Itaconate Shunt Part 2

SPEAKERS
Janet Dafoe, Robert Phair

Janet Dafoe
Hi, this is Janet Dafoe again, with my series of videos with research updates on ME/CFS research. And today, I have the pleasure of having Rob Phair here again with us. And he is going to talk about...do a second part of his itaconate shunt hypothesis, which is very exciting. And I haven't seen this before, so I'm really looking forward to it. So just want to say that sometimes it gets really detailed. And some of you will love that, and others might have your eyes glaze over a little bit. So I just want to encourage you to just let that flow past you and listen to the whole thing, because you'll get the main idea, which is very interesting and exciting. So welcome, Rob, and take it away.

Robert Phair
Thank you, Janet. And I'm happy to be here too. And so let me share my screen to start.

Robert Phair
Those of you who have already heard part one of this presentation will remember that the whole project started in discussions between me and Chris Armstrong, back in late 2019, just before the beginning of the pandemic. And it's a joint effort between Chris and me and my group at Integrative Bioinformatics, as well as Ron Davis's laboratory at Stanford University. We've lost a couple of the people on this list to industry since the last talk I gave. But we're recruiting and hiring people to replace those experimental positions. We're funded by the Amar Foundation to our company IBI and to the University of Melbourne. And this, as Janet says, is part two of the interferon alpha itaconate shunt hypothesis for ME/CFS. So I'd like to start with a relatively familiar part of metabolism. Let's switch to that.

Janet Dafoe
So you have a new name for your hypothesis?

Robert Phair
I do. And that's because part two is the immunology part of what Ron always calls an immuno-metabolic hypothesis. And so we have to have recognition in the title for both the immunology part, the interferon alpha, and the metabolism part that's itaconate. So let's switch to the...All right, this will be familiar to many of you.

Janet Dafoe
You could make it a little bigger, you could expand it a little bit. Well, thank you. Yes, thank you.

Robert Phair
So, this is the citric acid cycle, or the Krebs cycle, or the TCA cycle. And as I say, many of you will already know this as the central part of carbon metabolism in all the cells of the human body. It consists of a cycle of reactions that convert citrate, to cis-Aconitate, to isocitrate, to what some people call alpha-ketoglutarate. And this diagram refers to as 2-oxoglutarate, and then on to other molecules that eventually get converted back to citrate. This cycle of carbon metabolism takes place in the mitochondrial matrix and is responsible for producing the reducing equivalents, mostly NADH that are used to drive ATP production in the mitochondrial intermembrane. The reactions that supply those NADHs are shown in red here. So IDH, 2-oxoglutarate dehydrogenase, and so forth, make NAD, or in one case, they actually make an ATP directly. And this is an important part of central carbon metabolism because it's here that carbohydrates, fats and amino acids - those are the constituents of proteins - are converted into energy. And that energy is then used to drive ATP production. So we are in effect, converting energy from one form to another. First as the foods we eat - carbohydrates, fat and protein - then into NADH, and finally into ATP.

Janet Dafoe
So ATP is the energy we need to live, and breathe, and exercise, and lie there reading a book, or have our heartbeat or blood flow through our veins, all of that requires energy, which is ATP, correct?

Robert Phair
Yes, of course, you wouldn't have that energy if you didn't first have the fuel that you eat as your dietary input, the protein, the carbohydrate, and the fat. So this is where that conversion takes place. How do you get from the foods you eat, to the ATP that you need to drive all the reactions that Janet just listed, and a lot more.

Janet Dafoe
Let me also say that without enough of that energy, it would affect every system in your body, not just your muscles for exercising, but every system in your body needs energy to work.

Robert Phair
Right, this cycle takes place in every cell type. And so every function that is carried out by cells of the body is driven by this, what we call the central carbon metabolism. Okay, now, what happens in the periphery of the TCA cycle? Well, we can look at all of them together. Here, make it a little smaller. And so now you'll see carbohydrates coming in from glycolysis in the form of pyruvate. And they get into the Krebs cycle by pyruvate dehydrogenase, forming acetyl-CoA. The fatty acids get into the Krebs cycle by coming through beta oxidation, which again needs CoA, and results in acetyl-CoA, which then joins to oxaloacetate to form citrate. And so this is two of the major inputs to the Krebs cycle. The others shown in green down here at the bottom are the pathways by which amino acids, that is the constituents of proteins, get into the TCA cycle. And so what we want to see now is a picture of what happens when the itaconate shunt is turned on. And that's here. So shown in blue now is the detour that's taken when the itaconate shunt is turned on. And just to annotate what is gonna happen in the second part of today's talk, this pathway, which turns on the itaconate shunt, is triggered by the innate immune system. So this is why we're interested in tying immunology to the TCA cycle, because that's how the itaconate shunt gets started.

Janet Dafoe
And the innate immune system is the immune system that you have when you're born, that is not B cells and T cells. It's the innate response that you get when a foreign something attacks you and you respond with your innate immune system, while you're waiting for the adaptive immune system, which is T cells and B cells, and other things to show up.

Robert Phair
Right. I'm going to talk a lot about that in a few minutes. But yes, that's exactly right. So, what we want to see first, though, is the consequences of the itaconate shunt. And the consequences are pretty extreme. Because the itaconate shunt in blue is skipping the red processes in the TCA cycle that produce NADH, that's the energy that's going to be used to make ATP. So you can see that if your carbons coming from citrate get to cis-Aconitate, and don't go on to isocitrate, but instead go to itaconate, you are skipping many of the important reactions of the Krebs cycle, and instead are making itaconyl derivatives like itaconyl-CoA and citramalyl-CoA. So now, those do get back to pyruvate and acetyl-CoA, but this set of reactions is very slow. And that results in the accumulation of these two CoA-containing molecules in the cell. And not only is one of them, itaconyl-CoA, a poison for vitamin B12, which is required in this pathway from branched chain amino acids into succinyl-CoA. But also, the other pathways that rely on CoA are slowed down by having it sequestered here in the itaconate shunt.

Janet Dafoe
What do you mean it's a poison for B12. What does that mean?

Robert Phair
That means that itaconyl-CoA results in nonfunctional B12. So, the final enzyme in this pathway, it's called MUT, because it's a mutase, is completely dependent on vitamin B12. And without vitamin B12, it can't run, so the branched chain amino acid pathway cannot run to get molecules into the TCA cycle. Okay, so now this sequestration of CoA in the itaconate shunt results in turning off the CoA-dependent processes, or at least slowing them down to the extent that you've lost CoA to the itaconate shunt. So let's turn them off and see what the consequences are. Well, now, you can no longer get pyruvate into acetyl-CoA. Because you don't have any CoA to make that reaction go. You also find that there are two breaks in the Krebs cycle itself. You can't get from OAA to citrate anymore. So you've broken and you can't get from 2-oxoglutarate to succinate anymore, because both of those reactions require CoA. And you'll also notice that whole beta-oxidation of fatty acids is gone. That's gone because you need CoA for every two carbons in the fatty acid that you're breaking down to get into the Krebs cycle.

Janet Dafoe
That means you get energy from fatty acids anymore.

Robert Phair
Yep, just like you can't get energy from carbohydrates. And you can't get energy from branched-chain amino acids. Now, fortunately, there are already enzymes in the TCA cycle, or in the mitochondrion that allow you to branch to cover this bridge between 2-oxoglutarate and succinate. And between oxaloacetate and the rest of the cycle over on this side. And I've talked a lot about that in part one. So if

you haven't seen that, you can learn that by looking at the YouTube video on part one of the system. There are consequences here besides just being able to bridge the gaps. And they are that the whole system is vastly less efficient, because you've lost several of the NADH producing enzymes. And you get back one, which you can learn about in part one. And the other problem is that this remaining cycle can only burn glutamate-type amino acids. Those produce ammonia, which is a known neurotoxin. But you still...you can make some energy, you just can't make much.

Janet Dafoe
That sounds like an ME/CFS patient to me.

Robert Phair
Yes, it is. It's important to realize, though, that these cells that are caught in this shunt are not every cell in the body. They are the ones that were nearby the original infection trigger. So the cells that were infected, as I'll show you in a minute, will die off because the innate and adaptive immune systems are succeeding in killing them off. But the hypothesis here is that those cells are not the ones that are keeping the ME/CFS patients in the disease state. That instead it is the cells that are nearby the initial infection and are becoming stuck in the innate immune response.

Robert Phair
Okay, so my cat is standing in front of my notes here. So this system can be repaired with the enzymes in the mitochondrian. And there's GAD2, that's Chris Armstrong's favorite enzyme. There's GAD, that's glutamate decarboxylase. And there's GABA transaminase and SSADH. All those enzymes allow you to have a cycle again, but it's inefficient, and it only burns amino acids, which in the brain is a really bad idea, because two amino acids, glutamate and GABA, are the major of neurotransmitters of the brain. So now, instead of making lots of ATP, you are making less ATP, and you are burning molecules that otherwise would be signaling from one neuron to another in the brain, which is probably where brain fog comes from, if this hypothesis is correct. So again, there are details about all this in part one. So this summary of part one leads us to part two. And there's a lot of biology hidden in these indications in the upper left that say the innate immune system triggers the itaconate shunt.

Robert Phair
So let's move on to the second part, which is here. And let's start with that initial infection.

Robert Phair
So here we have a cell that's been infected, and it could be infected by a virus, or it could be infected by a bacterium. And LPS is a molecule that's sensed by the innate immune system. And that molecule, that is called lipopolysaccharide, is a molecule made by bacteria. So either one of these molecules, the virus itself, usually the RNA or the DNA, is sensed by a toll-like receptor, a TLR. And there's another TLR that can sense the bacterial signal, LPS. And then what happens next is that you turn on the NF Kappa B (NF-kB) inflammatory pathway. And NF-kB is a signal that is usually inhibited in the cell by a molecule called Inhibitor of Kappa B, I-kB. And this complex that's made up of the viral or the bacterial signal is able to break apart that complex, by converting I-kB to a phosphorylated form, which no longer will bind to NF-kB. And NF-kB is a transcription factor that can drive the transcription and translation of many molecules, but I'll just show you three that typify the classes of molecules that are used. First is

TNF Alpha, tumor necrosis factor alpha, that's an inflammatory molecule, one of many that will increase the permeability of the vasculature so that cells of the adaptive immune system, T-cells and B-cells, and also cells of the innate immune system, like neutrophils can cross from the blood into the tissue where these infected cells reside. So that's one element of the inflammatory response. Another one is to secrete chemokines. These chemokines, like CCL2, make their way out towards the blood and provide a pathway, a signaling path, by which cells that are in the blood are drawn into the infected tissue. So that's the same cells we were talking about a moment ago, the TNF-alpha opens the door, and the chemokines inform the cells in the blood that there's an infection out here, we need your help. And then lastly, there's a molecule called CD80, which it remains on the cell that was infected. It's now on the plasma membrane, and it signals that 'I'm a cell that's infected'. So it's a co-stimulator of cytotoxic cells coming from the blood. And those cells will recognize CD80, and bind to the cell that's infected and kill that cell.

Robert Phair
So this is the inflammatory pathway, the first of two mechanisms by which the innate immune system responds to an infection. What's the other one? The other one is the antiviral state. And that consists of the same signals, the virus TLR and LPS TLR, same signals activate phosphorylation of IRF-7. This is another immune transcription factor, and it drives the secretion - well, first, the transcription and translation, and then the secretion - of interferon-alpha. So why is that happening? Interferon-alpha is not part of the inflammatory response. It's called the antiviral state, because it's passing out of the cell that was infected and entering the extracellular space, where it's going to talk to the neighboring cells that aren't infected. And it's going to send a message to those neighboring cells, which says, "There's an infection nearby. You are just the bystander cell, but to the extent you are able, we want you to get ready for this infection. And it's by getting ready that these bystander cells initiate the antiviral state. And the whole rest of the story of part two, is that the antiviral state in the bystander cells becomes chronic. And that by becoming chronic, it puts all those cells, the bystander ones, into a disease state that we think represents ME/CFS.

Janet Dafoe
And that's the itaconate shunt?

Robert Phair
No. So the itaconate shunt is the metabolic part, and it is turning on the itaconate shunt, but that's only one part of what makes it a disease situation, which is what I'm going to talk about next. Okay, so let's go to this next map.

Robert Phair
So in this interstitial space, in the extracellular space where that interferon was deposited, it's now going to signal to the bystander cells. And it does so by binding to the interferon-alpha receptor, which is in the cell membrane of the cells everywhere in the body. Interferon-alpha receptor is present on all cell membranes of cells that have nuclei, so not on the red cells of the blood, but elsewhere. And so once it binds to the receptor, which is called IFNAR, it signals through the JAK kinases, JAK1 shown here, to phosphorylate two other transcription factors, STAT1 and STAT2. This pathway that I'm starting you off on is referred to as the JAK-STAT signaling pathway. These two phosphorylated

transcription factors then bind to IRF9 and form this trimer, a three-protein complex called ISGF3. And this is the beginning of interferon signaling.

Robert Phair
So once you're here, and you've made ISGF3, you can now signal to the nucleus. This is all in the cytosol of the infected cell. Over here is the nucleoplasm. And if we now add the ISGF3 to interferon-stimulated genes, we can see that ISGF3 has to get into the nucleus, and then it promotes the transcription of many genes that are referred to collectively as interferon-stimulated genes, or ISGs. And these genes, these are all the mRNAs that are produced. There are lots more than this, I've shown 1, 2, 3, 4, 5, of them. And there probably are 100, at least, interferon-stimulated genes, but I wanted to just show the core of the hypothesis. So you can see that this pathway, starting at ISGF3, and turning on the transcription and translation of IRF1 here, then synthesizing IRF1, the protein, which returns to the nucleus, and turns on other interferon-stimulated genes. One of those is ACOD1, and ACOD1 is the gene that codes for the first enzyme in the itaconate shunt. So this is how the immune system communicates to the Krebs cycle, that I want you to start up the itaconate shunt. It does this, at least, it's theorized that this happens, because, by turning down the production of ATP and other molecules that depend on the Krebs cycle, the resources that the virus needs, or the bacterium needs, to reproduce become unavailable. And so you're slowing down the reproduction of viruses and bacteria, in order to give the adaptive immune system enough time to get up to speed and to kill off the infected cells.

Robert Phair
So, the point here is that starting up the innate immune response in neighboring cells, or bystander cells, allows the immune system to both turn on the itaconate shunt and turn on many other genes. One of those genes, as you may have noticed as we went by, is interferon-alpha. And so there is a positive feedback that's produced, and we think this is critical. So now, you have interferon-alpha being produced in the nucleoplasm, or the mRNA is being produced in the nucleoplasm. Interferon-alpha itself is translated on the ER membrane, and then is secreted from the cell out into the extracellular space. And you can see now we have a loop, in which we started with a little bit of interferon alpha from the infected cell. We started up a signaling pathway, and that signaling pathway ended up producing more interferon-alpha. Normally, in a cell, this will not go on forever, because the cells that were infected will be cleared by the adaptive immune system, and those cells will stop because they're now dead, thus stop secreting interferon-alpha. And so the neighboring cells will turn off this signaling pathway. And we're concerned, and we're working on this diligently, as Janet says. We are concerned that the pathways that normally turn off this signaling do not turn off in ME/CFS patients. So we're not exactly sure which ones are failing to turn off at this point. But we are...measuring interferon-alpha itself. And the idea here is that this hypothesis predicts a couple of things. Let's go back to the other set of slides from...

Robert Phair
And here, we've measured interferon-alpha in the plasma of CFS patients and healthy controls. And I have to admit that these are only preliminary data, and that the assay does cover the same range for both healthy controls and patients. But you can see that the median for the healthy controls is fourfold below the median for the ME/CFS patients. So the prediction of the model is that there should be some

more interferon-alpha in the blood for patients, as compared to controls. And this is, to some extent, borne out by the experimental data. This is a very sensitive assay. It's developed by a company called Quanterix. And Layla and Anna produce the set of samples to send to Quanterix to measure interferon alpha. This is-

Janet Dafoe
Could you say something about those two high ones on the healthy control side?

Robert Phair
Sure. This is a problem with these data. That is that these two individuals, even though they are not ME/CFS patients, have concentration of interferon-alpha in the blood, that's not very different from of our most severe patients. So, as Janet suggests, this is a concern, because we wouldn't want to try and distinguish between patients and controls, if some of the controls are going to be as high as our severe patients. But it is nevertheless an important distinction between the healthy control and ME/CFS data, that the medians are quite a bit higher in the patients than they are in the controls. We think that these two individuals are going to be producing interferon-alpha in their blood cells, not in peripheral cells. It's important to realize that these concentrations of interferon-alpha are vastly smaller and would be required to turn on interferon gene expression. So we think that the high concentrations of interferon-alpha that are causing damage are outside the blood and in the tissues. If, on the other hand, your blood cells like plasmacytoid dendritic cells are producing interferon-alpha right in the blood....then you very well might have these relatively higher concentrations of interferon alpha in the blood from patients or from individuals who are producing interferon-alpha locally in the blood, as opposed to peripherally in the tissues. So what we're going to do to address that question is to measure interferon-alpha in the cells of these individuals, in the blood cells, and see if these are just bad controls. In other words, you wouldn't want to use controls in which the blood signal is not coming from peripheral tissues, because these are hypothesized to come from peripheral tissues. So that's the direction we're going. The next-

Janet Dafoe
Could you also say something about those five little ME/CFS guys that are down low?

Robert Phair
So there, that's an additional problem, these are all really low ones, are down near the lower limit of detection for this assay, so it's hard to take these very seriously, because they are below the limit of detection. On the other hand, if we have a more sensitive antibody for this interferon-alpha, we might be able to push this limit of detection further down, and see whether those are really at this point, really at low levels, just like the values in the controls.

Robert Phair
So this is interesting. It's, as I say, very preliminary, but it does point to the possibility that ME/CFS has values of interferon-alpha that are on average higher than they are in the healthy controls. Another interesting plot, from the same data, separates the patients from the healthy controls by Bell score. Bell Ability Score is a numerical score that's given to an individual based on what they can and cannot do. And so if you're a very severe ME/CFS patient, and you have a Bell score of 0, meaning you have trouble doing any of the normal tasks of daily living, and if you're a healthy control, then you have a Bell

score of 100, which means you can do all the tasks of daily living. And again, these are the same data points, just separated by Bell score. And you see again, with the mediums...which are indicated by the horizontal lines in each bar, that there's a trend to move from very high Bell score to very low Bell score while you move from very low interferon-alpha to very high interferon-alpha. And the idea would be here that in the long run, we will see a straight line from high to low, from high over here, at severe patient, to low over here, at healthy control, and it will go through the medians for the other Bell scores, these all being scores for ME/CFS patients that are not as severe as 0, but are intermediate between there and healthy controls. So that's the experimental side of what we're doing at the moment. And our plan, to go back to here, is to...

Robert Phair
Is to ask the question, whether the pathways that are supposed to turn off the interferon-alpha positive feedback loop are actually doing that. And so we're going to measure the messenger RNAs for not only SOCS3, which is thought to be the major pathway by which the interferon signaling via JAK-STAT pathway is turned off. So we need to know whether it's SOCS3, and there's another SOCS protein called SOCS1, stands for Suppressor of Cytokine Signaling. And that's what it does, normally. But we're hypothesizing that the positive feedback loop is present in ME/CFS cells, and not in normal healthy controls who get an infection. So the pathway waits long enough for the adaptive immune system to get going. And then is turned off by one or more of these suppressor pathways, is sometimes referred to as checkpoints. And there are other checkpoints as well, here's the ZBED2 checkpoint. It's produced in response to IRF1 in the original JAK-STAT pathway, and ZBED2 is a kind of a negative transcription factor. It binds to many, if not all, of the interferon-stimulated genes, and competes with IRF1. So it turns them off - that too, could be a checkpoint. There is also a checkpoint run by a protein called USP18. And that acts much more proximately at the cell membrane to disrupt the interferon-alpha/interferon-alpha receptor complex. And so it turns off the pathway by acting early in the pathway. And there are other pathways as well, there are phosphatases that can de-phosphorylate these transcription factors. And there are...there's a molecule called IL10 that could turn off this pathway as well. And so it's important for us to be able to check whether this is the mechanism by which normal cells will turn off interferon-alpha signaling, and CFS cells, which we also have from our patient volunteers, turn off the signaling in normal cells and not in ME/CFS cells.

Robert Phair
We're thinking too that, if this positive feedback loop for interferon-alpha is indeed what's going on, then the experiments I showed you just a moment ago will eventually show us that there is more interferon-alpha in the blood than there is in healthy controls. And that that interferon-alpha is responsible for many of the phenotypes, or the symptoms that we see in ME/CFS patients. And if that's true, what we need to do to heal, or to break this positive feedback loop is to interrupt the signaling that I showed you in this pathway. There are already drugs on the market that can interfere with interferon-alpha, by binding to it as an antibody. And they also are...there are other drugs used to block the JAK-STAT pathway. So there are possibilities on the horizon, by which we could turn off the positive feedback loop. We always worry about the side effects, and they'd be significant if you had to have these drugs present all the time. For example, this interferon pathway is required to fight off viruses or other sorts of microbes that might want to infect your tissues. So it's important to emphasize that blocking this pathway would only have to be for a short period of time. Because then the positive feedback loop

would be broken, and you'd return, gradually, to the normal steady state. So that's our long-term hope. And that's the story that I wanted to tell you today with interferon-alpha and the itaconate shunt over here.

Janet Dafoe
Could you say a little bit about if you block it, it could stop your immune system from working?

Robert Phair
That's what I just said. You don't want to keep it blocked forever. But fortunately, when you have a positive feedback loop like this, you will...you can stop the positive feedback loop briefly. And you will end up back in the normal steady state, rather than in a long term requirement for the drugs that block the loop. So you turn it off, and it can't sustain itself when you remove the drug. And the idea then, is that by having the drug which blocks the loop for maybe a day, or maybe three days, then you will no longer have the positive feedback loop. And when you remove the drug, that positive feedback loop will not reoccur.

Janet Dafoe
So during that time, would you want to stay away from people?

Robert Phair
You'd want to do what other people do, who are suppressing their immune systems, for other reasons, like cancer chemotherapy, or even interferon treatments for other diseases, like hepatitis C. You don't want to suppress the immune system forever. That would not be an effective way to cure anything, you just invite all the other microorganisms that would like to live in a human body to do so. So it's the short-term treatment with a blocker of the interferon JAK-STAT pathway that might be useful. If that turns out not to work, then we're going to need to know which of these checkpoint pathways is not working. And that will require investigations on the molecular biology of SOCS3, or of ZBED2, or of any of the other checkpoints that I mentioned a moment ago.

Janet Dafoe
That's doable.

Robert Phair
Yeah, we have the tools, we're already able to specify which messenger RNAs we want to measure, and then we can use the tools of modern molecular biology to measure. So I was gonna say at the end that the importance of these low concentrations of interferon-alpha in the blood is something like the problem of ocean pollution. We treat the blood as the ocean, and the sources of interferon-alpha as the rivers of the world. Then, we think of interferon now as a pollutant, we can see it in the ocean, at very low concentrations. But we don't know where in the world, a river is supplying that pollutant. And so the concentrations are very low. And they indicate only that somewhere in the world, there is a lot of that pollutant being produced. And so, in fact, it's probably 10,000-fold lower in the blood, that it is in a place where interferon-alpha is causing all the reactions that you see on-screen right now. So that's why it's the low concentration of interferon-alpha in the blood is not a concern. It's not causing the disease. It's just an indicator that the disease is present somewhere in the body. Thank you.

Janet Dafoe
Wow, thank you. That's a lot. I wonder, could you say a little bit about how this intersects with Bhupesh Prusty's work on reactivated viruses.

Robert Phair
Absolutely. He and I have been corresponding on this since the September symposium at Stanford. And there are two ideas here. One is that the microRNAs that get produced by his reactivated HHV-6. These microRNAs are RNAs and they may be sensed as foreign by the innate immune system toll-like receptors, TLRs. And so instead of a positive feedback loop, you might have a constant production of molecules, microRNAs, that are sensed by the innate immune system and keep the innate immune system turned on. Another possibility is that those microRNAs that are produced by the reactivated HHV-6 could be targeting the portions of the innate immune system that turn off JAK-STAT signaling. So, it could be, for example, that this SOCS3 mRNA is attacked by the microRNA that is produced by reactivated HHV-6, and in being attacked, it's degraded because now it's a double-stranded RNA. And you no longer have a switch that turns off the interferon pathway. And so that could be, actually, the cause of the positive feedback loop. So those three general ideas are what Bhupesh and I have corresponded about since last September.

Janet Dafoe
That's interesting. So now I'm going to ask you a question that I'm pretty sure you can't answer, but everybody asks it. And that is, do you have any idea of the timeline for when there might be something for patients to try? Anyway, I know, that's a terrible question, but everybody asks it, so...

Robert Phair
I'm happy to answer it. And indeed, I've already answered it. Yes, it's possible to break the positive feedback loop with either an antibody to interferon-alpha, or with a small molecule that blocks the JAK-STAT signaling pathway downstream, most of which are FDA approved drugs. Then the treatment would not be far off at all. But we have to establish that this is the problem, that this interferon-alpha positive feedback loop is exactly where ME/CFS originates. We can't just go treating the disease because we have a hypothesis, we need to demonstrate that the hypothesis is ,experimentally, at least partially visible. And so that's why the experiments measuring interferon-alpha in the blood are so important. And you pointed out yourself that there are some inconsistencies in those data. And so we have to make sure we understand those inconsistencies, and demonstrate that in the patients, we see a consistent signal telling us that there is an interferon positive feedback loop going on in patients. Then we can go on and say, "Oh, this is a good hypothesis, it's supported by the data. Patients for other diseases are already being treated with interferon-alpha antibodies, and with JAK-STAT inhibitors. Maybe we should try one, maybe we should do a clinical trial for ME/CFS and these drugs." But we don't know that yet. So when we do, though, you're all going to be the first people who know.

Janet Dafoe
You probably want to caution people not to try this themselves.

Robert Phair
That's the question that you're going to generate when you say, how long will it be before we have a treatment?

Janet Dafoe
I get asked that every day by somebody. So...

Robert Phair
Yeah, of course. So this is my answer. Given that the hypothesis is correct. But I don't yet know that it is. We're testing.

Janet Dafoe
So I presume you would want patients to wait until you know that it's true before they try these things.

Robert Phair
Well, I'm not a physician, I'm a basic scientist. So I'm not going to tell anybody what they should do. But yeah, I mean, if somebody came to me and said, "What do you think is the best bet?" Then I would say not, "I think it would be worth trying an antibody to interferon-alpha or a JAK-STAT inhibitor. But I can't tell you to do that." And I would tell you that because this hypothesis is just that - a hypothesis - that I'm not going to recommend that. And I'm going to recommend against it until we know.

Janet Dafoe
Great. Thank you very much.

Robert Phair
My pleasure.

Janet Dafoe
You want to unshare your screen, so we can just sign off? So I thank you for joining us today. And thank you so much, Rob, for all the work you're doing, and your amazingly coherent descriptions of these very complex things.

Robert Phair
There's a lot going on in that system.

Janet Dafoe
Yeah, it's pretty amazing. And it's pretty amazing how little, you know, the medical profession understands about all this. So we're really making progress, you know, not just for ME/CFS, but for the understanding of metabolism and the immune system in general, it's going to apply to a lot of diseases, I imagine.

Robert Phair
It might well. Ron often points out the values for interferon-alpha concentration in lupus, for example, are in just a little bit higher than they are for ME/CFS patients in our data.

Janet Dafoe
That's really interesting. Well, thank you for all your work, and it's really helpful, and I really appreciate it. Thank you very much.

Robert Phair
I think the next time I give this talk, it'll be better than it was today. I'm glad you enjoyed it.

Janet Dafoe
I did. This is amazing. Thank you so much.

Robert Phair
Take care, Janet.

Janet Dafoe
I'll see you next time for part three.

Robert Phair
There's a part three?

Janet Dafoe
Well, I assume someday, there'll be a part three.

Robert Phair
I actually have notes on a part three already.

Janet Dafoe
Oh, great. Okay.

Robert Phair
Take care, goodbye.