're doing with it. But where it works, it works in a lot of different places, because it works in the gut as well. But talking about the brain for a moment, it quiets glial activation in a safe way. It's not like a hammer. It's a little bang, not a giant bang, but a little is sometimes enough. And it's used in very safe dosing strategies. And it's used in other fields as well, for other things. But in LDN and ME/CFS, we often see patients respond to it clinically. And those of us that use it a lot have gone to the big meetings and talked about case series, 300 and more patients in our case series. Somewhat nice literature developing, aside -- I'll have it here. Because it also has effects on other things. And it has an effect on NK cells, another targeted treatment. And this is our Australian colleagues who have done a lot of work on NK cell function and ways to try to repair the damaged NK cells. And they're using LDN in some of their preliminary studies.

So, what are the clinical implications of neuroinflammation? Well, I had this old professor, and he used to say to me, "Nancy, you don't have to get too crazy here about the immune system, something turned it on. This is an antigen-driven system." And if there's ever a mantra in an immunologist's brain, it's, "It's an antigen-driven system, find the antigen. Where you see inflammation, where you see immune activation, something has got it turned on." So, what's on? It's either self, meaning autoimmune, or it's non-self, meaning there's a bug in the system, bacteria, virus, fungi, whatever. But it's self or non-self or some combination of both. So, in my own clinics, I certainly do a big search for what's there that could be driving the immune signatures that we see. So, I think that's a good point. I think that's a good point. there that could be driving the immune signatures that we see. And we sort of went through a couple of decades of looking at viruses and then got chilled down on them when HTLV turned out to be not true. And then we started to rev up again. Why? Because the Long COVID literature is clearly showing people that reactivate the Epstein-Barr virus during their acute infection are at high risk for having Long COVID. Well, that makes sense. And one of the problems we've had from the beginning of time with ME/CFS is that we have very few cohorts that we were able to follow at the beginning of their illness.

Most of the stuff we see is after it's been well-established. And so, understanding the space of what's happening in those first few weeks that set someone up for a long-term chronic illness after an acute infection that's typically very limited, that's a really important research space. But Long COVID is saying to us, "Well, let's think about viruses again." And the other thing Long COVID is telling us about is that the viruses that we didn't think were persistent or latent -- we know some viruses are latent forever. You have a herpes family virus like Epstein-Barr or the chickenpox virus, varicella, you have it forever. And it's latent, and it can reactivate later in your life and give you all kinds of grief. But we don't think of these common viruses that cause colds

mostly, which is what the SARS viruses come from, that family that caused about a third of the acute cold type viruses, and we don't think of them as being able to persist. And yet there's now a growing literature that SARS-CoV-2, the COVID virus, actually is still in the body in some of these patients that have had Long COVID for at least months and perhaps years. So, that's an important area to understand because perhaps we've been looking for the wrong viruses, perhaps in our interest in looking for what we know, reactivation viruses. And then I should say in ME/CFS, we mostly look at herpes family viruses, but Coxsackieviruses are also evident in the gut biopsies in some really excellent work that viruses can reactivate. But maybe viruses can persist and that would be -- and not even the viruses that we think of as being able to persist. So, there's a whole session, not this session, on bugs.

But I bring it up because they're immune activators, and this is the session on the immune system. What's there that keeps the immune chronically activated and inflamed? Well, I mean, there's been a lot of literature. But I'm going to just say, in 1990, we published this paper that says the things we're still talking about, which says something. That we haven't moved this all the way to clinical intervention in these 32 years. Oh my gosh, this is so frustrating to me, personally. Because we published this paper in one of the very earliest papers in ME/CFS on impaired NK cell function, impaired B-cell, impaired T-cell, reductions in the function of these cells in vitro, chronic immune activation, and evidence of EBV reactivation in one of the earliest papers in the field. And yet, we're still digging deep into these very areas with new tools and better, which is kind of cool. One thing 30 years gives you is new tools.

But I want to point this out, just a heads up everyone, and as we're thinking about research priorities, that men and women are different, not just in the testosterone and estrogen levels, but in their actual self-functions and the things they express and so on. So, this was a paper that we published, and we have a number of these gender-based studies. Dr. Nathanson and our group have a lot of genomic work if you want to look up somebody that's doing some real cutting-edge work on the difference between men and women in ME/CFS. But this is just a paper I pulled. It just shows some nice gender differences in NK cell function. The healthy controls are different, and the sick patients are different in these two populations. So, we can pull this out for T-cell activation markers for anything you see. And you'll see gender differences.

And you will see differences with age. So, I think that's a lot of that modeling work does -- a lot of modeling work that splits men and women and by age. And we have a pre-menopausal model, a post-menopausal model, and then with normal testosterone and low testosterone models. We have four different models. Now, having emerged from a lot of work that says, "As we design clinical trials, be careful you don't sweep everybody together. Look at the things that will give you the most likelihood of having early success in these trials. And then later do the generalizability studies where you sweep more people in."

But these initial trials, when they're super focused, are most likely to yield both proof of concept results and clinical results that would allow us and encourage us to keep going down a path. So, I spoke for a moment about viruses and how we're thinking about them differently. Our modeling group is also approaching viruses using these computational models to see if we can deal with the fact that there are different ways to think of them. And they can be in different tissues. So, for instance, the study that was done, that was the B-cell depletion study by our Norwegian friends. And we'd hoped it would drop EBV because that's a reservoir for latent EBV infection. But in fact, the parotid glands and the salivary glands are sitting there holding quite a bit of EBV. So, you just re-inoculate your system if you target one single tissue. You need to understand all the different tissues that this virus might be in. Now, that wasn't the point of this study, but we'd hope to buy that little side benefit of them doing a targeted study. And in fact, I think it just made us think more about these compartments that viruses hide out in, but also whether or not they're persistent or latent.

And again, in Long COVID, this idea that how many of these Long COVID patients have reactivated EBV and how they have T-cell-specific exhaustion of the cells that are trying to survey and control that EBV reactivation. These types of papers are coming out and are very well-crafted studies. And again, this is a Long COVID one. It talks about EBV reactivation predicting this ME/CFS-like presentation of Long COVID. I'm not going to speak a lot about autoimmunity because the next speaker is the most amazing speaker on autoimmunity. But I will mention that autoimmune diseases can be triggered by viruses. And so, the immune system should be preventing this by containing these viruses. And if they don't and there's a wrong exposure to something that can drive autoimmunity, well then -- and here we have some diseases that have a tremendous literature on these types of viruses, EBV, HHV-6, Coxsackie. Is this stuff familiar? The very viruses we think a lot about, and ME/CFS doesn't have evidence for reactivation, driving autoimmunity. And so, when you think about autoimmunity, we think mostly about antibodies, autoantibodies, because we can measure them very easily in the bloodstream if we know what we're looking for.

And now even with some tools, when we don't know what we're looking for, which is pretty cool. I mean, these big panels of autoantibodies that are now available, one out of UCSF that has something like 10,000 epitopes on it that can look at the immune system, I mean, the body's autoantibody response very thoroughly. And then there's a cytotoxic T-cell response that can be autoimmune. And this is the one we broadly ignore, but it is very important. And you'll see the MS literature in particular has really worked through a lot of these cytotoxic T-cell mechanisms and has targeted drugs just on this. And so, again, we can learn from other literature that could inform our literature. So, Dr. Scheibenbogen is speaking next about this, and I will keep on rolling past this slide because you're going to have such an excellent talk in a moment. Now, we are doing this study on comparing Long COVID to ME/CFS. And it's too soon for us to give you any really big things about it. But I am going to just say it's underway, and it's an excellent study

funded by the CDC. And it's going to compare the Long COVID cohort that we've developed to the MCAM study, which was a big ME/CFS study. It was done by a lot of different people that are in the field at seven sites and 750 or so patients.

So, we have a beautiful data set to compare to. So, we lined up those kinds of assessment tools so that they could be compared. So, then we have big questions, but the big one is how much is Long COVID like ME/CFS? Where do they differ? Where are they the same? And can we find targetable mediators? And what I'm most proud of about the study so far is the diversity. And this is something I need to point out is what we need to do. This study is a Long COVID study. And if you can just see that we have 16 percent African American. We have 51 percent Hispanic in our South Florida community that's about representative. This is a great representative sample for our community. And I'd love to in another time to talk about how we do that. I don't say this has been easy, but this has been successful. So, that's good news.

Now, this is a slide I threw in at the last minute. Because I was thinking to try to say, what do we know, what don't we know, and what do we already do? Because perhaps as a clinician, I can say, "Is it what do we already do?" Well, based on established treatments in comorbid conditions, because there are guidelines for treating IBS and POTS and fibromyalgia and so on, we apply all of that in ME/CFS. All of the doctors in the field will take those guidelines for the comorbidities, and we can help people. But we have to be aware that the study that Dr. Rowe did with the NIH way back when on fludrocortisone, an established, well-established, very effective treatment in POTS, failed to meet the primary outcome variable in ME/CFS. So, you have to think as you're assuming that your comorbid conditions that you can borrow from will actually have the same kind of response in ME/CFS, that's not a good assumption. We need to do the study. So, I'm grateful to Peter forever for pointing this out to our field because this was a really important thing that came from this study.

But the other thing is that we don't have big studies on immune-based therapies in ME/CFS, very, very few studies. And so, we are forced as clinicians to use the things, the tools we have in our hand, and the ones that are safe, and that we're not afraid to use like Omega-3s and curcumin and glutathione and NAC, things that we use all the time in integrative medicine practices. We've been using low-dose naltrexone without a Phase 3 trial, but we widely use it in clinical practice all around the country. And there are some discussions on that in the ME/CFS Clinicians Coalition webpage, which I recommend. And I suppose in very early studies in the field using Imunovir and Isoprinosine to enhance NK cell function.

More recently, though, most people are using mitochondrial approaches, meaning antioxidants and so on, to try to get some more oomph out of the immune system. But it's very limited. We do not have guidelines that are based on clinical trials for immune-mediated points of intervention in ME/CFS. So, what don't we know? Well, we have biomarkers, but we don't know really the

best way to use them. We know lots of things are wrong with the immune system. We know how to measure it. But clinically, how do we use them? And in our clinical trials, how do we use them as entry criteria so that we target -- grab the right patients into a targeted intervention using proven therapies that target specific immune mediators? If you know you have a TNF-driven population and you can measure that, why aren't we using TNF inhibitors in that population in clinical trials, for instance, just one example, or any other of the biologics? Are the findings that we have to date generalizable to the larger population? Because we don't have African Americans, Hispanics, men, women, blah, blah, blah, the older pediatrics in our populations, even when we do studies, I'm not certain that we should think that we can generalize them to the people that weren't included, so inclusiveness in our trials.

Using subgrouping strategies that actually define people by the mediator that we're trying to target. It only makes good sense. And you're more likely to be successful. But you won't be able to generalize until you do the next study. But at least, the first study makes it a very tight entry criteria that really makes it more likely to be successful. Studies that focus on improving immune function or reducing T-cell exhaustion make very good sense. Studies that help us understand better how to use the inflammatory profiles to define their patients, but also to use as targets of therapy. Autoimmunity I'm deferring and neuroinflammation never -- I forget the incredible role of oxidative stress in neuroinflammation in addition to the same things that are affecting the systemic immune system. So, what do we need to know? Are the things we already know valid if they've been retested in very large groups? Well, we can't retest things in very large groups. To be aware that the way we get funded in clinical in these days is to be innovative. A new idea that you're testing, not validating somebody else's idea in a larger cohort.

Those are very hard studies to get funded. And the way they are funded is by setting up a program announcement that's specifically to develop a network, a biorepository, a data way of doing that, and a way to fund by pulling things from that repository these well-powered studies that validate these earlier observations. So, the NIH, the NINDS has done a really good job of trying to develop this by repository, a data repository. It's very exciting. And then we have to have a way to pull from that and validate some of the really important findings in the field. Defining subgroups, we've talked about that. And here's one that we aren't doing, not anyone's doing yet, which is a long-term follow-up cohort to understand if these findings of neuroinflammation and inflammation are risking people's brain health or body health as they enter their older years. We have lots and lots of patients in our practices that are in their 70s and 80s. It would be a really good idea to understand the trajectory of their illness. And we have people that we can describe as having very early neurodegenerative illness in our practices, but we haven't counted them correctly. They don't know the epidemiology on that. We could really do a better job in understanding what aging is going to do in a brain, in a body that is immune-activated, and same thing with the mitochondrial dysfunction things.

And then finally, the chicken and the egg, what came first, and then what happens? How do we think about treatment in the earliest phase of the illness versus in the middle or later? And is it changing? Do we have to do things differently in the first three years where we see more obvious inflammation, or later on as we see more and more oxidative stress and immune senescence? So, the priorities, trials, trials, trials, trials. And I've suggested that perhaps the NeuroNEXT program could be expanded to include some sites that exported ME/CFS to allow the ME/CFS trial program to roll out in an existing structure instead of having to create a new structure for that. But no matter what, we need a trial structure. And that would be extremely useful. And then validation, a mechanism to validate the small end studies. I was on the IOM panel that said, "Oh my God. There's so much good stuff here, but none of it's been validated." All these studies that were powered at the earliest stage of pilot are just beyond, but never validated with large numbers. And then how this might help us with clinical consensus guidelines based on what we do know, even if it's just clinical experience and Level C or whatever evidence, to get some guidelines pulled together. And here the ME/CFS Clinicians Coalition, which I've put here, does make an effort to do some of this by having pulled together some tremendously experienced physicians from across the world to try to pull some of this together. But again, there's a lot more to do, and we haven't gone far enough there. And then please do not forget about the need to do gender and age-related studies. And that's it. Thank you very much.

Derya Unutmaz: Thank you, Nancy. I think we're moving into the question phase, and Vicky is going to moderate that.

Vicky Whittemore: Yeah. Thank you, Nancy. Lots of questions for you here. So, the first question we got was, if ME/CFS is autoimmune, or in some cases autoimmune against mast cells or endothelial cells, how would you demonstrate this? How would you demonstrate that? What would you look for?

Nancy Klimas: I can defer a lot of that to the next speaker, the autoimmunity questions.

But I will say mast cells have a way to look that you can use urine or blood looking for the mediators of mast cells, tryptase, and histamine. And there's a standard way of doing that. A positive is meaningful and a negative needs to be repeated because these are mediators that are flash in the pan. They just come and they go. So, you do the urine test when someone's really symptomatic, and you're likely to pick it up. Or the blood test in the moments that they're symptomatic, and you're likely to pick it up. So, the mast cell has a standard, and if you go -- actually, I have them right there in my slide set. There are some references to where to go clinically to get those guidelines. But also, the ME/CFS coalition has it up on their website.

Vicky Whittemore: Great. Thank you. Is low secretory IgA a common finding in ME and/or Long COVID?

Nancy Klimas: It's not been proven because no one has ever published it, yes or no, okay? So, to say it's common or not, clinically, we see it, and we see it fairly often. Actually, we see low IgAs in general and secretory IgAs often do come up low. And also, we're doing research where we have some genetic data that points us in the direction of whether the mucus itself, the mucus and the mucosal barrier, might be affected and making people more vulnerable to cross-gut immune activation. So, we're doing that kind of work as well, but it's all early stuff.

Vicky Whittemore: Another question, is anyone using migraine medications, for example, Nurtec, to reduce neuroinflammation secondary to migraines? And I assume that's related to migraines in ME/CFS.

Nancy Klimas: So, migraines are very common in ME/CFS. Lord knows that that's one of the most disabling symptoms. People with daily migraines are really disabled. They really have a tough time. But again, what we know about these drugs is just what we see in our clinical observations without any proper scientific tallying of response.

Vicky Whittemore: Okay. So, someone's saying they've anecdotally heard stories about individuals with -- let me see if I can get this right before I bungle this question here. Okay, regarding cytokines. If you have COVID, this individual is saying their fibromyalgia and ME/CFS, even ADHD symptoms went into full remission for about eight weeks. Anecdotally, they've heard similar stories from other individuals. "I'd give anything to feel as well as I did when I had COVID." That's very interesting. Is there any study into this?

Nancy Klimas: That is not the only person, and it's not restricted to COVID, okay? I mean, I have a patient. Every time he gets the flu, the day before the flu, his ME/CFS just melts away. That's interferon, okay? That's your natural interferon driving the hell out of every virus in your system, including the one you're trying to get rid of at the time. So, that's pretty exciting.

And it would predict perhaps a clinical trial that's never been done using your body's own natural antiviral, which we do have. We use it in MS and other diseases, as a potential treatment. That's a trial that needs to be done. But that's your own body saying -- and people say after a vaccine they feel better or worse. I mean, let's get real now. Some people have terrible relapses after vaccines. But other people say, "Give me a vaccine every two weeks, and I would just be great." Because the adjuvants in vaccines really drive a nonspecific immune response that's hidden. But that would really suggest a pathogen to me, not autoimmunity, a pathogen.

Vicky Whittemore: But I'm also aware of individuals who had the SARS-CoV-2, COVID vaccines who became much worse.

Nancy Klimas: Absolutely, same thing again. And so, I harp on this. And since I have an audience for the moment when you're going in for a vaccine, if you have ME/CFS, please take care of your mast cells. It's something you can do. You can pre-medicate with some antihistamines. You can take some quercetin or some other mast cell stabilizer. And you can go in a whole lot safer into the vaccine. I will also say, as vaccines go, the mRNA vaccines are the least immune-activating as compared to general vaccines that have big adjuvants in them. I mean, the Zoster vaccine is amazingly immune-activating. So, I pre-medicate all vaccines, not just COVID vaccine, with things that mop up mediators of mast cells and try to stabilize mast cells. That's my clinician now, I got no trial. I hate talking like a clinician in an NIH meeting, but here I am.

Vicky Whittemore: You are a clinician, indeed. All right. So, for individuals with ME/CFS with autoimmune disease, would CAR T-cell treatments like those from Kyverna address the chronic activation in ME/CFS as well?

Nancy Klimas: That's interesting. CAR-Ts are very specific. You're manipulating that cell to do a very specific thing, so you better have the right target. Bottom line is, yeah, not off the shelf. But perhaps it could be a designer CAR-T that could do the right thing if we had the right target.

Vicky Whittemore: So, I'll come back to that one because I want to have a conversation about how we move clinical trials in general forward. So, I'll come back to this one question. So, has there been found a link between IBD and ME/CFS, in particular the disabling neurocognitive sensory symptoms in severe, very severe ME/CFS?

Nancy Klimas: So, there's irritable bowel, IBD, and there's inflammatory bowel. They get the same initials, and a lot of people confuse them. So, if I were speaking about irritable bowel, the most common thing in ME/CFS in the gut, in terms of comorbid condition you can diagnose, I would say that that has a lot to do with dysautonomia. And I can't be confident that the immune system's really playing a major part. I mean, I don't know that for sure. Inflammatory bowel, of course, is quite different, and it can happen late. So, remember that with ME/CFS, where it's a diagnosis of exclusion, if you had inflammatory bowel, you'd have it excluded the first day you walked to the doctor. They say, "No. It's all being driven from your gut," which may or may not be true. It's just the nature of our case definition. But if you got it later, if you've had ME/CFS for 10 or 20 years and then you got inflammatory bowel, yeah, that happens. And we do see a lot of inflammatory stuff happens late. Again, Carmen gets to talk about autoimmune stuff, but MS and other things that we see downstream, that can happen.

Vicky Whittemore: The next question is regarding the split in male-female differences, and specifically in female's pre-post-menopause. Has there been research that looked at individuals with polycystic ovarian syndrome? And do you think that would be informative? And just before

you answer that just to let everyone know that we will also be addressing some of these issues in the webinar in January, looking at lesser studies issues. So, some of these issues may come up in that webinar as well. But I'll let you answer that.

Nancy Klimas: So, I don't know. That's a good answer. I will say that in our models of pre-post, the level of estrogen is in the network. And it's something that's in our models of how we would treat. And again, was just kicking one of the studies off in the next couple of months, and our reboot studies. We actually adjust the estrogen level as a part of the treatment in the peri-and post-menopausal people. But it's a study, it's research. We'll find out.

Vicky Whittemore: Yeah. No. That's excellent. So, is there value in prostaglandin D2 and F2a in urine tests for mast cell activation syndrome?

Nancy Klimas: Oh, you could learn so much. But yes. There's value in all these tests. There's also value in the clinical trial. I'm going to say as a physician, I know these mediators miss a lot. There's a lot of false negatives. I mean, maybe as much as 50, 60, 70 percent of the work we do as a clinician to try to nail that down at a single point in time, we're going to miss it. And so, I use clinical trials a lot. I mean, I use mast cell stabilizers to see if people are better, that's easy. We also have some flow cytometry panels that address mast cells and mast cell activation. That might be more direct.

Vicky Whittemore: Okay. Are interleukin deficiencies a common finding in ME/CFS and/or Long COVID?

Nancy Klimas: So, interleukin is a whole family of cytokines. There's a whole bunch of different ones, and they do different things. And when they're up or they're down, they point at things. So, there are some inflammatory interleukins, and they're part of the inflammatory cascade like IL-1. And now interleukin-2 helps cells grow, and it's a Th1 cytokine. So, it helps cellular immune responses. And then there's IL-4, for instance, that mediates allergy, and there's IL-2. There are all these different interleukins, and the pattern of them is very informative. We actually use a lot of cytokine testing in our clinical practice. Because I can look at it and go, "Well, your gut's messed up. You got a lot of inflammation. And the system that's driving allergies away is way whacked out right now." So, it can tell me as a clinician what's -- it can give me hints. When I can't name that tune, I can at least name that mediator.

Vicky Whittemore: Right, right. So, I think this is an interesting question. How do we address comorbidities within research like mast cell activation, likely impacting results yet most researchers are unaware of them, and they could potentially be affecting the results that they're getting?

Nancy Klimas: This is one of the great reasons to have the biorepositories that you're developing, I think, as everybody looks at the same samples through different lenses and then drops all that data into a central database. So, that you can try to understand when you perhaps missed it or there's another thing that would have affected it. And then some other superpower comes into all that data with some computational AI approach and discovers all the new data that's buried in the old data. That's how it works. Using the same set of patients that are well ascribed in huge enough numbers to matter and look at them through every lens we know how to look.

Vicky Whittemore: So, we just have four minutes before the next speaker. So, Nancy, can we talk a little bit about moving clinical trials forward? So, you did mention using the NINDS NeuroNEXT platform. And the way that works at NINDS is that NINDS is supporting an infrastructure of clinical sites, clinical trial sites across the country, and coordinating sites. And if a study comes in, for example, on ME/CFS, then the investigators proposing that trial can propose to add ME/CFS sites. And then each of those clinical sites that are already supported can sort of opt-in or out depending on the study and their ability to carry out that study at their site. So, that certainly is an infrastructure that exists that the ME/CFS community could take advantage of. Absolutely.

Nancy Klimas: That's good news because I would love -- and I know the ME/CFS Clinicians Coalition -- we've been talking about it since before the pandemic, how to put together a big LDN trial because it's widely used. And we should either be using it or we should not. But we don't have even a Phase 2 study in our ME/CFS population that could give us guidance. So, we were all -- we sat and said, "What would be the most important top three things we'd like to study in the field?" And LDN was the top. So, I would love to come back with that same group, any one of us to be the lead. I don't have to be the lead. But I would like to be in that group that proposed to NeuroNEXT. We can raise the flag and see how your neurology sites respond to an ME/CFS trial. That would be great. And how much you need to include other sites to pull that one off. So, now I've got another mission. We'll be writing you a nice protocol. But if that worked, I think that's maybe -- that's exactly -- I mean, I do think it's easier to take something that exists than to try to -- the politics alone of trying to get something from scratch. And I know the advocates have worked very hard to try to develop a clinical trials network mechanism. But if there's a mechanism in hand, we should use it.

Vicky Whittemore: Utilize it, yeah. And just with two minutes left, one of the questions was regarding how to involve individuals who are bed-bound, severely impacted, severely impaired with ME/CFS in any clinical trials going forward. How could that be considered or worked into a clinical trial?

Nancy Klimas: Kind of a good time to ask because the pandemic really challenged us, right? We had to start doing studies that no one had to come to our office. So, we've got a lot of experience in the last couple of years on changing our whole platform strategy to home-based interventions, and including having home visits and other kinds of strategies to be able to draw the blood and do assessments and so on. So, in essence, the pandemic forced us down this road. The low-hanging fruit is easy, the things with very low risk, particularly nutraceuticals because they don't even require a prescription. And there's a lot of regulatory stuff state by state that we have to deal with when it's a prescription-based intervention. Not that we can't handle it, but it needs a lot of regulatory work. But the easy ones to plough out are the nutraceutical ones. And I have, in Gulf War Illness, we have seven big studies going on that are home-based, and they get with the clinical trials network that's doing it. But it's exciting. And they have people go in if they need an image or something as a part of the trial just to do those parts, but as much as possible, do it at home.

So, for home-based people, I think we've changed the platforms. Still, they are the hardest patients, and they need their own, perhaps, types of trials to include them in the stuff from moderate to mild illness. It's nice, but is it going to get them out of bed?

So, having an intensely focused for the most severely ill patients absolutely needs to be done, can be done. But again, we need the -- what are we testing? We need the design.

So, perhaps this modelling work that includes the more severely ill in the models and starts distinguishing the subgroups within severity of illness, that might be useful.

Vicky Whittemore: Great. Well, thank you so much, Nancy. This has been really excellent. And with that, I'll turn it back over to Derya.

Nancy Klimas: Thank you.

Derya Unutmaz: Thank you for an excellent talk, Nancy. So, it's my pleasure to introduce the next speaker, Carmen Scheibenbogen. She is a professor and internal medicine physician at the immunology department, also a chair at the Institute of Medical Immunology, University and Hospital Charité, in Berlin, Germany. She is well known for her focus on role of infections in immune response during ME/CFS. And as Nancy mentioned, she is an authority in autoimmunity. So, look forward to her talk. Thank you, Carmen.

Carmen Scheibenbogen: Yeah. Thank you very much. So, I'm very pleased to be invited to this excellent webinar series. Actually, I would like to present my own slides, because I made a few changes to have not too much overlap to Nancy Klimas' presentation. So, it would be great if you can allow me to share my slide. Well, that's possible now. Okay. Now you should see my files. And I'm going to talk about the evidence we have for autoimmunity in ME/CFS. And my background is I'm a clinician too. I'm a trained hemato-oncologist and clinical immunologist, and I'm in charge for immunodeficiency patients. And when I became responsible for this clinic 15 years ago, there was already an outpatient clinic for ME/CFS. So -- and that has become my major clinical and research interest during the last 15 years. And since 2020, of course, post-COVID disease is another big focus. So, I'm going to talk about ME/CFS as part of the post-COVID syndrome, and then the evidence we have of -- for a role of autoantibodies in ME/CFS and clinical trials targeting autoantibodies. So, I'm trying to stay within 35 minutes, and then we will have 5 minutes for questions and answers.

And -- well, just briefly, to give an overview on the disease, ME/CFS is, in most patients, severe and chronic disease. And the major symptom is the exertion intolerance and the post-exertion malaise upon minor activities. We have another key symptoms of brain fog, of pain, and of orthostatic intolerance. And what we know well is that various viruses can trigger ME/CFS. Among the best studied is the herpes virus, Epstein-Barr virus. Enteroviruses are a common cause, especially in the U.S. Influenza virus can trigger it, many others. What we didn't know in -- at the beginning of the pandemic, if SARS-CoV-2 can trigger ME/CFS.

And there were -- we had initiated an observational trial to analyze patients who suffered from moderate to severe fatigue and exertion intolerance following COVID. These were outpatients, and these patients had no evidence for other comorbidities. And the results of this study were published last year already. And the main finding is that in the subgroup of post-COVID patients in 50 percent, we could diagnose ME/CFS according to the Canadian Consensus Criteria. And epidemiological data was similar to what we know from patients with other post-infectious ME/CFS. The median age was 43. Two-thirds were female. Patients were severely impaired. Most unable to work; a subgroup, house- or bed-bound. And we have now data for more than two years, and we face a chronic course of the disease in the majority of patients. And that has just published in another paper, the follow-up data.

And I just want to show you one figure from this paper. And what we did here is we analyzed the physical functioning using the SF-36 score. And when patients came to us at baseline that was around month six, they had, on average, quite impaired physical function. So, 100 means healthy, and 0 means bedridden. Blue dots are patients with ME/CFS diagnosis. Red dots are post-COVID syndrome patients with similar symptoms, but not fulfilling the Canadian criteria. Although, some fulfilled the IOM criteria, which are not that strict. And over the course of two years, we see that the most patients stay severely impaired -- those without ME/CFS diagnosis, we found at least some improvement. And what we found in the study too is that the impaired hand grip strength at month six is prognostic for a worse outcome during the next two years. Just another look into this study on biomarkers we analyzed, we found at baseline elevated interleukin-8 levels in most patients. We determine interleukin-8 bound to erythrocytes, which is more sensitive than just the plasma interleukin-8. And that was elevated in the majority of both ME/CFS and post-COVID patients. And upon follow-up, we saw that IL-8 levels decreased in patients.

We also observed decreased serum phosphate in a subgroup of patients. And this is a complex finding, most likely indicating metabolic disturbance. And what we found is that this improved in the post-COVID syndrome, but not in the ME/CFS patients over the two-year follow-up. So, now, I will come to the evidence we have for role of autoantibodies in ME/CFS. And to start with, I summarize here the pathomechanisms -- pathomechanisms, we know that play a role in post-COVID syndrome, and compare it to pathomechanisms for which we have study data in ME/CFS. And the main message is that we face very similar pathomechanisms. We have learned a lot about mechanisms in post-COVID. And what various studies showed is that persistence of SARS-CoV-2 is found in a subset of patients. And this is associated also with post-COVID syndrome. And as Nancy already said, EBV reactivation during acute COVID is a risk factor for post-COVID. And we know -- I said in the beginning that EBV triggers ME/CFS. And we do have some evidence that EBV reactivation is also frequently found in ME/CFS patients not triggered by COVID. Then inflammation, Nancy gave a great overview.

So, I have little to add. I only wanted to say that we have some evidence that is more relevant -- at least the systemic inflammation early on in the disease. Then autoantibodies, I'm going to give an overview on autoantibodies on the next slides. We have evidence for autoantibodies triggered by COVID as well. Actually, there is not the only specific antibody, but there are various antibodies which were found to be associated with post-COVID. And then a major finding is endothelial dysfunction and a resultant hypoperfusion. Nancy showed data about the microclots. We clinically see hypoperfusion in, for example, this Raynaud syndrome, and with cold fingers in a subgroup of patients. We can also assess the diminished perfusion in the brain by modern functional imaging techniques. Here on the left side is a healthy person, on the right side, a post-COVID ME/CFS patient. And the red areas mean well perfused. So, it's a clear finding. And then autonomic dysfunction is a major finding in ME/CFS, which is probably a consequence of these

pathomechanism as well as the mitochondrial dysfunction and the oxidative stress. At least there is no clear evidence for primary mitochondrial disturbance in ME/CFS. So, here, I summarize the evidence we have for autoimmunity in ME/CFS. Clinically, it's already in the family history of increased member suffering from various autoimmune diseases and the comorbidity with autoimmune diseases within ME/CFS patients; the most frequent is the Hashimoto thyroiditis. There are a few studies showing associations with autoimmune disease risk genes. We performed one study showing elevated frequencies of variants in CTLA4 and PTPN22. These are two important immune genes which can lead to a better activation of T and B cells. And then another important finding is an association with MHC molecules. Although, these are not a black and white study, so this is still little evidence. And much more work should be done on this. And then we do have evidence for mimicry sequences with EBV. That means that there are protein sequences in EBV which look similar to proteins in humans. And there is antibody response against such sequences in EBV, which is higher in patients with ME/CFS.

I cite here a paper from Nuno Sepúlveda. And then regarding autoantibodies, most data we have is on the so-called G protein-coupled receptor. Autoantibodies, I'm going to explain in a minute. And actually, there's a nice overview on all autoantibodies found in ME/CFS in MEpedia. And to sum up the data, actually, there is very little data for other autoantibodies. These are mostly small studies. Many are from the 90s. So, there is no good evidence that there are antibodies targeting organs, tissues, in ME/CFS so far. And we have first data on the efficacy of autoantibody targeting. Therapies in ME/CFS, I will give you an overview on this too. So, here is a comparison of the evidence for autoimmunity we have in ME/CFS and in post-COVID syndrome. I'm not going to go into these details now.

There are also some data on B cells already. Here are the studies listed of autoantibody targeting therapies. First study is with IVIGG and immunoadsorption in post-COVID syndrome as well. And I'm going to focus now on the data we have for autoantibodies against the so-called G protein-coupled receptors. And this has a lot to do with the autonomic nervous system. And in ME/CFS, we know from the clinic that we have often a severely disabled autonomic nervous system. So, what is autonomic nervous system? So, it's actually all we do without directly controlling it -- means that we breathe, that our heart is beating, that we distribute the blood in our body. And in ME/CFS, we have an overactivity of the active part of the autoimmune -- of the autonomic nervous system -- means the stress response or sympathetic activity. And we have various symptoms in ME/CFS which are a consequence of this overactivity of the stress response. And here comes the G protein-coupled receptor antibodies into this scenario, because this is a group of antibodies which play an important role in governing various functions in the body of the immune system, but also of the nervous system.

And part of this group of antibodies are those to adrenergic or stress receptors. And we know already that levels and function of such antibodies are altered in many diseases. And there are

associations with various things like severity of symptoms disease course. In ME/CFS, antibodies against stress receptors are especially interesting, because this is the major clinical impairment we see. And there are two major stress receptors called beta-1 and beta-2 adrenergic receptor. But there are also alpha-adrenergic receptors, which are sort of the antagonists of these beta-receptors. And then we have also acetylcholine receptor antibodies, and they are the rest response. So, they are the counterplayer of stress receptors. And what do we know about antibodies against these receptors in ME/CFS? We know from a few studies that such antibodies are elevated in a subset of ME/CFS patients.

I cite here the first study we did almost 10 years ago. And what we have learned too is that levels of such antibodies to beta-adrenergic receptors and also acetylcholine receptors correlate with symptom severity and with alterations in the brain detected by MRI in ME/CFS. We also know that beta-2 adrenergic receptor antibodies are active receptor-stimulating antibodies, and that their function is impaired in ME/CFS. And just to give you an overview on some of the most important data, so these are two studies analyzing these antibodies in the larger cohort of ME/CFS patients. And the summary is that in a subset of patients, such antibodies are elevated. This shows just one example. On the left side, patients with ME/CFS; on the right side, control. And the levels are higher in a subgroup of patients. This is a study we performed on the function of such antibodies in both healthy and patients. And this is one figure from this paper. And we used here as an assay a cell line transfected with beta-2 adrenergic receptors. And then you stimulate the receptor. We get a light signal. And what we learned from this study is that in all healthy people, we do have antibodies binding to this receptor, and that receptor is stimulated by these antibodies.

In a similar way, we find stimulating antibodies in ME/CFS patients with normal levels of beta-2 receptors in contrast to those patients who have elevated levels. In these patients, the stimulation of this receptor is less. And therefore, we can conclude that the function is impaired in these patients. But that is only one study from our group so far, and has not been reproduced by other groups. And then another study I want to show you is on the correlation of the levels of these antibodies with various symptoms. That is a very busy slide. I just want to make the most important point. And here, we correlated levels of antibodies with the severity of fatigue. And the higher the bars go up, the higher positive correlation we have, so more fatigue. We analyzed here patients with post-infectious ME/CFS and compared it to patients who got ME/CFS following other triggers -- for example, an accident or psychosocial stress. And what we found is that especially the levels of the adrenergic receptor antibodies, but also of acetylcholine receptor antibodies do correlate with the severity of fatigue.

We did a similar study recently in our post-COVID cohort again comparing patients with post-COVID ME/CFS and those who did not fall through the ME/CFS criteria. And again, in the ME/CFS patients, we found correlations with severity of fatigue and the levels of various -- of

such antibodies, but not in the post-COVID patients. And here, we had no correlations with this not-infection-triggered ME/CFS patients. And another finding from this study I think was very interesting is also that -- the Raynaud symptoms -- means the bluish cold fingers many patients have, indicating a microcirculation disturbance. Correlates with beta-2 receptor antibodies and also with these M3 acetylcholine receptor antibodies -- again, only in patients who fulfilled ME/CFS criteria, but not in the other post-COVID patients. This is all published studies, if you want to look into this data into more detail. And I just want to mention another study from a German group analyzing in a similar manner such autoantibodies. And here, the microcirculation in the eye. And similarly, they found that elevated levels of these antibodies are associated with an impaired perfusion. So, to sum up these findings, we know that ME/CFS is, in a major subset, an infection-triggered complex disease with immune and autonomic dysfunction, and we have evidence for an association of autoantibodies against adrenergic and other so-called G protein-coupled receptors with disease severity.

However -- and that is an important point I want to make -- association is not necessarily meaning causative. Thus, we need to study these antibodies of course in more detail. And here, clinical trials will give us important information. So, I will, in the last 10 minutes, talk about clinical trials targeting autoantibodies in ME/CFS. And actually, there have been few trials performed during the last decade. And the pioneers are the two physicians from Norway, Øystein Fluge and Olav Mella. They were the first to perform trials with the B-cell depleting drug rituximab, and also, more recently, with Endoxan. And they published several papers showing impressive evidence for activity in a subgroup of patients. Unfortunately, the multi-center trial then was negative. There are various reasons to discuss why this was negative, and I think the answer is still not clear about the possible role of B-cell depletion in ME/CFS by this negative study. Then we performed trials with high-dose IgG, and there were also a few trials performed in the past providing evidence that this has efficacy in a subgroup.

And we also performed trials with immunoadsorption, and I would like to show you more data on this. So, immunoadsorption is a technique by which you deplete the whole IgG of a person. And by this you also deplete potential autoantibodies. And it's sort of an apheresis technique. So, you take out the blood from the patient and separate cells from plasma by centrifugation, and then the plasma goes into a column. And in this column, there are peptides in this specific type of separation, but there are other filters. Here, the peptides bind the IgG, and then the plasma, which is cleared from IgG, goes back to the patient. And this is actually a technique which has been used for decades and is effective in various autoantibody-mediated disease. And in 2015, we initiated the first small trial in patients with post-infectious ME/CFS with elevated beta-2 adrenergic receptor antibodies.

We performed another small trial. These were just small trials in 10 or 5 patients because of the lack of funding for larger trials. And to sum up the most relevant finding is that five-day

immunoadsorption. So, that means patients were treated at five days. One immunoadsorption session takes around four hours. And this led to a rapid symptom improvement in 7 of these 10 patients. And we observed a sustained improvement in three of these seven patients for more than two years. And based on these studies, we initiated last year a trial in patients with an ME/CFS following COVID. And again, we selected patients with elevated beta-2 receptor antibodies, because that is the best antibody we have at the moment to justify immunoadsorption, and we need to show that an anti -- an autoantibody is elevated. This was an observational trial without a control arm. The primary endpoint we used is the SF-36 physical function I showed you already, which is a good marker to assess severity of exertional intolerance in this disease. And we included patients fulfilling Canadian Consensus Criteria having elevated beta-2 adrenergic receptor antibodies. And we performed immunoadsorption now with another device, with TheraSorb for five days. And in responders who were relapsing, we have the opportunity to do two further immunoadsorptions. And I can show you already our first results, because it's an open-end trial. And due to the urgency of finding therapies in post-COVID and ME/CFS, we published data from the first 10 patients already. So, this is the paper. And what you see here is that this technique is very efficient to deplete immunoglobulins. Here, not only IgG, but also, the other major classes, IgM and IgM, were nicely depleted. And the first point is a level before immunoadsorption, and then after five days of immunoadsorption. But you see antibodies come back in serum rather fast. We could also show an efficient depletion of beta-2 adrenergic receptor antibodies of all three subclasses. And coming to the clinical data, we observed an improvement of physical function in 7 of the 10 patients treated -- in some patients, a really good response.

So, before treatment they had SF-36 physical function score around 40, with a dramatic increase in four patients. So, these are housebound, mostly, it was 40. And if you increase to 60 or 70 or 80, you can participate in life again. We, of course, looked into other symptoms. And patients reported improvement of several symptoms including pain, cognition, and immune symptoms. And this was significant here for muscle pain and the immune symptoms. The absence of significance of these other two symptoms is probably due to the fact that these are really small numbers so far. But we included now 20 patients, and we will have more data also from other trials which are ongoing in Germany. In Germany, we got funding last year from the government to do clinical trials in ME/CFS and post-COVID syndrome. We actually got sufficient money to start five clinical trials, and this includes also a clinical trial doing controlled trial with a placebo control of immunoadsorption. And this trial has been initiated now, and we are -- and this is the data from the repeat immunoadsorption study I just showed you.

We have also a trial studying a drug, vericiguat, which improves perfusion. And we have a trial ongoing in post-COVID patients with neurocognitive symptoms with hydrosteroids. We have an ongoing trial of hyperbaric oxygen therapy. And a part of the funding goes into a biomarker platform and a diagnostic platform. So, we combine clinical trials with a comprehensive research

testing all the biomarkers we know which may play a role, and also doing functional MRT of the brain and assessing vascular function. And another trial is planned to do immunoadsorption and combine it with B-cell depletion therapy. And this is a huge platform with researchers and physicians from various disciplines participating. And we have funding until end of next year. We have also published a paper on the concept of this clinical trial platform, and we have already some of the first reports presented at a meeting we had in May in Berlin. And you'll find these talks which were digitally on this site.

And we have also published a paper summarizing this meeting, and also the data we have from our first clinical trials. So, now, coming to the summary, and answering the questions you sent me. So, what do we know? We know that these G protein-coupled receptor autoantibodies do play a role in ME/CFS. And we have first evidence for efficacy of autoantibody targeting trial. We don't know yet if there are further autoantibodies who may play a role. We do not know which role T cells play in potential autoimmune dysregulation. And we have no final proof yet that ME/CFS is an autoimmune disease. And we also do not know if this is probably only autoimmune disease in a subgroup of patients. So, what do we need to know? We really need to show that such autoantibodies are causative. And how can we get this proof? And here, I list the research priorities, I think, which are crucial to answer those questions. So, I would put more money into research on G protein-coupled receptor antibodies in ME/CFS to analyze further their function and their signaling, and to further potential autoantibodies. Then the role of B cells, which are the cells which produce antibodies, and autoantibodies, their function, the B-cell receptor repertoire, will tell us -- will provide us further evidence of potential autoimmunity. The role of T cells, which are important to activate most of the B cells.

Then it would be very nice to have an animal model of serum transfer. We still look for biomarkers for autoantibodies. This is especially important to select the right patients for clinical trials. And then the big challenge is, of course, to do large, controlled clinical studies targeting autoantibodies. And there, the good news is we have already so many licensed drugs for all these other autoantibody-mediated diseases like multiple sclerosis, like connective tissue diseases. And these drugs either target antibodies or destroy B cells or plasma cells. So, we just need to get funding and convince pharmaceutical companies to do these clinical trials in ME/CFS. And this is to acknowledge my team and the funding we get. And with this, I'm at the end of my presentation. And we -- I think we have some more minutes to answer questions.

Vicky Whittemore: Yeah, thank you very much, Carmen. It was really an excellent presentation. So, I -- we have some time to answer some questions here. So, I just have a question -- a sort of a clarifying question about the study they did in Norway, the negative study with rituximab and the other drug. So, it's my understanding that there were some responders, some individuals that responded. But overall, the results of those -- the trial was found to be negative. Is that correct, Carmen?

Carmen Scheibenbogen: Yeah, that is the multi-center trial. There were many problems with this multi-center trial. For -- one was that they had to halve the dose because they had not enough money. And then they added four centers who had probably not that much expertise in ME/CFS. So, probably, the wrong patients were selected. Because the first trials they did, the phase one and phase two trials, they were very convincing. They had really patients who were huge with long-term remissions. And these are really very -- these are excellent clinicians and researchers. So, this is very convincing data. So, I think this question is still open. And now, we have more efficient antibodies which are more efficient to deplete B cells. And now, the two guys from Norway have initiated clinical trials targeting plasma cells. Because if you target B cells, you may miss plasma cells, which can also produce autoantibodies. And there are also drugs which can destroy these plasma cells. And at the Berlin meeting, they showed the first data from their ongoing trial, and showing, again, impressive clinical responses in ME/CFS patients with this approach.

Vicky Whittemore: Yeah, thank you. So, one question we have is, if immune absorption removes antibodies, both auto antibodies and good antibodies, do individuals who undergo immune adsorption for long COVID develop herpes virus reactivations after the procedure?

Carmen Scheibenbogen: Yeah. Actually, we have no evidence that this is the case. And we do not completely deplete the immunoglobulin stores, so we cannot get rid of antibodies, especially on the mucosal surface and within the tissue. And the antibody-producing cells are, of course, continuously producing immunoglobulins. So, we have little immunodeficiency which is caused by such an approach. And immunoadsorption is, for us, more a proof of principle. So, we cannot really cure the disease just by washing out antibodies. But we learn -- we see very fast too is getting better. And the plan is that we combine immunoadsorption -- in those who respond, we then do B-cell depletion. However, we still think that we probably can get more efficacy also by indirect targeting of B-cells. Because that's what we learned from clinical studies is if you deplete the immunoglobulins very strongly, then, of course, the body tries to compensate this.

So, B cells proliferate more, and this may be a stress, especially for newly performed autoanti-producing B-cells. So, we have some evidence that also B-cells get destroyed by this procedure -- a certain subgroup of B-cells. But in post-COVID, we learned that it's definitely not a cure. It's an important answer we get that it's effective in a subgroup, and -- but we need other therapies. We need really drugs targeting either antibodies -- there are newer drugs, for example, FCAR Tigimot [spelled phonetically]. This is a drug which disturbs the metabolism of immunoglobulins. And there's just a trial ongoing in POTS with this trial. And then we have all these various antibodies targeting CD20, CD19, CD38 -- and CAR T cells were mentioned. Actually CAR T cells can also destroy B cells. Actually, they can do this very efficiently. And in

other autoimmune diseases, for example, in lupus patients who do not well respond to that B-cell depleting antibodies, CAR T cells are very effective.

Vicky Whittemore: Great. Thank you so much, Carmen. There's some additional questions, but perhaps we can come back to some of these questions at the discussion at the end. But thank you so much. That was really great. And thank you for your answers to the questions. And back to you, Derya.

Derya Unutmaz: Thank you also. This was really a fantastic talk. So, our next speaker is Dr. Maureen Hanson. She's a professor in Department of Molecular Biology and Genetics at Cornell University. She is also currently director of the Center for Enervating Neuroimmune Diseases. She was recently elected to National Academy of Scientists, and is really a leading researcher in the ME/CFS field. She's also the director/co-director of the ME/CFS Center NIH-funded at Cornell. So, looking forward to your talk, Maureen.

Maureen Hanson: Thank you. So, I'll get started. My theme I decided on was immune cell-type approaches to identify mechanisms of ME/CFS. So, I thought I'd start with what we don't know about immune cells in ME/CFS and point out that most of us have been studying PBMCs. Now, these are fairly easy to isolate. They're reasonably easy to store and keep them functional and happy in the freezer or liquid nitrogen. And as a result, people have not really studied granulocytes -- neutrophils, for example, that Nancy mentioned. And there also have not been a lot of studies of erythrocytes. There have been some studies of the red cell deformability, but there needs to be more studies of red blood cells as well. The problem being that you often -- for the granulocytes, you really need to have fresh samples. And that is often very difficult to have and to work with immediately.

So, another type of cell that we don't know a lot about in ME/CFS is tissue-resident immune cells. We've been studying, again, blood cells, because it's very easy to acquire blood from individuals, and then look at their immune cells in the blood. It's less possible and more difficult to acquire biopsies in order to look at tissues where there are, in fact, resident immune cells, which can do a lot of bad things. And one of the bad things that -- a type of cell that, if it's in a bad state, can do you some harm are mast cells resident in tissues. And as Nancy mentioned, a number of individuals with ME/CFS have mast cell activation that is probably causing some unpleasant symptoms. So, we really have to learn more about mast cells. I would also like to point out that there could be a particular abnormal immune cell that is driving the symptoms of ME/CFS. And when one analyzes all PBMCs together or all granulocytes together, you could miss the cells that are actually causing the big problem. I'm showing the percent of typical -- a typical person might have 10 percent of their PBMCs being B cells, 80 percent T cells, 5 percent natural killer cells, although there is variation in the amount of the cells in different individuals. But there are other cells like dendritic cells that might be in your preparation that represent a very small percentage of your PBMCs. And there are other types of cells that perhaps Derya will be talking about that are also in very low abundance. So, there could be this evil, aberrant cell here that might be causing some big problems, that if we combine everything together, we will miss, because it's representing such a small proportion of the cells. And this is also a point I'd like to make. There are huge numbers of different types of T cells, for example, and maybe one of these is driving the bad symptoms of ME/CFS.

There are, however, methods for analyzing individual immune cells or individual cell types. And these need to be used more than they have been used. So, one can isolate particular types of cells using magnetic bead isolation. You can perform single-cell or single-nuclei sequencing and get the RNAs that are present in each cell so that you can find out if there is that one abnormal type of cell. Another type of individual cell-type analysis is flow cytometry, which I believe Derya will be telling us about. You can also do mass cytometry, which is related to flow cytometry. And you can also do flow sorting where you are able to obtain particular types of cells by having markers on those cells, and then acquiring a lot of those cells that you can then further analyze in a great detail. A type of single-cell analysis that has recently attracted some interest in ME/CFS is Raman spectroscopy. And this is, again, a single-cell method where you are looking at the spectroscopy of different -- of individual cells.

And this interesting paper from Karl Morten's group showed that there is significant differences between ME/CFS and control when they used this method. I'm not going to discuss this anymore. This is a topic that will be covered in the metabolism webinar. And I look forward to that talk. So, there are a number of tissue-based microscopy methods for analyzing individual cell types that need to be used in ME/CFS. Now, these are often extremely expensive methods, and that's one reason that it's not been used. But another reason is that many of these methods are very new. These are essentially looking at sections of tissue that you might have on a slide. There's really four different methods here, but they're all related with the idea that you're looking at a section of a tissue on a slide where you can see individual cells. So, for example, this one over here is 10x Genomics. You can visualize the gene expression in individual cells, and that can give us a lot more information about what's happening in individual cell types in that tissue, as well as the immune cells that might be resident in those tissues. So, the immune cells that are present within tissues can also be analyzed by taking the tissue, dissociating the tissue, and then having single cells that you can do the single-cell RNA-seq on, or you can take the heterogeneous tissue and obtain nuclei from the cells, and then have single nuclei analyzed by single cell -- the single-cell RNA-seq methods.

So, there is -- I only know of one report so far about single cell RNA-seq and ME/CFS. And this work was done by Andrew Grimson's group, which is one of our collaborators in our NIH ME/CFS Center. And as he is out of town, I am going to be giving this presentation about some of the work done in his lab. This work was -- and he provided me with some slides. This -- the work is available as a pre-print that you can consult, and it's under review for publication. This study used 30 ME/CFS and 30 sedentary control subjects. And for each subject, about 4,500 cells had their RNA sequenced and then analyzed using the 10x Genomics platform. So, this is showing a dimensional reduction method to separate different types of cells according to their gene expression. So, each one of these dots that you might see here, that's a different cell, and they can be grouped together based on their gene expression pattern. And you can then -- using that pattern, identify what type of cells these are. So, for example, here, we have naïve CD4 T

cells, naïve CD8 T cells. But the one that was turned out to be extremely interesting was the classical monocytes. The classical monocytes exhibited the greatest difference in gene expression between the cases and controls.

To make a long story short, I'm going to summarize a great deal of work that you can see in the pre-print. But the pathways that were found to be dysregulated by looking at the gene expression in these classical monocytes are listed here. These pathways all have something to do with monocyte function and development. And the aberration in these pathways are consistent with increased monocyte migration to tissue, which would then result in increased macrophage activity in ME/CFS. So, in order to look more at these tissues, these cell types, once you have gotten one that you think is particularly interesting to look at, you can do flow sorting, as I mentioned earlier. You can sort a batch of immune cells and obtain the particular type that you're interested in. So, this is something the Grimson Lab did. They made classical monocytes from four ME/CFS and four controls, and performed total RNA-seq on those to see if they could validate this finding of the signaling of the monocytes to enter tissue. And what's interesting is that this graph here shows the most dysregulated -- in this case, upregulated -- genes expressed in ME/CFS versus controls. And these CCL4 chemokines are ones that are known to attract immune cells to go through the endothelium and migrate into peripheral tissues. So, that's consistent.

And the other interesting thing is this GM-CSF that's also known as CSF2. This is granulocyte-macrophage colony-stimulating factor which stimulates the stem cells to produce granulocytes and monocytes and signals them again to develop into macrophages. So, if you look -- we looked around to see if there was any other evidence from prior work of macrophage activation at the protein level. And in looking at a -- at the proteins in plasma using the SomaLogic assay, we published a pilot study of 20 cases and 20 controls in 2021 and found that this TIMD3 protein was upregulated. And this is known to regulate macrophage activation, and interestingly, also is known to suppress NK cell-mediated cytotoxicity. And as Nancy said in her talk, that is one of the common findings that, long ago, she -- her group showed that the NK cells were less active. The finding of elevated GM-CSF has been seen in multiple studies. On the left here, I have a study -- a graph from a study by Montoya's group, and at Stanford, Mark Davis. And when they compared the amount of GM-CSF in mild, moderate, and severe patients, they found a higher amount in the severe patients.

We also, in a collaboration with the Columbia group, found that the higher the GM-CSF you have, the worse physical function you have on SF-36. So, this is also consistent with the single-cell RNA-seq. To summarize then all the data so far on this -- in this study indicates that the monocytes from ME/CFS subjects express genes indicating they're being signaled to move into the tissue, become macrophages, and the purpose of these macrophages is to surround or kill pathogens or infected cells. So, maybe they're killing some virus. We don't know what that virus

might be that they could be acting upon. Another very interesting aspect of this study was that -- was the comparison using machine learning to examine the gene expression data. So, using machine learning, each of the cells could be classified from its RNA sequences, whether it was "normal" at a normal profile, or a disease profile, an abnormal profile. And this just shows an example of one case and one control. But this is a typical example of each, where the case has a number of these blue predicted diseased cells, as well as some predicted normal cells among the classical monocytes.

Well, this control has mainly normal cells and only a few of these predicted diseased cells. In fact, if you look at the population of subjects, the controls in general had pretty low predicted diseased classical monocytes, while the cases were clearly having a lot more of these predicted diseased cells. But I think what's even more interesting is that there was a correlation of the number of predicted diseased cells and an important symptom. So, this is fatigue. As fatigue is higher in a subject, they have more of these predicted diseased classical monocytes as measured by the MFI-20. So, the other thing that single-cell RNA-seq measured and found was that platelets were dysregulated. And now, platelets aren't -- you know, this was not -- we didn't deliberately isolate platelets in these preparations. But some of them were present in the PBMC preparation that was put through the single-cell RNA-seq. And so, some could be seen. And to summarize, there are -- we -- the evidence is that the -- there are pathways activated in platelets that indicate that the platelets are in a state of activation.

And the -- this is what this shows here, the dysregulated genes. This is not -- this just shows what happens when a platelet is activated. You get a clot. And I am not going to talk about this any further, because we're going to have a talk on -- in the circulation webinar by Dr. Pretorius, who's been studying these microclots and abnormal platelets in both ME/CFS and long COVID. So, I'd like to turn to the fact that there are a number of T-cell abnormalities that are caused by chronic antigen exposure. So, in the -- in autoimmunity and cancer and chronic viral infections, you can have T-cell abnormalities, which might be a loss of self-ignorance or self-tolerance and autoimmunity. You can get anergy from autoimmunity, but also from cancer and chronic viral infections. And you can also get senescence from those three. But something you can get from cancer and chronic viral infections is T-cell exhaustion, which is what I'm going to talk about some. There's really little known about these abnormal states in ME/CFS. I'm aware that in addition to us, there's some other groups that are analyzing T-cell exhaustion. But with regard to pre-2020 ME/CFS, there's really very little known about T-cell exhaustion. So, is there CD8 T-cell exhaustion in ME/CFS? And again, I'm going to present some of our work, because I haven't seen other work published, although there are some studies ongoing. T-cell exhaustion is being considered in long COVID, but I think it's important to know whether this is happening in pre-2020 ME/CFS. So, T-cell exhaustion results in abnormalities in immunometabolism. And this is something else I'm not going to talk about. And the reason is that it's going to be covered in the Metabolism Roadmap webinar by Jessica Maya at a later date.

So, if you have chronic antigen exposure, what can you end up with? You can end up with hypometabolism, which is what I'll be talking about, or you can end up with hypermetabolism, which is what Carmen Scheibenbogen talked about. There are some published reports that are relevant to T-cell exhaustion. And maybe Derya will be talking about some of this. He -- in his preprint, he provided some evidence for chronic activation of the TH17 cell subset, which is something you would expect in T-cell exhaustion. There's also a transcription factor that is likely to be upregulated because of hypomethylation. And then there's a report from a Spanish group showing higher exhaustion markers -- PD1 and CD95 -- in ME/CFS. So, you can identify the existence of exhausted T-cells by the presence of the inhibitory receptor, PD1, and also by three transcription factors that are known to be expressed in the nucleus of exhausted cells. The protein TOX is an example of one of these transcription factors that is driving exhaustion, and it drives exhaustion by either decrease -- by expressing -- causing genes to be expressed that decrease the immune response, or to -- by increasing the expression of immune suppression markers, such as PD1. So, another thing that I'd like to point out that we don't know much about is that circulating exhausted immune cells have been analyzed in most diseases more than tissue-resident cells. And of course, the tissue-resident cells have really not been analyzed in ME/CFS for their -- whether or not they are exhausted. But we are going -- I'm going to be presenting some work about T-cell exhaustion in circulating cells, but it would be very important to know about cells in the tissue. And you can use spatial transcriptomics, as I mentioned earlier, to detect exhausted cells. This comes from a review about cancer cells, and this is a diagram of a tumor, in which, by using spatial transcriptomics, you can identify whether or not you have exhausted T cells in this tumor.

You could also do this in ME/CFS biopsies to find out if there's exhausted T cells. So, I'm going to describe an unpublished study of exhaustion markers in circulating T cells. The flow cytometry work was done by Jessica Maya in my lab. And using 15 cases and 14 controls, she isolated CD8 T cells by magnetic beads, and then subjected them to flow cytometry to examine the levels of PD1, TOX, TCF1, and T-bet, the three transcription factors. Now, one thing that is interesting is there were no differences in the subset frequencies of CD8 T cells. It's possible to use flow cytometry to identify these different types of CD8 T cells. And really, there was no difference between patients and controls. So, assay, I'm only going to show a little bit of this data. Manuscript is in preparation. We hope to have this out soon because I really don't have time to go into a lot, but I just wanted to indicate some of the data that is making us suspect the existence of T cell exhaustion. So, here in total T cells, the number of cells that express PD-1 and TCF1, that transcription factor, are higher in ME. The number of cells that are expressing the PD-1 and the three transcription factors are also higher in the ME subjects. In the case of early differentiated CD8 T cells, there's higher expression of Tox, as you can see here, and there's a higher expression of PD-1, Tox, and T-bet -- higher numbers of cells. Sorry, I was saying that wrong. Higher number of cells expressing PD-1, Tox, and T-bet in the ME preparations. Finally,

the last thing I'd like to just show is these terminal CD8 T cells in which the higher percent of the terminal cells are expressing this Tox transcription factor.

Now, I've just shown you flow cytometry data, but it's also possible to look in the single cell RNA-seq data at the particular -- the same type of cells that are showing some exhaustion phenotype by flow cytometry. You can also look in RNA-seq. This was done by David Lu and the Grimson lab. And he was able to detect that in ME, this transcription factor called EOMES is elevated relative to controls. This is a transcription factor that is known to promote T cell exhaustion. And a receptor protein called SLAMF7 also is upregulated, as is Tox in these cells analyzed by single-cell RNA, by RNA-seq. And then also this receptor protein PDCD1, which is a key player in exhaustion, is also upregulated in ME. So, I'd like to acknowledge the unpublished data that I've presented. Andrew Grimson's lab and the people in his lab who did all of the work to get the single cell RNA-seq, as well as Jen Grenier's lab that did a lot of the analysis, and then Jessica Maya, who did the flow cytometry work. And of course, our funders were key. But I'd like to end with the research priorities. I was asked to come up with five research priorities, and these are the ones that I am suggesting that we really need to know what the signals are that are driving this dysregulation of these immune cells and the platelets. Are the immune cells in ME/CFS exhausted? And if they are exhausted, if so, why? Is it herpesviruses? Is it the inciting virus that's still present? Is it endogenous retroviruses? Some of that will be explored in the Chronic Infections Webinar. Because there's been little work on granulocytes, it would be good to know if they were functioning normally.

And what about the immune cells in tissues and organs? Are they abnormal in ME/CFS? It would be useful to know how are monocytes and platelets contributing to immune abnormalities and symptoms. Because certainly, there are some potential treatments that one could use if you have documented T cell exhaustion or abnormal activity of platelets. So, I'll end here and take questions.

Derya Unutmaz: Thank you for the excellent talk, Maureen. I'll refer to Vicky again.

Vicky Whittemore: Yeah. Thank you very much, Maureen. It's all -- picking up on that very last comment that you made. One of the questions is what are potential treatments for T cell exhaustion? How is that addressed clinically?

Maureen Hanson: Yes. So, there have been a number of very effective cancer treatments because T cell exhaustion does occur in cancer. There's a number of monoclonal antibodies that are being used to overcome T cell exhaustion. The issue is that clinical trials really are needed and that these anti-exhaustion drugs are not something to be taken lightly. They do have a lot of side effects. So, this is something again, I think that we need clinical trials and some physicians

who are really experts in the application of these, if we have proven that someone has indeed T cell exhaustion that is driving symptoms.

Vicky Whittemore: Thanks. So, another question is, are there any cell surface markers or ways to identify, I guess, and inform which tissues the monocytes are entering? So, I guess the question is, how do we know or do we know what tissues may be impacted by the monocytes?

Maureen Hanson: I think that's where we need to get biopsies and look. We don't know. We don't know where they're going. So, we need to find out. Obviously, well, there are many tissues we'd like to know about. We'd like to know about muscle. We'd like to know about brain. But again, most people don't want to give you a brain biopsy. So, we may need to use some more accessible tissues and find out if we have evidence for exhaustion.

Vicky Whittemore: Are these same kinds of things being seen in long COVID?

Maureen Hanson: I don't know that classical monocytes have been specifically examined yet in long COVID, but I don't know. I'll say -- wait a minute. I will say one thing. Of course, platelets are definitely known to be abnormal in long COVID, and we're going to hear about that from Dr. Pretorius. But as far as the cells, I don't think we know yet in long COVID.

Vicky Whittemore: Okay. Let's see. Have you been able to follow up the monocyte single-cell RNA-seq data with protein expression data?

Maureen Hanson: Yes. The reviewers wanted protein expression data. And so, that's something that the Grimson lab has worked on. And just to make a long story short, the protein data is consistent with the finding of the single cell RNA-seq.

Vicky Whittemore: Has anyone looked at ME/CFS -- individuals with ME/CFS with bleeding disorders, for example, von Willebrand's? Or maybe this is a question more for circulation or another?

Maureen Hanson: Yeah, certainly there's some protein level information, but I guess this would be for circulation, the circulation.

Vicky Whittemore: And then another question is, has anyone looked at individuals who have both ME/CFS and are cancer survivors?

Maureen Hanson: Maybe the clinicians could comment on that. I can't comment on that. Although I have heard people comment that their cancer treatment was far easier than having ME/CFS.

Vicky Whittemore: So, let's see. This is -- can thymosin alpha one or TA1 overcome T cell exhaustion so TA1 improves the fatigue level in some individuals? Is there any evidence to suspect ME/CFS proposed biomarker of -- that this could be a biomarker of NK cell function in ME/CFS?

Maureen Hanson: I can't answer that. Maybe Nancy would be able to answer that. She's welcome to break in if she can.

Nancy Klimas: Could you repeat -- I didn't hear the question, I'm sorry. Say it again.

Vicky Whittemore: So, the question is about thymosin alpha one. And can it overcome T cell exhaustion? And then the rest of the question is, or maybe it's a separate question actually. Is there any evidence to suspect the ME/CFS proposed biomarker of NK cell function might fit the classification of NK cell exhaustion?

Nancy Klimas: So, I mean, thymosin has been our focus early in the field. In the 90s and maybe around 2000 or so, there were some thymosin-focused interventive studies, a very small pilot that never went any further. But of course, at that time, we weren't even talking about T cell exhaustion, so we wouldn't have done those markers. So, it'd be interesting to look again, using perhaps better products and better biomarkers. I'm not sure that I can say that -- NK exhaustion is interesting because it's a new word, the exhaustion, but we published some papers to show depleted perforin and granzymes, which is a nice way to look at NK cells without having the -- there's a lot of problem measuring NK cells because they really lose their function, hour by hour, once they're in a tube. And so the normal range is change over 24 hours. And after 24 hours, they're toast. There's nothing worth measuring there. So, they're really difficult to do in studies because you have to have an expert lab, and you have to get the cells there really quickly. And it's difficult. So, if we had a flow measure or something that was stable and you could measure a day or two later, it's better. So, we had thought perhaps the perforin-granzyme content would be a nice way to look at a surrogate for function. But now it would be nice to look at all over again with the exhaustion markers and see if it's tied to NK exhaustion. In other words, we don't know, but it's a cool study.

Vicky Whittemore: Right. Those are all the questions that came in for you, Maureen. Does anyone have any additional questions, or Derya, do you have any questions?

Derya Unutmaz: Maybe we can have a discussion on this. But I think the word “exhaustion” does not really reflect what's going on. I know this is very commonly used, but in reality, these cells actually become much more dangerous. Therefore, the immune system is trying to regulate them by upregulating these checkpoint inhibitors. They're not exhausted at all. They are fully

armed and ready to go and can be very harmful cells. I just wanted to clarify them. Because exhausted implies they need to retire or something, but that's not the case.

Maureen Hanson: Well, it's certainly a mechanism that the immune system is using to keep the damage down. But the result of that could also be that they're not responding to, for example, a virus as well as they should be.

Vicky Whittemore: Right.

Derya Unutmaz: For sure.

Vicky Whittemore: Right. Okay. Well, I think with that, thank you, Maureen, very much. It was really excellent. A lot of really excellent work. I think we'll take a break now, and we'll reconvene at 1:30 with Derya's talk. So, we'll see you back in about 15 minutes. Thank you.

Vicky Whittemore: Hey. Well, I'd like to welcome everyone back. I hope you had a chance for a short little break. But it's my pleasure now to introduce the next speaker who is Derya Unutmaz from Jackson Labs. Take it away, Derya. Thank you.

Derya Unutmaz: Thank you very much. I'll get started right away. So, of course, I'm going to talk about the immune system and immune perturbations in ME/CFS. So, first I'd like to start a little bit with a little bit of an introduction and what we know about the immune system in ME/CFS. I think I don't need to convince any of you that there's now overwhelming evidence that multiple parts of the immune system are perturbed or disturbed during ME/CFS disease. We heard some fantastic talks about this. But the question is how and why this happens, and what does this really mean in the context of ME/CFS? I think that's something we should try to answer during this webinar. So, just a very brief background on how the immune system works. It's a sort of an army within ourselves. It's very complicated. There are many, many compartments because it's essential for our survival. But at the same time, it's very dangerous, as we realize from many different chronic diseases that result from immune system dysfunction. Sometimes could be deadly. Sometimes even during infections like we've seen during COVID in the late stages, we really needed to suppress the immune system to prevent mortality and severe disease.

So, we have generally two compartments of the immune system: one, we call the innate immunity. These are sort of the first responders. The guys that respond right away when there's a bacteria, virus, or whatever threat that's entering into our body. And you heard about them. This includes also mast cells and macrophages. And even the epithelial barrier cells have sort of an innate resistance capabilities that tries to stop the viruses. And this happens within hours. And the goal of this is that --to really slow down the sort of -- the enemy that's entering you and give enough time for the adaptive immunity, which is really the main part of the immune system that will allow a very specific response to that given pathogen. We heard about the autoantibodies. So, antibodies, they're sort of like smart missiles that will be generated by these B cells, bind to viruses, bacteria. But, of course, you know, if they bind to our self-antigens, our cells, then that causes a disease called autoimmunity. And that's not a good thing. And then we have these T cells that are very, very complicated. They have many parts.

And I'll mention a little bit more about that. And they have multiple roles. They're sort of like the generals or the regulators of the immune system. They give orders to other cells, what to do. And some of them are cytotoxic, are sort of like assassins. They will go and seek out and find virally infected cells and kill them. And we actually use that capability against cancer. They have anti-cancer effects. They can kill tumor tissues. But again, they are very dangerous cells. So, they can damage normal tissue. So, we have to control them. Then the immune system has built-in mechanisms as we heard in the previous talk, that will have checkpoints. Okay, that's enough. You shouldn't continue to kill other cells.

Or we have what we call regulatory cells. They're sort of like the bureaucrats of the immune system that tell the immune response to slow down. So, in general, this is what happens when you're infected with something. You have cells, what we call naive cells, that have never seen this virus, bacteria, or other antigen before. As soon as the virus enters you, it's picked up, and it's passed. The small parts of the proteins are picked up and presented to these naive T cells. And out of the millions and billions of cells, a few of them will actually recognize these small parts in what we call peptides, and will expand. They will proliferate, create a clonal army. They will differentiate. If it's a B cell, it will turn into a plasma cell. If it's a T cell, it will turn into this, what we call effector cell that has now very potent functions, whether it's cytokine production, whether it's killing, whether it's regulation. And this happens within days.

So, within seven to 10 days, you have this response. And once the virus or the threat is gone, most of these cells die off because you don't want to keep a lot of these cells around. They could be dangerous, and we would be full of lymphocytes after a while. So, a few of them remain, and those few of them survive for years. And that's the basis of immunological memory. So, if you are exposed to the same virus or bacteria again, they can respond very, very quickly. So, it's a very, kind of, elaborate system, but unfortunately sometimes it doesn't work. And we have problems. So, this is the good part of the immune system, right? So, it's evolved to clear the infections, as well as repair tissue damage after the damage is done. But then you have the bad part. And that is if it's not controlled, if it's against our self; you get autoimmunity, allergies, septic shock that could be really detrimental for our health. And then you have the ugly side of the immune system. And that's something that's not always apparent, what we call chronic inflammation or -- which causes chronic illnesses.

We think that ME/CFS is part of that. But, you know, development of atherosclerosis or dementia and other chronic problems, especially during aging; we think are largely due to this, what we call chronic inflammation. The immune system thinks that there is threat or these cells, what we called exhausted in the previous talk, they are actually kind of become like mercenaries. You know, they keep on doing damage even though there is no threat. They think there is a threat. So, as I mentioned, this is a very elaborate system. There are many parts in this, and I'm just going to mention about the T cells. This is what I've been studying for the past 30-plus years. And there are many, many subtypes of T cells because you have -- you can have many different threats, viruses, parasites, bacteria, different types of it. And then you have to regulate these T cells through these cells that we call the regulatory T cells.

But again, each of these have different functionality. They can cause autoimmunity, allergies, and other tissue damage problems. And we can also divide these cells into what we call conventional and unconventional T cells. Conventional ones are the ones that recognize antigens or peptides, but the unconventional ones are also very important. They don't discriminate between the peptides from the bacteria or whatever, but they recognize as constant parts, such as

lipids or small metabolites. These cells are called MAIT cells, natural killer T cells, gamma delta cells, and they probably evolved to recognize constant regions that are either produced by bacteria or viruses or other cells. And we think that they're also involved in regulating the microbiome, which I will talk in a bit. And so this is sort of -- shows that, you know, classical cells recognize peptides.

For example, MAIT cells recognize these metabolites derived from bacteria in our gut. This is actually a metabolite produced during vitamin B2 production. And so, MAIT cells, for whatever reason, recognize that and can be activated. Natural killer T cells recognize glycolipids, for example. And what about NK cells? Well, NK cells do the opposite. So, if there is no recognition through these MHC molecules, they recognize that as a threat. For example, certain cancer cells downregulate these MHC molecules that allow T cells to recognize them so that they're trying to escape, but then natural killer cells come in and say, "Oh, okay, you can't escape from us. We know that you're enemy." Or some viruses do that, like CMV. Some herpes viruses can downregulate this MHC to avoid response from the T cells, but then NK cells will recognize them as infected cells. So, they're kind of like a backup system along there. So, how do we measure all that stuff? And Maureen mentioned a little bit about this. Our main tool is what we call flow cytometry. We can measure all these populations, different compartments, different functionalities, using this machine that has a bunch of lasers in them. And then when we do that, we can generate lots of lots of data.

And this is just an example from some study that we did in the past few years where we can identify different subpopulations of immune cells. And we find major differences in ME/CFS patients. But what is really important? That's not the surprising part. What was surprising, at least in our studies, when we divided the cells into two major groups, one group, patients who had the disease for less than four years, and then those who had more than 10 years, so more chronically infected. And we found differences between these two groups of patients. So, the ones that had -- in some parameters, the ones that had disease more than 10 years, had disruptions, but not less than four years. You can see some examples here.

The other thing that turned out to be really important is dividing the patient groups based on age. For example, some of these markers were different if the patient group was less than 50 years. And we compared them, of course, to healthy controls less than 50 years old. Whereas, they were not different in patients that were over the age of 50 years old. And we had vice versa conditions, too. But most of the markers that were different actually became more apparent in less than 50 years. So, we think that this might have to do with -- again, in the previous talk, we mentioned, and Nancy mentioned this too. There could be some sort of an accelerated aging of the immune system too. So, that's what also we mean by exhaustion. The cells are becoming older, faster, and that's causing chronic inflammation. So, you know, we have generated hundreds of parameters. Others have done, as Nancy mentioned, you know, many, many studies. And this is not a

complete description, but there are lots of compartments in the immune system that are disrupted. We have chronic inflammatory cytokines, immune activation. These are things that happen during infections, of course, but they should not persist. If they persist, then we have chronic activation conditions that cause damage. Disturbances in NK cell function, I mentioned about NK cells.

We heard a beautiful talk from Carmen about autoantibodies. So, that suggests B cell dysfunction because the B cells should not be making autoantibodies to our own antigens. And then we have lots of different subset of T cells that seem to be functionally disrupted, not just frequency-wise, but their functions are either lacking or too much. For example, CD8 cells that respond to viruses, Th17 cells --I'm going to mention a little bit more about those -- and then unconventional T cells, such as MAIT cells and gamma delta T cells that are more trying to stop or regulate the trillions of bacteria that are in our bodies, and perhaps some viruses as well. There are disruptions in energy metabolism, mitochondrial abnormalities in T cells. We have some data on that, and others have published on it. We heard about neuroinflammation, gut immune compartment disturbances. I'll get back to the gut, and we'll have a very nice talk after this. And as I mentioned, there could be some sort of an accelerated aging of the immune cells that turns them into more sort of dangerous populations that cause chronic inflammation. In fact, you know, in one of our studies, we were able to put together a bunch of these immune parameters actually. When I say bunch, we had thousands and thousands of data points, and we did machine learning analysis. And by just looking at different immune differences between healthy and ME/CFS patients, we could tell who is a patient, who is a healthy control, up to 90 percent sensitivity and very high specificity.

So, it shows that, you know, there is something really major going on in ME/CFS, and perhaps some of this could be used as a biomarker in the future as well. Again, going back to the question, what does this all mean? Why is this happening? So, this has been our hypothesis, and others have been working on this as well, that there is a very intricate link between the microbiota, the trillions of bacteria that live in our bodies, that actually we need them. They produce a lot of metabolites. They digest our food. And that has a lot of different effects on the immune system. We need it, but sometimes it has a bad influence. So, depending on what this is, during infections or stress conditions which trigger the immune system, you might have a good, the bad, or the ugly outcome, depending on your microbiota, metabolism, and of course, somewhat, genetics as well play a role.

So, microbes are, as I mentioned, are very important for us. And we had actually been analyzing -- and when I say we, not just our lab, but other labs as well. And our major hypothesis was that if we could understand the microbiome, then we might be able to link that to the immune disturbances, because the link would be through the metabolism, actually, because about 20 to 30 percent of metabolites in our -- in our body are derived from the bacteria. They affect all our

physiology. And then maybe doing that, we could discover not only biomarkers but novel actionable targets for treatment of ME/CFS. And one difficulty is that everybody's microbiota is personalized. So, they have different levels, different frequencies of different types of bacteria. And there are probably thousands of strains in each person, and then trillions of them. So, you have to really sort of look at all of that in every individual because, you know, there is a very fine equilibrium between the bacteria in our gut. So, you have those that are very, very beneficial to us. You have those that live in a commensal, you know, peace treaty. And then you have those that could be potentially dangerous, but they are kept under control probably by the other bacteria. But if there is some sort of an inflammation or some sort of a trigger diet or infections, these guys start to overgrow, and that causes gut inflammation. And gut inflammation causes all kinds of immune disturbances. So, to measure that, we actually sequenced these bacteria from the fecal samples of the patients and healthy controls. We try to do analysis of these thousands of different strains and come up with different profiles.

And there's quite a bit of work going on here, and very, very interesting work that suggests that the microbiota is disrupted, including one from our group, another from Columbia Group with Ian Lipkin, and so on. I won't -- this is not an exhaustive list. There are dozens of studies now suggesting that there are disturbances in the microbiota, and therefore, metabolic disturbances. So, is there any link between this disturbance and the immune system? There are several actually. So, one -- again, Maureen mentioned this -- one subset of cells that we and I think Nancy's group have found and others have found, is a subset of cells called Th17 cells. Now Th17 cells produce a cytokine called IL-17. And this IL-17 does a lot of things, a lot of good things, but also a lot of bad things. They seem to play a role also in autoimmunity, in chronic inflammation. So, they need to be regulated. And they are also very responsive to gut microbiota composition, the type of bacteria you have. In fact, in ME/CFS patients, especially those who had more than 10 years, we find very consistently, reduced numbers of IL-17-producing Th17 cells, suggesting that they are disrupted. And one reason that could be happening is the microbiota differences.

And we have a couple of other examples mentioned about the MAIT cells, for example. And then they seem to be disrupted functionally. And that also links to the microbiota. Of course, we don't have direct evidence, but this is circumstantial evidence that's pretty convincing in some ways. And then so finally, the thing that links the microbiota and the immune system from the outside world is the chemical space, so what we call metabolome. So, of course you have the proteins and you have all kinds of other things. But a lot of things are happening in the metabolome. Lipids, sugars, amino acids and all kinds of small metabolites really have -- some of which have a huge effect on our immune system and our physiology. And a lot of them we don't really understand. We were able to measure a thousand different metabolites in our blood. We don't know which ones are important or not important and what kind of effects they have. But clearly, this is an area that requires a lot of investigation. And you'll -- we'll have a great

webinar next week on -- specifically on metabolism. So, I won't go into details of it, and there will be a lot of talks on that.

But at least to say that there's a lot of evidence that there's a metabolic dysfunction during ME/CFS, by many, many groups. We have some very interesting results that we're in process getting them into papers. So, just an example. For example, this, what we call tryptophan pathway or metabolites are disturbed in ME/CFS. And in fact, this is from our data set. We also find components of this pathway to be disturbed. And the reason why this is important is that this pathway directly affects the immune function, both directly through the tryptophan catabolism, but also through this molecule called aryl hydrocarbon receptor or AHR, which is involved in regulation or activation of many immune cell functions. And we have some very interesting data we soon -- we hope to soon publish on that. Okay. So, that's what we know, but what we don't know is also quite a bit. So, what triggers these immune disruptions, whether it's microbiota, or is it infections as we heard about for EBV or SARS-CoV-2, and why it happens in some people but not others? We don't know if or which components of the immune perturbations are the reasons for ME/CFS symptoms. You know, again, whether they're the consequence or the cause, we don't understand.

For example, metabolic dysfunction, is that really a disease progression or is just a side effect? We don't know the impact of contribution of immune set points. I showed you the age. It turns out to be really important. And sex was mentioned, female versus male. There are huge differences in the immune system. We know that females get more autoimmunity, and they are overwhelmingly more in ME/CFS cohorts. Infection history, genetics, lifestyle that triggers disruption in this homeostatic balance, both in the microbiome and metabolism, but also in immune response. So, if you have a different set point, maybe you are more or less susceptible to getting ME/CFS. Something that would be very, very useful to understand.

And what do we need to know on complex interplay between the immune system, metabolism, and microbiota that I just mentioned? We need to know if microbiota changes are due to immune changes or vice versa. You know, that's not something we understand because the immune system controls the microbiome. Microbiome controls the immune system. We need to know which metabolite secreted by the microbiota that impact the immune system, whether these are abnormal ME/CFS. We have evidence -- I mentioned about tryptophan. Another one is butyrate pathway. We find decreased butyrate-producing microbes in our body, which by the way could be an immune suppressor. So, that also links directly to the immune responses. Tryptophan pathway also could be an immune regulator as well, but we need to know more of that. We need to know the role of energy and lipid metabolism contributing to immune perturbations, immune patients. We have evidence that energy metabolism is very important. In terms of metabolism, we also find very interesting links between the lipids and the immune cell disruption, something that we are preparing for publication very soon. We also need to know how infection, stress

responses, or other environmental factors trigger immune response, also chronically impact microbiota and metabolic pathways. This is not something that happens in a matter of days, weeks, or months. In years, these things build up. That's why it's important to look at patients who have the disease for many years versus less.

So, what are our research priorities in understanding the immune disruption, development of biomarkers and therapies? I think we really need to identify actionable metabolic microbiota targets linked -- that are disrupted in ME/CFS patients. These are really actionable. You can't change your genetics, but you can change your metabolism. You can change the composition of your microbiota. We need to identify and measure immune outputs during actionable, targeted interventions. So, what happens if you do a clinical trial using these? So, to assess that very rapidly, if we can find some biological markers, that would be really, really important.

And we need to design and implement small intervention trials. Nancy mentioned this. Really, clinical trials are very important, and measure systemwide changes of immune response, metabolic pathways, and microbiome. We can learn a lot even if the trial doesn't work. And we need to develop highly sensitive and specific biomarker sets based on immune metabolome data sets so that we can objectively diagnose who have ME/CFS, and how long they had it, and so on and so forth.

And we also need to design research studies and specific cohorts of subjects to determine the biological heterogeneity of ME/CFS patients. I mentioned about those who had the disease less than four years, more than ten years, females, males. They are probably maybe a dozen different subgroups that we can identify. So, this is a very heterogeneous disease. Some might have autoantibodies disruption. Others might have microbiota disruption, and so on and so forth. So, you know, if we do that, we can develop ontology-based personalization or grouping of the ME/CFS and target specific problems in each subgroup.

So, my -- I'd like to acknowledge all my group at Jackson Lab and the really great contributions of the clinical team, Cindy Bateman, Suzanne Vernon, Microbiome, Julia Oh. We're doing metabolism with Xudong Yao and Shuzhao Li, and competition work with Duygu Ucar. And of course, the funding from NIH that made all of this possible. I'm going to stop there and take any questions in the last few minutes.

Vicky Whittemore: Yeah. Thank you very much, Derya. I don't see any questions that have come in through the Q&A, so please enter questions if you have any questions there, or if anyone from the panel has a question. Let's see. I just see a couple that just came in. Hold on. Let's see.

So, what is the best way to include individuals who have had ME/CFS for years or decades? Sometimes this population is left out. I know another concern there is that sometimes the cut off is, say, 60 to 65, so how to also include individuals who have aged out of the eligibility of a trial?

Derya Unutmaz: Yeah. This is something that we debated with Cindy Bateman and a couple other clinicians. It's not very easy because I think we need to rely on the history of the disease, but I feel that -- you know, I'm not a clinician in this field, so I can't really answer this question. But I can say that this is really important because I think the long-term disease seems to be quite different. In fact, we see that from long COVID. You know, when we talk about long COVID, it's actually months. We see a significant number of long COVID patients getting better after a year. A few are not. So, you know, how is that similar to ME/CFS becomes a little bit questionable at that point. There's more acute disturbance in the immune system that sort of normalizes and you lose the symptoms. But then something else is going on in these patients who have disease for -- especially for decades.

Vicky Whittemore: You know, one of the things I think will be very interesting to look at in the RECOVER study, which is a study of individuals with COVID who then go on to develop long COVID, is that transition from the acute infection phase into the long COVID and then being able to compare those who are diagnosed with ME/CFS versus those who are not. So, I know from my involvement in the RECOVER project that biospecimens are being collected across that whole timeline. So, I think some of those -- when those biospecimens become available for research, that will be really, quite interesting because it's a very unique opportunity to have those biospecimens, unlike, you know, what we've ever had access to for ME/CFS.

Derya Unutmaz: Yeah. If I can just add one more thing. I think, you know, age is, as I mentioned, is a major factor because as you also age, your comorbidities also increase. You know, even in seemingly healthy people, the immune system starts to get disrupted because of the aging process. So then that sort of overlaps, and we need to sort of dissect, you know, what is actually, you know, that long term effect is purely ME/CFS effects or some of these comorbidities or the aging effect.

Vicky Whittemore: I see, Nancy, you have your hand up. Do you have a comment or a question?

Nancy Klimas: Yeah. Sorry, I couldn't find a way to type it in from the panelist login. Yes, I wanted to know whether or not you could postulate hypothetical ways that you could design studies that would affect immune senescence or immune competence. Are we talking about stem cell studies? What do you think is the type of approach that we're going to go down for that?

Derya Unutmaz: I wish I could answer that because then we would solve aging process as well. So, that's something that -- it's something we've been thinking for the past 15-20 years. Actually, this started with HIV infection because, you know, in HIV-infected individuals who live, you know, decades now because of treatment, you see this accelerated aging of the immune system. The senescence start about a decade earlier than we would expect. So, how can we reverse that? That's the million, billion, trillion-dollar question. But hopefully we will figure that out.

Nancy Klimas: Thank you.

Vicky Whittemore: So, with regard to the microbiome. So, there are some interesting questions that have come in. So, have disturbances of the microbiome been teased out from that found in various locations of the GI tract? So, are disturbances, in other words, found in one -- part of the GI tract versus another? Or say if you see a change in some of the microbiota, is it sort of consistent throughout, or do we know?

Derya Unutmaz: Yeah. Very interesting question. Of course, we don't know because we actually use the fecal samples. So, that's probably mostly colonic bacteria. But we don't know unless you do biopsies or, you know, you have to go into the small intestine with endoscopies or things like that to collect samples from there. Some people might be doing that. We haven't done it. And I don't think this has been done in ME/CFS, but it's a really interesting question.

Vicky Whittemore: Yeah. And a question I would have also is, you know, you talked about sort of increases in certain types of microbiota and the dysfunction that causes. What about when you have a decrease, for example, individuals who are on, say, chronic antibiotics where you're wiping out the microbiome?

Derya Unutmaz: Yeah. It's really kind of the ratios of different types of bacteria. So, for example, you have these phyla called Bacteroidetes versus Firmicutes. You know, people generally find that if you have too much -- if that ratio changes in the favor of Bacteroidetes, you tend to have more chronic inflammation. Of course, there are different -- you know, thousands of different strains in there. The antibiotic treatment is a double-edged sword, right? So, because it's not selective to the good guy -- bad guy. So, you might be killing off the bad guys as well -- I mean, good guys as well. So, are you going to affect the ratios of that? That's unclear.

So, more sort of maybe microbiome transplantation could be a more interesting target for therapy. So, in order to change that ratios, you actually try to replace more Firmicutes, if that ratio has been disturbed. You know, that that has been done in some infectious diseases, but I think, in a very crude way, literally, from fecal samples. But if you can identify these bacteria, grow them, and then turn them into literally drugs actually, and replenish them, that could be a very interesting therapeutic, perhaps.

Vicky Whittemore: Yeah. So, I'm aware that there are fecal transplant clinical trials starting in, I believe, both UK and Norway, for ME/CFS.

Derya Unutmaz: Okay. That's cool.

Vicky Whittemore: So, I think that those will be interesting. And one of the other questions was, are we aware of any studies or trials where they've used specific diets to try to impact the microbiome in individuals with ME/CFS?

Derya Unutmaz: So, diet is the number one reason that can affect your microbiome. So, this is well-established because the bacteria there respond to the carbohydrates versus lipids versus proteins. I mean, they digest these. And if you limit certain parts of your bacteria, for example, if you think -- I would advise people to eat less carbohydrates because they seem to promote sort of the troublemakers more or, you know, processed meats, for example. And that definitely affects your microbiotic composition, and in turn, that will affect loss of your biology.

Vicky Whittemore: And the last question, I'm aware of studies like this that are being done in other diseases, and especially epilepsy where people have taken the microbiome from individuals with the disease and put them into animal models. And what is the result of that? Do you develop that disease, or how does that impact an animal? Have those kinds of studies been done in ME/CFS? I'm not aware that they have, but...

Derya Unutmaz: I'm not aware in ME/CFs, but for example, in our institution, people are trying -- during aging, you know, trying to collect, you know, microbiota from aged people or younger people, and do this transplantation experiments, or within the mouse populations, old or young. They have not been very conclusive so far. It could be because the mouse biology is pretty different than the human. But if we could do that in human trials, you know, transplant a young, healthy person to an older, chronic, infected people, or inflammation, that could be very, very interesting. And if can measure quantifiable outputs in the immune system, would be very fascinating.

Vicky Whittemore: Yeah. All right, thank you, Derya. There's a lot of really other interesting questions here that we'll come back to in the discussion toward the end.

Derya Unutmaz: Okay. So, I will introduce our next speaker, Armin Alaedini. He's an assistant professor of medicine at Columbia University Medical Center at the Institute of Human Nutrition. He actually is a great segue from my talk because he also works on microbiota, our guts, in this junction of metabolism and the immune system. So, look forward to your talk, Armin.

Armin Alaedini: Hello and thank you for the opportunity to present some of the work that my lab has done on ME/CFS in the past few years. I do want to apologize in advance for not being able to do this live. I am right now serving on an NIH study section, so I will try to join for the Q&A later though. So, much of the data that I will show you in this talk is from an NIH-funded study that we recently published, this particular study here, that really explores this gut-immune metabolic interplay in the context of ME/CFS and its potential relevance to ME/CFS symptoms.

So, I don't need to give much of an introduction on ME/CFS, I know that, but I want to start by mentioning that there is, you know, a large body of data pointing to the presence of various immune system abnormalities in ME/CFS. And something that a lot of us might not think about is that ME/CFS patients also complain of various gastrointestinal symptoms that are mainly of unknown etiology. Now, little is known about the nature of the relationship between the established symptoms of ME/CFS, the gastrointestinal symptoms, and the immune system abnormalities that have been documented in patients. There are a small number of studies that provide some evidence for gut microbial dysbiosis, elevated circulating microbial DNA, and increased immune responses to bacterial LPS in circulation. Suggesting that there may be some level of gut microbial dysbiosis and possible alterations to mucosal barrier function in ME/CFS patients. But the contributing factors and the pathogenic relevance of these findings remains mostly unclear.

So, the overall objective of our research has been to really understand the relevance and contribution of the gastrointestinal system to ME/CFS and to also explore the relationship between gastrointestinal dysfunction and the various documented immune abnormalities in ME/CFS. We're also particularly interested in identifying biomarkers that can be used for ME/CFS stratification. And that's really aimed at improving diagnosis, but also helping to develop new therapies that could be considered sort of, as precision treatments for subsets of ME/CFS patients.

I will show you data from two sets of cohorts, one set of patients and controls at rest, and one undergoing an exercise challenge. Samples from the first set of cohorts came from a study that was done some time ago, a collaboration between SolveCFS and several ME/CFS clinics and GlaxoSmithKline. And that's the study you can see on the right, the original study. But the patients and the healthy controls in this particular study were not statistically significantly different in terms of age or sex or race or BMI. And at the bottom, you can see the inclusion

criteria and exclusion criteria that we used to include patients and controls. The data on gastrointestinal symptoms for these study participants was not very exhaustive, but still, it was clear from the data that ME/CFS patients have a lot more GI symptoms than healthy controls.

So, for example, you can see here that over 80 percent of ME/CFS patients had one or more GI symptoms in the past six months, whereas in controls, it was reported in only about half of the study participants. If you look at individual symptoms like abdominal pain, bloating, and nausea, again, you see that both severity and frequency of symptoms were significantly greater in ME/CFS patients than in the control cohort. Even more impressive was a difference in the frequency of diagnosed IBS between the ME/CFS patients and controls. Close to half of ME/CFS patients had also been diagnosed with IBS compared to only seven percent of controls. The difference in other GI conditions like celiac disease, Crohn's, ulcerative colitis, et cetera, those were not different between the two groups.

Now, we were particularly interested in celiac disease, and one reason for that is that celiac disease is or has been historically underdiagnosed. And so, we wanted to see whether we can find biomarkers of celiac disease. So, we looked at markers are the most sensitive and specific markers of celiac disease. And as you can see on the top, we found no association between ME/CFS and these celiac disease markers. However, we did find elevated levels of antibodies to gluten, and gluten is, of course, the dietary trigger for celiac disease. And these were IgG, IgA, and IG antibodies. All three were increased in ME/CFS patients. And by the way, we also looked at other dietary antigens like casein, and it was a similar picture, not as -- perhaps some of them not as -- statistically as significant but antibodies to other dietary antigens were similarly elevated. Interestingly, levels of IgG antibodies, these dietary antigens, particularly gluten, correlated or were associated rather with GI symptoms. Patients with GI symptoms had significantly greater levels of these antibodies than those who did not have GI symptoms. And the antibody responses were not limited to those against dietary antigens.

Looking at antibodies to microbial antigens that we assumed would have a gastrointestinal origin, we saw similarly elevated levels of IgG, IgA, and IgM antibodies. So, here you can see antibodies to LPS and flagellin being elevated in ME/CFS patients. And one thing I should mention is that, I think I took that slide out, but IgM antibodies to dietary and microbial antigens correlated strongly with flu-like symptoms in ME/CFS patients. And I think, as I go through this presentation, it may become clear as to why that may be. But all this data points to a potential breach in the intestinal barrier. And so one of the things that we have been looking at for a long time in my lab has been a marker of intestinal cell, epithelial cell in particular, injury, and that the intestinal fatty acid binding protein or FABP2. And as epithelial cell injury occurs, more of this FABP2 is released into -- eventually gets into circulation. As you can see here, FABP2 levels are higher in patients with ME/CFS. Now, with this microbial translocation and breach of the intestinal barrier, we would also expect to see greater levels of acute-phase inflammatory

markers, particularly the soluble CD14 and LPS binding protein that are quickly released in response to circulating microbial. But to our great surprise, we found neither levels of soluble CD14 nor LPS-binding protein to be elevated in ME/CFS. That was not even a trend towards higher levels. And a few years back, we had also found that levels of CRP are also not elevated in ME/CFS, as you can see on the right plot. So, there appeared to be a lack of optimal acute-phase responses despite presumed increase in microbial translocation. Why is this? Well, it could be because perhaps the lack of significant translocation at the time of blood collation, or maybe there are actual defects in the acute-phase responses in ME/CFS.

To answer those questions, we decided to study cohorts of patients and controls undergoing an exercise challenge. Several studies have demonstrated that intense exercise is associated with gut permeability and increased microbial translocation in generally healthy individuals. So, we thought that exercise challenge would be an ideal model for assessing immune response to microbial translocation in ME/CFS patients.

So, here are the exercise challenge data. As you can see, the LBP and sCD14 levels increased significantly in the healthy control group as we may expect. However, the LBP levels did not increase at all in ME/CFS patients. And the soluble CD14 levels increased less significantly in the ME/CFS patients than in the healthy control group. And we saw sort of the opposite picture, when we looked at IgM antibodies to LPS. As you can see here, levels of IgM antibodies to LPS increased in ME/CFS patients. Whereas, they did not increase significantly in the healthy control group, and this may be a compensatory immune response to make up for the lack of LBP and CD14 expression. This is an innate-like IgM antibody response. Of course, we would not expect an IgG or IgA response. But this compensatory antibody response, as we assumed, was apparently not adequate because there was still an increase in LPS levels in the ME/CFS group, but there was no such significant increase in the healthy control group, as you can see. And what was really interesting was that the increase in IgM antibodies to LPS correlated with an increase in IL-10 levels in ME/CFS patients. There was no significant increase in healthy controls. So, IL-10 promotes T cell independent secretion of IgM antibodies, and it may explain what we were seeing in terms of an IgM antibody response. But IL-10 also inhibits activation of monocytes and limits inflammation. So, this increase in IL-10 may also explain the lack of LBP and CD14 responses that we were observing.

Now, because metabolism clearly has a profound effect on immune responses, we also were doing a lot of metabolomic analysis. So, when we did a sort of global metabolomics, we saw changes that were clearly consistent with physical exertion as you can see in the plots on the left. See the pathways are very consistent with physical exertion. And in the principal component analysis on the right, you can see that there is a shift for both ME/CFS and the healthy controls after exercise, with some stratification. As you can see, ME/CFS and healthy controls sort of stratify separately.

But the most significant differences between the healthy control and ME/CFS patient cohorts were for levels of glucose and citrate in the metabolomic analysis that we did. As you can see here, levels of glucose and citrate both increased significantly, quite significantly in the control group, but they did not increase in the ME/CFS patient groups. Now, this could be very relevant to some of the immune data that I just showed you. So, glucose deprivation has been shown to induce IL-10 production, and IL-10, in turn, can actually block aerobic glycolysis in immune cells, which would then further abrogate a pro-inflammatory antibacterial response. On the other hand, citrate has been shown in several studies to play a very important role in sustaining macrophage inflammatory responses and activation of M1 macrophages.

And what was also interesting here is that the levels of glucose and citrate correlated with levels of LPS-binding protein, but they correlated negatively with levels of LPS-binding protein. So, this could be very relevant to the immune data that I just showed you. And I want to throw out this idea here that the observation, the microbial translocation, and the inadequate or suboptimal immune response to neutralized circulating microbial antigens could perhaps be relevant to neuroinflammation in ME/CFS. Now, we know there's data showing that circulating microbial products can actually bind to TLRs on various cells to trigger a localized inflammatory response. And there was this really beautiful paper that came out several years ago showing that LPS can bind directly to TLR4 on the luminal surface of brain blood vessels. And that, in turn, can result in local cytokines secretion in the brain that has been shown to actually activate the microglia to displace synapses. So, this data could be very relevant to neuroinflammation and perhaps some of the symptoms that patients are experiencing.

So, there has been objective evidence for neuroinflammation ME/CFS emerging in the past few years through imaging studies. Here are a couple of recent studies. For example, one is from Japan. The other one on the right is from Jarred Younger's Group in the US, showing evidence for neuroinflammation in ME/CFS patients. And we may be able to draw some parallels with other conditions. So, for example, HIV infection where there is clear evidence of microbial translocation, that is linked to gut epithelial cell damage. There is clear association also with cognitive deficits and fatigue. And so a pathway like that could also be at work in the context of the neurocognitive symptoms that are experienced by ME/CFS patients. So, I had to be rather frugal with showing you data. More data is in the paper that I mentioned to you that was recently published. But I think the overall -- if I can summarize -- the data, I think, do start to provide some basis for understanding the gut-immune-brain interaction in ME/CFS and start to sort of make a link between gastrointestinal symptoms possibly, and some of the immune abnormalities that we are seeing in ME/CFS patients. In this study, what we saw was a downregulation of acute-phase innate immune responses which actually, we think, is relevant to both intestinal and extraintestinal symptoms. And this immunosuppression, along with an actual enhancement of compensatory B cell responses to neutralize or counter antigen translocation, perhaps is mediated

by specific alterations in metabolism and an IL-10 immunoregulatory response. So, I'll stop here. I want to thank the funding organizations, agencies, that supported this work, and members of my lab, as well as our wonderful collaborators who made this study possible. And I also want to thank you for listening, watching. I will hopefully be present in person for the Q&A. I look forward to it. Thank you.

Derya Unutmaz: Thanks. I think, Armin, you just joined us. We don't have much time, but maybe one question, Vicky, if you want.

Vicky Whittemore: Yes, I don't see any questions specifically in the chat yet, or the Q&A. But I guess my question, Armin -- and thank you so much for the talk and for being able to join us today. So, I guess my question would be -- and I've discussed this a lot with individuals with ME/CFS. And the people I've talked to have said that the gut dysbiosis or GI issues they have didn't occur right away. So, they had an infection. They stayed sick with whatever, didn't get better, but the gut issues came later. And so I guess my question is sort of what is the cart and the horse here? Is the changes we see in the GI and potentially microbiome, not cause, but essentially an effect of something that's happening systemically between that immune-gut interaction?

Armin Alaedini: Yes, I don't think it's been studied well, sort of the chronology of the GI symptoms. That's a really good question. I think we really should study that to better understand the connection, the link between the causality issue. I think that's really important. And also, there may be some heterogeneity here. So, you know, there may be patients who have the GI symptoms even during, for example, an acute infection. And then after that, you know, eventually it develops into ME/CFS, and others who may develop the GI symptoms later, perhaps because of other issues related to, you know, metabolic defects or other things. So, we may be talking about different mechanisms here. That's possible as well, but definitely worth studying.

Derya Unutmaz: Okay. Thank you so much, Armin. If you are able to stay, we'll have, at the very end, a discussion, about 15 minutes. But I know you're in study section so, no worries. Thank you.

Armin Alaedini: I'll try to join. Is that that at 3:00?

Derya Unutmaz: No, it's actually 2:40. We'll have research prior to this discussion, for 20 minutes, coming very soon.

Armin Alaedini: Okay, I'll try to join for that.

Derya Unutmaz: Thank you. So, our last talks are from -- actually we call Lived Experiences -- from two patients. These are going to be short talks. The first one is from Angela Termini. So, go ahead, Angela, and then we'll have one more.

Angela Termini: Hi. Thank you. I'm Angela, and I have chronic fatigue syndrome. I'm going to talk about my experience today, and everything I share is based on my own experience and opinion. I'm not speaking as a doctor in psychology or a mental health therapist, just as a person living with CFS. So, I jumped at the opportunity to join this working group because I wanted to join the team that takes CFS down. And I wanted to join the immune group in particular because I've watched enough crime dramas to know that the bad guy is often an obvious suspect. And what I mean by that is I have a history of autoimmune disease, so I suspect my symptoms are rooted somewhere in my immune system. So, here's a little bit about my journey.

I got CFS in the summer of 2017. It was an ordinary day. I was on vacation, and I woke up with the symptoms I have now. You know what they are: fatigue, post-exertion malaise or PEM. My PEM is pretty immediate, and I get it from any type of exertion. And I get lightheaded when I'm upright. I also have heaviness in my body and behind my eyes, and I feel like a drunkenness in a way. I get sore throats when I exert myself, and I feel fevered a lot. I call them phantom fevers because they don't show up on a thermometer. And there's the word-finding problems and memory haze. You know the deal. I also want to mention that I had some weird mystery symptoms years before the CFS. I was always a healthy girl, aside from the occasional flu or cold. But in 2010, I started getting bouts of really bad fatigue, like someone dropped me in a pool of glue.

In 2011, I started getting lightheaded spells when standing. Then in 2012, I had just finished a run. I got pain and weakness in both of my quads, and it just never went away. For years, I had trouble lifting my legs, walking upstairs, and standing from a seated position. It eventually spread to my arms, and I struggled to lift even lightweight things over my head, like a blow dryer or a curling iron. My entire body was stiff and inflamed, and I had pins and needles in my feet and hands all the time. When I would get up to walk or run, my heart would thump really hard. I knew things were bad when, in 2014, I got bronchitis, and I was prescribed prednisone to help me breathe, and the prednisone completely relieved every single symptom. After I finished the bottle, every single symptom came back. I was scared. Like, what is happening to me? Then in 2015, they found celiac antibodies. So, I went gluten-free, and unexpectedly, my symptoms improved by about 80 percent. And I continued to get better. Then in 2016, my PCP prescribed me Vyvanse to help me lose the weight I had gained all those years I was sick. I loved Vyvanse. I started working out like a machine. I lost weight rapidly. I was feeling great. I had taken it for about a year when, bam. That day at the beach, I woke up with the symptoms I have today, and the Vyvanse stopped working after I got CFS. It was like taking a sugar pill. So, I discontinued it completely. I was really sick. I went to all sorts of doctors trying to get better. I eventually got

diagnosed with CFS and some comorbidities. I think that they're important to share. I have small fiber neuropathy, white matter disease, SVT, hyperadrenergic POTS, FODMAP intolerance, and IBS, and possible lymph issues. My high sensitivity CRP is very high, ranging from five to 11. And I have an elevated IL-10 and a mildly high GDF-15. I also have persistent IgM and IgG antibodies to a common virus called CMV, but no PCR. I don't have mast cell disease, and my blood lactate and ammonia are normal. Perhaps these are all clues to the bigger picture. So, what else do I know? I know that water exercise seems to be the only activity that doesn't trigger immediate PEM. I feel really good in a pool of water.

I also know I'm intolerant to alcohol and intolerant to beta-blockers and statins. I get flushed a lot during a crash, and I stop getting sick with common illnesses like the cold and flu, after getting CFS. Maybe it's a coincidence, but maybe it's not. And one thing is for sure, everything I do requires negotiation. If I push beyond my threshold, which is quite low, I will feel sick. I'm on the brink of the flu all of the time, so I feel sick with the flu most of the time. I try to take care of myself, but everything requires energy. Even resting requires energy. For years, I spent days in bed. No one could tell me what was happening to me or if I get worse. So, I was afraid that exertion would cause permanent damage, but all that resting triggered my PEM too. Because I'd be lying down and reading, that's mental exertion. I'd be laying down and talking, that's physical exertion. Or I'd be lying down feeling stressed, angry, and sad for being sick, that's emotional exertion. And all that resting deconditioned me too.

So, I had to find balance between rest and activity. And it's an imperfect equation, but I do the best I can. CFS affects every demand of your life. I went from working 50 hours a week to not being able to work at all. I went to school for 11 years to pursue my dream career, CFS came and took it away. We live in a culture that valorizes grit and working yourself to the ground through exhaustion. I think one reason I got so sick was I tried to work through my sickness. I didn't rest or take care of myself. I was in denial, and I figured it would go away. So, I crashed all the time. When I crashed, my body becomes leaden in a way. It just stops. I don't know how else to put it. I get to a point where there's no more energy to consume, and that's a bad place to be. CFS takes no prisoners. It changes you. I'm an extroverted, bubbly person, but now I'm more of an introvert because bubbly takes energy, and it's frustrating.

I can't tell you how many times I've had to cancel outings with friends or leave phone calls unreturned, and some people take it personally. They don't necessarily see me as a sick person. I think that's why I spent so much time trying to explain to my friends and family about my illness. It was important to me that they understood my experience because it's so invisible. I look healthy yet I was suddenly absent from their lives, and I felt so alone. I wanted them to know it wasn't personal. I wanted them to know I'm not a fraud, that I actually feel really sick. And I guess I wanted them to fight in the trenches with me. I wanted them to say things like, "Don't push yourself. I'm sorry this happened to you. Let me help you get better." I wanted them to read

the literature and research the doctors with me. But people don't really do things like that. I don't think it's personal. I just think they have their own battles to fight. Or maybe they assumed I'm okay because I look okay. Or maybe they're suspicious of CFS because it's hard to believe in something you cannot see. More than that, I think CFS is just hard to understand. I can walk and talk. I can tell jokes, and I can laugh.

And you know, there's a bit of agency involved in CFS. And for me, it comes down to how sick I'm willing to feel in order to live my life. For instance, there are things I want to do that bring me joy, like calling a friend, and I'd rather feel sick during the phone call than not have that phone call at all. It's a cruel disease because you're in constant negotiation with yourself. So, I do things in moderation. I pace, but I have to tell myself no to most of the things I want to do. I often wonder what all this sick feeling is doing to my body in the long run. Will it cause permanent damage? Will it cause other problems like cancer? The not knowing really scares me, and the mystery of it is hard to embrace. I replay the day I got sick over in my head, searching for clues, any explanation for my symptoms. Where was I? Who was I with? What was I doing? What did I eat? It's just a big mystery. Maybe I need more testing. Maybe I have an undiagnosed rare disease. When you don't know what's making you sick, you become suspicious of everything. All toxins in your hair products, in your food, in your cleaning products. So, I went completely clean, but it didn't have much bearing on my CFS. I still do it though because it's important to be as healthy as you can, chronic illness or not. It took me a long time to come to terms with my illness.

It steals your life in an instant, and you're left with the pieces. Fortunately, I have a fiancé who picks up a lot of my pieces. But we had dreams to have a family and get married and have two incomes, but CFS really changed the landscape for us. We're learning how to roll with the punches, but for years, I could barely contain my rage and sadness for our losses. And grief isn't linear. It still hits me in waves and knocks me down, but I won't burden you with my losses because I know you have them too. There's no blueprint for managing chronic illness, and for me, caring for myself took a lot of trial and error. I think things got better when I realized I can't see if that's a way. I can't fight it or defeat it. I had to learn how to manage it and pace my activities. I stopped taking advice from people who aren't familiar with the literature, and I joined a CFS support group. For the first time, I was in a group of people who got it. I also found supportive medical doctors, and I started taking LDN, five months ago. It’s helped with my fatigue, but not so much with the PEM or the flu-like stuff. Aquatic exercises helped me regain some strength. And I started working with a therapist to help me navigate life for the chronic illness. Lately, I've been well enough to start working again.

I work five hours a week. I may never be able to work full-time again, but I'm slowly able to add more things to my life. I'm redefining my purpose, what I want in life, and how I experience joy. I'm still figuring it all out though. I'm not an expert, but I'm getting better at it every day. Also,

important to note, desperation for wanting to get better is breeding ground for people who want to take advantage. You have to be careful who you accept treatment from, and you have to be conservative with who you share your story. Some people, even with good intentions, will make you feel like you're not trying hard enough to get better. Or they'll project their own stuff onto you or make you think you have diseases you don't actually have. Or they'll push treatments that can actually harm you. So, build a community of trustworthy, supportive, and positive people. And most importantly, be kind to yourself. This is my experience with CFS, and it might be different from yours. But that's the conundrum, right? There's variation in our symptoms and our narratives and in the literature. That's why research and symposia like this are so important. We need to learn from each other. I hope my story can provide some insights as we work towards clinical trials. To the doctors and researchers trying to help us, thank you. To all those living with CFS, we may not win every battle, but let's win the war. Let's get through this together. Thank you.

Derya Unutmaz: Thank you for this heartfelt sharing of your experience, Angela. Really appreciate it. So, the next talk is another lived experience from Tracy Duvall. Tracy, go ahead, please.

Tracy Duvall: Hi. I've been given the opportunity to briefly tie my experience as a person with ME/CFS to future directions for research. Next slide. Here's some of my lived experience. I wanted to be part of this immune system webinar because all of my lasting downturns have resulted clearly from infections made easier by being immunocompromised or allergic reactions. It started in 2010 when I developed ME/CFS suddenly as a result of something like a norovirus, and much of my improvement has been due to immune-related treatments. I've not only participated in several research projects as a subject, including at NIH, but I've truly read every research abstract on ME/CFS in English, and sometimes the whole article, for the past few years. Finally, I've tried scores of treatments with some success. Indeed, I've gone from not being able to do anything, to being able to walk for about 30 minutes on most days, and I'm able to do part-time work from home, barely, while lying in this zero-gravity chair. Next slide.

What I'd like to address here includes what we know. We know that treatments already exist that can help people with ME/CFS. But tragically, the majority who don't see specialists are unlikely to know about them, or they have to struggle mightily to get them prescribed. What we need to know includes whether it's better to think of ME/CFS as having subsets or being divided into multiple diseases. Practically, this boils down to knowing which patients will benefit from a particular treatment without excluding any from our concerns. Here, I'd like to take a moment to illustrate my frustration as a very interested bystander. If I remember correctly, multiple labs have found that cells from healthy people act sick when exposed to blood from ME/CFS patients and vice versa. But it appears there's no systematic program to search specifically for the substance in the blood that makes this difference. As the years of my ruined life crawl along, I

fear that this promising therapeutic target is being neglected. Or perhaps there's a great explanation that I, as a layperson, don't get. This is just an example. There have been other exciting announcements that seemingly don't seem to be explored, similarly don't seem to be explored. Biomarkers galore. It would be great to have some authoritative resource like a giant table that would list findings and what has happened to follow up on them. Next slide.

Given all that, here are my suggestions for research. First and foremost, it would be helpful to perform clinical trials of affordable drugs that have an excellent chance of success so that the FDA will approve them specifically for this disease. Then ordinary doctors will prescribe them to the many patients without specialist care, and insurance will be more likely to cover them. Ask the Clinicians Coalition, but candidates might include low-dose Naltrexone and valganciclovir, which I have to take at a low dose. More generally, in any kind of research, I urge the NIH to impose a more systematic and comprehensive approach. I believe that Dr. Scheibenbogen and her colleagues recently announced such a program in Europe. My lived experience as an anthropologist suggests that you'll get farther trying to explain the differences among people than by reifying their average.

For example, potential subsets should always be included and analyzed rather than excluded as often happens now. I recommend that subjects be asked whether they had a sudden onset via infection, like me, and the results for this group should, as a matter of regular procedure, be compared to the results of people with gradual onset. Similarly, the same thing should be done for people with delayed PEM versus sudden onset PEM. Is the process the same? In addition, please establish whether PEM actually occurs from emotional or cognitive exertion. And if so, whether it follows the same pattern as PEM from exercise. Other issues might be included. The bigger point, again, is to encourage a more systematic and comprehensive approach. Finally, I wish more research attended to transitions rather than simply checking off symptoms. For example, how is it that some people experience extended periods of recovery? Mine lasted a couple of months until I got a camplyobacter infection. But some go for years before returning to full on illness. Any hypothesis explaining ME/CFS should directly contend with such changes. Or what about the timing of PEM? How many hypotheses -- so many hypotheses explain why fatigue might occur, but they don't explain why it could take two days to switch on. Or what changes in the brain when I go from lying down to standing upright. Whatever it is you want to investigate, I highly recommend that researchers search through the extensive discussions on online forums. I think you could get a head start by seeing, by doing qualitative research to see how people with ME/CFS describe their experience and what they have already tried. Thank you.

Derya Unutmaz: Thank you so much, Tracy. This was great. I think we conclude this speaking part, Vicky.

Vicky Whittemore: Yeah.

Derya Unutmaz: It's up to you now.

Vicky Whittemore: Yeah. So, I'd ask all the panelists to turn their cameras on. Anyone who would like to participate in discussion now. Just a couple of general things before we get into more specific conversations. I saw a question or comment about sort of what do we want to include in the research roadmap in general? Is it just clinical trials? And my answer to that would be no. What this whole exercise is intended to do is to really identify the gaps in the research. And so, if we think that we need to move forward with clinical trials to impact the immune system, are we there? And if we could say, "Okay, tomorrow we could put up a protocol to study this treatment, to look at this, and think this is how it would impact the immune system. And here's the subset of patients we want to look at, and here's how we would do that," then fine. But if we don't have the answers to that, what is the research we need to do to get there? And so I think that in general, just to set the stage, we're not talking just about clinical trials, but what is it that we need to know to be able to do those clinical trials and to get to treatments? Derya, you have a comment?

Derya Unutmaz: Yeah. Just quickly, since this is an important point, I wanted to also add that even when we do clinical trials, I think we really need to know what parameters to measure as well so that we can learn from the clinical trials. I think Nancy mentioned about thymosin. You know, about 20, 25 years ago, we didn't know about exhaustion and all these concepts, so they were not looked for. Perhaps we could have learned something really important in that trial. So, I think, you know, developing biomarkers and parameters to analyze during the period of the clinical trials could be very useful. I don't know if Nancy wants to comment further.

Nancy Klimas: No, I agree. And also, some of the things that we did early on that seemed so innovative and cutting edge were also premature for these very reasons. And we're not done in a powerful way. And so, I wouldn't disregard earlier work. For instance, there was a study, a Journal issue that published a high dose IV gamma globulin therapy that was done, I think, at the NIH, against a low dose -- now, one was in Australia. One was in in Michigan. It was a low-dose, or rather, I should say, replacement dose and high dose. And the editorial compared them and said one was efficacious, and one was not, so don't bother. They disagreed. They weren't even the same therapy, you know? And we've never gone back there until the work of Carmen and her team really. We haven't even gone back to the simplest approaches to autoimmune. And the big one, subsets. For heaven's sakes, identify the group so that you have the best chance of getting a response.

Vicky Whittemore: Armin?

Armin Alaedini: Yes, and I think what just Nancy just said, it sort of underlines the issue of heterogeneity in ME/CFS and the need to stratify ME/CFS into subsets that actually would respond to treatments like IVIG. So, there would be, you know, patients who would respond to that IVIG treatment. So, if we identify those patients through biomarkers, et cetera, we can then target that patient subset and actually get efficacy. So, I think, going back to your question, what do we need? Are we ready for the clinical trials? I think, you know, this would be a big part of that if we want these clinical trials to be successful. A lot of great work has been done in the past, like Nancy just mentioned, but I think now we need to put the pieces of the puzzle together and identify patients who actually would respond to those treatments.

Vicky Whittemore: Other comments?

Nancy Klimas: There's a lot of chat in the Q&A about clinical trials networks, and you and I talked before about this NeuroNEXT idea. But I can really see the benefit of a way to do phase one, phase two translation rapidly with this much understanding now of subsets and punitive interventions. The idea that we could do concurrent multiple studies is a really important concept and a task we might want to try to undertake.

Vicky Whittemore: Got a question, but I'll go to Alain first. Do you have a comment?

Alain Moreau: Thank you, Vicky. So, first of all, congratulations to all panelists. So, I think you did a great job, and I really enjoyed the presentation today. I think we need to bring ME/CFS in the precision medicine era. So, we know -- and clinicians seeing a lot of patient. I'm not a clinician, but I know from Cindy Bateman and others, that they can also distinguish -- and the same for Nancy. When you see patients in your clinics, you can differentiate them based on the clinical phenotypes that you can see from them. And we know on the molecular side that we can now more -- we develop tools. So, we have the tools. We have drugs. And I think now it's time to associate the best responders. Okay? And we can validate on some subset of patients. So, that's why I'm pushing the concept of precision medicine. Whether you are looking more about the immune system or the system, at the end of the day, we should be able to share our data and try to further distinguish who will be a good responder. And when I say responder, meaning that the drug will do some kind of positive reaction and not a negative reaction. Because we need to remain on the safe side even when we reposition drugs. Because it's one thing to claim that repositioned drug is faster, but we need to remain on the same side.

And again, because patients came to you guys, the clinicians, at different stage of their disease, it's very difficult to capture the early stage. That can be a game-changer if we can do that, but we need to deal -- to be pragmatic. So, that's why I truly believe that provocation maneuvers that can be applied in a standardized way that can further set apart the different group, can reveal more.

Okay. And we can apply through precision medicine, and eventually, select the best treatment options. And that can become a combination of several drugs.

Vicky Whittemore: So, that really feeds into actually what I was going to ask, and that's -- oh, sorry, Derya, I'll let you comment first.

Derya Unutmaz: No, just to follow up on what Alain and Nancy said. I think, you know, we can also learn from other interventions like changing diet or taking nutraceuticals. Because, you know, some patients do benefit, others don't. Why? You know, why that person's symptoms get better? Angela shared her experience, for example, that some treatments made her better and wasn't long-lasting. So, on a personalized basis, what's really going on could be very useful sort of road marks.

Vicky Whittemore: So, my comment was going to sort of pick up on what Tracy said and what I think I'm hearing and would just like your comments. Since we're focused on the immune system in this webinar, I'll focus on that, but that's not to exclude everything else regarding ME/CFS. But I think, I guess what I'm suggesting, and I think where Tracy was going with his comments, was really a much more holistic look. And if you have -- what I'll put out there is what I think we need is a standardized protocol that very systematically and carefully phenotypes and probably genotypes, which we'll hear more about the genetic susceptibility in an upcoming webinar. Phenotypes, genotypes individuals with ME/CFS, and then slots them into what might be the best clinical trial based on that profile, that personal profile. Because there's just, I think, so many different ways and things that can be perturbed and dysfunctional in ME/CFS that part of the problem with a lot of the studies in the more distant past, not more recent, but is, is that everybody just got thrown in one bucket, male, female, old, young, you know, and very small ends. It's very hard to make sense of that data. Clearly that has become much better, but I think we just need much more data on each individual, sort of this precision ME/CFS, to really understand the full and holistic picture of an individual. And then, you know, from that, how can we develop targeted treatments for these different individuals and/or subsets of individuals with ME/CFS. Just your thoughts and reactions about that.

Tracy Duvall: Well, so I just want to quickly follow up. The reason I put in the immediate onset PEM versus not delayed PEM is basically along those lines. There might be things that don't occur to you that you might see, if you looked at the descriptions people have of -- like Angela gave of hers. That communicates subsets that might not occur to you if you don't have the disease, and you're not involved in these discussion groups online.

Vicky Whittemore: Right. No, I appreciate that very much, Tracy. And actually, it comes back to a question I saw in the Q&A about how to involve individuals with lived experience. And that's exactly the point, right? Is to really understand people's experiences and what are triggers,

what are things that -- and what are those differences between, you know, sort of immediate onset and delayed onset of PEM. Sumeeta you have a comment.

Sumeeta Varma: Yeah. And it actually connects to involvement of patients and their experiences and views. One thing that I am now really interested to know in the patient or potential research participant population is about risk tolerance versus risk aversion. I'm hearing a lot of risk aversion from the investigators and clinicians, and I've experienced this in my own care as a person with ME/CFS. My professional background is in oncology where we treat people with all of the dangerous drugs that all of the ME/CFS clinicians and researchers are afraid of. But we do it because we and the patients feel that it is worth it. My personal experience as one individual is that I would be happy to take or participate in a trial of a higher risk intervention, given the impact on my quality of life of the disease itself.

I don't know how widespread my view and my attitude about risk is among the patient population in general. I'm guessing that there is a variation. But I think that if there is willingness of patients to pursue maybe some bigger swings in terms of biologically more potent interventions, I think that's important to know, important to perhaps plan around, and I think also tells us something important about what the -- another way of understanding what the impact on people living with this disease is. What would you be willing to do to potentially get rid of it? I'd be willing to do a lot more things than have actually been offered to me. And I worry sometimes that -- because as far as we know, this is not an illness that shortens people's lifespans dramatically -- the degree to which it is impairing people's lives and quality of life isn't fully appreciated.

Vicky Whittemore: Now, thank you for those comments. I think, you know, that's a very interesting perspective, I think, and something that really does need to be explored in terms of the perspective of individuals with ME/CFS. From the other side of it, for any kind of treatment or treatment trial, there needs to be strong scientific rationale, and there needs to be -- you know, the regulators don't want you potentially harming individuals. So, there has to be really careful thought about the safety of potential and any potential concerns about any kind of treatment. But Nancy, you have a comment?

Sumeeta Varma: I'm sorry. As far as -- I don't know about regulators' views about this disease, perhaps, but again, in the oncology world, people regularly die as a result of their cancer treatments, as a result of cancer treatments that are under study. We have a toxicity category of grade five, which is fatal toxicity from the treatment given or under study.

Vicky Whittemore: No, but I think the difference is --

Sumeeta Varma: Risk has to be managed thoughtfully, but desperate times call for desperate measures, and those situations exist in medicine. I don't know for how many ME/CFS patients this was -- would be one of them.

Vicky Whittemore: Agreed. But cancer is often fatal in of itself, so that's why there's the ability take those risks. Nancy, you have a comment?

Nancy Klimas: I was sort of going to echo that it's almost a bigger responsibility to the investigator when they postulate potentially toxic drugs, knowing that the informed consent process, that you're talking to someone so desperate that they would do anything. And that does happen. And so, when I'm sitting there writing these informed consents, I'm like, man, I have a bigger responsibility to assess risk-benefit ratios than ever before. Because even though my partnership with the study subject or the patient is a partnership and an informed one, it's intense, you know? And I feel a great deal of responsibility when I'm proposing things. Nonetheless, I propose biologics. I propose big gun things. I think this is a bad illness that needs a big gun treatment if it's appropriate and targeted and extremely well-managed on the safety side. So, you'll see the trials on our webpage. We have simple stuff, probiotics, nutraceuticals, but we have big gun stuff too. Or I should say safer stuff, not simple stuff because neutraceuticals actually have a lot of potential that there are a lot of agents that are targeted, that are considered nutraceuticals rather than drugs.

Vicky Whittemore: Yes, we need to wrap up this discussion. Thank you, Nancy. I think, I'm sorry, I didn't see that David Kim had his hand up. David?

David Kim: I think Roshan had his hand up before me.

Vicky Whittemore: Did you as well, Roshan? Sorry.

Roshan Kumar: Yeah. I was just wondering, Nancy, you mentioned, is it with your fellow clinicians, you talked about trials you'd really like to see, and you mentioned, I think, LDN at the top of that list. I was wondering what are your top three or five, and what do you think are robust enough markers to enroll on? Like if you were trying to subset patients for this, for a trial?

Nancy Klimas: Well, I mean, there's been a failed IL-23 trial, but I still think that we have to use biologics that are targeting TNF and the inflammatory cascade, the cytokine inflammatory cascade. That would be a very meaningful, but using people with elevations, even if you have to kick it with the exercise challenge to see the signal. And that is a little trouble because the baseline, a lot of people are subtle, but man, you give them a minor exercise challenge and their signal cranks way up there. So, I would say, in the immunology world, which is where we are today, I'd be thinking pro-inflammatory cytokines. My second one would be, how much does the

mitochondrial disruption that we're going to see in another one of these sessions, how responsible is that for the immune dysfunction and the functional defects that we see? In which case, are mitochondrial interventions going to be immunomodulating enough to see better immune competence? And then finally, DNA repair and all the immune senescence stuff that there are some early interventions that could potentially be appropriate in our field. So, you know, inflammation, function, and immune surveillance and senescence, those are the focus areas.

Vicky Whittemore: David?

David Kim: I wonder if -- you know, we're discussing interventions and drugs. And I'm wondering if stem cell comes into -- have a little place in this. One of the presentation there was a stem cell work done to address the long COVID. I don't know where that study's at right now. But also, there was a presentation, I think was in a kind of science meeting where the Lyme Society said that stem cell was inconclusive. And if stem cell is a way to reset your immune systems, and it seems to be, from what I'm hearing, perhaps a milder therapy; is the findings that the Lyme Society was mentioning the stem cell was inconclusive, perhaps a result that, that was a kind of a literature search. And so, we don't know the quality of the stem cell that was being used. And so is it appropriate to bring that into this forum as a possible clinical trial option?

Nancy Klimas: And the other part of that, exosomes, stem cell-generated exosomes. So, yes, we're in negotiation with one of the stem cell companies right now. I know another group is as well. I think it's very appropriate, but we want until we do the work.

Vicky Whittemore: So, we need to wrap up the webinar. And so at this point, I would like to give my sincere thanks to all of the speakers and panelists and everyone who participated today. The webinar and transcript will be posted on the website, hopefully within about one to two weeks. It takes a bit to edit the webinar as the recording, as well as to clean up the transcript, and so watch for that. And we hope that you'll join us for the webinar next week on metabolism. So, with that, I will sign off and say thank you to all the participants, all the panelists, and everyone who's on our working groups. So, thank you very much.