PATIENT-LED
RESEARCH
FUND FOR LONG-COVID

# PLRF Webinar 2025 transcript

[Gina]
Hi everyone. I am Gina Assaf. I am the co-founder and co-lead of the Patient- Led Research Collaborative and I'll be hosting this first part of this session.

I'm a woman with medium brown skin and medium length dark hair. Today I'm wearing a white t-shirt with the Patient-Led Research Collaborative logo and black text and colorful icons along with small drop earrings and a simple necklace. I'm also wearing eye glasses. First of all, I wanted to say welcome everyone and thank you for joining us for the Patient-Led Research Fund Webinar. I am Gina Assaf, one of the co-founders and co-leads of the Patient-Led Research Collaborative, or PLRC for short. Back in 2022 when there were still very few Long COVID biomedical research studies and even fewer that involved patients, the idea for this fund stemmed from our community. People with lived experience knew what questions needed to be asked and we saw the huge gap in research that included our voices in a meaningful way. So we imagined something different. A research fund entirely led by patients. One where patients were involved at every step from deciding what to fund to helping shape the studies to sharing the results. That vision became a reality through the Patient-Led Research Fund with support from Balvi Philanthropic Fund, now called Kanro.

Today, we're excited to share what came out of that vision, the outputs and updates of the 10 biomedical research projects supported by the fund. You will hear from patient researchers who consulted on these projects who will be here to answer questions during the Q&A. You'll learn more about patient engaged research from experienced patient researchers and we'll reflect on the power of patient-driven funding. Thank you for being here with us. We're really looking forward to sharing this with you.

A quick overview of PLRC. We are an international group of people living with Long COVID and other infection-associated chronic conditions. We come from a wide range of professional backgrounds and bring both lived experience and interdisciplinary expertise to everything we do. We first came together in April of 2020 through the Body Politic support group where we published one of our first papers documenting Long COVID. Our mission is to represent people with Long COVID around the world through participatory research, medical education and advocacy - always grounded in live experience. As I mentioned, we come from a multi-disciplinary background and everyone on the team lives with Long COVID and/or other infection associated chronic conditions. At any given time, we have three to five co-leads.

Currently, that's myself, Hannah Davis, and Letícia Soares. We also have a small number of part-time staff supporting operations and communications. The rest of our team members are volunteers, although some have been compensated through specific projects, funding for their engagement and expertise. We have a mission and vision that guides all of our work and helps keep us grounded in our values and goals. These reflect our commitment to centering the experiences and expertise of people with Long COVID and infection-associated chronic conditions.

And now I'd like to introduce our first speaker, PLRC co-founder and co-lead Hannah Davis, who will discuss the talk about the patient research fund and the process we took. Hannah.

[Hannah]
Hi everyone. My name is Hannah Davis. I'm a white non-binary person with shoulder-length dark brown hair and I'm wearing a black sweatshirt. I'm going to talk a little bit about how we implemented the Patient-Led Research Fund. So, quick facts about it. It was a total of $5 million in cryptocurrency. It took 8 months from creating the idea of the fund to funding the actual grantees. There were 15 patient panelists with Long COVID and other infectious onset chronic conditions and we ended up giving grants to people over five countries and funded 10 studies. Each study had at least two patient representatives on each study. Some even had four and we designated funding specifically to create patient engagement for each team. Four of our priorities in doing this fund were: first of all fast funding. We needed efficient funding. It was necessary to make quick progress toward finding causes, diagnostics, and treatments for Long COVID particularly during the time Gina referenced in 2022, a lot of Long COVID research was still on characterizing symptoms and prevalence and we really focused only on biomedical research for this fund. We wanted to focus on patient-decided research. So, we prioritized areas of research identified by patients as being important to them. Followed by a rigorous panel review and process to select grant applications. We really wanted integrated patient participation. So, patient engagement was included at the study design, recruitment, analysis, and paper writing stages of each study. And the fourth one was open science values. So we really believe in this and that it's important to create movement and urgency in the field and require that the teams make their scientific findings public providing transparency and communication by sharing their research outputs. The admin part of the team was [led] predominantly by the leads in PLRC at the time. Lisa McCorkell was the operations lead of the panel and grantee administration and subsequent follow-up; and myself, Hannah Wei and Gina Assaf and other helpful volunteers worked to further the conceptualization securing funds and donor relations around the fund. We particularly want to thank again the Balvi Philanthropic Fund for funding this study and taking a chance on us. I mentioned research areas of focus identified by patients. We put this out in the request for proposals because these were some of the areas that patients really cared about but were being understudied at the time. This included things like the role of hormones, mechanisms, driving connective tissue disorders and connective tissue damage, therapeutics to repair and strengthen the connective tissue and structural spinal conditions like CCI and some of the ossification disorders we see in Long COVID overlaps with conditions like endometriosis which we know are very common. How the menstrual cycle and how pregnancy impacts Long COVID. We put a focus on antivirals both

against COVID and against reactivated viruses like EBV and others. We focused on vascular dysfunctions and clotting including microclots, the mechanisms of cerebral and general hypoperfusion and hypovolemia, which is lower blood volume. Mitochondrial and metabolomic studies. Focus on sleep, including why patients have unrefreshing sleep and circadian rhythm disorders. Focus on everything brain fog-related, especially neurocognition and neuroinflammation. Anything to do with the immune system and mass cell activation. Anything to do with autonomic nervous system dysfunction, mechanistic overlaps between Long COVID and related comorbid illnesses. Additional triggers of ME/CFS and connective tissue disease, and then of course treating and researching post-exertional malaise and fatigue but particularly what the pathophysiology of PEM was, which was not being studied at all. So in addition to the topics, we also limited the type of studies to what patients prioritized. So diagnostics and biomarkers were eligible, pathophysiology studies were eligible, drug repurposing studies were eligible and treatment and clinical trials were eligible. But we specifically excluded survey based studies, prevalence studies, qualitative studies, occupational therapy studies, anything on behavioral research including CBT and of course graded exercise therapies. There were 15 patient reviewers with Long COVID or an infection-associated chronic illness that reviewed and selected the grantees and, crucially, I mean we always run into the issue that that people think patients aren't qualified but these patients are qualified both from a lived experience standpoint but also a huge range of very strong domain expertise. So these are people with backgrounds in molecular biology, neuroscience, science, immunology, pulmonology, human- centered design, computer science, infectious disease, biostats, etc. So, we're well equipped to be able to read pretty advanced scientific proposals and make a judgment on them. So, we had seven areas that we looked at for the proposal evaluation criteria. The first was, of course, the study design, how well it was put together, whether we thought it was viable, and was asking a significant question. We looked at whether they were informed in infection-onset illnesses. Especially in the early years of the pandemic, there were a lot of people who were kind of on a learning curve about virus-onset illnesses generally. And so we're not asking very strong hypotheses about Long COVID. And so we rated proposals higher where people had a background in HIV or in ME/CFS or dysautonomia or Ebola or other kind of long-term issues from viruses. We gave extra points to those that were researching a patient prioritized research topic. Whether it was one on the list we mentioned earlier or one that the patient panel decided was a patient priority. We rated their understanding of patient engagement and their proposed use for patients in their proposals and if they would include patients at every stage. We included the impact and scalability of the eventual results, whether it would be applicable to patients all around the world and how significant the question or the answer would be if it came out. We looked at diversity, equitable impact and open science. We really wanted people who were thinking large scale about the impact of the proposal and the values of putting their research forward, and were including appropriate patient groups and considering diversity in their study recruitment. And then we also included extra points for inclusion of an IACC or comparator group. In addition to control groups, healthy control groups, we looked at anything that was looking at several IACC cohorts -,got a few extra points. So we ended up funding 10 different different grants, one clinical trial, two immunological studies, one on T- cell exhaustion and one on immune profiling, one microbiome study, one on PEM physiology leading to a possible biomarker, one on sleep dysfunction mechanisms, one on microclots, one on surgical management looking at the effects

of a spinal operation on ME/CFS symptoms, one AI repurpose drug identification study and one multi-systemic analysis. And as a patient organization comprised of people living with Long COVID and related conditions, we're at a critical juncture and are aiming to raise substantial funds to continue our work. So please consider donating today and sharing our funding page with your network. We deeply appreciate whatever you can give. We have a range of projects that require funding. We're hoping to run PLRF 2 again if we can raise at least $2 million but are also looking at clinical trials, endpoints, biomarkers, and others we'll talk about later in the webinar. Thank you.

[Gina]
Thank you so much Hannah for a great overview of PLRF fund. This is the upcoming next hour of our agenda. You have the full agenda shared in the chat for reference, but we'll have two breaks during this full session. So, we're going to start off with Braeden Charlton presenting on “Skeletal Muscle Abnormalities in Long COVID.” Braeden, feel free to turn on your camera and your audio. We look forward to hearing your talk. Awesome.

[Braeden]
Well, thank you very much for the lovely introduction. I originally wanted to just give this presentation on the skeletal muscle adaptations that we've observed in Long COVID but well I felt as we've been wrapping up some research here in Amsterdam particularly related to ME/CFS. I thought I'd go ahead and share some of these results with you all today. So one of the big topics of debate so to say is the hypothesis that some skeletal muscle adaptations that we see in Long COVID and ME/CFS patients might be mainly attributed to deconditioning or just bed rest. And so as a muscle physiology lab with prior experience in bed rest studies we actually wanted to tackle this head on. So we were very fortunate enough to be able to recruit a cohort that involved healthy controls that had been infected with COVID but recovered age- and sex-matched Long COVID patients and age- and sex-matched ME/CFS patients. And fortunately, we also had a large data set from a previous study done with the European Space Agency that was conducted prior to 2020, so before COVID, in which people underwent a 60-day bed rest. So, they were simply head down in a bed for 60 days, not moving. So, it's our extreme example of what deconditioning can do to the human body. We have both biopsies and exercise data to be able to see how these muscle adaptations occur over time. So with that maybe unsurprisingly deconditioning does result in this decrease in maximal aerobic capacity or our maximal endurance basically and we do see a very similar thing happening with both the Long COVID patients as well as the ME/CFS patients. However this is very superficial because there's a lot of systemic changes that can contribute to our whole body exercise capacity. When we look at a marker of lactate accumulation or the gas exchange threshold. So beyond this point in exercise is when we start to accumulate lactate and ultimately this signals our body that it's essentially time to stop exercise. What we can see is that there is a decline following deconditioning. However, with our Long COVID and our ME/CFS patients, we see essentially a stepwise reduction in this sense. So we can already start to see that there's some small dissociation from what we would consider classic deconditioning. Further we have this mark of the VO2 heart rate response. So for every watt that our body needs to put out we need a certain amount of oxygen to come into our body and our heart has to be able to circulate that. So our

heart rate should increase with the amount of oxygen being consumed and what we can see is following bed rest is actually fairly conserved. However with the Long COVID patients and the ME/CFS patients, this is an increasing slope - meaning the heart is having to essentially pump faster to try and supply the same amount of oxygen. And there can be two reasons for this: either the oxygen isn't getting to the muscle necessarily or it's not being utilized appropriately at the muscle. Further, one of the biggest hallmarks of deconditioning that's been shown throughout multiple, multiple studies is this muscle-wasting effect that we see and this typically occurs within 3 to 6 days and again we see this within our bed rest participants. However, if we look at our Long COVID and ME/CFS patients, we don't see this necessarily, which again argues against this idea of deconditioning playing any role in either of the diseases. Further, when we shrink muscle fiber size or grow muscle fiber size, this should be accompanied by a change in capillarization. And so what we see is even though the muscle fiber size changes, it's matched with an appropriate amount of capillary still within the bed rest participants. However, if we look towards our ME/CFS group, we actually see that even though they're maintaining muscle mass, it appears that they're actually losing capillaries in this sense. So less fibers are being supplied adequately with either oxygen or nutrients and less waste is being removed. Further we can also look at mitochondrial content. So our ability to generate ATP or energy within the muscle and what we notably see after bed rest is this decrease in mitochondrial content marked with SDH activity and while the Long COVID patients don't actually seem to differ from healthy controls in this sense the ME/CFS patients kind of exhibit this trend. We can look a little bit deeper into this. So SDH is the marker for how many mitochondria there are. But it's not enough just for mitochondria to be there. They also have to function properly. And so we have this other marker that's the oxidative phosphorylation or basically how well the mitochondria are functioning. But this is a combination of the quality and how many mitochondria there are. And so what we can do is we can take these two parameters, divide them by each other and we get basically our mitochondrial quality. And what's fairly evident is, during bed rest even though we're losing mitochondria, the quality of them isn't necessarily decreasing. However, the mitochondrial quality in both Long COVID and ME/CFS patients is significantly reduced. Thus explaining this decrease in mitochondrial oxidative phosphorylation capacity. So kind of to illustrate that a little bit what should happen in a healthy human is oxygen comes through the blood it diffuses into the mitochondria here and then ultimately gets used to generate ATP or energy. What we're seeing with the bed rest is there's just fewer of these mitochondria and so the ability to generate ATP at the end here or energy is decreased. With Long COVID and ME/CFS they might have the same number of mitochondria but they're not functioning properly. So those were just kind of a few quick points. We have a long list of things but these are kind of the most evident arguments we have against this idea of deconditioning playing a significant role in either Long COVID or ME/CFS progression. Most notably, both Long COVID and ME/CFS patients show these signs of reduced oxygen extraction at the periphery and this earlier lactate accumulation which we don't necessarily see following deconditioning, as well as the patients retain their muscle mass and with respect to the ME/CFS patients, they typically lose capillaries relative to the amount of muscle mass they have; as well as both Long COVID and the ME/CFS patients have this reduced mitochondrial function and quality rather than this mitochondrial loss that we see with deconditioning. So with that I also want to highlight some of our ongoing work in our upcoming projects by Anouk at the top, Jelle in the middle and

[ name unclear ] at the bottom. We are continuously trying to kind of understand the pathophysiology from skeletal muscle perspective as well as how this integrates into PEM. So Anouk recently has been working very hard trying to understand the endothelial function and how this might contribute to that oxygen extraction problem I was talking about earlier. Jelle has been very busy looking at some electron micrographs trying to quantify different morphological changes that we see at a very small size. So using these images, we can start to look at that mitochondrial morphology and whether there's signs of deranged mitochondria that might explain this decrease in the quality; as well as we can also look more into the endothelium here and whether there's this thickening that might also be contributing to some sort of oxygen diffusion problem or even problems to vasoconstriction or vasodilation and [ name unclear ] and myself have been recently working on some proteomic, metabolomic and lipidomic data sets in the muscle and blood to hopefully find out more about biomarkers and kind of the environment in patients. So we wouldn't be where we are without our patient representatives also lending a hand in suggesting where the projects necessarily need to go. So I'd like to extend a big thank you to Anil, Jessica, Patricia, Eelco, Sophia, and Justien for all their all the work that they've done in helping guide us through the patient perspective. And last I'd also like to thank also all the funding agencies as well as all of our lab mates and particularly all the patients and controls that we've been able to analyze through this process that have contributed to all these results so far. And yeah, I'd be happy to take any questions at this point.

[Gina]
Thank you so much, Braeden. We have I think time for one or two. I'm going to ask you from some of the questions that we have, and from the audience. A question we have is: how do the findings of this study contribute to understanding overlapping disease mechanisms across infection associated chronic illnesses?

[Braeden]
Yeah. I'll try and keep that a very succinct answer but from at least some of the results that we've seen in the skeletal muscle there does seem to be a little bit of an overlap particularly when it comes to the mitochondrial and endothelial functionality in patients. Now whether that mechanism remains the same or not is potentially up for debate as different viruses will interact with different interfaces within the body. So it might be “multiple roads lead to Rome” in this case. But I think we're inching closer to what exactly that is. Great. Thank you for that. Another question we have is: do any of these findings feel like they could become a potential biomarker? Oh, that's a very good question. I think if anybody was a keen eye on one of those slides with the endothelial markers we've seen some kind of remarkable results in terms of what we think is basal lamina thickening. However, we're looking for other cohorts to hopefully test this against, as we've only been able to show it in our cohort so far cuz that's the only one that we've had access to. So in that sense, it definitely could be. We've seen some other potential candidates through the metabolomics analyses as well and then we're also trying to balance the whole with some of these biomarkers that we see in the skeletal muscle. They're a little invasive because you have to take the biopsy. So preferentially we try and find something with the blood,but it is a real balancing act at the moment

[Gina]
Thank you so much Braeden. We really appreciate the talk and we will now bring on Dr. Caroline Dalton with the talk, “Fibrinoloid microclot quantification in plasma from people with Long COVID and ME/CFS.”

[Dr. Dalton]
Thank you very much for inviting me to present our work. So I'm going to talk about fibrinoid microclot quantification. I'm just going to call them microclots in plasma from people with Long COVID and ME/CFS. So, so the aims of our study are to develop a method for quantifying microclots and we're going to use automated imaging microscopy which I'll explain briefly what that involves and then compare microclot counts - so count the number of microclots in the plasma samples from people with Long COVID, compare this to controls, and then we have also taken some other groups so people with recent COVID infection, people with ME/CFS and also a small group of vaccine injured people. And then really I think from our patient representatives really what they're really interested in is whether we can use microclot counts as a biomarker. So just to remind you that for something to be a biomarker like Braeden mentioned that it needs to be sensitive. So you hope that almost everybody with a condition you're trying to identify has the biomarkers. So in this case, that would be race microclots and then you exclude… the biomarker needs to be specific, so excluding those who don't have the condition. So therefore the controls perhaps would have low microclots. So bear that in mind when we look at the data in a bit. So this work came about because out of some papers which I'm sure quite a lot of people in the audience have come across or seen from Resia Pretorius and Doug Kell which came out in 2021/2022 showing that the spike protein from the COVID virus induces aggregation of fibrinogen. So fibrinogen aggregates naturally but when it aggregates with the spike protein it seems to form structures and misfolded proteins that are particularly hard to break down and they termed these microclots, and they showed that there were increased numbers of these in people with… in samples from people with Long COVID and this paper from JACS also showed that even on its own the spike protein is very very susceptible to misfolding, forming amyloid like structures. So amyloid, we're using the word amyloid here to mean a kind of misfolded protein as opposed to beta amyloid in Alzheimer's, which is where people are more familiar with the term amyloid. So at the time that we applied for the money for this grant the method that Doug and Resia were using was not quantitative. So so we aim to make a quantitative method and this slide from a paper the following year kind of shows their hypothesis which is that fibrinogen forms fibrin normally with with thrombin but when when the spike proteins integrated into those that fibrin structure you get these microclots that seem to be resistant in a subgroup of people to break down and so… and these seem to be more common in people with Long COVID so and Doug and Resia's method originally used Thioflavin-T staining which is a very standard method for it staining amyloid and we had expertise in my lab for… we'd worked on Parkinson's and we had expertise in staining for amyloid and we're using the THT stain to stain for amyloid so we were encouraged… We were already doing a bit of work on Long COVID, on wearables and symptom tracking and we were encouraged by our patient partners to go looking at microclots as well since we have the expertise in amyloid, staining for amyloid. So this is the instrument we use. It's called a cytation and what you do is you put the sample into a slide I'll and I'll show you a picture of slides in a minute and it's a fixed

volume and it does what's called Z stacking. So it it measures in three dimensions scans the sample across and then also up and counts the number of objects in in a fixed volume and then give that gives you a number and you can identify you can look at the size distribution of the microclots, how many you've got and what size and shape they are. And these are just the slides we use. So there's so you can see there's a fixed small volume and then the microscope will automate the counting and tell you how many objects or how many microclots there are in each of the samples and this is the sort of data we get. So A and B are the pictures off the slides and then C and D are a closeup of some of the microclots and we can distinguish between a high and a low count. I know it's maybe a little bit difficult for you to see. So this is very reminiscent of the pictures or of that Resia and Doug saw down their microscope, but the difference is that our automated method and high throughput method can count the number of objects and microclass. So okay, so on to the data. So we've got four groups. So we had a group of samples that we took really quite early on. So this is back in ‘22 where they were pretty sure they'd not been infected with the virus, with COVID virus, and then we had a group who'd had been infected and recovered and then our Long COVID group and then our recent COVID group who'd had covid within the last four weeks but had recovered and were not reporting any ongoing symptoms. And you can see that there's two things I really want you to notice from this graph. One is that the recent COVID are the highest as a group of the microclots. And the second is that there's a whole load of people with… although there's a whole loapd of people with Long COVID who've got higher microclots, there's also a whole load of people with Long COVID who have what you might call in the normal range. So going back to thinking about specificity and sensitivity, we're not picking up all the people with Long COVID with if you're just counting microclots. So we also looked at how long it took for the microclot numbers to drop down after you'd had COVID. And it takes quite a long time actually for levels of microclots to return to what you might call a normal level. So several weeks or even months. So far we can say people with Long COVID they have microclots in their plasma but some of the controls also have microclots but so is their presence diagnostic or not? People who've had a recent infection definitely have high counts but they don't have the same symptoms as people with Long COVID. So and then as a group people if you take the group compare the group with Long COVID with the controls there's definitely a difference between the groups but there's a significant overlap and about half of the people with Long COVID don't have particularly raised microclot counts. So, and also one of the things we observed because we asked people about what medication or supplements they were taking was that these depending there were some supplements that knocked the counts down and yet they didn't necessarily always make people feel better. So, two things that are a problem if you're thinking about microclots as biomarkers is that you can feel very unwell but not have raised microclots. But also you could have raised microclots and not have symptoms of Long COVID if you've just had an infection or at least just had COVID. So then we moved on to look at and so each of the dots on this data I should say represents a person. So then we recruited a much larger cohort and repeated the study with controls, Long COVID, and this time we also recruited an ME/CFS group and a vaccine injured group, and you can see that we see the same pattern that as a group the Long COVID and also the ME/CFS and also the vaccine injured group are all significantly different from the control as a group. But the ME/CFS group and the Long COVID group, we still have these like maybe a half of the group that have what you might call a normal range or and microclot counts in the

normal range. And the vaccine-injured are actually the highest out of the whole lot and actually the vaccine-injured are significantly higher not only than the control, but also the Long COVID and the ME/CFS groups. So what can we hypothesize or what can we… how can we interpret this data? So I think one interpretation would be that any exposure to COVID or the spike protein could cause microclots. So whether that's through vaccination, whether that's through infection, it can cause microclots and we could speculate that raised microclot counts are actually an indication of inflammation. So this could be inflammation because you've just had COVID. It could be inflammation or it could be persistent inflammation associated with Long COVID, associated with ME/CFS, associated with vaccine injury. So normally this inflammation will clear after you've had an infection. So in some people it persists. We don't know if other viruses have the same effect. So obviously a lot of people or a subsection of people with ME/CFS attribute or or their ME/CFS onset was associated with, for example, Epstein Barr virus. So maybe the same mechanism is going on. So I think we could say that Long COVID involves microclots but it needs plus… it needs something… an additional factor that having microlots is not sufficient. Yeah. So last slide: what are we going to do next? We're going to investigate whether the microclot are all the same. Are the recent COVID ones the same as the ME/CFS ones, the same as Long COVID. So we're going to do proteomics and have a look… and look at relationships with other markers. So fibrinaloid microlots are raised in people with Long COVID, people with recent COVID infection, ME/CFS, vaccine/injured compared to the controls but there's a limitation of the biomarker just by using them… as using it counts just the actual number of microclots… but I think we believe that raised microclot counts are an indication of ongoing inflammation and looking at the contents may lead to a better biomarker. We've published our data in this preprint and when we've got the proteomics data we'll then send it to a journal, and I'd like to thank all our collaborators from various universities about the UK but especially Long COVID Support and our patient representatives: Michael, Jo, Margaret and Asad, and Patient-Led Research Collaborative for funding the study which is just completely amazing.

[Gina]
Thank you so much Dr. Dalton. We will now introduce also Michael Natt, the patient rep in this study who's joining you for joining Dr. Dalton for the Q&A session. Michael, what motivated you to be involved in this process and the research project as a patient rep and maybe talk about how this has influenced your work in advocacy efforts.

[Michael]
Yeah, absolutely. Thanks. So I think I initially made contact with Carrie because I wanted to understand what was going on with myself. So I caught a very mild symptomless - almost - COVID infection then started developing the usual rigmarole of symptoms and I I wanted to understand what was going on with myself and luckily Carrie's lab was local to me and they were doing this work on microclots and at the time there was a lot of buzz around: you know, what are these things? Are they diagnostic? And because within our healthcare service here there were zero tools tests to try and figure out what was going on with many people with Long COVID, and then after meeting Carrie I realized I can give more than just my blood to this study, and that there's an opportunity here to actually inform research practice as a patient rep through

my experiences, and that was something I found rewarding because my background is in scientific research, but I now had uniquely unfortunately this vantage point of being both a researcher and understanding that world, and now as a patient and understanding that world, and then the second part of your question. So, how's that influenced my advocacy effort? So, from this first experience with patient involvement, I know I now have a good idea what it should look like. It doesn't always look as good as this and I know that because I've been involved in lots of other projects now which haven't done it quite as well, but it has helped me inform those. And for example, one of the tools that I take to those groups is the PLRC scorecards. They're very useful if you're trying to convince a research group that potentially their culture around patient involvement could be improved. And then the other couple of things is I now work with Long COVID Support who are a big UK charity and we have a research involvement consultancy where researchers can come to us and we'll give them advice on how they could do their patient involvement often using resources that PLRC have made. And then something else I realized not particularly from this involvement but in my involvement in general is that there's a lot of new researchers to the IACC space and they need to calibrate quickly and one way of doing that is… so a guy called Rory Preston who was involved with Visible made a website called CrunchME, which somebody will put in the chat I'm sure and I'm involved as one of the lead contributors to that and it's sort of a place where new researchers or older researchers can go and find a list of all the places you can get funding all of the top researchers, even the names of journalists who have faithfully covered a work. So, it's sort of a one-stop shop. Amazing, Michael. Thank you for that. That is really a great answer. Dr. Delman, we do have a one question for you from the audience. I am a Long COVID patient with confirmed microclots and made improvements on triple anti-coagulation therapy over a period of 15 months. I stopped it and quickly got worse. Then on restarting a few weeks later, never returned to previously high baseline. Any thoughts on what is driving persistent clotting in Long COVID patients who have the persistent improvement on triple anti-coagulation therapy, but deteriorated back to their previous baseline? Yeah, I mean I'm not a clinician so it's hard for me to advise on drugs. There's no doubt at all that taking triple therapy knocks down the microclots right way down to below control, because we've tested people before and afterwards and seen that, but they don't always necessarily feel better on triple therapy, but then obviously some people like you mentioned do… so I think… I'm not sure if getting rid of the microclots with the triple therapy is the thing that's driving the improvement, and then also driving the relapse. I think it might be coming along for the ride possibly. So yeah, I just think it's really complex and recovering back to baseline or not recovering back to baseline is also really complex and yeah.

[Gina]
Thank you, Dr. Dalton, more studies are needed in this area and more information definitely. Just for us to stay on time now we really appreciate - thank you so much Dr. Dalton and Michael I'm going to hand it off now and after I introduce Dr. Liisa Selin with the talk, “Altered T-cell responses in Long COVID, PASC, and ME/CFS.”

[Dr. Selin]
I'm Liisa Selin and just in all openness as this is patient-led research, I am an ME/CFS patient of 50 years and I had my last very severe episode following the COVID vaccine in 2020. And that

is obviously one of the reasons why I have a very strong interest in what drives ME/CFS. And we have strong data here on the fact that the CD8T cell response is highly dysfunctional. And we were able… We had already started studying ME/CFS patients prior to the PLRC funding which allowed us to add Long COVID which we suspected would be very similar and what we think is happening is there's an aberrant response to an immunological trigger, like infection, and this leads to a dysregulated immune system with overactivation of the CD8T cells and subsequent T-cell dysfunction particularly CD8. Now there's actually two parts to this work. I'm going to present the first part here talking about the CD8T cell dysfunction looking in functional assays, looking at cytokine perforin, and a unique subset of cells that we're seeing in patients that are actually CD4 CD8 double positive. Later on today you're going to hear from Roshan Kumar who also we were fortunate enough to get a collaborative PLRC grant and in the same patients he was doing C single cell RNA seq and and re-confirming our findings about these dysfunctional, exhausted CD8 T cells but also we'll give you some preliminary data we're able to look at T-cell repertoire so we can potentially identify the antigen because we do believe this is an antigen driven disease. We have published a paper already based on some of the findings we've derived from this funding looking at what we consider this to be a potential marker for ME/CFS and Long COVID. But in addition we did a case study on eight patients that were treated with nebulized antioxidant, antipathogen treatment where we found improvement in symptoms and in this biomarker. Other teams have found a similar type of dysfunction in the CD8 T cells during the time that we've been working. And this is probably the most important slide of the talk. If you tire, just to explain the overall concept: first of all T-cells start out as double positive expressing CD4 and CD8 in the thymus and then they come out into the peripheral blood and they're either single positive CD4 or CD8. The CD4s do all kinds of important cytokine functions. One of them is making this cytokine 9 that we'll talk about a little bit later. And the CD8 cells are predominantly known as cytotoxic. They make this perforin granzyme that's very important for killing particularly virally infected cells. They make interferon, gamma and TNF. There are also these double positive cells that are very poorly understood. They were identified over 25 years ago. But little is still known. They're known to increase in autoimmune, postviral, during inflammatory processes. They have very diverse functions that cover both CD4 and CD8. And what we found was: in our studies as I'm going to show you, is that the CD8 T cells are highly dysfunctional. They're decreased in frequency. They have poor production of interferon gamma TNF and perforan granzyme - suggesting they're in a what's called a T- cell exhausted state that has previously been seen in tumor environments or following persistent virus infections and this exhausted state is associated with generalized metabolic problems, oxidative stress, as well as very dysregulated cytokines including including inflammatory and inhibitory. The double positive cells we found to be increased in the majority of patients and they were spontaneously producing this IL-9 cytokine and I'll show you a little bit of that data. What's unique about our studies compared to many of the others looking at dysfunctional T-cells is we collect the blood, do our symptom questionnaire, and we do it over time - although I'm focusing today really on the first visit. We enrich for the CD8T cells that we found to be abnormal and then do the functional assays that I was talking about, we look at the frequency of CD8 and the double positives and then we have sorted these cells by flow cytometry and sent them in our collaboration with Roshan to do single-cell RNA seq and actually look at the T- cell receptor and because of my long experience and Roshan's

experience in T- cell repertoire and our collaborator Dr. Ghersi we think we will be able to work out what their antigen specificity is. Here's an example of a facts plot for an ME patient, and a Long COVID, and a healthy control. This is the peripheral blood high frequency of CD4, low CD8. But when you sort for the CD8s this high ratio, 10:1, results in seeing that the frequency of double positive CD4 CD8 is actually very high: 18%. A healthy control is about 1%. And what we find is that there's an increased ratio in ME patients overall and an increase in this frequency of double positives. And we found the same to be true for Long COVID patients. When we looked at, and this was published in that manuscript, when we looked at the functionality here, interferon gamma after PMA stimulation, you can see strong interferon gamma production in the healthy controls but poor production both in the Long COVID and the ME and the same was true for TNF, and we have done now similar studies this is unpublished that perforin is very highly expressed - it's constitutively expressed even before stimulation in the healthy control compared to both the Long COVID and the ME/CFS and these are T- cell pair-wise plots of pre-Inspiritol treatment for looking at interferon gamma in eight patients - red: ME, blue: Long COVID, and you can see there's a significant improvement in interferon gamma production, but we also now have data, although this is not published, there's a significant improvement in perforin expression at the same time as there is a significant decrease in overall severity of symptoms. This is just a new type of analysis we're starting to do because we have all these functions in one test tube. We can do what's called the tSNE analysis which is a scatter plot used in single-cell RNA seq and look and see what the populations look like. And I'm just going to focus on this one here: the left. This in, circled in red is what the ME/CFS patients cells are doing and look like. The cells on the, outside that box are what the healthy control looks like. So you can see that they are really completely different in their T- cell - CD8 T- cell and double positive function. What we then did was a principal component analysis of the ME and the Long COVID patients and found that the healthy controls are in squares, and the patients are in circles, that the PC1 the patients completely separated from the healthy controls based on function, and these are all the functions to the right - you can see for the loadings, but not only that we found the patients formed two clusters. Cluster one, which is predominantly female and cluster two, which had most of the males and they are immunologically different. One of the most…two the most significant differences was cluster one tended to have higher CD4 CD8 positive frequency and lower CD8 frequency, suggesting this might be beneficial. And actually when we looked at it in our large correlation matrices, we found that the higher your double positive frequency, the lower your severity of ME/CFS was. And at the same time, the higher your CD8 frequency was, the higher your ability of your CD8 T cells to express perforin. Which is very consistent with a melanoma model where they've found in the tumor environment the T-cells are exhausted and there's an increase of double positive cells making IL-9 which helps to support the CD8 T cells and in fact that is what we found in cluster one: they are spontaneously making much more 9 than cluster two and cluster 2 interestingly enough has this group of CD4 positive perforin-expressing T-cells compared to healthy controls or cluster one. This appears to be, I think, their compensation to try to control pathogens and our model side is that infection or toxin or something sets up an inappropriate immune response. This leads to overactivation, exhaustion. This can be treated with immune modulators such as the nebulized antioxidant Inspiritol or JAK STAT inhibitors. We think this leads to difficulty controlling all the persistent pathogens that we have and such as the herpes viruses, enteroviruses, and that's

why antivirals are probably an important component of this. The state of exhaustion as I mentioned has tremendous dysregulation both in your innate and in your inhibitory responses. So your immune system is really a mess. You're in a state of oxidative stress and there are treatments for that including the nebulized antioxidant and some JAK STAT inhibitors and all of this I think leads to the neurological symptoms, the autonomic instability, the brain fog, and the other things that we see like the increased double positive increased cytotoxic CD4s. We're also seeing increased MAIT and gamma/delta - I think these are compensations trying to control all these persistent pathogens and and and will help us perhaps identify which pathogens and I think a lot of this data helps to explain the heterogeneity of the disease because I think different pathogens and different compensations are occurring in the patients once this dysregulation occurs. And I just want to thank my collaborators and again: none of this would be possible without my longtime collaborator Anna Gil. Also, Nuray, Rivka, and Megan are patient collaborators and other patient collaborators have joined us: Roshan and Dario. And the Inspiritol people: Carolina Ionete and Chris Hemond who have helped set up Long COVID/ME/CFS clinic at UMASS. and of course PLRC for everything that they've done.

[Gina]
Thank you, Dr. Selin, very much. I'd like to also introduce Dr. Megan Fitzgerald, a PLRC member and a patient rep in this study. She'll be joining you for a Q&A session. Hi, Megan. So, I'm going to ask my first question to Megan. Megan: What has been different about this engagement over more conventional patient advisory panels?

[Dr. Fitzgerald]
I think, and I'll speak a bit more about this later as well. so I can talk specifically about working with Dr. Selin as she had mentioned she is a patient with longtime ME. So, on top of being able to discuss, you know, the research and and her just having this innate knowledge that patient contributions are important to the research and asking me a lot about long COVID and and the onset and how that might differ from ME and and that sort those sort of really fantastic discussions that we had. It was really inspiring to me to see how much her own lived experience had informed her research, not only in the PLRF-funded work, but in the work that she's done for decades before this. You know, as someone who's dealing with new disability, who was interested in research before I had a PhD, I mean, I was conducting research, you know, at a high level before this. One of the big things psychologically for me is that I was afraid that this was the end of my research career, you know, but then I got paired with Dr. Selin and it was just - I'm getting chills talking about it. It was so inspiring to see all the work that she's done for so many of us. So, it was really on a personal level incredibly good for me to to be a part of her work and her study.

[Gina]
Thank you so much, Megan. And Dr. Selin, we appreciate you and in the interest of time, we're going to move on to the next talk, but we'd love for you to answer questions in the chat if you can, if you have the time for that. Thank you both, Megan and Dr. Selin. And for now, I'm going to now move to the next speaker who is Dr. David Esteban who couldn't be here today but

recorded his talk, “Alterations in tryptophan metabolism in the gut microbiome of people with ME/CFS and Long COVID.”

[Dr. Esteban]
Hi everyone, my name is David Esteban and I'm a professor at Vassar College and I'm going to tell you about research that we're doing on tryptophan metabolism in people with ME and Long COVID. So first let me talk about the gut microbiome. It's this complex community of bacteria, viruses and eukaryotic microbes that reside in the gut and affect host physiology and function. Gut dysbiosis which is a change in the community of the microbiome has been reported in a number of diseases including ME and Long COVID. Along with these changes we see things like reduced production of butyrate and other short- chain fatty acids. These are important molecules that communicate and affect function in the host. We've seen changes in intestinal permeability. So there's transllocation of bacteria and bacterial components across the wall of the gut into the bloodstream that can cause things like chronic inflammation. There are some findings looking at changes in tryptophan metabolizing bacteria in the gut microbiome, although these are a little bit inconsistent, as well as identification of changes in microbial metabolites that are found in the bloodstream. So my interest is in specifically in tryptophan metabolites produced by the gut microbiome. And the reason is that many of the products that are made from tryptophan by microbes in the gut are ligands for a host cellular receptor called the aryl hydrocarbon receptor or AHR. AHR is expressed in the gut epithelial cells, in cells of the immune system, and in cells of the brain as well as other tissues. AHR is a transcription factor. So when it binds these tryptophan metabolites, it activates gene expression in these different cells. And these genes that are activated have a variety of different effects, including regulating gut permeability and gut homeostasis, regulating the development of T- cell subtypes in the gut mucosa, and regulating mucosal and systemic inflammation, as well. They can travel to the brain and regulate mast cell activation and degranulation, regulate microglia function as well as astrocyte function. These metabolites produced from tryptophan by the gut microbiome have an effect both locally in the gut and distally in other tissues. And you can see that many of the systems that are regulated by AHR are ones that are affected in ME and in Long COVID. So we were interested in looking at the production of AHR agonists in people with ME and Long COVID. I will start with talking about our study on people with ME, which is largely complete and our study on Long COVID, which is not quite as far along but for which we have some preliminary data. So the design of the study for ME: we used the SolveME Solve together registry to recruit participants. Participants filled out a variety of surveys including medical history and so on and we sent them a stool sample collection kit so they can collect a stool sample at home and ship it back to us to the lab. In the lab, I can analyze the microbiome to look for the community structure and composition. We did some metabolomics to look at different concentrations of metabolites in the stool. And then I have two different assays that I can use to measure AHR activity as induced by microbes in those samples. So one of them is measuring AHR-induced gene expression in an intestinal epithelial cell line and the other is a reporter assay where I have cells that express luciferase under control of AHR. So when they're exposed to AHR agonist they produce light. Let's first take a look at the microbiome in people with ME and controls. And this has been shown before, but it was nice to see in our study again, repeated, that the microbiomes of people with ME have lower diversity on ours by all our

measures of diversity, as well as a shift in the community structure. So the way to interpret this figure is we're looking at clustering patterns and shifts in the position of the centroid. So the big blue dot here, the centroid, for the ME cluster and the gray dot for the cluster of control samples and you can see that they've shifted. That indicates that there's a change in the community structure of the two, control and ME communities. We also see a change in the firmicutes to bactaroidetes ratio which is typical of dysbiosis. So all of this together indicates that the microbiome of people with ME is less diverse and differs in composition from controls. And of course this has been seen before. So this confirms that we're also seeing these kinds of changes in our study sample, as well. For metabolomics, we used targeted metabolomics to look at 79 different metabolites in the stool samples, and we found nine that were significantly different between people with ME and controls and those are shown here, individually. What's particularly interesting to us is that three of these metabolites: indole, indoleacetate, and tryptophan, are AHR agonists and they're all elevated in these stool samples. We then looked at which microbes correlate in abundance with tryptophan metabolite concentrations and we identified a subset of a sub community of the microbiome that correlates either positively or negatively with one or more tryptophan metabolite. This sub community is heavily dominated by clostridia and it differs between patients and controls both in structure and diversity. So these are likely to be functionally relevant changes in the gut microbiome with respect to tryptophan metabolism. Finally, for looking at AHR activity using our two different assays in the top here, this is our reporter assay and the bottom two are looking at the expression of two different genes that are upregulated by AHR. What we found is that there was no difference in AHR agonist activity between patients and controls, regardless of the assay system that we used. However, when we instead looked at splitting our study population based on the presence or absence of cognitive symptoms regardless of underlying disease, what we found is that people who reported cognitive symptoms have elevated AHR activation. And again, we found this regardless of the assay system that we used. So this indicates that people with cognitive symptoms have microbiomes with an increased capacity for tryptophan metabolism. Our study with Long COVID is not as far along. However it's identical in design and with the same hypothesis of altered tryptophan metabolism. At this point, we have recruited all of our participants and we have collected samples from everyone. Let me show you a little bit of our study population. This is very preliminary data. I haven't done all of the analysis on this yet. And we actually have a few more participants than is actually shown here in this table. We have approximately equal numbers of patients and controls. 70% of our participants are female. Unfortunately, we weren't able to recruit a very diverse study population. It's overwhelmingly white and non-Hispanic. We tried to get a more diverse study population, but were unable to do so. None of the demographic measures so far seem to be different between patients and controls. On the microbiome side, we have some preliminary data. It's not ready to show yet. We want to rerun our microbiome sequencing just to get some higher quality data. But that should be coming soon. On the metabolomics side, we have a new collaborator, Andrew Gold at Ohio State University who's going to be doing targeted metabolomics for tryptophan metabolites and we'll be able to do untargeted metabolomics for numerous other metabolites. We do have some preliminary data looking at AHR activity and what we have found so far is that in our qPCR assay looking for AHR induced gene expression, we see elevated agonist activity in stool samples from people with Long COVID. We see that when we look at the expression of one

gene AHRR this other one CYP1A1 seems to be slightly upregulated but it didn't reach statistical significance This is quite preliminary. We need to repeat these experiments, but it's exciting to see that we may be detecting a difference here. So, in conclusion, we've shown that there's microbiome dysbiosis in people with me. That's been shown before, and that specifically that dysbiosis includes the sub community of tryptophan metabolizing bacteria and that we see elevated AHR agonist activity and AHR agonist in the feces but also elevated activity in people with cognitive symptoms. We think this is relevant because AHR is a known mechanistic link in the gut microbiome brain axis. Therefore, altered tryptophan metabolism and altered AHR agonist activity may play a role in the neurocognitive symptoms in people with ME and potentially Long COVID. So, we should consider AHR as a potential therapeutic target. I want to thank a lot of students that have worked on various parts of this work. So Vassar is an undergraduate liberal arts college. I do not have grad students or postdocs. So the work here is all either done by myself directly or an undergraduate student. And I think one of the things… one of the real benefits of doing this kind of work at a place like Vassar is that these students go on to be doctors or researchers or other health professions. And hopefully having gone through research in my lab, they have a better understanding of the importance of really understanding diseases like ME and Long COVID. I'd also like to thank folks at SolveMe for their access to their database and surveys and assistance with recruitment as well as my PLRC research patient researcher partners Megan Fitzgerald and Tess Falor. My collaborator for metabolomics Andrew Gold at OSU. And of course, a big thank you to the study participants. Finally, I'd like to thank PLRC and SolveMe for funding as well as some internal funds from Vassar College and a crowdfunding campaign that was run by Megan that raised some additional funds for metabolomics. So, I want to thank those who donated to that crowdfunding effort. Thank you.

[Gina]
Thanks Dr. Esteban for pre-recording his presentation for today. And we have Dr. Tess Falor who is a patient rep in this study will be answering one question from the audience and actually we have a question that we want to ask you: What made this research study particularly interesting to patients?

[Tess]
To patients I think it was decentralized which I think is really important for patients. The decentralized sample collection at home which allows people with different severities to participate, you know when people have to go into people they can get post-exertional malaise afterwards. So I thought that was great. Also Dr. Esteban mentioned that it might inform treatments. So there are med meds that are HR agonist and antagonists and you can also you know modify the microbiome. So I think those two things are patients will appreciate.

[Gina]
Thank you so much Tess. We appreciate you being here to answer your question. Anybody has any other questions for Tess, if she has the energy and time, she can try to answer them in the chat. And now we are honored to welcome back Dr. Megan Fitzgerald, a PLRC member and patient rep for multiple PLRC projects with the talk, “Nothing for us without us. aligning research funding with patient priorities.”

[Dr. Fitzgerald]
I'm going to be starting with my own personal story here. This picture was taken in March 17th of 2020. This is a St. Patrick's Day selfie that my family and I have taken every year and it's actually really painful picture for me to look at now because that was the last day of my life in good health. It was right after lockdown and, I didn't know it but I was at that time incubating COVID 19. I had been exposed and became very ill after that. And this day represented the transition of my life from kind of a healthy person to somebody with a long-term disabling and severe chronic illness. But it was also the change in my life and career - a change that moved me from being a researcher in biomedical sciences to a patient, and then back into the research community as a patient researcher with a completely new perspective.
Just to speak a minute about the history of PLRC as well. PLRC emerged out of a patient support group on Slack called Body Politic that I was involved in, and really the root of this patient movement was self-preservation. I don't think that any of us, none the leadership, none of us anticipated that this is what we would be doing with our lives. In the early days we even all counted our illness duration in days, which was in retrospect very naive. But this was based on the information that we got from medical and the research establishments at the time. COVID was thought of as a disease that you either died from or you recovered from within 2 weeks and only really the ME advocacy groups were sounding the alarm that this was going to be a mass-disabling event. These are comments that I took from PLRC's… the page where we have po where PLRC posted their initial research report on the symptoms of Long COVID. Now at that point a lot of us were unable to obtain care for our condition through our healthcare providers and a lot of us, even worse than being unable to obtain care, were being gaslit about our condition. Here are some quotes. “Doctors have no idea what it could be to the point where I worried it was just me. I have lost lasting heart and lung damage. I stopped mid-sentence because the word won't come to mind in any language. I was fit before I got this virus. I just found out about your work and I feel validated and not alone any longer. Thank you for putting a voice to the otherwise almost forced silencing of this debilitating postviral reaction.” In the beginning, as Hannah had mentioned, there was a real misalignment between the funding priorities and patient needs. Some examples of this misalignment was the lack of knowledge, of the people who were being funded, about infection associated-chronic conditions and illnesses such as ME or ME/CFS. There was a focus on studies on cognitive behavioral therapy and graded exercise therapy - instead of on studies on the pathobiology of Long COVID or clinical trials of potentially curative treatment. And cognitive behavioral therapy, it can help with some chronic illnesses, but it's not a substitute for actual curative care. And graded exercise therapy had been shown with ME to actually have long-term harm. So the fact that this was being considered was really upsetting to those of us in the patient community. Another example of misalignment were studies that focused only on patients that were hospitalized with a severe acute COVID infection, where most of us who have Long COVID were actually… initially we had a mild or or moderate infection and were not hospitalized. So patient researchers emerged on the scene and we were advocates for ourselves and to bridge the gap between the patient community and researchers, scientists, clinicians and pharma. This is an illustration of how patient researchers can bridge the gap between the patient community, researchers, scientists, clinicians and pharmaceutical companies. This segment of the illustration under the bridge here

in the hazardous water represents hazards that research studies were falling into like psychologization of illness study design that doesn't account for post-exertional malaise or PEM which is one of the big hallmarks of ME as well as Long COVID. There are specific issues facing marginalized communities that were not addressed in a lot of these studies such as lack of access to health care and healthcare providers and [...] there's also an assumption that anybody who had COVID had access to a test. You know, right at the time that they were sick, this was not the case. A lot of us, including myself, who were sick in the first week, there just wasn't testing availability. And then this continued really throughout the pandemic. Even now, home tests don't appear in the EHR. So, we had to push back. The role of the patient researcher is honed by our own lived experience and this gives us unique insights that are crucial to ensure scientifically valid study design. We are not tokens on these studies. We are really integral parts. In my own role, I have been a patient representative in the Selin labs and with N3C and NIH Recover as well as with the Esteban labs. And some examples that we can help out with as patients and being patient representatives on these studies are to develop better inclusion exclusion criteria for each study design. As I had mentioned - access to testing, should this be an inclusion criteria or should it be an exclusion criteria? If you have, for example, one of the big problems with testing for COVID infections, if you're using a negative test as an exclusion criteria, and yet someone couldn't access a test until 2 weeks, a month after the initial illness, then they get a negative test, but they had all the symptoms of COVID and they might be part of the no-COVID control group because they had a negative test, but that doesn't make any sense. So, we've had to, you know, it seems like common sense when I'm saying this, but this is something that we've had to reiterate over and over again. And that really can only be learned through live experience. Post-exertional malaise or PEM: researchers need to take this into account when they're designing studies. Or it can lead to symptom exacerbation for participants or participants dropping out of the studies. So this is something else that we discuss frequently as patient representatives in these studies. Dysautonomia, which is a condition.. a syndrome a cluster of symptoms that many of us with Long COVID struggle with, can lead to specific testing parameters such as, you know, if you do a cognitive test with somebody who has dysautonomia it that results of that cognitive test might might change based on body position and blood flow. So these things need to be taken into account and often researchers who have not experienced this firsthand aren't aware of these things. Just speaking to symptom burden and symptom complexity to researchers is also really important. And in terms of researchers, also help with recruitment and in the publication process to make sure that the scientific information is relevant and communicated clearly to patients. Including us as patient researchers can lead to better science, and not including patient researchers can lead to irrelevant or even unusable findings and kind of a wasted investment. So what does patient-led funding look like? We're seeing some of the results of this today, which is so exciting. And the PLRF, as Hannah had mentioned, was created with $4.8 million in funding from the Balvi Philanthropic Fund. And Hannah had shown the patient researchers on the patient-led research funding panel before. So I'll go through this quickly, but again just to reiterate, not only were we patients, most of us had really important and extensive records in healthcare and data sciences before becoming patients. So you're hearing about the studies that were funded and you heard from Hannah about how these proposals were evaluated and chosen. So what I wanted to do with this slide is to make the additional point that we're making a difference not only with these specific studies that are

funded but we're creating a virtuous cycle of Long COVID research. If you fund studies on things like CBT and GET or studies that psychologize illness that leads to more studies being funded on psychologization of illness. But if we fund studies that are really looking for biomarkers and for the pathobiology and for curative treatments, then this is also going to lead to more evidence showing what's wrong in the condition and more funding for these types of studies that will actually lead to better quality of life, and hopefully a cure at some point. So I think one of the most important long-term contributions to PLRF is that we are really a crucial part of creating a virtuous cycle of Long COVID research. Going forward, now that these studies are wrapping up, I don't want the momentum from this to cease. Funders should continue to require patient involvement at the priority-setting stage, not just, you know, at the review stage, but: what do patients think are the most important things to fund within the grant review process? And they should incorporate patients as research partners throughout the life of a study. Researchers and clinicians need to continue to build real and respectful patient partnerships both with individuals as well as with patient advocacy groups and, crucially, to provide the accommodations that these patients need. A lot of people with Long COVID are quite severely affected and there are accommodations that need to be taken into account in order to ensure their participation, but their voices are imperative in order to build better studies and to have better outcomes. Patients: we've done a great job and I think we need to continue to advocate that the studies that we want to see funded are funded, particularly in the current funding environment and to educate our communities, our healthcare providers and the rest of the patient community on what these research studies are finding. So to reimagine the future of research, and this is a change from where I started off over 5 years ago, when my journey began and I was a biomedical researcher doing research on conditions where I had very little interaction with patients. I want to envision a future where patients are trusted partners in research. Now this is a quote that will differ a bit from those in the beginning in terms of what it's like to be a patient representative. This is from a quote from an article in eLife. “I found myself amid a large group of researchers, clinicians and other representatives like me who were also concerned. This also allowed me to soften to take a breath and realize that after a full year of my family struggling with debilitating illness while not being believed by practitioners and others, this condition was finally being recognized and legitimized”. It's just very rewarding to participate as a patient in these studies. It's a lot of work. and it's energy draining at times, but it's also something that I think we need to continue to fight for and to dedicate ourselves to. And just to reiterate that biomedical research studies without patients really risk producing results that don't matter. But when patients help lead, that's when science can really become life-changing.

[Gina]
Thank you so much, Megan. We are pausing for 10 minutes. We'll be back at 2:45. Thank you so much to all the speakers from the first part of this.

[Katie]
Okay. Hello everyone. Welcome back. I think we're good to get started, my name is Katie Drackert. I am the partnerships and advocacy coordinator here at PLRC. I am a white non-binary person, wearing a white button-up shirt with a black and silver bow tie and some glasses. Thank you again everyone for being here today and really excited for this lineup we've

got for you. So, up next we have Dr. Roshan Kumar going over “T- cell dysregulation revealed by single cell profiling of ME/CFS and Long COVID patients.” Hello Dr. Roshan.

[Dr. Kumar]
Thank you all for inviting us to present here. It's a real privilege. And this is a collaborative project with Liisa Selin's lab and you heard from Liisa earlier in this session. So we've been funded by the PLRC. So Liisa talked to you about T- cell abnormalities in ME/CFS and Long COVID. What we're working on here is understanding these abnormalities at a single cell level. So we're performing single cell profiling on T cells from ME/CFS and Long COVID patients and that includes gene expression profiling and it includes sequencing the T- cell receptors of these cells. Now our goals here are to identify disease-specific cell states that are associated with ME/CFS and Long COVID to characterize the heterogeneity of that unique double positive CD4 CD8 T- cell population that Liisa described, and that is sort of special because they have the capability in principle to interact with both MHC class 1 and class 2 presented antigens. So these have been observed in other autoimmune diseases and there's the hypothesis that they might be more prone to auto reactivity. So by characterizing these cells at a single cell level and profiling their TCR repertoires we hope to identify disease associated cell states and by knowing the T- cell receptor sequences of those leverage that information to ultimately be able to identify the target antigens driving these dysfunctional immune responses. So we're trying to build on work that's gone before and by doing single cell immune repertoire profiling get to the antigens and underlying causes of these diseases. Now you may be familiar with this slide or these disease models that you know there are multiple potential disease models for Long COVID and associated infection associated chronic illnesses. There could be a persistent pathogen driving it. There could be cross reactivity and autoreactivity leading to an autoimmune response. There could be direct tissue damage from the immune system. There could be reactivated viruses. Importantly the specificity of the immune response in each of these cases would differ. So by understanding the immune repertoire in patients, we want to ultimately move to understanding targets recognized by the immune system and disease mechanisms and use that for biomarkers and to guide treatment decisions. So this is our data set. It's a relatively small data set. These are all patients and controls in Liisa's lab. So six ME/CFS patients, six Long COVID patients, and four healthy controls. Importantly, we're focusing in on particular T- cell subsets. So in addition to sequencing unsorted peripheral blood mononuclear cells we are sequencing enriched populations of single CD8 positive T- cells or double positive CD4 CD8 T cells. So these can be relatively rare but by sorting for them we're enriching for them and we can identify clusters of cells we can identify T- cell receptors we can identify expanded T- cell clonotypes. here just to give you some of the highlights of what we're finding. This is integrated data from all the patients and controls from the sorted CD8 T- cell population. So we can identify different groups of cells. We find this very interesting cell population in some patients that is this cluster of cells that show features of both exhaustion and activation which suggests this continued antigen driven response. And we find expanded clonotypes here which again suggests that they're responding and continuing to respond to something that's driving them to exhaustion. We find this unusual population of CD4 like T- cells that express the transcription factor data 3 among this population of CD8 like T- cells, so given that there's expanded populations of these CD4 CD8 double positive T- cells, it's possible that these are cells in some sort of transition

phase and we're trying to understand that more. We find among cytotoxic T cells in the patients, we find signatures of dysfunction, impaired responses, which is consistent with Liisa's lab's functional data. Now we can identify T- cell clonotypes that show these impaired responses and we see expanded populations of naive cells that have their own distinct expression patterns. And you know one when we were putting together this proposal one of the main concerns of the review panel rightly so was would we be able to see disease specific differences in such a small cohort. Because Liisa's lab had this functional data being able to see these differences in small cohorts that gave us confidence that we were able to observe these differences but here we we can see them and this is just a little more detail on some of these you can see green is the healthy controls and you can see some of these cell populations are just more abundant in patients both ME/CFS and Long COVID these GATA 3CD4 like T- cells the exhausted cells these naive cells. If we look at what's shown here is the top 20 most expanded clonotypes in these patients an ME/CFS and a Long COVID patient healthy controls and expression of certain factors. So if you look at these highlighted clones, you see this unusual phenotype of simultaneous expression of inflammatory factors like interfering gamma in TNF, but also multiple exhaustion factors like PD-1, LAG3, CTLA4. You don't really see that in the healthy control. So it suggests that these T-Cells are being driven to exhaustion by certain factors. So we can zoom in on these sets of cells, we know their T- cell receptor sequences and right now we're working on trying to understand what motifs are enriched in these cells and those are what ultimately drive recognition of particular antigens. So we finished basically our sequencing cohort. We're working on the analysis and for next steps and working with our collaborators Dario Ghersi who's an expert on TCR repertoire analysis and a new collaborator we're hoping to do some screening with. We're really trying to get to understanding what these target antigens are. This shows in the cytotoxic T-cell populations that ME/CFS and Long COVID patients show more expression of immune activation markers, less expression of these factor molecules enzyme A and perforin. Again, this is in line with Liisa's functional data. Importantly, different patients show different profiles and I think this is part of the value of this sort of high resolution phenotyping. You know, I've been a caregiver as well for my wife who has had ME/CFS for many years and one of the most frustrating things was, you know, going to all these doctors and as many of you might have experienced getting many test results back that are basically normal and wondering how can someone so sick have all these normal test results. But these are the sorts of processes that can be happening basically under the hood. You can test for them in the lab, but we don't really have clinical tests for them at present. So being able to understand these responses at a high resolution, I think is critical to developing biomarkers for these diseases. We're applying analytical tools to identify dysregulated modules of genes. We see expanded or elevated TGF beta signaling in patient cytotoxic tea cells but decreased cytotoxic T- cell functions. And here's showing the GATA3 expression in this population of cells. This is from a particular patient they seem to form this distinct cluster of cells and expanded clonotypes suggesting an antigen driven response and within the double positive cells we can identify multiple distinct cell subsets and really the heterogeneity of these cells has not been characterized. We're able to apply this module analysis to identify gene expression programs that are distinct between patients in controls. And so for the first time we're really able to understand the underlying heterogeneity in these cells and how they differ between patients. And as I said, we're very excited about moving to the next stage of trying to understand the

antigens that are driving these responses, working with our collaborators. These are a couple of expanded clonotypes or types of TCRs that are very expanded in particular patients, intriguingly this TRBV24-1 TCR family has been associated with multi-inflammatory system syndrome in children and with more inflammatory COVID. So, we're now looking through all the data for patterns of these T- cell receptors. So I'd say takeaways are: we've identified these disease specific cell states. We see certain clonotypes that show these signs of activation and exhaustion. We can characterize heterogeneity within these double positive T-cells. And big picture I think we hope to use this type of immune profiling to be able to classify patients and ultimately find some sort of clinically translatable biomarker. This sort of work is probably too complicated to apply in large you know at scale in the clinic but to identify simpler correlates that could be used to classify patients and guide treatment decisions and we're really focused on these target antigens I want to say a special thanks to our wonderful patient representatives Megan who you've heard from and Rivka and you know it's it's really essential to have that perspective and I think it's really helped us try and build a research program around this because they keep the focus us on what really matters which is how is this going to affect patients and patient care ultimately. Rivka has been a longtime patient advocate. Megan of course who you heard from has a neuroscience and neurology background. Rivka actually brought us together my lab and Liisa's lab because I had known Rivka through her work with Mass ME and we have a great group in a larger group including many of whom have had personal experience with these diseases. So, I'd like to thank the whole team and the patients and volunteers for our study and thank you for listening. Happy to take questions. Thank you so much. Yeah, I believe we've got time for one question and we have a question that you may be able to answer from Dr. Selin's talk. If someone is a responder to Inspiritol, does it follow that they likely would also be a JAK-STAT inhibitor responder? Is that a correct interpretation? I don't know if it's the same pathways that are impacted. Maybe Liisa can weigh in on that later if she gets a chance. I do know in that paper that she showed the lab had monitored patients longitudinally who were under Inspiritol treatment and they saw reversal or normalization of that immune dysfunction that they were able to measure with those functional assays. I don't think that kind of work has been done in a you know controlled setting with patients treated with JAK-STAT inhibitors but that would certainly be an interesting comparison to do and I know Liisa's lab is working on also just in vitro systems where you can treat with Inspiritol and you actually see an effect in vitro so that could be also a powerful system to dissect mechanistically what certain drugs including those two are doing. Amazing. Thank you. And I think we can we fit in another question. Does illness duration factor into your results? I think almost certainly. Unfortunately, we I mean we would love to be able to do like systematic longitudinal studies. We've only been able to profile a couple of patients where there were samples and we do see changes over time. You know, one thing there's patients I didn't really show this but who show much more of that naive cell expanded and dysfunctional cytotoxic phenotype whereas others show more of an inflammatory phenotype. Now it's possible that those represent different stages of the disease. Liisa has hypothesized that you know with prolonged stimulation in disease those cytotoxic cells could die out which makes it look like there's an expansion in the naive population but because that's what's left and not receiving the stimulation. That's a model right now. I think we would need to do those longitudinal studies to to understand that better.

[Katie]
Amazing. Thank you so much. Really, really appreciate your time and your work. Awesome, so up next we have Dr. Per Sjögren. Please correct me if I'm wrong on that pronunciation. And again, remember you can continue the Q&A in the chat if anything else comes up for y'all. And we also have Dr. Bo C. Bertilson and they will be discussing “Surgery for Foraminal Stenosis in ME/CFS.”

[Dr. Sjögren]
So hello everybody and thank you we are happy to be able to share this project with you at this occasion although it is unfinished business and we are very grateful to PLRC OMF and Balvi and others who have made it possible to pursue this work a work that we believe can make a difference for patients who suffer from ME/CFS in combination with abnormalities in the spine. So my name is Per Sjögren and my close colleague Dr. Bo C. Bertilson is also with me here today and we are both affiliated to the Department of Neurobiology Care Science and Society at the Karolinska institute in Stockholm Sweden and we are also closely connected to the Brigade Clinics in Stockholm that for many years had a specialized ME center up and running with about 2,000 patients registered all diagnosed with ME/CFS according to the Canadian consensus criteria. A key collaborator in this project is the Uppsala University Hospital which is in Uppsala. It's a town about 1 hour drive away from Stockholm which has complicated things a bit and actually this project has shown to be a rather tricky one and I will come back to that later. So a short background to this project. Based on numerous clinical visits at our specialized ME center, we can conclude that obstructions in the cranocervical area are common in patients with ME/CFS and we have also published some data about this a couple of years ago. And in this context it's also important to mention the study by Rowe and colleagues showing that cervical spine surgery improved me symptoms among patients with ME/CFS and cervical spinal stenosis a well-known publication. so the our hypothesis in this project are that cervical spine abnormalities such as foraminal stenosis may have an impact on the disease state and symptoms of MECFS. And also importantly, patients with ME/CFS may tolerate and profit from cervical spine surgery of foraminal stenosis as well as patients without MECFS. And this is of importance since these patients often are declined or refused surgery despite being clinically motivated just because their underlying ME condition. So we designed a pilot study that may be able to answer some of these questions including pre and post-operative examinations of 12 individuals about 12 individuals with MECFS and also with established cervical foraminal stenosis and associated radiculopathy. And for you who are not familiar with radiculopathy, you can find a Wikipedia explanation down here below. Some of you may ask why we chose foraminal stenosis and let's say not spinal stenosis. Well, the answer to that is that we didn't. Foraminal stenosis was predetermined because our collaborative surgeons at the Uppsala University Hospital were specialized in that area and conducted research of their own in the surgical procedures of foraminal stenosis correction. So we are fully aware that other spinal abnormalities could be of importance in this context as well. So the timeline here as you can see we... the surgery to correct for this foraminal stenosis this is part of normal care normal clinical care the research is the pre surgery examinations and the post surgery examinations using a rather extensive protocol including neurology orthostatic intolerance. We perform lumbar puncture with opening pressure determination, biosampling of both plasma and cerebra spinal

fluid as well as a range of prompts and health metrics using variables OURA rings in this case in which we can monitor activity patterns, sleeping patterns, heart rate variability and so forth. Very convenient tool. So, for some results, we don't have much to contribute with because we have had an considerable delay in this project and the main two reasons for that is that the ethical review board refused our application initially which was rather frustrating. However it was later approved after an appeal from us and subsequently we have had some recruitment challenges. We pre-selected 25 patients from our patient cohort that we found to be plausible candidates for surgery. However, when they met our surgeon in Uppsala only two of those fulfilled those stringent criteria for surgery. So at the moment we only have two patients have entered the study and they have both recently completed surgery without complications I would like to add and we are planning for the first physical follow-up examinations after the summer time but we do have some monitoring data that is health metrics that show some improvements post-operatively and we also made a recent phone checkup that verifies these improvements and Dr. Bo C Bertilson will guide you through these outcomes. So please Bo.

[Dr. Bertilson]
Thank you Per. Yes, it's very recent follow up actually. It's done two days ago. And the first patient is a female of 65 years old. And her own description of the process or the waking up from the surgically anesthesiological was that she had a new life. She had a new brain that was working. and she was chuffed I think it's the word in English and she says her whole new life she can do things she has not been able to do for 40 years like walking and working in the garden and without getting tired post no PEM. And she can walk stairs, she can breathe, her stomach functions, her arms function. her husband, I even talked to her husband and he says she's not only more social and vital, she has a new life and this was from actually from day one after surgery. So her story is it's very interesting. The other patients yeah of course the metrics you can see that below there she's doubled her steps per day after the surgery and her deep sleep is is increased by 50% and time laying down is decreased by 50% about the other patient has not had the same positive experience. The one thing she does point out very clearly saying it five times is my brain is functioning it's fantastic. Five times she expresses this and though the radiculopathy which was the reason for surgery is still there. So she has a lot of pain and dysfunction of her arms. However, the brain is functioning. And she says, "I wish I had done this long long long ago," so these are the summary of these two patients so far. Back to you questions. Yeah, of course we are working to recruit the the rest of the 10 patients and it's a big process because we need to have them from the right area and they need to have both the radiculopathy and the the ME diagnosis. We're very thankful for the the our co-workers especially Dr. Huhmar who is a neurologist and... Well you can see them all here and the surgeon Anna MacDowall and Pavlos and of course we thank you for the sustaining financing of this project.

[Katie]
Thank you. Thank you so much. That was incredible. Found myself getting a little emotional when you were talking about that patient who just couldn't stop expressing how much her life had improved. Thank you for sharing. We have patient rep for this study. Tess Falor, excuse me, would love to have Tess here. Got a question. Yeah. Hi, thank you so much for helping with this

study, my question for you is why is it relevant to study structural spinal conditions after infections?

[Tess]
How long do I have? This is something I'm very passionate about. It's a good example of a gap between patient anecdotes and what patients want to see researched and what is being done and being funded. So I think that this was a really good example of our fund panel coming together and say yes we we want to support studies like this. So the structural issues like I said patient anecdotes within the patient community there's been many people who have improved from surgery or non-surgical treatments for multiple different structural abnormalities. I'm one of them. So I've I have many of these different conditions during the beginning of applying the study I actually had an artificial disc replacement. I had radiculopathy. And so again I just I think that it's so important to study and it's just it's not being funded and I think there's so much we don't know but some of the work that has been done like the paper they mentioned their group in in 2020 just it shows that this could be a major issue. I worked on some numbers 50% of the people in that study were hyper mobile 80% had a craniocervical obstructions. So this isn't just like a a rare issue that some people might have these structural conditions. It could be a much larger issue and I think understanding how these can cause ME/CFS symptoms can also teach us about general hypotheses of the pathophysiology of ME/CFS like not saying that everybody's ME/CFS is caused by these but if it can lead to the same symptoms and issues then it teaches us a lot about what is happening what's causing these symptoms.

[Katie]
Amazing. Thank you. Thank you so much for that. And Dr. Per, I have a question for you. Let's see. Could the speakers address potential mechanisms for how for I'm so sorry, my brain fog is not allowing me to pronounce this word foraminal stenosis could contribute to ME/CFS. And are they still recruiting? How could patients potentially participate?

[Dr. Bertilson]
Well, as Per mentioned, we would have liked to have the spinal stenosis patients because one of our hypothesis is that it's the fluid, the flow of the cerebral spinal fluid that is actually the main problem causing all these dysfunctions and that's why in the previous article which also was mentioned We found that about 50% of our ME cohort had intracranial hypertension and 80% had some kind of obstructions in the craniocervical area that might cause this hypertension and without having the complete data we have performed 40 lumbar punctures on a new... Another cohort of patients where we follow them post lumbar puncture how they feel just by lowering the pressure or potentially lower the pressure intracranial pressure and regulating the flow and it's a very interesting study by itself which we cannot tell more about right now but it's foraminal stenosis should not be affecting the flow that much. But if you know like I worked as a surgeon to you know that if you have a foraminal stenosis you are very much likely to have also protrusions causing disruption of the flow in the spinal canal. It's very seldom it's just only the foraminal that is affected. So that's why we accepted the surgeons proposition to study for foraminal stenosis instead of spinal stenosis which would have been more interesting.

[Katie]
Thank you. And just real quick are y'all are still recruiting correct? And do people need to be local in order to participate?

Yes, it's a cost question because you know this surgery cost several hundred thousand Swedish crowns and we don't have the finance if we had finance we could include patients from wherever but but that would need considerably much more money. So we need to have patients who live within the Uppsala area.

[Katie]
Got it. Thank you so much. Appreciate y'all and your work, and we can continue as always the Q&A in the chat. Up next we have Dr. Wenzhong Xiao, and he will be discussing “Systems Biology Approaches to Uncovering Disease Mechanism and Drug Repurposing for Long COVID.”

[Dr. Xiao]
Thank you. First I'd like to acknowledge the PLRC organization and staff for your support funding this project and also organizing this symposium. I feel I learned quite a bit so far from the updates of other projects. So for the next 10 minutes or so I'm going to just briefly give an update of what we have done for this project you know trying to do drug repurposing computationally for Long COVID and MCFS. This project is co-founded by Open Medicine Foundation and Patient-Led Research. So just to first set up the stage if we look at a typical drug development process I'm sure many on this call are very familiar with this. It typically goes from pre-clinical research where one would study the disease mechanism like what we already heard from several presentations to identify target and then to test those targets in either individual and most likely animal models. And then that goes to clinical trials which would take a few years to accomplish going from phase one to phase three. And once the drug is approved for a particular condition then you go to postmark market monitoring either in the electronic health records of the patient or some of the other monitoring studies. The issue that we know for ME/CFS and Long COVID is that you know there's not really a well-established animal model for either one of the diseases and there's not really clinical trial results for us to leverage. So what we decided was to focus on the pre-clinical side by integrating computationally literature findings and research data of MECFS and Long COVID with the existing biomedical knowledge. In other words the findings on genes, proteins, drugs, pathways and diseases and we also started to look at the other side of the spectrum. The idea is that if we can actually learn from the real world data to not only discover new candidates that already patients have taken but also we can potentially use this as a way to validate and prioritize our computational findings in order to find better candidates for clinical trials.

So the particular computational approach that we use this knowledge based network analysis. It's not a new idea you know we ourselves have used it 20 years ago for other disease studies. So a way to understand what we're trying to do here is perhaps from this picture where as we know gray wolves exist in the entire world while coyote is exclusively in the new world. So if we imagine somebody who come to the new world for the first time and you see a coyote and

naturally we would examine this new animal and then in our mind we would leverage our extensive knowledge about the animal kingdom perhaps the exclusive perhaps the gray wolf which is well known in in in the world and then we would reach a conclusion that you know this coyote animal looks like a gray wolf and therefore we might deal with it similar to what we deal a gray wolf. So that's sort of the idea behind what we do computationally. Except here we're looking at you know the genes, compounds, diseases and symptoms and what we try to do is compare what's known in ME/CFS and Long COVID with other diseases that people have already studied at each one of these levels. For example, one could say what are the genes that are known to be important in ME/CFS and on COVID and compare that gene list with lists of genes that are known to be important in well studied diseases and based on that we can draw a conclusion that perhaps there's a set of diseases that would potentially be similar to ME/CFS at gene level and certainly we can combine the findings from all these levels and come up with a overall similarity between other diseases and ME/CFS. And then the next step is to say that perhaps once we identify those diseases that might share similar potentially underlying mechanisms with ME/CFS and Long COVID we can potentially look at the drugs that are already used in those conditions and perhaps look at whether those drugs might be potentially as candidates for clinical trials in ME/CFS. We did one of our early studies only looking at gene levels and listing all the authors of the publications just to acknowledge the contributions of patient researchers that we had the opportunity to work with. So this is a sort of a very simplified results of what we have found so far, the central circle shows some of the genes only actually a subset of those genes the entire list is on the right side that we found to be important in ME/CFS and Long COVID. The circle you know next to it are a set of diseases that we're examining to see whether those diseases would share similar mechanisms as MCFS and Long COVID and lastly the outside circle which are in green shows the drugs that might be relevant. So just to you know show some concrete results this is a set of genes that we identified to be potentially candidates for intervention.

It roughly separates into three parts. The first part is genes in the central nervous system. Those are... if somebody is a neuroscientist these are probably genes that are most familiar to you. The second set are the immunoinflammatory genes. I'm sure the immunologists are very familiar with this and the third part are the genes that are known to be important in metabolism. So right now we have generated several hypotheses in terms of potential modulations for each one of these systems and we're discussing with clinicians and partners and pharmaceutical companies to see whether some of these can potentially be of interest to move to the next step. Another I'm just using this as one example another part of our analysis really has to do with human metabolism and the reason is just because comparing to other systems human metabolism is actually better understood relatively in other words which metabolite get modulated by which enzyme or regulator and what is the outcome of that enzymatic reaction. So for example here each line is a metabolic reaction and you would have the metabolite that get catalyzed by an enzyme or a group of enzymes and then turn to products and so on and so forth. So the whole thing connects to a metabolic network. So we develop tools to do a more quantitative analysis on metabolic networks and you already heard in the first presentation of this symposium the study of Dr. Wust's lab on muscle biopsy of Long COVID patients. There's another study which I'm sure a lot of people are familiar with which is the NIH study of the deep

phenotyping of MECFS and their metabiosis were also taken. So we took data from those just as an example we took data from those two metabiosis studies and tried to identify in terms of the metabolic network that you see in the previous slide what is the common set of changes from both studies. And what we identified is as you can see in the blue lines essentially the amino acid metabolism seems to be suppressed in the muscle and therefore one could do what we call in silico knocking and knockout analysis. Basically assuming that we include particular supplements or we activate or suppress each one of the genes in the metabolic network and see what's going to happen whether the… For example total ATP production in the patients can improve and based on that you know we proposed some of the potential candidates for modulation. And that's also published recently. I'm going to switch gears a little bit to talk about our work at the other side namely real world evidence and that is a collaboration with Martha Eckey who's also a patient researcher. So in collaboration with Martha Eckey we did this study of treatment survey of roughly 5600 Long COVID and ME/CFS patients. Out of the 5600 responses roughly 4,000 responses have enough data for us to analyze in terms of the patient self-reported outcomes on the effects of roughly 150 treatments. So this give us a sort of baseline understanding of what are the treatments that ME/CFS and Long COVID patients have been taking and what treatments that actually worked for them. So because of time I wouldn't really have the time to actually go into the details but I do want to mention this work just in case when patients are going to see their physicians maybe this is one of the things that we can discuss with the physicians to help them pick some of these candidates for their treatments. So let me go to the next slide. So in summary what I showed you are these two separate work. One is based on existing knowledge from academic research to identify candidates for drug repurposing. And the second piece has to do with looking at the real world data to identify and prioritize drug candidates. And I think one of the things that we all want to achieve is whether we can actually correlate patient clinical information with their treatment outcomes so we can actually better recommend the treatments to the patients.

[Katie]
Dr. Xiao, we'll have to move into our questions with the patient reps. I really appreciate all of this incredible work that you've done and also noting, you know, things that patients should bring up when they go to their doctors. Always always super helpful info. So thank you so much. And I know that we have Chimére Sweeney and we also have Dr. Becky Taurog. Chimére Sweeney is a PLRC patient rep and also the founder of the Black Long COVID Experience LLC and curator and writer at the Blackest Side of Long COVID. And Dr. Becky Taurog has a PhD in biochemistry and a post doc. in structural virology. Hi y'all. Thank you so much for joining us today. I've got a question for each of you. Let me see. First of all, Chimére, love your work. Yes. And my question for you is what makes this research study particularly interesting to patients?

[Chimére]
The reason why... first of all I want to thank PLRC and Open Med Foundation. Wenzhong I want to thank you so much. Becky was such a great patient partner to work with. I miss you, Becky. It's so good to see you. For me as a Black woman, I want to speak from that point of view. it was interesting because it just I remember thinking when I first got Long COVID I thought to myself I was like there are so many medications being presented to me or at that time at the beginning

there weren't very many mentioned to me or suggested to me. I have a long history story about the reasons why. However, as I started to learn more and I started to talk to doctors, especially about neurological symptoms and so on and so forth, I learned about the many medications that doctors were prescribing to patients concerning these symptoms and others. And I remember thinking to myself, I wish that we could learn how to repurpose quickly drugs so that it will make it easier for all patients, but especially patients of color who did not have immediate access to internet who, you know, who may have never experienced this kind of chronic illness or disabling condition. And that's why I was interested in this study because it was like it even just what Wenzhong just presented so quickly, that is such a great charting and perspective of what each drug can do. And so as a teacher and an educator who likes to educate Black folks on how to advocate for themselves with Long COVID and disabling conditions, that would be something that I will bring back to, you know, my educational cohorts and talking to them because most of the time if you have a fear of the hospital, you have a fear of of medical treatment for whatever for one reason or another, most of the time for me it's because I've been racialized so much and many of the people like me. It is very comforting to know that there have already been drugs that are placed on the market that your doctor can say well this drug will work in addition to this according to your condition. Most of the time I felt like I could trust my doctor even my current one now from Mount Sinai Dr. Lee Hinnant shout her out because she'll say to me, "Chimére, this has been proven to do these four things. Would you like to try it?" And it's that precise medical care and question that Wenzhong just talked about that makes me more trusting of her expertise. And I know if I were to go back to people that I educate, I'd be able to say, "Oh, this is used for this this." And it makes it so much easier and so much more likely that they will actually go to their doctors and say, "How about we try this versus this?" And that's easier because that my last point is that that gives those kinds of patients an opportunity to feel like they're partnering with their doctor versus feeling like they are just being told to take this without any information. And so that's why I thought this study was very interesting and it just had a cool bunch of people. We it got along very well and it was just a dope experience. So those are the reasons for me.

[Katie]
Thank you. Thank you so much for sharing. And Becky, my question for you is what would you like to see being researched next in light of findings of this study?

[Becky]
Oh boy. I mean, there's so much still and I still actually just really enjoy I still attend group meetings with Wenzhong's group pretty regularly and it's been just been a really lovely partnership. I don't because I'm not well enough to do science on my own anymore. It's really nice to just like have a I guess like a little finger in a in a science pie. But yeah, I mean the thing is and what I find with Wenzhong's research because they do so much computational work, they're able to pull lots of ideas. So they have just like they're brimming with ideas and then what's hard is funneling that into the next step of like actual clinical trials that can get, you know, the kinds of data that would allow for a drug that would be used on the market for ME or for Long COVID, but I I feel like I mean as their group is starting to do work with medical records and pulling other forms of information. So they're always and and the muscle biopsies and

anything that they can any data that they can get there. I see them just trying so hard to, you know, pull out any useful information and move it forward. And I do want to say I mean continuing to maintain this relationship with them. What I see with Wenzhong's all of the people in his lab group are that they they're so dedicated. They so deeply care about the Long COVID and ME communities and they're really they want to bring about change and they're always looking for ways to use you know leverage their expertise in science to help patients. And I think that's just really beautiful. So yeah.

[Katie]
Thank you so much for sharing all of you. Really really appreciate your time. And our next speaker before we take a break and come back is Dr. Alain Moreau and he will be going over “The Epigenomics of Long COVID Insights, Breakthroughs, and Emerging Hope.”

[Dr. Moreau]
Thank you very much. I'm delighted to be here today to present you some part of our results part of the initial mosaic studies and due to the time constraint I will limit myself to the epigenomics, and I would like to acknowledge PLRC for their funding, as well as Open Medicine Foundation and Transmit Tech institute that play a pivotal role that allowed us to present a very interesting interesting proposal to PLRC. So before I start, I would like to acknowledge my team members and also people from my labs that are the artisan of this work, as well as all the participants and volunteer that contribute to the success of Mosaic, and and for the 2D presentation. Here are my full disclosure of who is supporting what in my research program and as well as other activities. I will focus today my talk on two epigenetic mechanism associated with Long COVID pathogenesis. The first one is the study of non-coding RNA, more particularly small micro RNA that are very very small and those micro RNA are known to interact with messenger RNA and they attach at the end of those messenger RNA. And what they are doing is that they by attaching to the 3UTR with specific locations, they attract a complex called the “risk complex” and this complex often leads to the degradation of messenger RNA. But also more importantly, prevent the translation of the messenger RNA so abrogate the production of proteins. One microRNA, and there's about 2600s mature micro RNA in humans, can target up to 200 different genes and one genes can be targeted by more than one single micro RNAa, so very very small molecules, but very powerful regulators in the human bodies. The second modification is DNA mutilation. So as you know, there are ways to prevent the activation of genes especially when there are some zone that are hypermethylated in the promoter regions that will turn off the activation of a gene or when it's hypomethylated is going the opposite. So that will favorize the increase of the expression of that gene. Of course those methylation  may happen throughout the genes and hyper or hypomethylation may have different effect. The third mechanism that I want to mention today is about the ... modification which summarize all the epigenetic mechanisms that we know so far. So we create a relatively large well- characterized cohort of Long COVID, and as you can see on this slide which summarize the clinical and demographic characteristic of our participants. So we compared them with pre-pandemic LC control. And you can see that this is a disease largely dominated by female. The only difference that you can see here, beside the fact that they are severely affected based on the Kenzen consensus criteria other criteria that we are using regularly, they are slightly younger than the

LC control we use here in this study. So this is a longitudinal study where we investigate non-hospitalized Long COVID. We select at least at 6 months post-infections to reduce the noise because as you know normally people infected by SARS-CoV-2 recover relatively rapidly, but sometime they may take some more than than two weeks. So at six months we know that the individuals suffering of Long COVID are well engaged in their pathological process, if you wish. And we have several visit and we follow them every 3 months up to a full years of follow-up. At the 1st and 5th visit, we have a stress test that we apply a standardized provocation maneuvers that we usually use for people suffering myalgic encephalomyelitis and and we also assure measure the cognitive function using the brain check, brain oximetry, using near infrared spectroscopy. We collect blood, urine and saliva. Well, the other visit between visit 2 and 4, we are just doing the same thing, but simply at baseline without the provocation maneuvers. So, we tested over 50 individual as I said before with 43 LT control at baseline and T 90 minutes after the stimulation. We did a brain check for the cognitive dysfunction questionnaires and different collection from the plasma collected. We were able to use a panel of macaron that we initially developed for diagnosing ME/CFS, and using the very same panel, we were able to stratify patient along six subgroup. So this is what we call the Long COVID with ME. So they have the very same signature and mimic ME symptom, fibromyalgia FM, those having both, and these three group represent 70% of our Long COVID cohort. But we define also three additional subgroup that are not mimicking ME or fibromyalgia, but they have other type of symptom like neurological, respiratory, and severe allergy. So the very same panel allowed us to make this stratification which is very important in the follow-up of the individual affected by Long COVID. Also, I forgot to mention that using this launches on followup, we know that up to 54% remain severely affected, while almost 30% of them will recover without any specific treatment, and unfortunately almost 17% of them will see their symptom worsening from visit one to visit five. When we compare, and this is a key question, whether the Long COVID patient having ME like symptoms or fibromyalgia like symptoms, are they looking very much like pre-pandemic individual with ME FM or ME plus FM? And this is what you can see on this diagram, where the only difference is the illness duration, which was much shorter for the long covid as expected, as opposed to prepandemic patient. But otherwise they are similarly affected. If you look about ME, FM, and other individual they are they are more or less affected the same way, with the exception of the Long COVID ME that are a bit more severely fatigued using the MAF20 questionnaire. What is interesting here and and because due to the time constraint I cannot go to one by one for the details, but we developed a specific micron and our signature for each of these subgroup of Long COVID. But this signature allowed us to make a demonstration that independently of the type of the viral trigger, okay, and this is what is illustrated in the cartoon A, we we may trigger a different micro RNA that will share the same pathway way or the same micro RNA that will of course trigger– target the same genes. And that might explain why different virus will lead to us to ME/CFS symptoms. And the panel B, you can see in the light blue these are illustrating the 11 micro RNA we use initially to diagnose with very high accuracy ME/CFS patient, and now we can use to stratify Long COVID patient along this subgroup based on a different signature, different response. Interestingly, and this is I think a very important question for the clinician assessing those Long COVID patient, as well as for the patient, is what is the likelihood to recover or to improve, versus the individual having higher risk to remain severely affected? So this is what we have done here in the panel. A, so we use a

first visit because we need a predictive test. And and and we compare with the outcome at visit four, and this is what you can see. We develop a panel of 10 novel micro RNA and that give us a pretty much accuracy to predict the lack of recovery of if you prefer the recovery potential of individual suffering of Long COVID. But at the very first visit where everyone is looking the same based on the clinical symptoms, I give you two example in panel C and D, where you can see the lack of recovery. The Long COVID recovering looks very much like healthy control as opposed to Long COVID in not recovering for these two micro RNA. We did the same on the methylation profiling. Okay. And we establish a very specific DNA insulation signature using PBMC's and saliva. We compare at that time Long COVID versus short COVID, people having recovered from their infection. As you can see here, we can completely split and separate the Long COVID with improvement versus those remaining severe. And you can see the same with this ET map. What is interesting in term of potential target and treatment, we identify a gene that we call for now gene A because we are still in the validation phase. But that gene A is differently methylated and is overexpressed at the first visit in the Long COVID improving over time versus those that remain severe. But you can see that in the LT control there is no change of that factor. And at the visit tree you see it's normalized. So why we think that the improved Long COVID benefit of this transient activation lead us to hypothesize that this gene can be the key regulator that sends a kind of wakeup call to normalize and to help the affected individual to clear infections to modulate inflammatory saki and to shift to adaptive immune and bring the individual affected with Long COVID to a healthy trajectory toward particles and what we're looking right now is can we use some drugs that we can reposition to artificially during few weeks to activate that gene on everyone to break this vicious circles leading to Long COVID and permanent squ. So in conclusion, we can predict Long COVID clinical trajectories and the likelihood of disease recovery using a combination of a macro panel and a differential DNA metilum signature. We identify epigenetic mechanism and those mechanism can explain how different triggers such as viral infection can lead to the development of ME phenotype. And so far we already have identified promising therapeutic target that should be explored in more detail in order to facilitate the recovery of Long COVID and prevent posal condition like Amy. Thank you very much. Thank you Dr. Maro., real quick, we have a question from an anonymous attendee., from your perspective, how close are we to a biomarker or biomarker or means of diagnosis that differs from elimination of every other possible disease that might contribute to the symptoms? I've had ME/CFS for 9 years and still have doctors trying to gaslight me saying it's I would say that we already have published in 2020 in scientific report those panel of micro RNA that we are using on a regular basis to diagnose me and to really make sure that we can differentiate people coming to us to participate whether they have me they have fibromyalgia or both but sometime we identify individuals that are not me using that panel so I truly believe that we have the tools, we have those biomarkers. Those biomarkers are clinically useful, but they can also on the research side try to reduce the heterogeneity of me and other related conditions that allowed us to do better proteomic better metabolomic studies because now you can work with more homogeneous subgroup and that's why I didn't have time today to to show you our proteomic and metabolomic results. But we have now a very clear view of who is doing what and that will lead us to better targets and will assist us in the development of better way to treat those individual using precision medicine. Thank you. And was there anything that came up during the process of the results that surprised you in any way and if so why? I was surprised

that is that the SARS cuff 2 infections leading to Long COVID allowed us to identify or make the demonstration what a lot of people suspect that different different virus can lead that bring you to develop me it's one is one thing to see it is another way to make the demonstration beyond any doubts and this is what this upcoming manuscript will show so we hope that those manuscript will be published in the next two months so stay tuned.

[Katie]
Amazing. Thank you so much for your work and for your time. We really appreciate you. Thank you. And we are going to go ahead and take a five minute break. We'll be back after that with a little bit more. And just a reminder, if you're feeling able right now to donate to PLRC, help us sustain this work that we do around Long COVID that would be fantastic. Share with your friends, let them know. and we'll see you guys in 5 minutes.

[Gina]
Welcome back to our third and final session of this webinar. We will have the two final talks of this webinar followed by a round table discussion. Our second to last talk is by Dr. Janet Mullington with the talk, “Characterizing Non-restorative sleep in post-viral disease to advance intervention innovations.”

[Dr. Janet Mullington]
All right. Thank you very much. Well, I'm very honored and delighted to be here with you and grateful for the support of the Patient Led Research Collaborative and the Open Medicine Foundation that has really helped to make this work possible. I'm going to start with some background. We don't actually have a lot of completed data to show you but I'll show you where we are and what we've done in terms of preliminary data. So just in terms of the basics, sleep problems are among the top symptoms that disturb or are problematic for patients with ME/CFS and Long COVID. The symptoms of insomnia- difficulties falling asleep, frequent or prolonged nighttime awakenings, early morning awakenings, and other sleep disorders. New onset hyperomnia or profound sleepiness that is around the clock sometimes. And REM behavior disorder, or an acting out of dream content. Sleep disordered breathing, circadian rhythm disorders, and sleep-wake instability have all been described in Long COVID patients. And this is an example of really profound sleepwake irregularity. And you can see 11 nights of sleep. You see the 00 being midnight. So there's a lot of irregularity in the timing of sleep and then you'll notice that the duration of sleep is also quite disparate from under 2 hours to over 8 hours. And the other thing to notice in addition to the duration and and the timing of sleep being abnormal, is the fact that here you see sleep is short two nights in a row and then on the third night there's no rebound which one would expect after shortened sleep for a period of time. So we think of this as a homeostatic deficit in addition. And pre-existing insomnia before one has developed Long COVID, so before they even have COVID and poor sleep quality or short sleep of less than 6 hours, has been shown to increase the risk of developing Long COVID in multiple studies. So one of the studies that that our group Monica Hack had an NINDDS R21 award and was looking at pain and sleep in Long COVID and followed, we followed the participants for 14 days before bringing them in to the hospital to record their sleep for a single night. And what you

see down here is smata sensory smata sensory testing. And they were also wearing an acttograph before coming in. And day-to-day fatigue intensity was considerably higher in Long COVID and overall much higher than in Long COVID or people who had had Long COVID and rec or sorry excuse me had COVID and recovered. So the pre-covid here is historical data, and the green is showing you long participants who had had COVID but recovered fully, and the red is people who developed Long COVID. And you can see here over 14 days the day-to-day sleep quality, and the average sleep quality are reduced in Long COVID. And in addition Monica Hack and Marissa Angghert are looking at some inflammatory inflammatory measures and pro-resolving mediators looking at low sleep disturbance patients with Long COVID, who have low sleep disturbance versus high sleep disturbance relative to control. And this work is ongoing and these specialized pro-resolving mediators are also currently being analyzed. Along with that we looked at the PSG data, and this is work that Haoqi Sun has just published with our group, and this is showing you SPO2. So the oxygen saturation through the night is lower in the Long COVID. It's not a clinically relevant amount but we wonder whether it has physiological significance and might be related to endothelial or other factors. The resting heart rates a little higher also in the Long COVID participants, and this is showing you the sleep across the night. The green is non-REM sleep, and the the mustard color is the REM sleep. What you can see here pretty pretty obviously is less REM sleep particularly in the first half of the night and more wakefulness. The black is wakefulness. So the Long COVID participants had lower sleep efficiency about 10% lower more than that percent lower sleep efficiency, and also more awake sorry stage one through the night. So there's they're waking up and having more lighter sleep disturbance, lightening of sleep. And they also have longer sleep onset latency, and and delayed REM onset. So this is the study that we're currently doing with that that's our non-restorative sleep and postviral disease study. We have completed 10 ME/CFS, 17 Long COVID, and 13 control. And we've got several more already scheduled to hopefully complete before the before September the cohort so that we have 18 per group and they are age and sex matched. And Jennifer Scott Sutherland has been working to help us with recruitment. Robert Thomas is our sleep expert who is seeing Long COVID patients and ME/CFS patients. Christine Howser is also working with us to help with recruitment and screening and our patient representatives, we greatly appreciate the work of Letícia Soares and Beth Pollock who have been incredibly helpful just providing their insights, strategizing on logistics and and data collection. Helping us with tools and and looking at ways that that we will be analyzing some of this data with with their input and and their collaboration. So just want to go over the protocol a little bit in more detail. And I see I'm running short on time, so I'm going to go quickly, but this is this is the protocol. There's–they come in and we do quiet wakefulness before sleep and after sleep. They have a a full PSG polysomnography sleep and then measured sleep and then they have the five multiple sleep latency tests, and then they have sleep the next night. And we are doing hourly recording of blood throughout. We're doing performance testing with the NIH toolbox. And then at the end before they go home or we can actually have them come back in we we will do for those who are are willing to participate a cerebral spinal fluid sample and or skin biopsy for measuring alpha-synuclein, and for the CSF we're interested in looking at a arexent which is related to sleep-wake regulation, and it's a marker of narcolepsy. So we have been measuring cortisol. We're measuring melatonin and act and in previous studies they have found evidence that there's a flattening of the cortisol rhythm in ME/CFS. And also there has

been evidence of in Long COVID, lower cortisol. But that was only measured at one time point. So here we have the full circadian rhythm and this is only five subjects per group yet, but they are matched and and we will hopefully as I said have 18 per group when we're done. So Haoqi Sun has been looking at some of the microstructural EEG aspects and the coupling of the slow oscillation at sleep spindles is especially important for sleep dependent memory consolidation. And recently actually Hayward at the NIH found a sleep dependent procedural motor memory consolidation deficit in patients with Long COVID. And so we've been looking at this and have some really exciting results, preliminary results, that we are going to apply these methods to our currently running study. But what we've seen is that the slow oscillation is shown here, and the spindle is paired with this the peak of the slow oscillation in the normal control participants. But in Long COVID and actually in another data set we have of ME/CFS, there is the spindle is aligned with the nater or the down down point of the slow oscillation. So this, we think is quite important because it is occurring in both of these conditions where non-restorative sleep is a significant problem, but not seen in the healthy control sleep. In addition the morphology of the spindle is is different. So in the ME/CFS participants, they have more deterioration of the the tight spindle quality in the latter half of the spindle which you can actually see depicted here. So the spindle morphology breaks down much more quickly in Long COVID than in the healthy sleep, and that's referred to as chirp. And one other thing is that the the there's a correlation of .43 with restorative sleep quality in a two week daily diary with this phase relationship between the spindle and the slow oscillation. So we are optimistic that that might represent a a biomarker potentially. So, I just wanted to end there and thank all of our wonderful collaborators. I mentioned Letícia and Beth, also Alicia Stokes, Mike Doyle, Rammy Deng, and several of other of our collaborators whom I mentioned many of them throughout and our support.

[Gina]
Thank you very much. Thank you so much, Dr. Janet Mullington. I'm going to ask Letícia, ...who was a patient rep and also co-lead on the Patient Led Research Collaborative. She's been helping, she's been doing a lot of the behind the scenes work on this webinar as well. Just wanted to acknowledge that we haven't seen her on camera yet. And my first question is actually going to go to Leticia and I'd like to ask you why do you think this research is what is relevant about this to the patients?

[Letícia]
Thanks Gina and thanks Dr. Mullington. I think that first of all sleep disturbance is a feature of ME/CFS and it's extremely common among people with Long COVID. And I I haven't had over the nights of sleep that I had since I got sick five years ago. I haven't had a single decent one. And I have experienced several of the symptoms that Dr. Mullington mentioned and it's extremely debilitating. It's debilitating. It affects other symptoms. You have positional malaise that can affect sleep and be affected by sleep. So it's something that affects other symptoms and possibly it could even hinder recovery, if not managed well. Right. So having some investigation you know description, characterization, understanding of what is going on with people with Long COVID and any sleep can route us to direction of how to manage this and considering all of the complexity of these illnesses. Thank you so much Letícia. I have one question for Dr. Mullington from our patient group and it is, how might these findings relate to

the glymphatic system dysfunction and issues with CFS flow that have been found in Long COVID? Well, the the slow oscillations are probably related to that glymphatic system. And the spindles, the spindles occur with and without slow oscillations. But we do know that there's there's significance on those on those coupled spindle and oscillations with regards to memory. So they may be important for memory consolidation and function and potentially brain fog. So I think that yes they may be related. I think there needs to be more more research in this area, and I was kind of excited and surprised really to see how alike the ME/CFS and Long COVID phase relationship was between the slow oscillation and the spindle. So we haven't really seen that relationship like we see here in other conditions. So I think it warrants further exploration.

[Gina]
Thank you so much Dr. Mullington and thank you Dr. Letícia Soares. We are now going to move on to the next, and actually last panel funded talk which is from Dr. Peluso, Michael Peluso. And it's a pre-recorded and because he cannot be here today and the talk is called “Monoclonal antibodies in Long COVID what needs to happen next.”

[Dr. Peluso]
Hey everybody, it's a pleasure to be here. I'm Michael Peluso from UCSF and I'm going to talk about monoclonal antibodies and Long COVID, results from outsmart LC, and what I think we need to do next. I want to begin by acknowledging both funding from the Patient Led Research Fund for this project and our team in San Francisco, who's been working on Long COVID for over 5 years now. As many of you know SARS-CoV-2 persistence has been linked to to Long COVID and there are case reports that have suggested that some people with Long COVID have experienced improvement after receiving monoclonal antibodies, either for treatment of a new COVID infection or prophylaxis. But this has really not been tested in any sort of rigorous controlled manner, which is what we need to you know eventually get drugs authorized or approved for a condition. Here we conducted the first placebo controlled randomized trial of a SARS-CoV-2 specific monoclonal in people with Long COVID. We hypothesized that treatment with the monoclonal to people experiencing Long COVID would result in symptom reduction as well as improvement in various biomarkers of inflammation, in comparison to those being treated with a placebo. We used a study drug called AER002 which was made by a company called Aerium Therapeutics. This is a SARS-CoV-2 specific monoclonal antibody that was active against all variants through the fall of 2022. Importantly, it had retained effector function which means in addition to binding SARS-CoV-2, it could theoretically promote clearance of infected cells by inducing a more robust immune response against the virus that it was neutralizing. And we theorized that since people with Long COVID had a prior infection with a variant that was susceptible, if they were infected before the fall of 2022, that this strategy would be expected to work because it doesn't matter what's circulating now. What matters is what virus they were initially infected with back in the day. This is the design of the clinical trial, but we enrolled people with confirmed SARS-CoV-2 infection who had significant Long COVID symptoms. We didn't specifically target any specific symptomatology, and we conducted a variety of baseline assessments. Then we did an infusion visit, where individuals got an infusion of the drug or about 250 cc's of placebo formulated in saline. We saw them at 2 weeks, 1 month, 3 months, 6 months and 1 year after infusion. And we measured a whole variety of parameters. So the

primary focus of the study, of course, was safety as this was the first time this drug was being tested in people with Long COVID. We measured a variety of patient reported outcomes common ones including PROMIS-29 as well as objective outcomes. And you can see here that we tried to match PROs with objective outcomes. So for physical function we used the Duke activity status index scale which is a PRO, and then did a 6-minute walk test. For cognition we used an everyday cognition scale, and a neuro neurocognitive test called CNS vital signs. And for autonomic dysfunction we use COMPASS-31 and then an active stand test. We measured a variety of different biomarkers and then people had the option of opting in to more invasive procedures including gut biopsies, pre and post PET imaging pre and post and cardiopulmonary exercise testing. And so I just want to pause here and acknowledge PLRC you know this study was picked up for funding by the group. It would not have happened without that funding. I'm really grateful for their support. And then also obviously Aerium provided the study drug so that we could test it in this clinical trial. The population of individuals that we enrolled is outlined here. So you can see we enrolled 36 individuals it was 2 to1 randomized. So 24 individuals got the active drug, and 12 got placebo. You can see the groups were pretty well balanced in terms of age, in terms of sex. This was a fairly diverse group. You know, similar breakdown in terms of total number of SARS-CoV-2 infections before the study. Similar time out from infection. People were about two years into their Long COVID. Everybody had initially received vaccine series and the baseline symptom count was quite similar between groups. It's not shown here, but fatigue and neurocognitive dysfunction were the two most common symptoms. So the primary outcome of the study was safety. And I can say that you know this was safe. The risk of adverse events was similar between the two groups. There were no grade three safety events that were related to the study, no study pauses, nothing of serious concern. I want to thank the safety monitoring committee shown here for helping navigate us through the trial. I'm sure many of you have seen by now the primary efficacy outcome. So that was PROMIS-29 physical health summary score, which was also used by a number of other Long COVID clinical trials. And unfortunately also in this study as in many of the prior studies we did not see a significant difference in the physical health summary score between the groups at day 90. This was also true of all of the secondary outcomes. So there was not a significant difference between the treatment arm and the placebo arm and any of the secondary outcome measures at day 90. That includes the biomarker measures. But I have to say, you know, this was sort of only skimming the surface of what we're measuring in this study. There are many many more analyses that are underway right now including assessment of antigen persistence obviously something that we're very interested in with the SARS-CoV-2 specific monoclonal more multi-dimensional markers of inflammation and immune dysfunction including really in-depth proteomics work etc. So when we post the initial manuscript for this, hopefully later in the summer, we'll have a lot more detail than what I've shown today. So I want to take a moment and say some of my thoughts about why it didn't work in this case. I think we all had a lot of hope and were quite disappointed that it was a negative study in terms of the topline efficacy result. So it's possible that the drug that we used in this study, which was the only monoclonal that we were able to access at the time, was just the wrong drug. Maybe it did not have the activity we needed. Uh, maybe the dose was wrong. You know there were a lot of limitations with this drug in terms of coverage of ongoing SARS-CoV-2 infections, and so you know there are a number of possibilities related to that. Maybe the initial virus that people had been infected

with had already sort of you know mutated in its in its micro environment had already escaped right the natural immune response and you know potentially the antibody mediated immune response. It's also possible you know almost everyone in this study had had multiple infections by the time that they enrolled. Even though the infection that caused their Long COVID, you know, was from a variant we knew to be susceptible to this antibody. It's possible that subsequent infections with more modern variants were not. And that people could have sort of a mixture of variants driving their Long COVID that we could incompletely clear with a drug like this. It's possible that we enrolled the wrong participants and by that I mean specifically that we didn't enroll enough people who truly had viral persistence. We don't know whether all Long COVID is driven by viral persistence and you know my bet would be that it's probably not. That it's probably one of the most important drivers, but is not present in all cases of Long COVID. The data so far suggests that somewhere around a third of people have this in a sort of detectable way, and you know with a small study like this it's possible that we just enroll too few people who ultimately have that as a driver of their symptomatology. And so that's up for debate. Another thing is that most of the evidence for viral persistence as a driver of Long COVID so far is up to a year post COVID. We're working really hard to kind of expand that time horizon but you know everyone in this study was over 2 years from their initial infection, and so it's possible that this mechanism is more active earlier on and that there's sort of a window in which you need to clear and and then once you're beyond that window strategy like this may not work. It's possible that a single dose is not the best strategy. And that we actually need to explore a testing multi-dosing strategy, you know, over months rather than just a single dose at a single time point. Or that we need combination approaches to target both the virus and the immune system simultaneously. And I'll make some more comments on that too. And then finally, it's possible that we simply, you know, had the wrong hypothesis here. You know, some people argue that viral persistence is just not a cause of Long COVID. I don't think that a few negative studies of short courses of Paxlovid and a negative study of monoclonals is enough to prove a negative. And so I really don't think that we should be giving up on this yet. But I think we need to be really careful in thinking through how we design the next phase of studies. The current state of the field is that there are now multiple clinical trials targeting viral persistence with single therapies that are all negative. You know, multiple negative studies of Paxlovid, this negative study of a monoclonal, albeit an outdated one. We're still waiting on the results of Vital which is much larger than any of the studies that have been performed so far, and I think will be really informative. I don't think that we should be abandoning this line of pursuit based on a few small negative studies. But this is what I think we need to do next. So first, I really think it's necessary that we collate real world evidence of people responding to these monoclonals when they receive them in the context of regular clinical care. This is really important in engaging various stakeholders including industry stakeholders and regulatory stakeholders, and there's a group that you may have seen online called Long COVID labs, that is making an effort to begin to collate these cases so that we can get a better understanding of what's happening in the real world. Alongside that, we need to be designing the next round of trials to target viral persistence. And I think part of that is going to, you know, be admitting that we need to be a little bit more selective for the next phase of trials. And what I mean by that is really trying to confirm that individuals enrolled in these trials have this mechanism as part of eligibility, or at least formally stratifying the enrollment and the randomization by the detection or not of persistence.

In order to do both of those things, we really need rigorous biomarker development now to support this. Many of you may have seen people from our group talk about this program that we're developing called Viper, which is really meant to address the biomarker problem for persistence and other mechanisms so that we can drive clinical trials forward. I think we also need to consider new approaches. So, we need to be testing modern monoclonals. I'm encouraged that there's a study of Sapavabart, the Astroenica monoclonal, which is funded by Silk and is being led by Nancy Climus' group in Florida. We also need to be doing studies of Pimeivart, Pangarda. Many of you may have seen the press release from Invivid, which makes that drug this week announcing the formation of this group, this task force called Spear, to think through a lot of the nuances of clinical trial design for monoclonals and Long COVID. And then I think we also need to be testing combination approaches and that that doesn't need to necessarily begin at scale. But we you know every study of Long COVID so far has pretty much been a single agent and I think you know wearing my hat as a an HIV researcher for HIV cure, a lot of the momentum in that field is combining different types of agents that target different types of mechanisms to see how we can alter the biology. And so I'd really like to see some studies of modern monoclonals in combination with antivirals and that includes antivirals beyond protease inhibitors. You know targeting the RNA polymerase for example. ANd then I also think that we need to be targeting both the virus and the immune system, together. There are studies now targeting each of those in isolation. But the next phase of this I think is studies of monoclonals and immune modulators. We also need to not just hang our hat entirely on this mechanism. A lot of people think that I'm dogmatic about this mechanism. I think that this is an important thing to chase down. And I really believe that this is probably driving a significant proportion of cases of Long COVID. But that can't come at the expense of testing all of the other mechanisms that all of you are aware of that we talk about as potential drivers of Long COVID. And so we should be launching trials just you know beyond viral persistence in parallel with trials that target viral persistence or in combination with them. So I'll end with a few acknowledgements. I definitely want to acknowledge the amazing study participants, many of whom you know have become like family members to our team. We spend a lot of time with them. They were very patient with us and we're very grateful. The study team who made this possible, it takes a lot of people to make a randomized trial possible and feasible, and really grateful to my team in San Francisco. Also to Aerium amazing collaborators we enjoyed working with them. Our funders, especially the Patient Led Research Collaborative. There was also supplemental funding for some of the scientific aims of the study provided by Polybio and the NIH, our patient advisers from PLRC Steve and Anisha, who were amazing to work with and just you know everyone else who contributed to making this possible.

[Gina]
We are grateful for Dr. Peluso for recording his presentation. Anisha Sekar was the patient rep for this study. However, due to our time and we'd like to end before 5, we are going to move directly to the next session which is, which Anisha will also be on. So we will get to hear from her and I am going to start by introducing Julia. However, we're wanted to say we're closing the webinar with a forward-looking discussion about the PLRC or patient-led research funding as a model for biomedical research. And I'm delighted to introduce Dr. Julia Vogel who is our moderator for this final round table named “Advancing Long COVID Research: The Power of

Patient Centered Science and Patient-Driven Funding.” Dr. Vogel is a contributor at the PLRC and a senior program director at Scripps Research. She's using her five years of lived experience with Long COVID and myalgic encephalomyelitis to conduct research that aims to address Long COVID and other infection associated chronic illness symptoms, through efforts such as the Long COVID wearable study and others. She has a PhD in computational biology and medicine and an MBA both from Cornell.

[Dr. Vogel]
Great. Thanks Gina. I am happy to be here to help moderate this panel. Super speedy. Anisha if you want to come on camera. Anisha Sekar is a patient researcher who has worked with several Long COVID and ME researchers including being a rep on that trial from Michael Peluso. And then we have three grantees who you've already met. Dr. Liisa Selin, Rashan Kumar, and Janet Mullington are all going to be part of this panel as well as Hannah Davis, the PLRC co-founder and co-lead who helped administer PLRF. All right. Is everyone? Yes. Okay. Great job. People just coming off camera. On camera, I mean. So, I want to start with Anisha., I would love to hear from you what you want future funders or institutions to know about your role in this process and how they could learn from this collaboration or build on it. Absolutely. So I think a lot of what I'm about to say is core to the PLRC and to PLRF, but involving patients end to end is extremely crucial. Not just in communicating results, but if patients are involved in setting the research question, if they're involved in study design, that means that we're focusing on big questions, big swings, things that are actually going to lead to treatment. And it's also ensuring that as the study is being built out, we are answering the questions that were we're trying to ask in the first place. So, I think the biggest thing is patients need to be fully involved and be co-creators from the very beginning, not just at the end. Love that. And then I'd love to turn it to the grantees. How do you think the PLRF model shapes research in Long COVID compared to traditional funding mechanisms? What advantages do you think this offers? And if you would like to give advice to other people who might have money to distribute for Long COVID research, we'd love to hear that. Maybe we'll start with Liisa. You're at the top of my screen.

[Dr. Selin]
Okay. So I mean I can personally speak it. It was a huge advantage to me in understanding the disease because I have the disease and I think you heard from Megan that because of that I have a very different perspective and I'm very fortunate and unfortunate because I am a physician, an infectious disease specialist as well as a researcher. So I also have the clinical perspective of the disease and I and I really think those are extremely important but on top of that it was very useful having the patients as part of our lab meetings and our our regular input to keep us focused and to give us information or to give their interpretations of what we're finding. And even though what we do is very basic science, as you can tell from my talk, what we do is really look at the immunopathogenic mechanism. And this is not just something trivial that I came up with. This was something in the back of my mind as I worked as a viral immunologist for my lifetime. And then finally I had that aha moment of seeing the T-C cell exhaustion and and luckily for me, unluckily for me, the place I saw it was in my own blood. I had just asked Anna to do an assay. It was well known NK cells and T- cells are not functional.

They don't kill well. That was known back in ' 84. And I thought, well, nobody's ever done the intracellular assay on it, so why don't we do that with a general stem? And she showed me my blood and I thought she'd forgot to add the antibodies. So that there's a tremendous advantage, yes, in patients being involved and having easy access to blood. They also are wonderful at advocating for us and also at recruiting more patients for us. So we have Rivka, we have Megan, but we also have Cynthia Adinig, we also have Giovani who just joined us. He's in college. He was knew some of the people. And then we have Andy Joliet whose son has it. And then of course Roshan's wife has it. So we all really appreciate it and it gives you a different perspective and respect when you're actually trying to do the bloods, do the research. Yes, I think and I and also from the reviewing point of view, I think your process is much more open-minded about what I was doing, which was taking off in a completely new direction at the time. Immunology was very poorly represented in the world of ME/CFS and then Long COVID. So yeah that's great very cutting edge right? Very yes so yeah I think I think it's a great model.

[Dr. Vogel]
Yeah thank you, love it. Drs. Mullington and Kumar anything you want to add to that about the strengths of the PLRF model?

[Dr. Mullington]
I would just like to say I'm very impressed and grateful for the work that the patient-led group has done and published. Some of those early patient reported symptoms and experiences were really amazing. To me as a sleep researcher seeing reports of increases in acting out of behavior during sleep increased experience of lucid dreaming things that might suggest a REM sleep process. So I think that really you bring insights, you know, with that private access to experience and being able to share that with researchers and clinicians, you really provide insights that can't be obtained in any other way. Love that. It's exceptional.

[Dr. Julia Vogel]
Go ahead, Dr. Kumar.

[Dr. Kumar]
Yeah, I'll just add I mean seeing all these talks, I, for $5 million or whatever that seems like a fantastic return on investment. I mean I know so a lot of these are sort of in the middle in terms of getting the results published but you know in a lot of diverse areas there's been progress and for for me personally I can say you know the process was so much smoother and so much less red tape and like Liisa said so much more open. I mean this is funding, an industry academia collaboration. There's no way we're getting an NIH grant from that especially, you know, coming from a different field. Now I have personal experience in this disease because of my wife and I think I've gained some perspective on that. But you know I don't think traditional granting agencies are going to value that at all. And you know company, we have certain capabilities that you know they would probably undervalue. I think for suggestions for the future, you know, I think this kind of model has the ability to act as a catalyst and you know, I think you already see it generating some data that can serve to, you know, form the basis for acquiring future funding from maybe more traditional sources. And if there's a couple of you know targeted areas that

you know donors would have, I think being able to have biomarkers that we can implement in clinical trials house is critical, and will really open it up and attract industry interest. Which I I think is really important, like like we have to make Long COVID, I I mean this in the best way, like another disease, like a regular disease that that industry works on that academia works on that the mechanisms that we have in place work to address this huge unmet need and and things like biomarkers and being able to run effective trials I think will.

[Dr. Vogel]
Okay. So, I am going to ask one more short question and then I'm going to ask all of you where you want to be, where you think we will be in the Long COVID field in 5 years? So, I'm giving you a second to think about that. I'm going to ask a short question to Dr. Selin and then Hannah, I want you to answer the five-year question next. Dr. Selin, I'm wondering if you think we need to concurrently treat with antivirals and immune modulators? This was a question from the audience.

[Dr. Selin]
Absolutely, that I do believe that. So I think you gather from the my model slide that I really think the instigating virus sets up the infection, but it doesn't have to be the virus that's causing the syndrome to persist or go on. And I hate to disagree with the last speaker, but. Healthy debate is good for the field. I think that it's a bit of a red herring trying to treat the persistence of Long COVID because it's reactivation I think is so tiny compared to all these other persistent viruses that we have in our body that are have been shown by many studies to being being reactivated. So I think if you do it's been shown that certain people will respond to antivirals but most ME patients for instance are on them for the rest of their life. So I really think you have to attack the disease actually on three levels. You have to attract– attack immune modulator antiviral and you need to give metabolic support. You need to be able to get antioxidants and try to help correct that oxidative stress, and there are very few.

[Dr. Vogel]
Sorry, I'm gonna stop you there because we're so short on time, but I love the triple therapy idea. Thank you so much.So we have like two to three minutes, but I really want to hear from each of you. Where do you expect the field to be in five years? Hannah, you go first.

[Hannah]
That's a great question, and I know that it's always a battle to maintain morale for both patients and researchers. You know this is such a long process, and it you know, for a patient being 5 years feels like forever. Cannot imagine you know what it has been like to be sick for decades. But I do think that we have made such incredible progress. You know, Long COVID is one of the most studied illnesses ever. You know, I have the Google research paper report on every morning. There's just a dozen new ones every day, because it's being studied around the whole world because everyone is affected. And I think the breaking point is going to be a really solid diagnostic and biomarker and then treatments. And I think we will see both of those definitely within the next 5 years. It may take a couple more, but I think that we're moving so close to that, there's a wide variety of possibilities. And also, you know, for better or worse, since we're

continuing to see Long COVID, I think we'll see a lot after this current wave. More and more people are personally affected by this. I've always said I think this is something that will affect every family, you know very very soon if not already. it's already surpassed rates of diabetes and I think just the scale of it alone you know means that research will happen but I do think it will involve you know more private funders and groups getting involved.

[Dr. Vogel]
Sounds great. Thank you for the hope. Anisha I'd love to call on you next.

[Anisha]
Yeah. I think we will have a lot more vocabulary around Long COVID. It is as currently defined very heterogeneous, and I think as time goes on we're already getting there but we will be able to separate out long co with this presentation Long COVID with this presentation. Which then leads to more targeted treatments and just a bit a better ability to understand Long COVID and its many presentations, as well as I think a greater ability to relate Long COVID to other conditions. I love what Dr. Kumar said about it just being another disease and treating it as such. I think as initiatives like this spread and there's more and more research, we're just going to gain a lot more ability to describe and relate Long COVID to other diseases.

[Dr. Vogel]
All right. The rest of you are going to squeeze into two minutes. Thank you. Dr. Kumar. I'd love to go to you next.

[Dr. Kumar]
Yeah, I hope we'll be at the point where we can be doing biomarker guided trials, even small ones, but I think where we're measuring a lot of stuff that will make a big difference. We have to acknowledge it's a very heterogeneous disease and you know if one approach works for 20% of patients, that's a huge win. I like I'm sure many of you are very concerned about what's happening to our biomedical research infrastructure but you know I think non-traditional approaches are even more desperately needed now.

[Dr. Vogel]
Agreed. Dr. Mullington.

[Dr. Mullington]
I agree and I think that we have an opportunity because there has been so much data collected and there are so many wonderful resources to mine and so much expertise developing in machine learning and approaches that will help us to really crunch that big data and make real headway. So I'm optimistic. It's a time that we might be able to really do individualized medicine in a way that helps to solve this very heterogeneous problem for many. And go ahead, Liisa.

[Dr. Vogel]
Okay. Optimism all around. Go ahead, Dr. Selin.

[Dr. Selin]
As you can imagine, after 50 years of ME/CFS, and I'm still here fighting, I realize this is I'm a I am a a glass half full person or I wouldn't be here. And this is an incredibly, speaking from the medical perspective, and research, a very complex disease. And that's probably why the medical system has ignored it. It's just been too difficult to understand. But we do have the technology now. We should be able to do it. And my time is up. So I think we should be in clinical trials within the next five years. I really really hope so.

[Dr. Vogel]
Sounds wonderful.

[Dr. Selin]
You need to get it trained in the medical training.

[Dr. Julia Vogel]
Yes. Absolutely. No, you're good. I believe I'm handing it back to Hannah now for a wrap-up. Yeah. All right.

[Hannah]
Thank you all so much for your time. Appreciate it. Thank you all so much. Thank you all for participating and attending our patient research fund webinar. We're very proud to have hosted this event that framed and contextualized the high-speed quality and impact of research prioritized by patients and supported with the knowledge of patient expertise. We hope this webinar underscores the importance of biomedical research that's grounded in patient expertise. Before we leave, I want to emphasize that PLRC needs imminent funding to continue our work past this summer. There are a lot of projects on our list that we see as vital to do, but need funding for. We'd love to do patient-led research fund 2.0, but need to raise a minimum of $2 million to do that. We'd like to increase the number of clinical trials and breadth of treatments trialed. Work on coordinating endpoints and outcome measures for clinical trials across groups. Identifying and validating biomarkers and diagnostics, advancing the patient prioritized areas of research that are very understudied but are very common in our population. We'd like to optimize our data repositories for sharing of PLRC generated data sets, since we have a lot of strong data and would like to re-release that in a clean format to the community. We'd like to foster studies particularly on cross-disciplinary research including HIV AIDS, autoimmunity, brain injury, connective tissue disorders, and mechanical basis spinal brain stem research. We'd like to expand our patient registry. We're hoping to enroll 15 or 1,600 patients by 2026 and do analyzing data on drug repurposing. And with that in mind, there are a few ways you can help us. Please consider donating (https://patientresearchcovid19.com/donate/) directly yourself, or monthly if you're able. Or sending our fundraiser to donors who are invested in Long COVID. And another way you can help is by circulating our menu of services (https://patientresearchcovid19.com/plrc-advisory-services/) to researchers, and other partners. We've worked with a wide variety of research groups, universities, experts across the world over the last 5 years and have a wide range of services we provide in that regard and the link to

those services will be put in the chat. But overall, we hope that this webinar shows a clear vision of what we can achieve and as always thank you for being part of our community. Thank you.