Thanks to 'thefreeprisoner' from the Phoenix Rising Forums for transcribing the first part of Dr. Mikovits talk on Prohealth on Jan 22nd.

Annette Whittemore:

...happy to see that the ProHealth organisation was able to get this online so a lot of patients who are too ill to make it are able to follow this online.

First of all I'd like to that Rich Carson, I'd like to think ProHealth for putting this together and the HHV-6 foundation and Kristin Loomis.

It's a pleasure to be here and to have an opportunity speak to you about the recent discovery of XMRV in Chronic Fatigue Syndrome patients. Thank you for inviting Judy Mikovits today. We've made a special effort to learn more about the most exciting news in the world of CFS since the 1980s when major outbreaks of this disease were reported in several locations around the US including three small towns in Nevada.

These reports came on the heels of the discovery of HIV and AIDS. During this time Dr Mikovits was at the NCI working in laboratories that were actively studying this new virus which she began her doctoral program at George Washington University. In fact she wrote and presented her doctoral thesis on HIV latency, presenting it the same day that Magic Johnson announced he was HIV positive. The good news is that he is still healthy after all these years, having had the opportunity perhaps to prevent a high viral load from ever ocurring.

Jumping forward to 1989 brings a critical event to the life of our family. Our daughter became ill and suddenly we found ourselves in a black hole of medicine where noone seemed to agree on anything having to do with a disease that had been dubbed 'Chronic EBV'. The problem was that she didn't have EBV or even the antibodies that would have indicated that she'd been exposed to that virus. Like you, we sought answers but instead we found confusing and even nonsensical theories about her illness. And thus began the journey for answers.

All of us have various milestones in that journey. Our first and probably one of our most exciting milestones was meeting Doctor Peterson who at that time was rpomising that he would do all he could to help Andrea and to help this family. Our second milestone was really meeting Dr Mikovits. And that was a wonderful and perhaps prophetic meeting. We were at an

international conference and she had come along as a guest of the HHV-6 Foundation which she heard a very important talk about a set of patients who were developing a rare form of cancer. She took a leap of faith with us to develop a medical research institute when we asked her to come to Reno and the rest is history.

Judy brought with her a scientific passion for discovery of truth, and a curiosity, with well-taught skills from the laboratory of Dr Francis Rosetti, the co-discoverer of the first retrovirus HTLV-1. Judy has a fiery temperament and a heart of gold. And when she is not counselling CFS and cancer patients, she teaches students, devises experiments and travels to major conferences and universities to educate others about the intricacies of the scientific methods used to find infectious and replicating XMRV in the blood of CFS patients.

Before I introduce you to Judy, I'd like to ask three things of you.

First, please stay involved and advocate for your rights; medical treatment and adequate funding of research. Your congressmen and senators need to hear from you.

Second, please stay informed and educated. Listen critically to what is said and who is delivering the message. Are they speaking on your behalf?

And third, I want you to know that the WPI is going to continue its promised mission. We are not going to stop until we find the answers, but we can't do it alone and so we do continue to ask for your support and your help. We appreciate so much all the donations that have come in, all the amazing good wishes that have come our way, and we wanted to thank you today all around the world for letters that have come in to support this effort; that really really helps.

So now it is my good pleasure to introduce you to Dr Judy Mikovits, research director of the WPI.

[applause]

Dr Judy Mikovits:

Well thank you Annette.
I too would like to thank Pro Health and particularly Kristen Loomis and

the HHV-6 Foundation for sponsoring this event.

What Annette didn't say is that it was Kristen Loomis who put us together by asking me to attend that meeting in Barcelona, Spain. I never did get outside to see Barcelona but I saw some amazing scientists and physicians there in the room and in the meetings. So I'm also honoured that you came out on this day and I understand how difficult it is for patients to get here, and I appreciate all the calls and letters we have gotten around the world since the publication of this paper. It's amazing the response that we've gotten and we're just delighted. We work for you. The institute is a translational research institute. You can tell the architects drew this because they're making a lot of money. We don't usually drive Porsche cars [laughter].

But at any rate, this is what building looks like. It is three-quarters built and will be open to serve patients in September this year. So keep your eye out for the opening ceremonies and the ground-breaking there. We're excited to see patients.

Before we could see patients, Annette knew that she would need to start a research program because there were no bio-markers, diagnostics, treatments or anything. So we started looking with the patients there, with the diagnostic acumen of Dan Peterson. So I came up right after that meeting in Spain in 2006 and spent the summer just meeting the patients.

I'd never heard of the disease before then and it was just eye-opening to me to see how sick these patients really were and to try and understand really what the disease was. So my dear husband when I talked to him about Reno, all he kept saying was 'Reno? That's not by the ocean' [laughter] but I'm a patient advocate as well in Ventura County with a Cancer Support group with Bible Fellowship Christian Cancer Support Group and they were kind enough to let me go to Reno because I said "These people are much sicker than you." [laughs]

So I'm going to talk to you exclusively about the retrovirus XMRV. All of these slides will be uploaded both to the WPI website and to Pro Health so you can look at them later on.

I understand that I've left a lot of the detail of the science because when we see the science you start to understand the implications of this discovery in not only this disease but perhaps a number of old diseases

where we might find a new understanding.

So this is the Cleveland Clinic rendition of the retrovirus and of course the electron micrograph that accompanied the publication in Science.

So I'm going to give you a little history lesson. We're going to walk through the publications.

Interestingly XMRV was identified by Bob Silverman and Joe de Risi in just 2006 so just at the time that we were meeting each other and making these fateful introductions, this virus came out, where Bob Silverman who was an immunologist at the Cleveland Clinic was looking at prostate cancer patients where there was familial prostate cancer. That is, it's hereditary, but that it's hereditary in a funny way where maybe brothers-in-law or distant relatives would get it and not direct father-son, things like that. We actually had a case in my own family where my stepfather died very young of prostate cancer and it's an aggressive cancer, and when you get prostate cancer very young it suggests there's something else going on environmentally.

So he looked at a single nucleotide change of a varient in an anti-viral gene known as RNase-L. This gene, the protein's job is just to degrade RNA from viruses and protect you, and turn on the interferon response. But he found a variant in that gene where that single base change that is in about 13% of the population makes this enzyme only about 20% as active, so it dysfunctional because it doesn't work. So he hypothesised that maybe these men were susceptible to a virus. And he met Joe De Risi at UCSF, who had a technology which is basically a chip. It's like a chip with a bunch of information on it. The information is just sequences of every known mammalian virus, so 20-30 maybe 70 base pairs of every known virus from [considerable region?].

He simply took the DNA from these men and applied it to the chip, and the red part you see right here shows that it exactly matched [if you want to match in opposite (?)] the sequences from this particular virus. So when they took this out and they sequenced the virus there, they found that there were retroviral sequences in 10% of those tumours, and those with that particular variance, and that those sequences were most closely related to what was xenotropic murine leukemia virus. It's a gamma retrovirus and we'll talk more about that later. They just number them alpha, beta, gamma for convenience because the number are known.

Xenotropic means it can no longer infect mice. Xeno means foreign. So what we know from the xeno family of viruses is that they look like murine leukaemia viruses but they lack a receptor and we'll talk about that later, so that they can't infect mice. So he named this virus Xenotropic Murine Leukaemia Related Virus because it wasn't exactly the mouse virus. Clearly it was something different, suggesting that this might be a new virus. So his laboratory did a little more work in the next 2 years. Again we're just talking about 2007-2008. Usually it takes a year just to get a paper published.

So what he at first identified; they knew that the mouse family of xeno viruses would recognise and bind and actually enter the cell through this receptor. So it sees the receptor; it's called Xpr-1 and this is a calcium channel type, an ionic receptor. They don't know the function of it... this is called the G protein it has a particular role in sequencing. We know there's a loop right here or so, where the mouse virus has two or three amino acid changes and that's why the virus can no longer infect mice. That's one of the reasons we know this is not from a mouse. This receptor is on every cell in the body. So it doesn't tell you a whole lot about the infection or what cells would get infected in the disease.

So the next thing he did was, he molecularly cloned this. He used techniques to write the virus, the entire 8,000 base pairs and put it in a vector which allowed him to multiply it and make it an infectious virus. So he made this infectious virus, he didn't actually isolate it. Using the infectious clone, he then infected various cells and found that the virus integrated. It inserted itself into DNA preferentially at the start site of genes. And that's the part of the gene that turns on and off their expressions, so a lot of the differences you see in patients culd be explained by turning on and off the wrong genes when a retrovirus integrates.

So let's do a little bit of retrovirology 101. This is the genomic structure of a retrovirus. Now, retroviruses have an RNA genome. We have DNA genome. We have nucleic acids; our genetic information is packaged in DNA. This virus has a single stranded RNA genome that's present in two copies in the virus. So it first has to be reverse transcribed by the enzyme reverse transcriptase. So you have to take the RNA back to DNA and then the integrase gene there, shown here; this is a pall, so all a simple retrovirus will encode is the structural proteins, gag, pall and the envelope,

and then the enzymes. They don't have any extra proteins like HIV or HTLV-1 which are complex retroviruses and they write a bunch of proteins that regulate different parts of your body.

The good thing about this virus is that it's a simple retrovirus. There's less that it can do to interact with your cells to have those go wrong. So that's the first piece of good news. It's the first ever simple retrovirus known to infect humans. So we can think a lot about that as scientists and how it might cause disease.

So once you make the virus, you go from your genetic information I showed you in the last slide into the envelope protein which has two proteins actually in the surface unit. This is what binds to that receptor, and then the trans-membrane unit that sets itself into the matrix of the capsid. This is the capsid protein there, and that's known as gag, so you're gonna see capsid and envelope throughout this talk so you'll understand that when you have antibodies that develop, these antibodies are recognising these areas of proteins, and this is depicted here as that double stranded RNA nucleus, and the polymerase which simply writes the RNA into DNA and then packages it all up and leaves the cell.

Now let's talk about how you detect retroviruses, because that's important in thinking about how we found this virus and how we study it in the laboratory. So the viral life-cycle as I just described; once you have the DNA integrated into the chromosome, once it's integrated to the reverse transcriptase integrated it into the chromosome. It's there and it replicates every time your cell divides and your DNA replicates. So if your cell isn't dividing, theoretically it's just latent. It's just there in the DNA and it's not making more viruses, it's not making copies of itself. It's not infecting more cells. This is a good state if you have a retrovirus is to just shut down the transcription.

As Annette said when Magic Johnson was found to sero-convert, they found an antibody in his blood so that's very shortly after he became infected, so they were able to give him therapeutics to prevent the virus from making many more copies in his body, so theoretically the reason why he never got sick is because he is maintained on those anti-retroviral therapies as well as the immunomodulating therapies. He's kept that virus down so that he never theoretically will get AIDS. We'll talk more about that later.

Once the cell starts dividing and you start writing that DNA and transcribing it into all of the proteins we just discussed, the envelope will then package the core of the capsid there, that looks like this, in the RNA genome, that double stranded genome, and it actually uses your cell membrane, cholesterol and lipids to leave the cell then and look like that artist's rendition of the viral particle. So when you're looking for retroviruses, and there are only two known, are the HTLV-1 family -- there's a one and a two -- and HIV, the human immunodeficiency virus.

As Annette mentioned, Frank Ruscetti discovered this virus and reported it in 1980. At that time there was no PCR so he couldn't look for an infected cell by a sensitive method so he looked for that enzyme reverse transcriptase because reverse transcriptase is only in retroviruses and not in human cells, so it's easy to look for the activity of that protein that would then transcribe and make the virus. And sometime maybe if you've learned the history; it's amazing the small small signals they found in the early days to describe the virus.

But then you're going to do what we call a western blot which is to run out the proteins of a cell on a gel electrophoresis and just blot it and look for antibodies -- and we'll show you those later -- for the viral proteins and test for specific antibodies to the envelope and the gag proteins and just look for the presence of virus in infected cells.

The first thing you do clinically is you look for serology -- that test that shows you that your system is making an antibody to that virus. That was the test that Magic Johnson got. You have a virus in your body and your immune system's job, to distinguish self from foreign. So we know this is foreign because you have made an antibody to it and then finally, it's rarely done clinically, to identify HIV or HTLV-1, is to isolate the virus and actually purify it in cell culture.

So that leads us up to the next paper. So after the first bit of work that Bob did in describing this virus, there wasn't a lot of excitement about it in the scientific community, because they didn't know that it was an infectious virus. It was just sequences in prostate tissue tumours, and it wasn't meaningful to the scientific community because we all have sequences of viruses in our body as we all know, maybe as much as 15% of our genome is made up of viruses that are silenced by our immune system so that they can't be expressed.

So the work that we did then generated a lot of excitment. What we did was we detected this infectious retrovirus and showed that it was infectious in the blood cells of patients with Chronic Fatigue Syndrome. We're going to walk through exactly how we did this to show the virus.

At first we did PCR because at the time this paper was done, the only thing that was known was Bob Silverman's specific PCR technique. So we had not validated or identified any antibodies. It was not known that it was a pall virus, an infectious virus. So that's what this work was, it was serendipitous really, that we happened to have the patients who had this virus because if we did not have a well-identified cohort of CFS patients and we were just looking at the general population, retroviruses aren't highly expressed in the general population. HTLV-1 is 0.2% in the US population, and we'll talk a little bit more about what that means too. Retroviruses are not ubiquitous. It's not like EBV and CMV where everybody has them.

So we had these well-characterised patients who had been sick for many years. I think it was a large part of why were able to isolate this virus.

We'll start at the beginning and that's the cohort, who they are. When it came to the Institute, what we talked about was really important was having a repository of samples from all of the patients so we could look at the RNA for their gene expression, at the DNA for maybe what was different about the genetics of some of you that might make you sick.

Then we look at the plasma for proteins to see if we could identify immune modulators called cytokines that tell your immune system and tell your brain how to function. So we made these samples across RNA, DNA, protein and plasma, and then a culturable cell, so we kept some frozen such that we could grow them up and make more of them any time we wanted of your peripheral blood mononuclear cells; that's your white blood cells.

So we used patients who came literally from around the world and this was actually not correct in the Science paper because I didn't know there were international people in the repository at the time. When they come to Incline Village it's assumed that they are from Nevada, and when we decoded this over the Christmas holidays we found 12 or 15 states, the UK, Ireland, Germany and Australia as well. So we had both international and people literally from all over this country, not necessarily Reno,

Nevada, where the associated outbreak that we know occurred there in the early '80s.

So the inclusion... all you had to do to be a sample in our repository was have a CDC diagnosis of Fukuda criteria or the Canadian definition diagnosis which is more stringent for various immune defects and inflammatory defects. Regardless of severity, the samples in the repository are from people aged 19-75. We don't have any whole bodies yet of people; though people do offer to donate whole bodies, however I don't think we need them at this point [laughs].

The study characteristic, like the disease was 67% women, reflecting the gender bias in incidence of CFS. The mean age was 55, but some of these people had been sick since they were children or early 20s or early 30s so they had a long haul with this illness.

The 218 control samples were de-identified samples so we don't know who these people are. They came from two places; they came from a medical practice in Reno, they came from a doctor identified people as healthy and these were collected from people before I came to the University in 2007, and they were looking at the immune systems of healthy people to identify some of the dunctions so we were able to use those samples under IRB approval. There is also a paternity diagnostic company in Reno where they get samples from all over the world from mum and dad, so we tested from those 100 or so samples too, so we were at least able to zip code match. We have regional areas for the geographic location so that it was matched for location.

This is a PCR gel; I simply run them out for electrophoresis and that gives you a different size so you can look at the exact size of the fragment of DNA that you're looking at. Again, this was done by Bob Silverman who is our collaborator in the study. Today is actually the first anniversary of January 22nd when I called ... we saw some of these data right after the Christmas holidays and we had promised Bob for a long time that we would look at this because RNA cell is a major defect in our patients, whether it's underactive or overactive, something is wrong with the RNase-L pathway in CFS patients according to decades of research.

We promised Bob that we would simply look, although we had done a micro-ray technology and we had not found the virus there, we had his specific primer pairs so we could go in and look for that gag structural and

that envelope gene so that we could see viral sequences in the cells that could make viral proteins theoretically. What we found was that 67% of the patients we looked at, we could find sequences in both the gag and the envelope gene or just the gag depending on the virus life cycle at the time. This was astounding because we only found the sequence in 3-4% of the healthy control population. It's also interesting; I said 68 out of 101 patients. On some of these patients we looked...

Transcription by Kim

Judy Mikovits
Section 2 (Video #1: 27+ mins to 40:08)

On some of these patients we looked three and four times for the DNA in the unstimulated cells. So this is just that pellet that I made when I sorted all the various samples. I just held one as white cells so that I could make DNA later or RNA later, depending on the technique I wanted to use downstream. So, it's important that this was in 68 out of 101 samples. It was 68 out of 101 patients and it clearly says that in the paper. So, at any given time, depending on the viral life-cycle, we might not find this virus in the unstimulated group (inaudible). And I give you the example of that is: follow this patient 1118 throughout the talk and you'll see that this patient, if you only use sequences, would have been called 'negative'. So, we were concerned because PCR is a technique that is fraught with contamination. If you're looking for a needle in a haystack, just a few sequences in a million bases, you might make an error in your enzyme and it might put the wrong base in there.

So that…Jaydip Das Gupta in Bob Silverman's lab, cloned and sequenced three of these patients – and that's shown here – and what it's intended to show is: If you compare the isolates that they had from the 3 prostate cancer cases, where they had actually cloned these, you can see, if you compare it to the reference strain, known as VP62, that's the reference strain of what this virus looks like, the CFS samples here were clearly different, but they were highly similar - 99.7% - there were maybe 8 bases different across the entire 8,000 base pairs. So, this virus isn't like HIV theoretically. It's not changing. We don't find quasi-species in patients when there are lots of different

viruses, because HIV mutates so much. Therapeutically, that's something that we can take advantage of and suggest that it might be easier to develop therapies because the virus is going to be largely the same.

So, Rachel Vagny, my former student at the National Cancer Institute – I asked her if she could construct what is called a phylogenetic tree of this virus so we could understand where it came from (hopefully). And so that's shown on the next slide. And what a phylogenetic tree is - is you take all of the sequences of all the Murine Leukemia viruses - they're called Ecotropic viruses – all the families of virus that they've ever identified, Mason-Pfizer Monkey virus, all the sequences, and you put them into the computer, and then you put into the computer at the same time the sequences of our 6 isolates – the 3 prostate cancer and the 3 CFS isolates that we had at that time. And you do what's called 'blasting'. You ask the computer to find similarities. And when it doesn't find similarities, you get what's called a new branch on the tree. So, clearly, these diverge here, and we don't know when that is in time, but these data suggest that the prostate cancer – that XMRV both in prostate cancer and in CFS – form a new distinct branch. That it's a new *human* retrovirus. It doesn't have any of the sequences of mouse in it. And when we blasted it, also we did the same thing against the human genome - because I told you, we have a lot of endogenous viruses that don't actually come out of our bodies as infectious particles – we blasted it against the human genome and found that it did not match any sequence in the human genome. So, it's clearly a foreign, exogenous virus that can now, theoretically, be infectious. And that's what we'll show in the next slide.

So, here are our sequences. And you can see, they're clearly not contaminants. We didn't have this – we weren't working with this in the lab, actually, at the time. But we didn't have this, and maybe spread it through the sample in any way. It was there – clearly different isolates. We now have more than 170 isolates, because we isolate from every single patient in all of our studies. And we're actively looking for funds and going to sequence those viruses because it might give us clues as to some of the differences in what we see, maybe something, you know the

various symptoms, because CFS is quite a heterogeneous disease.

So, at any rate, we next went to – I'll summarize that – So in summary, what is XMRV then? These data suggest, at this point in time, we have sequences related to XMRV that were not found in any mouse strain. So, it's a new human retrovirus. The origin of XMRV remains unknown. We don't know how it got into the human species. We don't know how long it's been – 40 years is the guess of John Coffin, who is a mouse retrovirologist working on these families of viruses for more than 40 or so years. And that XMRV is not a mouse virus – clearly from these data. So it's a new human retrovirus.

So we next asked: Could we find those proteins I mentioned? So we took advantage of.. Sandy Ruscetti, Frank Ruscetti's wife had been in retrovirology as long as he has, but because they didn't want to work on the same thing, men usually get the credit for what women do, so Sandy worked on mouse viruses and Frank worked on human viruses and I don't think they actually ever published together. But we were thinking about it and saying: None of the reagents that were out in the world, so far nobody had found viral proteins from XMRV, even though it had been discovered 2 or so years earlier. In January we started looking. So Sandy had saved a box of antibodies – this is really a tribute to the value of your tax dollars going to basic research – because they created this mouse retrovirology program and put a lot of money into trying to understand – if you can understand how viruses cause cancer in mice, you might understand how it causes cancer in humans. And this was in the late '70s and early '80s. And somewhere in the early 2000s, they were going to throw out all of these reagents that they developed and Sandy said, "No, I'll keep them in my freezer." Frank always says that the reason they're still married is because Sandy never throws out anything. So, at any rate, she gave us these viruses, I mean these antibodies, and we screened our samples there for protein in our samples. So, we looked at the activated peripheral blood mononuclear cells. And what we do is, we stimulate these to divide, and add T-cell growth factor, or now known as IL2, which was actually the discovery that Frank made that preceded the identification of the first human retroviruses. Retroviruses grow

and divide in cells, so you have to divide the cells in order to get the virus to replicate to levels that you can see with the technology of the time. And that's important in this study too.

So, what we've got here is we looked a number of her antibodies – these are all family members of the virus – this particular antibody which you'll hear a lot about is a spleen focus forming virus. It's a mouse virus that causes various diseases including a neurological disease and erythroleukemia – red blood cell leukemia. So, its envelope is both a neurotoxin and an oncogene. It causes cancer and causes toxicity. So this virus itself – she had this antibody that was highly specific. It recognizes all known polytropic and xenotropic viruses. We hypothesized that it would recognize this virus and clearly high levels in some patient's cells, but not in others. Interestingly enough, if you look, and use a panel of antibodies, this is a gag antibody to a gag protein I showed you there that structural gene and this virus, this antibody is a polyphone virus that recognizes the entire MULV. And you can see when you use a panel of antibodies to the viruses, essentially everyone, 68% now of 50 people we tried just one time, you could see their proliferating blood cells. You can see evidence of viral proteins.

So we next asked if we could see this in normal cells, because of course you want to make sure that it's not in normal people. And you can see clearly here in the 24 normal donors (now up to 60 or 70 that Frank's done) at the NIH clinical center where they have a good donor program – they're all negative. So, these proteins, these viral proteins are expressed specifically in the CFS patients and not in normal donors.

So we next asked if we could transmit that. Is there any evidence that it's an infectious virus? So the first thing we did was we took plasma – so that's the plasma, the liquid off the white blood cells there – and we took their plasma and [this becomes essentially the key to the whole study] we co-cultured it. We simply put it in a flask with the cells known as LNCAP and that comes from lymph node-cancer-prostate. So this came from a lymph node of a 62 year old man who had metastatic advanced prostate cancer. And these cells grew by themselves in the laboratory so that you could use them as a tool for studying prostate cancer. And, in

one of my lives, I developed prostate cancer drugs, because, when my stepfather got ill, I became interested in prostate cancer and had been working on this. So, I knew LNCAP was also deficient in RNase L, and the type one interferon pathway. It had no interferon response. So, we always look for biological multiplication of the virus instead of the multiplication you would use with PCR. So, actually replicate the virus or multiply the virus in cells. You have to find a cell that will grow a lot of virus so that you can study it. So we took that plasma from all of these patients you see high levels – now 84% of the plasmas contain infectious virus that we could not see. I sent all of these plasmas to Bob Silverman and he said, "Sorry Judy, I don't see the RNA of the virus" there when he looked for the two copies of RNA in the particles which suggested there were very few copies of actual particles of virus in these cells. But again, we could transmit it.

And the next question we asked is: Is this a whole virus? Is this an infectious virus? Kun…Shima, my friend at the NCI who is an expert in Electron Microscopy, did this electron micrograph for me, and what you can see here is the budding of a virus from the cell. It shows you again that it's not a contamination, it's actually a transmission, because you've got a budding particle. And that particle is called a C-type retrovirus, because in the old days, when we used the word, they called them 'C' but they changed the name to gamma, but we're old-fashioned, so we keep the 'C' type. [Ends at 40:08 in video #1]

Judy Mikovits talk Section 3 (Video #1: 40.08 to mins to 55.40: transcribed by Froufox)

Section 3 (Video #1: 40.08 to mins to 55.40)

And what you can see here, characteristic of a gammaretrovirus, you can see this budding - remember I showed you it takes the cholesterol and buds itself out of the cell to form the outer membrane. And heres that capsid that encloses where the viral RNA is, to protect it. So you can see both immature particles and many mature particles in those LNCaP that have just been

exposed to patients' plasma, showing there is infectious virus there. So the next thing...so we were pretty happy with this and we sent it off to Science in early May of last year, and they came back to us and they said, "We're 95% convinced, but show us an immune response...if this really is an infectious, non-self virus, not an endogenous virus, your body will make an immune response."

So again we went to Sandy Ruscetti and um this part was funny too because we were struggling to do this, because you don't want a whole virus infected cell, you need to have just a part of the virus in order to get the noise out of there. And what Sandy had developed when she was studying the spleen focus-forming virus was this antibody again to the envelope protein. And she expressed it on the cell lines - used two cell lines. This is a mouse b-cell line that expresses the erythropoeitin receptor (its just for red blood cells), and when she co-expressed the envelope, you see high levels of the envelope on the surface of these cells. So we took these cells and put them in whats called a flow cytometer where a laser will see the fluorescently tagged antibody on the surface of the cell and count the infected cell as it runs through the instrument, the channel and single cell. So you can see that the cell line went out the envelope protein being expressed, you see the white and the black are superimposed showing that theres nothing reacting specifically with that. If you then take that antibody I showed you, to the envelope, its called 7C10, and expose the cells to it, they all light up, virtually 100% of these cells have the antibodies that are recognising the cells with the envelope protein. If we then take a patient sample and do exactly the same thing, you see there theres an antibody, this is for patient no 1104, thats one of the sequences we have, and there it is, theres the immune response in the plasma showing now we have an infectious virus with particles that can exogenously infect and is non-self.

So, the next step in what happened in the literature is work in prostate cancer again. So this comes from the lab of Ila Singh, whos an MD PhD at Utah, and she was looking at XMRV in malignant prostate cancer tissue in the tumour cells. One of the other reasons why the oncologists in the cancer community weren't excited about Bob's discovery of XMRV sequences was

because when they looked at those, they only found them in the infiltrating stromal cells - the microenvironment. But those of us who think a little deeper than most oncologists about cancer, know that 50% of all tumours are actually your immune system, your white blood cells going in to try and clear the cancer because thats their job is to recognise tumour cells. So we werent concerend, we were excited that it was, and it made sense to us that it wasn't the tumour cell itself harbouring the virus, but the immune cells that were inside the tumour.

But Ila showed that XMRV WAS present in the malignant tumour cells and that it was associated with that high grade tumour, that tumour that my stepfather died of, that you get younger and they get really sick really fast. And what was different in the advance in her study is she developed an antibody specifically to XMRV, to the whole virus, another polychromal antibody. And she showed that she could recognise with that antibody, in whats called Immunohistochemistry when you send a biopsy to the lab, they look at it, at a tissue block. So she did that and she showed that 23% of the prostate cancer tissues she looked at had a protein to XMRV, a lot like our study but she saw a lot less DNA sequences than she saw proteins. So this paper came out about a month before our paper but we knew about it from about mid summer when we first met.

So again in her study, the limitation in her study, was that again that there is no evidence of the infectious virus that I just showed you. So we had evidence of infectious virus in CFS...can we see evidence of infectious virus in prostate cancer? So Frank did this, this is again that antibody, looking for the antibody in the patients. And here he used, this is called a prostatic secretion, so they're just looking at the prostatic secretion and when they had a person who had sequences of the virus, positive in the prostatic secretion, you can see there that there are antibodies in that patient, so that patient is infected. In an XMRV PCR negative patient we don't see antibodies, so that person is unlikely to be infected with XMRV. And again in the plasma of this integration here, so that now they have actually found in this patient exactly where the virus integrated into the cell, and that patient has a significant amount of antibody. So in prostate cancer no-one had ever transmitted virus and shown that it was

infectious that way. So I show you the exact same study where we took the plasma from the prostatic secretions there and found high levels of the virus when we put it on LNCaP, showing now in both prostate cancer and CFS, XMRV is an infectious virus. And in a significant portion now they are finding in prostate cancer patients.

So why bring that up today, is because if we look and we do a summary table of the technologies that I showed you that we used to find the virus, what you see is that patients here in red are clearly infected when you look at plasma antibody responses, and you look for tramsmissions through infectious particles in the plasma, you can see the red patients both in the prostate cancer and in the WPI patients. These patients were PCR negative, I bring back to you 1118, but we found plasma transmission of that virus that I didnt point out, pardon me when we passed that slide...but ALL of these samples were negative when you did the most sensitive PCR that Bob and everyone developed in unstimulated cells. So those white blood cells, fresh out of the body, not dividing...very low copy numbers of this virus, but clearly these individuals are infected.

So going back to the literature now, two studies have come out since then, and one was in October, right around the time our paper came out. And this was from a German group led by Norbert Bannert and he found a lack of evidence for the virus in over 580 prostate tumour tissues, when he used the sensitive nested GAG PCR techniques that me and Bob and everyone is using right now. And he had developed his own ELISA which is looking for an antibody in the sera - its a similar test to what I showed you for looking for antibodies to that. And he couldn't see any of the evidence of the virus in those sera, and so he concluded, and they concluded that XMRV was not in prostate cancer. And then earlier this year, a similar study came out by a group in England that showed a failure to detect XMRV in CFS. And they looked at 186 DNA samples and they did nested GAG PCR and they found nothing.

So what could be the reasons for the discrepancies in these studies and what we've shown you in the studies of Ila Singh. So first of all, the prevalence of XMRV, thats the distribution around

the world, is unknown. The studies that we've shown you today is all we know about XMRV prevalence - that its in the US and in several hundred people including those with both prostate cancer and CFS. But I remind you that retroviruses are not ubiquitous, they're not everywhere. The sensitivity of the assays in these studies were not the same because both of these studies didnt rely on **???**, they relied on PCR, they didnt look for infectious virus. Of course the Bannert group didn't know our study because they were under consideration at the same time. And then also that XMRV has an extremely low copy number that I showed you, that even if it is there, you could miss it by these sensitive techniques. And mostly importantly, and something that didn't occur to me until I saw all of this data, was that we don't know anything about the viral reservoir of XMRV. I assumed its lymphocytes because thats what I know about HIV and HTLV1. But what if the plasma virus was coming out of the tissues and then the cells that were actually in the peripheral blood were not the main reservoir of the virus? What if there is another tissue reservoir? We don't know what that is, so these are all possible explanations for why we saw it, and we see a lot of it as you see in the plasma of these people, not a lot by copy number, but certainly there is infectious virus there. So thats what we're thinking.

So if you look at data that suppoorts these arguments, what you will see is the distribution here of HTLV1. Now HTLV1 infected people are 10-20 million across the world, and I bring up this one point that HTLV1 causes a neurological disease known as HTLV1 Associated Myelopathy...they have trouble walking and balance and almost like a paralysis looking disease. And that occurs only in about 20% of the infected individuals. And then of course HTLV1 was shown to be causative, satisfied Koch's postulates as we know them for viruses - for an adult T-cell leukaemia, and this is a very aggressive leukaemia and the mechanisms for how it causes that are still largely unknown. But at any rate 10-20 million people are infected, but you see very few - only sporadic cases occur in the US or Europe and the US incidence is only about 0.2%. They dont even test for it in the blood supply because its just simply not a problem in America, its endemic in the regions that are shown here today.

And the second argument that supports maybe whats different between these studies is the transmission from the actived PBMCs .. so if I take the white blood cells, some of which where I can't see virus and just put them on LNCaP, I can transmit the virus to this indicator cell-line that has shown you because its defective in RNaseL (theoretically because its defective in those, but we learn more about it later), will amplify and replicate high levels of the virus. So there are scientific reasons why there are differences between these studies, but I dont think there is any doubt that XMRV is a new human retrovirus, and since both HIV and HTLV1 are associated with neurological diseases and cancer, and now we have associated them with a neurological disease and cancer, that this a real human pathogen.

So recent publications after those publications (I'm just walking through the literature off the last few years) might give us a clue to the pathogenesis - how XMRV might cause disease. So this paper by Steve Goff's lab shows that XMRV establishes in an efficient infection, and spreading infection, thats enhanced by transcriptional activity in prostate cancer cells. And what that means is, I told you the receptor is on every cell of the body, but clearly every cell doesnt have the machinery necessary to replicate the virus to high levels. In fact we see that the peripheral blood mononuclear cells really don't, and thats why we dont know where the tissue reservoir is. So he simply infected a lot of different cell-lines and he found that the expression was very very low level except in essentially one cell-line and thats LNCaP. So we got very very lucky in that this was the only cell-line I thought about as an indicator cell-line....we could have screened the hundreds of cell lines I know of that we do regularly when we're looking for viruses because if you can't grow it you can't study it.

So LNCaP turned out to be really serendipitous and I think the key technical advance to being able to make that discovery, its just clearly luck. He showed that LNCaP responds to androgens, I told you it lacks interferon and RNA cell anti-viral responses, and I'll show you whats called the promotor, the response elements, that might give us a clue as to the pathogenesis. And then Bob Silverman's lab showed the same thing, he showed that androgens stimulate transcription which is the replication and

division of the virus. So here's a clue to the disease, because we know the only two diseases so far that are associated with this retrovirus are prostate cancer, a hormone responsive disease, and CFS, one thats thought to occur primarily in women. Interestingly that I didnt say that I knew is LNCaP is androgen responsive, so you can make it do a lot of good things and thats why we use it in drug development for prostate cancer. (ends at 55.40)

Transcribed by Sproogle

...the response elements that might give us a clue as to the pathogenesis and then Bob Silvermans lab showed the same thing. He showed that androgens stimulate transcription (the replication and division of the virus). So here's a clue to the disease because we know the only two diseases so far that are associated with this retrovirus are prostate cancer (a hormone responsive disease) and CFS (one that's thought to occur primarily in women).

Interestingly that I didn't say that I knew is LNCaP is androgen responsive. So you can make it do a lot of good things and that's why we use it in drug development for prostate cancer. So lets look at I showed you that organisation of the gag col and envelope of this simple retrovirus. This U3 region is highlighted because this is sort of the on/off switch of the virus. This turns it on to make more of the particle in your genome so this signals your cellular machinery to start making more virus and what Steve Goths lab showed (and he graciously gave me these slides about mid summer) was that there's only three responsive elements that turn on this virus that he can find so far.

Two are called glucocorticoid response elements and their shown here. When a protein actually recognises that exact sequence and sits down it tells the virus to turn on replication. And so interestingly enough, what turns on the virus? Hormones. Progesterone, androgen receptor and testosterone and we don't know all the other hormones. There are a lot of oestrogens and oestrogen like compounds even in our environment these days which might tell us maybe there's an oestrogen compound that's not a naturally occurring oestrogens in a plastic in the environment that is actually turning on the virus.

So we don't know all of the things that turn it on at this point. And the

other thing that turns it on is cortisol. So what is cortisol? It's the stress hormone and so right there your turning on the replication so it's an on/off switch for the virus with the stress response. When your told that you respond poorly to stress there might be a reason for that if your replicating a retrovirus! (laughs).

Sorry I shouldn't laugh.

So then we went back into thinking about this virus, we thought about the clinical research findings that had occurred throughout laboratories around the world throughout the years. What it mentioned in part was we know that CFS is a multi system disorder (and in Spanish I say sequelae) but there's lots of inflammation going on, you have allergies, multiple chemical sensitivities there's a lot of inflammation and increased numbers of activated T cells and the production of these inflammatory molecules I mentioned known as cytokines and kinokines. Also a key dysfunction in the immune system of CFS patients is this low natural killer cell activity and sometimes numbers.

The natural killer cell has two jobs in the body, kill tumour cells and kill virus infected cells. In CFS it's long been recognised (I think first identified by Nancy Klimas and her colleagues more than 20years ago) that natural killer cells in CFS patients don't function normally although the dysfunctions not known, but that again gives us a clue to the pathogenesis. So this suggested to us that this chronic infection with a retrovirus (retroviruses are associated with immune deficiencies) might lead to the creation of actually immune deficiency that has patients succeptible to opportunistic infections and more likely to develop cancer.

So I've schematically drawn our hypothesis on the next slide and I basically just lifted the graph of what happens in HIV and changed it to what we know happens and changed it to all the data that we have so far. In HIV what happens is that there's an early infection, the green line is actually the plasma viral load and it goes up in a spike. This might be a flu like syndrome or it might be nothing at all, you might never know that you were actively infected at this point and get sick. But then you have multiple other infections, stress hormone, advance inflammatory responses that cause these various spikes of the virus throughout a time course which we don't know.

I've heard the incubation period of this virus is 21 days. We don't know anything about the incubation of this virus we've just discovered it! So at any rate, all these events operate to set the viral load higher because every time you divide a cell, that your white blood cells, the cells in your immune system and actually our paper shows its the TB and NK cells are infected. Those cells are getting infected, more and more and more of them and some of them are long live memory cells that you need or they're going to the tissue then and they're infected and they're spreading the virus to other cells and we don't know where that tissue reservoir is and as I said the receptor theoretically is on every cell.

Not every cell can replicate the virus but virus can get into every cell. So it's infecting more and more NK cells as the load keeps coming up and at this point something happens to your NK cells, this envelope antigen comes to very high levels like we see in our patients plasma and white blood cells and we know that that in animal models or in animal viruses of this family is actually a noctogene and a neurotoxin. So we hypothesise that the envelope alone is creating some of the neurological sequelae and that they're different from the virus replicate. So it can be sort of the envelopes around a lot more, I showed you the defective particles we less infectious virus and more defective virus but those proteins can affect your body.

So we know you're making antibodies but some of the sicker patients don't make antibodies and CFS patients are known to have problems with antibody production for whatever reason, we're not saying that's direct to the virus but you know it's not a great leap of faith because that's what we saw in the early eighties with AIDS patients we had no idea how long those men had the virus.

All of a sudden there were getting Pneumocystis and Kaposi's Sarcoma (a form of cancer that only occurs in older men in Italy) and that's because as you age your immune system loses effectiveness too. So all of a sudden we're seeing a virus that is not endemic in the United States, well actually from these patients they actually indentified HHV8 (Human Herpes Virus 8) which actually is causative for Kaposi's Sarcoma and that virus, I led a drug development program about a decade ago just before I came to California and we were going to make drugs to target AIDS associated malignancies and we found as soon as we got the highly active anti-retroviral therapy and got rid of the HIV and silenced that the Kaposi's Sarcoma went away as did the HHV8 so they cut the budget for that drug

program and rightfully because there's no need to develop these drugs because they learned that at that point all you have to do is control the retrovirus, get the immune system back to functioning, and also the good news is most of those men their immune systems are functioning well. You can get a lot of them back to at least a level of health even though they have to stay on various drugs the rest of their lives at least they could cure the immune deficiency.

So in summary then of the science part of the talk:

XMRV is the first simple human infectious retrovirus. It's a gamma retrovirus it's not complex so it's the first one known in this family and we know nothing about the pathogenic potential other than the two diseases that we've seen it in. We know that human retroviruses are not ubiquitous I've shown you the distribution can be quite low in various places in the world. We don't know how it spreads across continents.

They're not benign, meaning they cause disease. All three known human retroviruses are associated with the neurological diseases and cancer. And importantly they are not airborne, retroviruses are not contagious you don't get them in the air. We know that for instance with AIDS patients that it's not a problem to kiss AIDS patient and hug AIDS patients and so that knowledge is there for this virus as well. So there's three known now, the complex and now the simple and I've mentioned that a number of times.

Interestingly and something we should think about in light of the replication studies and the other studies as we're going on, I say HIV and HTLV but I've been saying one but there are variants of HIV there's a HIV2 that is less pathogenic, there's a HTLV2 that is less pathogenic in fact hardly pathogenic at all. And these are clearly different and have different pathogenic profiles and just a short extension of that suggests that there could be variants of XMRV there could be subtly different sequences of viruses out there that are associated now with different phenotypes, so the way the disease looks, and different cancers or different neurological diseases.

So I know that the scientific community is actively looking for variants so that's another good news about these studies is that there are a lot of exited retro virologists and immunologists who started as soon as these learned this in July to the put the world resources and the best minds on

this virus associated with CFS and that's probably the first time that's happened in the world so they're excited about that.

So a lot of the questions that I got, and I wrote this talk around the question that I got, had to do with reasons to be tested. You know we don't have the best diagnostic test yet because we still haven't validated that serology test. That serology test is done in a labratory it's very cumbersome we need to validate it clinically in order to look for antibodies in the population against this virus and that is the number one test when you go look for HGLV. But that said there are opportunities to get tested and you might have your own reasons to get tested. Now generally a physician won't test because there are no treatment options. There are no known anti retrovirals currently that are known to be good for XMRV so why go get a test for it if you can't treat it?

But it can give you additional validation that your illness is an organic illness and that can have a huge psychological boost because you can begin then to think about immune support and things you might do and changes in your life style where you may be able to support your immune system in the meantime while we develop drugs. And importantly you want to protect your personal family and public health, we need to know where this virus is. And it does help, physicians then start to see, physicians like Dr Peterson will know how that might relate to your other infections your other immune issues if you have cytokine profiles some of the tests he does. It might help him or some of the other physicians with your therapy to know that this is a player in the game now.

And again it underscores the more people that are infected, that 3.75% is 10 million Americans, so that I didn't have to say anything the drug companies called me the next day and said “Gee we'd like to help!” and so we're actively working with them and they are helping because there's another piece of good news which is that there are drugs that were on the shelf that were developed all the way through phase 2 clinical trials so they were shown to be safe in people but they just didn't work as well against HIV as the drugs that were out there so why spend a lot of money developing them? So there are real targets that you can go after that can serve regions between these viruses right now and maybe come up within the next year with a drug and a clinical trial for that drug that would go along way toward treatment.

So right now we recommend to prevent the spread of XMRV, if you have

CFS and you wanted to be as prudent even if you didn't get tested say "Okay I might be infected". So what would we recommend? The HIV precautions because it's a retrovirus we know it's spread we found it in blood in the body fluid secretion prosthetic secretions so you just want to assume that these precautions that are very stringent, and have prevented the spread of HIV in some countries, that if you don't donate blood or sperm (this virus can infect sperm cells) so if you have CFS or maybe a history of aggressive prostate cancer in your family you might think about not being a blood donor.

Follow the HIV precautions. Don't share toothbrushes because you can have bleeding gums or razors. Use safe sexual techniques and I say here do not breastfeed. It's don't breastfeed after six weeks when the maternal antibodies go away. When they did that in Japan where ATL (that aggressive leukemia) was rising in the late seventies and early eighties, all they did was say "Okay no breast feeding!" and 40% reduction of ATL rates in Japan. So prevent the spread of this virus and you can reduce the disease and protect your family and your children.

Part V Transcribed by Garcia

So what are our research priorities?

At the WPI we're actively working with the federal government to develop that next generation of tests. We expect that serological assay (Rachel will get mad at me but) within a month. She told me yesterday that the data were looking really good.

And we want to investigate the prevalence of XMRV. The federal government, the National heart, lung & blood institute actually called as soon as the paper came out and we set up a blood working group to investigate what is the true prevalence. Prevalence means the presence, the distribution, not necessarily the disease, we use incidence with disease, and prevalence of XMRV in the blood supply. Our numbers were small they were only 2 or 3 hundred that's 4%. And so, but 4% is still 10 million Americans, so you want to look at that, and they actively are. And they're working on that second generation test as well.

We want to understand those tissue reservoirs and clearly it may not be the PBMC's. Is it the lymph nodes? Is it bone marrow? It's possible (I don't expect it) but it could be the brain. We don't know at this point.

We are actively working as I said with drug companies to develop anti-retrovirals and immune based therapies.

We want to understand how it's transmitted. We've got a family study going on in the research plan, it's just getting IRB approval and ready to start so hopefully we can get families who have any number of diseases across the spectrum, fibromyalgia, other neuro-immune diseases, maybe a higher incidence of cancer, but we need healthy people as well, so we'll take essentially anybody into that protocol. And as I said that protocol will help us investigate the incidence of XMRV in other neuro-immune diseases.

Important questions that the field is working hard to answer and we are as well but we won't be able to do all this: Is XMRV a causal factor in CFS and possibly some aggressive prostate cancer? And we'll talk a little bit about how you think about a causal factor. One way to do that is, we have several patients who came to Dr Peterson and they said "I was fine until I got into a car accident. I got a blood transfusion in the hospital and I got CFS" or "I had a surgery and had a blood transfusion." So if you can identify a blood transfusion exchange of an acute infection that causes the disease and the virus wasn't there before in the human and it's there afterwards and it's in the donor, then of course you've got causality and that is one way that causality was shown in HIV as causing AIDS. I think we all know the tennis player Arthur Ashe and that is how he got HIV/AIDS and subsequently died.

So how does XMRV enter the human population? Is it a zoonotic [from animals] transmission? We know its not a mouse, at least not any of the mice we know. It could be a field rodent of some kind, but we've never found the virus in another animal. This is the first animal that is the "Xeno" and that is man. So how does it enter the population and when did it enter? What's the worldwide incidence of XMRV disease that should say or prevalence of XMRV. Where is it? Is it in England, in Europe, at what level? We know it's 1.7% in Japan because of a study done earlier this summer.

And does it alter the risk of cancer development? Because HIV & HTLV1 both by causing immune deficiencies do.

So a lot of people wanted to know are we working internationally to replicate the studies. Everyone you see on this slide, a lady in Canada, part

of the blood group in Canada also had called me since the study came out. We've been working with Jonathan Kerr and we have a 5-year RL1 with him, but Ellie Barnes in MRC in Oxford. Norbert Bannert on that German paper, he was working with a advocacy group led by Regina Koch I think and they found a few samples that were maybe positive. So he called me and said "Can we work together and have that antibody?" Again everybody you see on this list, Jonas Blomberg in Sweden. Norway, Germany, the Netherlands, Italy, Spain. We can't handle the samples we've got so far, but we'll try and we'll send the reagents out to anybody to replicate the work and find out more about the disease.

We also know of additional incidence studies that we're not involved with but are occurring at Kiel University again in Germany. And here in the USA, Sam Chow is working and has identified the virus in China and I do know that Richard Huber has had success at finding virus in CFS and other patient groups and of course I mentioned that blood working group that's working throughout the United States and I didn't list the number of US collaborators we have. There is a lot interest, a lot of the world's best labs are working on this and we're going to get there a lot more quickly than we would because of everything we have learned from HIV.

So what about Diagnostic Tests? I said should your physician or you want to be tested currently (the last time I looked online) there were only 3 companies offering the diagnostic test. Of course the WPI licensed the technology to VIP Dx, (we're a non-profit institute) who is using our proprietary culture method and PCR in combination along with a Western Confirmation. So we look for both antibody and PCR positivities in the cultured and co-cultured cells. And we use 20mls of blood to do this and make sure of the accuracy of the result and the price is $450. Clongen lab has a real time PCR that is just looking for sequences on 1ml of whole blood and there price is here [$375]. You won't find this virus in 1ml whole blood by PCR. I think I've shown you that with the negative cases in the prostate cancer. And also a company known as Cooperative Diagnostics in South Carolina. We don't know what their PCR method is but their using a drop of blood on a piece of paper so they tell you if you put a drop of blood on a piece of paper, you don't need a doctor or anything, just send a cheque and of course they won't find anything.

Your help is critical really to advance this science. At the WPI we either want your money or your blood. That's the only two choices! You decide what you'd rather do. But we need you to participate in these research

studies and we do have a form online: www.wpinstitute.org and you can email me. We've got a form online to register.

We're asking for some clinical characteristics but we're asking for those more to help us put it in a study. We won't turn anybody away. We will look for the virus if we can get those studies. We're waiting for the IRB approval, that's the human assurance to make sure we're not hurting you and we're protecting your privacy. So we expect that this week.

Donate funds to the WPI research and clinical programmes that will be established later this year. The clinical programmes will really come of the research and the diagnostics. And then write to your government officials and encourage them to support XMRV research. This is an infectious disease. Why isn't the National Institute of Allergy and Infectious Disease considering this virus? They've been pretty quiet haven't they? We haven't heard a word from them. So we need our government agencies to look at this virus because it's an emerging infection as I said of unknown pathogenic potential.

I'd like to thank the people who, we couldn't have done this study without them. This has been a 3-way collaboration between the National Cancer Institute and its contractor SAIC, Cleveland Clinic and the WPI. As I said earlier when Vinny Lombardi and I together with Max Pfost first saw the few sequences of the virus I called Bob [Sliverman] because obviously we were doing the work with him and then I called Frank [Roscetti] and said you know "I need you" and he said "I won't go" and I said "I'll pay your way to San Diego on the beach for a week! Whee!" and he said "Not any more of your schemes Judy! I'm not going to do that again!"

So at any rate we met at a restaurant and we showed Bob, and he didn't know what I was going to tell him. Interesting Frank's a bit cantankerous because I gave him about a week's notice because we'd had 3 weeks and I was pretty sure I knew what we had. We had to get a 3-way inter-institutional confidentiality agreement.

So Frank called the office in the government and they said: "No we're not going to do that" and he said "Look we're talking next Saturday. You can either have a confidentiality agreement or you won't", but they got one. So Frank and his lab, Dan Bertolette did everyone of those beautiful Western's that I showed you, just a magician. Mike Dean & Burt Gold sequenced the entire RNAse-L gene in more than 100 patients. Ying Huang did all of the

PCR that we had done totally blinded where samples never came to our labs to show it wasn't contamination.

And of course I've mentioned the lab of Sandy Ruscetti, Charlotte Hanson & Jami Troxler who were key in providing all of those reagents without which this study clearly wouldn't have been done. Cari Petrow-Sadowski developed that immune-response assay in a real hurry this summer and I mentioned Kunio & Rachel who did bioinformatics support and electron micrographs.

We couldn't do it without Dan Peterson's diagnostic skill. I mean he biased the patients such that we could find the needle in the haystack but these are the patients that come to the institute. They have classic symptoms of CFS. When we have taken patients that have emailed us with exactly those same symptoms, we find the virus every time we look including in Europe, in England, Ireland. We couldn't do it without the CFS patients and advocates. We do so appreciate the support all along.

This was a tremendously difficult year in trying to keep quiet. Knowing what you had and don't say anything until you are sure you're sure you're sure. Every day was just are we sure? And so we were able then just with the small crew you see here and this supportive staff. Vinny, Katy and Max they pretty well have worked 24/7 for at least the last year and we have our lab meetings at the bar. They said they were going to make a drinking game based on my talks, I'm not sure if it's how many times I said "umm" or whatever! So at any rate, with that I'll thank you for your attention and take questions.

Transcriptions by Advocate, JAS, Lily, thefreeprisoner, Kim & garcia of the Phoenix Rising Forums

Question: Before any of the questions I just wanted to say thank you because through your work and your collaboration, you've bought more excitement to the CFS community and your collaborators have than in 2 decades. And you've also brought more interest in the illness than we've probably seen ever, so I thank you.

Judy Mikovits: You're welcome. [Applause]. That's interesting, because the

reviewers of the paper didn't really know what CFS was. They said: "oh that's a poorly understood and complex disease" and they went on to the virology. So we were able to get that reviewed without any kind of bias. And I think that was significant as well.

Question: What percentage of the population has this virus?

Judy: We found it in 3.75 percent of the U.S. population and it was from across the United States. And in Japan they found it, just screening the blood supply of a couple thousand people, they found it in 1.7 percent. So we don't know the true prevalence.

Question: So, only in 3.7 percent? What about the other 96 percent of CFS?

Judy: No, that's the healthy population. It's in ninety-some percent of the CFS population. I stuck this slide in here and Frank keeps taking it out, but you might have heard in the press after the paper came out, we didn't do all of the tests, all four tests, on all of the people prior to the submission in May. We just looked for evidence of infection and looked to see if we could isolate the virus, looked to make the point, and it wasn't so much about the CFS. So what we did after the paper was published, is we went back and we looked with all four assays for evidence of XMRV in those PCR negatives. Because now we know that indeed those negative samples may have evidence of infection and what we found was that 19 of the 33 had antibodies in the plasma. We found transmissible virus in the plasma of 33 of those people, and then we looked at that latent virus because the company I used to work at here in Santa Barbara was called Epigenics, and it was developing methylation-inhibitors for epigenetic silencing, and that's what happens to viruses, and so we used Decitabine, which is a demethylating agent that opens up the genome and turns on the virus, and found that there was latent virus in 10 of those people. And when we summed it all up and tabled it out, 99 of the 101 patients in the Science paper had evidence of XMRV infection.

Another way that HIV/AIDS causality was established was by saying… So the statistics of this means there's a 10 to the minus 35 chance that you had CFS in our study without having XMRV.

So I'll go back to the AIDS analogy. You can have HIV… so we wrote in the paper "virtually impossible", the editor took it out. We wrote, "highly

significant." The editor took it out. Finally we said, "significant." But at any rate, 35 zero's in front of a 1 would tell me it's virtually impossible to have had CFS without having XMRV in this study.

So you can be infected with HIV and not have AIDS. We know that. People are being treated, a lot more elite-controllers are coming out. But you can't have AIDS without having HIV. So if we can establish that XMRV is to CFS as HIV is to AIDS, which is what we're trying to establish through that immune system understanding. So HIV kills CD4 cells and leads to AIDS. XMRV does what to the immune response? To the T, B and NK cells to lead to CFS? To turn your question around, sir… What about, the incidence of CFS in this country is 1 to 2 million…is that more or less what is said? By the Canadian or the Fukuda criteria. So I said 10 million people were infected. Where are the other 8 million? Do they have cancer? Do they have nothing? I showed you that only 20 percent of HTLV-1 infected people were actually sick with one of those diseases.

So you can be infected with retroviruses and be carriers and not be sick. And so that's one reason to be tested so if there is a genetic susceptibility, which we're looking to, maybe a reason, an immune defect that was unknown as to why some people get sick and others don't. You certainly want to know where the virus is so if you're a carrier so you can protect your family.

Question: Do you know how many have tested with VIPdx and how many are positive?

Judy: I don't work with the company. So they only take samples two days a week because it takes three days to do that, so they've done hundreds of samples in the last couple of months, and at least half of them are positive. Or 40 percent. And again, their doctors are looking at… the doctors who are well versed with CFS, so they're immediately sending off… Dr. Cheney, Dr. Klimas, a doctor in Canada, Ellie Stein, maybe even Susan Levine in New York. I'm not sure because it's illegal for me to know those data because there's confidentiality between the patient and the physician. But quite a number and, yes, it's there. CFS is a heterogeneous disease. I mean anything based on fatigue. So certainly everything is not going to be this virus. But maybe there is a disease, and of course that's what we're looking for, biomarkers for understanding how you can get sick and be sick forever and not have drug targets, not have diagnostics, so certainly there are going to be lots of people who have what might be called CFS today,

and that's why we've also coined the term XAND, for XMRV-Associated Neuroimmune Disease, and that would be because we've seen…

I had done a number of studies with family members after the paper came out and prior to now… where I just said there's a family member where the children have autism, there's fibromyalgia, there's excess cancer, and when you look, you find the virus. So we've found the virus in Atypical MS. Atypical MS is a non-demyelinating MS, it looks like MS, it has some brain lesions like MS on SPECT scans. At any rate I'm not a physician. Just looking at families with different types of neuroimmune disease, we started seeing that the virus was there, and so that's why we started thinking it might be involved in a broader spectrum of neuroimmune diseases, with overlapping symptoms because in fibromyalgia, pain is the primary symptom, but in a lot of people it's this body-numbing fatigue, so fatigue goes along with it.

In fact, Cindy Bateman, who's a fibromyalgia expert at the University of Utah, she says fibromyalgia is CFS with pain, and she can distinguish those who get better with fibro with certain therapies and compounds, and she takes the others away who don't respond at all, and puts them more into the CFS group. So there's definitely going to be a lot of things where XMRV's not going to be everywhere. It's not, it's 4% but the people who are infected are sick.

Annette Whittemore: Earlier you said that 40% were positive. So describe the fact that if you're positive, you're positive. But if you're negative, you're not necessarily so.

Judy: Yes, that's correct. So I answered that question based on the samples that came through there. Everyone who is positive is definitely positive for having the virus. But we don't know what the people are, what the doctor's sending in, so the people could not have that disease. So it could be a clearly, distinguishing delineating marker - biomarker - or diagnostic at that point for various diseases. So a doctor might see a spectrum and say "I don't know maybe I'd better check." Because the earlier you catch it, just like cancer. Early detection. Make sure the reservoir is … make sure you don't have that virus multiplying, and you can live a normal life. Don't let it get … you know the commercial out right now is HIV doesn't have to equal AIDS, well XMRV doesn't have to equal disease. If we keep it down we keep the immune system strong.

Question: So what you're saying is you may test negative but not be negative?

Judy: That's correct. If you do it by the PCR. If you do it by VIPdx, at least right now, it's running along the lines of… We've got the antibody, and we've got three of the four tests. We'll license it to anyone. We're a non-profit institute, so everybody pays the same royalty, so any diagnostic company could do the gold standard. But right now if you test negative you're not necessarily negative, even at VIPdx. Because we want to go do that serology test. Maybe we can't find evidence of the virus. But you've been exposed which would be a good thing because your levels are theoretically low and you've just now made the antibodies so you can prevent disease, as we did with Magic Johnson. But we don't know anything about the immune response to the virus.

Question: What about transmitting it, when you get pregnant, to a child? I mean you were talking about breast feeding?

Judy: Well, it is theoretically possible. We don't know. Gamma retroviruses are vertically transmitted, so the egg and the sperm can be infected and you can actually vertically transmit gamma retroviruses. But this is the first … we've only studied it for two months. We don't know. Theoretically it's possible. If we make those data it will certainly fall out when we start looking at family studies.

The horizontal transmission is the only thing we know about right now. Because of course you have to find somebody who just gets infected in order to understand the disease. And since a diagnosis of CFS is being sick for six months, well, if only. If that's the only thing we can do is to stop that practice, making somebody wreck their immune system or be totally sick for six months that would be a great thing with the discovery. If that's the only thing that comes of it.

Question: Would you not recommend getting the test again if you did [test negative]? I just took the blood test with my doctor …. and he's part of a study. He didn't tell me who he sent the samples too, it's a 20-patient study, so if that came back negative …

Judy: It depends on where it is sent.

Question: Take for example VIPdx?

Judy: If it's at VIPdx, as soon as we have the serology we'll go back and do all the negatives. We save them. So we'll go back and we'll… We do isolate virus from all of them, but it costs a lot, so… So if they're really negative, we won't isolate virus either. That's correct. At VIPdx we're going all the way to virus isolation, because we want to make sure we are sure.

Question: So at a non VIPdx?

Judy: They're only doing PCR. All bets are off. I showed you almost 60% of them are probably, they're all false negatives, because very few people can find them.
And participate in our research studies, because as I said, there's no hurry. There's no real reason to pay for a diagnostic test because we can participate in research studies and fund those studies and you get better data. Because I'm not privy to those data. I can't answer the question of where they came from, where they are in the United States. Those patients and doctors have to release that information, and that's not the job of the diagnostic company. So the information is not useable in that way. Now we're pretty good right now. We've been as responsive as can be. By the way, if anybody thinks they might have been in our repository, if you simply e-mail me we did decode that study over the Christmas holidays, so we can tell you if you're positive or not. We can tell you whether or not you were in the study, because everybody. There's more than 500 samples who are patients in the repository and we only pulled about a hundred of those for this study so we don't know if… I don't remember everybody, but we did decode it so we know who the positives are at this point in time, and we are sending letters, but you have to ask me first for our human-assurance protocol, so just ask me and we'll tell you.

Question: In the beginning you mentioned that the virus uses lipids to grow?

Judy: It actually uses the cell lipids to make its lipid membrane. It's an envelope virus and it has lipids, so it pulls cholesterol in to make it's… so it uses all the cell machinery. It only codes the enzymes and the envelope proteins and cells, so the lipid bilayer of the envelope virus…there's lipids in it. It's from your cells, and cholesterol's a part of that.

Question: Because I'm wondering, I've met a lot of other CFS patients who

like me have high cholesterol. That just made me think, might there be a correlation?

Judy: Yeah, and nobody's ever looked. It's certainly something that they could look at correlating. I can't think of a reason why. You might presume you'd have less if you're using it up for another purpose.

Question: What about children?

Judy: We do have a little bit of data on that because we have two children in a study who have a genetic disease of cholesterol. It's called Niemann-Pick's Disease. It's also known as Childhood Alzheimer's. And these kids, you know it's a cholesterol metabolism disease where eventually your brain, you'll eventually die of it because if you get too much cholesterol and it messes up your brain and everything.

And those kids have been treated by James Hildreth in Nashville, Tennessee, at a small college, I can't remember right now. And he's using Cyclodextran and some of the cholesterol drugs. He's actually an HIV drug developer, and the kids are showing some improvement when he modulates that pathway and stops the virus from entering or exiting the cell, so we don't know anything about XMRV. We just know what other viruses do, so he is having success, suggesting that there's some opportunities there.

Question: Are you working with him?

Judy: Yeah, we're working with him as well. That's why I didn't list all the collaborators at the United States. We're providing reagents and whatever intellectual knowledge we have and whatever physical abilities and instruments we have to these collaborative efforts as well.

Question: Until forthcoming therapies are established for those who have XMRV what are people doing to once they are tested? Are they taking immunomodulating therapies or whatever?

Judy: Some of what I showed you here, that turn on/off switch, suggests non-steroidal anti-inflammatories. So non-steroidal anti-inflammatories could well help. Things that will balance cortisol. Maybe... these are just thought processes... because you know inflammation turns on the virus, and I don't know much about hormone therapies and how lowering

hormone levels might help, but I do know anecdotally that a lot of women in a particular time in their cycle get much, much sicker and can't get over it. I do know in the laboratory progesterone really upregulates the virus, so if you have a birth control pill… and again I don't really know anything about this... I'm not a physician... you might think about just keeping the levels balanced and avoiding the fluctuation.

So certainly supplements can help a lot. Retroviruses cause a lot of oxidative stress. So things like N-acetylcysteine and glutathione, the detox type… People do take supplements. I know that a lot of people have had success with immune modulators, just helping their disease, or supplements, because they know about them. I caution against taking too much or taking a bunch of things.

Try to learn as much as you can, because supplements aren't controlled by regulatory agencies, and therefore if you're not using a high quality you could actually be putting poisons in you, and since we don't know much about the virus you could modulate the wrong way.

But things that upregulate NK cell function, and there are known compounds out there that do that, that are marketed in our state, so that could help you, so… I'm really not… We don't know a lot about it, but that's how people are actually starting to help themselves.

The other thing is to stay out of stressful situations. It's hard to do. In fact, we've seen a lot of people get worse just with the stress of the discovery, which is sad. Just the stress of the discovery has people freaking out. That's why I want you to call me. Because we don't want you to think: "Oh, no I have a retrovirus!" We want to talk to you because it's serious and you can have untold…

I mean most people say: "Wow I got it!" you know their congratulating people when they come up positive, which is really strange… [laughter] Then they get really scared, because they don't know anything about it. And we're here as much as we can to help, but we don't know anything about that retrovirus. All we can say is that… the same thing I've been saying today. I hope you would walk home and say: "It's not a mouse retrovirus. Retroviruses are not ubiquitous, and they're not benign. So I have to think about those facts." So it's wide open.

The drug companies… the one thing, if you do get tested, and we know

you're positive, a confirmed positive -- we'll get you into the earliest clinical trials. And they'll have things pretty soon because all they have to do -- and it's major Pharma -- all they have to do, and I've given them the reagents and the cell lines -- we've made several cell lines from people that make a lot of virus. And so we've given them those cell lines, and all they have to do is take something off the shelf that rationally might inhibit a particular, say the integrase gene or another gene that's conserved across the three retroviruses [HIV, HTLV, XMRV] and show that they can see the efficiency, efficacy and knock down levels of the virus in the lab to levels, which they're the known blood levels of the drug can achieve, and they can submit and do the paperwork for a clinical trial. And it's already known to be safe because it's already passed Phase 1, or safety trials, in humans. So we looked at those first. And there are a number of companies who, as I say, they are high quality companies, and they are more than interested. They are doing it now, and have been doing it since October.

Question: What about cancer, especially the hormone-responsive cancers?

Judy: We very much expect that some of the breast cancer incidence -- we hypothesize that inflammatory breast cancer a lot like what we saw with the inflammatory prostate -- but yes, it is a very real hypothesis because the incidence of breast cancer in young women that you've never used to see before, is rising at levels that suggest something environmental, and not necessarily genetic. You know we never had cancer in my family and you see young women that way, so it certainly is something that we're looking at … the we here, I always say we and it's everybody but me usually… it's the National Cancer Institute. We're also looking at lymphoma, because CLL (chronic lymphocytic lymphoma) is a lymphoma, a B-cell lymphoma and it's also been going up and up, and it suggests to us some kind of role of an infectious nature, so we are looking at a number of lymphomas with a group in New York, a group in Florida, and the Nevada Cancer Institute. I don't have a breast cancer study set up.
Question: There's actually an anti-viral…

Judy: Anti-retroviral.

Question: …anti-retroviral vaccine that's been used

Judy: Yeah, a vaccine is a real opportunity, and we know that they still don't have an HIV vaccine yet, that's efficacious, but HIV is a complex

retrovirus. So when you're thinking about the reason why you have to take a flu vaccine every year, it's because the virus changes. Well, an HIV virus in a person in a week will change too much even. They call them quasi-species.

One of the really interesting things about this study is we only isolate one thing out of these people. When we do the sequencing, it's clean. We don't isolate quasi-species. We don't have the virus have these changes here in one week or one year… we have patient samples across dozens of years. We isolated XMRV from a 1984 plasma sample from a patient. So we got it in 2008 and we got it in 1984, which again suggests that the virus has been along at least 25 years and it might have a role in the disease but is not causative, so yes indeed, it could play a role in other things.

Question: People with CFS have shown a lot of evidence in other studies of being immune-suppressed. How are you certain that it's XMRV that's causing immune-suppression as opposed to say 8 other viruses like Kerr is suggesting or other viruses that are causing immune suppression and making XMRV opportunistic?

Judy: Well, there are a couple of things for that. First of all, we're not certain of anything. So I'd say it's a hypothesis. It's because of what I know about HIV, and HHV-8, so these herpes viruses, where it's the underlying immune deficiency. The other viruses aren't retroviruses, the other pathogens too, the bacteria, and they don't live in your immune systems forever and replicate and have reservoirs. They're across the board, so everybody's infected. Probably 90% of this room has an EBV infection. But very few people express EBV, have chronic active EBV. That suggests that your immune system has something wrong with it. It could certainly go either way, but retroviruses don't do that.

The CFS world has looked at any of those pathogens, so here's chronic Lyme and here's EBV and here's… It's never one place, something that unifies all of those. So it's certainly a testable hypothesis, and that's one of those things that will just happen. If you get an anti-retroviral and the chronic EBV goes away, and a lot of the symptoms go away… I'm not saying that the EBV doesn't cause a lot of those symptoms. That's what makes it so hard to figure out the disease. But if there's an underlying immune deficiency that's created… that's not simply depression… but is getting worse every year, could be an explanation. So we're happy that we

can test that, because we do have different populations where we can see what the role of the co-infection is. We've never looked.

We're looking with various groups at big cohorts of chronic Lyme and big cohorts of chronic EBV, Q Fever… things like that have been associated with… Jonathan Kerr, in fact, he's working actively with us to see if it makes sense, that you need the combination or you need one or the other, but… in the general population the incidence of XMRV is something between 2 and 4% right now, so… whereas it's 90% of some of the herpes viruses, and most of us are exposed to some of these other pathogens, so I certainly don't have an answer, but again, this gives us a testable hypothesis to look.

Question: So I'm XMRV positive. And I have a son who is. I was told that he also has lyme when he was 7. Explain the chronic Lyme connection in him?

Judy: Well, again, we don't know -- it's my thought that -- it's our hypothesis that the Lyme Disease, especially in Lyme Disease, where it goes away and it's almost cured and you only see some proteins that don't necessarily; you know; it suggests you almost cure it with the antibiotics but you have to keep the antibiotic there because there is a low level that your immune system can't clear, and maybe it can't clear it because you've created an immune deficiency with the retroviral infection. And we've never looked at a Lyme cohort yet. Again, we're setting that up, but we don't know the connection. But the hypothesis is, if we can treat the retrovirus, then the chronic Lyme will go away, is the thought. And you'll treat with both.

Question: But he was untreated until about 7, and was bitten in Europe, and no-one understood that there. He had the rash on his legs and no one believed me that that's what it was.

Judy: Well, we can still clear the Lyme. For instance, in the AIDS population you treated the pneumocystis pneumonia. You treat it with the appropriate antibiotics because you don't want the co-infections to kill him, and then do the anti-retrovirals too. There's no reason…for instance, one of the questions that I got online was, "Well, I'm taking antivirals. Do I need to stop in order to get tested." No, because antivirals don't target retroviruses. Retroviruses are very distinct viruses, so no you don't need to stop. We'll still find the virus.

Question: But even 10 years later, because he was never treated. That was 10 years ago. You're still saying you would still treat for Lyme?

Judy: Yeah, well you probably should be at this point. Treated for both the Lyme and once we have a treatment, for the retrovirus.

Question: If XMRV is transmitted sexually, how come it's not seen as CFS in couples?

Judy: The possibility is that it's transmitted sexually, but we've never actually shown human-to-human transmission meaning we caught the day when the other got sick. I don't actually have an answer for that other than that I know that it might well be more in couples than we think, because it's a milder form of symptoms, or maybe this person's a carrier. There's still a lot we don't know about why prostate cancer and why CFS… what is the hormone component that so turns on the virus? They may be carriers and not know it, and certainly there's a lot to study there to understand the gender differences in these diseases.

Annette Whittemore: Maybe part of the answer is that If XMRV turns out to be the cause, would you have said that you wouldn't have CFS without it, given you can have the virus without having CFS?

Dr. Judy: We have looked in a limited number of families that we've done, and in fact, maybe only one member of the family has CFS, and so it will be an interesting year, but, once we really get to the data of looking at these families and the various diseases and trying to understand – it's a very low replicating virus so it really just sits there for a long time.

So if you can keep the reservoirs low, you might have the virus your whole life and never get sick. We don't know how long it's been in the population. We think like with other retroviruses, the younger you get sick, the more severe the disease.

When Sandy Ruscetti gives the rats the viruses when they're neonates, they get cancers. When they get it when they're 30 or 40, they don't get anything.

So the immune system is educated and grows as you go along. It can be more fragile at different times in your life. We've seen a lot of puberty,

boys and girls alike. There's a lot of infection or at least apparent infection, disease occurrence at 12 or 13. And I do know that's when Andrea got sick [to Annette Whittemore]. And yet presumably, your family was in the same space, but there was no child who had hit puberty at that point and that might have been the difference.

Retroviruses don't infect people differently. You can't go to Germany and say the reason they don't find it is because they have hardier genes in Germany (although my husband might argue that) [laughter]. Because everybody gets infected. It's just which immune system can control the virus and keep it down.

Since we've been able to treat HIV/AIDS now, we found elite controllers. People who are walking around with HIV who never knew they had HIV, and the copy numbers are all low. Their immune system is fine, and they have no idea when they got it.

Question: So a lot of patients say they had a flu, a weird flu-like condition, a week or two before they got CFS. So what you're saying is that flu-like illness is a bug that came along and allowed the XMRV to create CFS?

Judy: Well, no. What I think is happening is – I know almost nothing about CFS – but what we think is happening is – remember the slide where I showed you those little events? You know, what was the event that was the straw that broke the camel's back?

Where did the balance tip between here you've got your immune system working well and the virus and the immune system are co-existing just fine, then some other, that other bug, whether it be lime, a flu, a anything, gets you. And then the virus, the cells divide, and so do the B and T cells you need to mount an immune response. And now you've got your memory population that might have been harboring the virus and it's replicating because it's seen that same pathogen before, so it could be a common everyday pathogen, and then you just tip the scale to where now your immune system can't handle it or anything. And every day you're seeing more infection because you're NK cells aren't working, your B cells aren't working.

We put that antibody up there for a reason. We haven't been able to correlate the levels yet, because we haven't been able to find high enough numbers. But we do see these infected families where infected spouses

and things have very high levels of antibodies, that suggest maybe antibodies in this retrovirus can be protective, and maybe there's an immune therapy on the horizon as well.

So you can think about it in that way. It doesn't have to be the insult. You might not know how long you harbored that virus.

Question: So what you're saying is XMRV was there it wasn't the insult, then something else came along that was the tipping point?

Judy: Yes, that was the tipping point, that's correct. That's our hypothesis. And again, it's testable, we don't know.

Question: Is there differences between – because you've got a big population of patients with sudden onset and then you've got a big population that had gradual onset. And a lot of the gradual onset patients are worried that "Maybe I don't fit this equation." So what would you say to that?

Judy: That little bump is smaller, so it's not a huge burst. So that it was little insults over time. I think, for me anyway, I know only a handful that I've looked at, that I know the patients and I know what the onset was. But the gradual onset there's no real difference between – it just depends on that environment, what the other triggers and events were that spurred it on. So I don't see if you are gradual onset, it doesn't mean that you might not be infected as well.

Questioner: That's an important thing to know.

Question: Have you tracked any of the inflammatory markers in the blood with the XMRV virus?

Judy: Yes.

Question: What are you finding?

Judy: We find a signature suggesting a viral infection, an unclear viral infection. So we have 5-10 inflammatory cytokines and chemokines that will cluster in an infected person. The problem is we don't know if it indicates active infection. Certainly when the virus is quiet that inflammation will go down and those cytokines will change, and it might

be a nice biomarker for following active infection, but we haven't analyzed the data in that way yet. So it very well could sit, and some of those go down and up very quickly. So like 3 days you're IL-8 and your IL-6, & some of the chemokines are elevated and then they go back , like an EEG. Just like you might assume the retrovirus could be an EEG, depending where in the body that is. We don't know the reservoirs, so we don't know what's controlling it.

Question: I'm confused about something. You say you can't find it very easily by PCR, so you are culturing it. Then how did you find it for the Science paper?

Judy: Well, because I found it in 67%, by PCR, of the patients, but I looked several times, at any given time. I just said it's like an EEG. So I got lucky and I found it at a time when the patient was high. They'll come to the doctor when they're sick which might mean they're replicating more WBCs and there's more virus in their WBCs.

Question: So you checked these patients multiple times over time?

Judy: No, I did them all at the same time because they were in the repository. So we collected those samples multiple times over time.

Question: So you took like 10 samples from one patient?

Judy: Not that many, usually it was 4. A Poisson distribution, the copy number could be as low as 5 or 10 copies per mil of blood, so there's a statistic called a Poisson distribution, where you might find it one out of three times so we went more than one of that and went 4 on most people when we found it.

Question: So the UK paper had studied patients, if they had looked at 4 or 5 per patient, do you think they might have found it?

Judy: They might have certainly found more, yeah.

Question: They essentially accused you of contamination.

Judy: Right. But why would I have contamination in my sick people and not my healthy people? How would I do that? I did it in three different labs. I did it in Cleveland Clinic. When I first came to the Institute, we

didn't have a lab, so everything we drew from around the world, I sent to Frank's office. They processed it. I went there, I put it in the microarray or did whatever I was going to do, and we worked there for a couple of weeks, you know, while we were building our lab and it got started.

So from the way we did the study there's just NO possible way there was contamination. And that's what the reviewers concluded. And the phylogenetic analysis was one of the things they asked for (they asked for 3 things after the initial submission). The phylogenetic analysis proved two things. It wasn't a mouse virus, it's a human virus; it wasn't a contaminant from a lab. We never do mouse work, but it wasn't a contaminant from mouse feces or something in the lab and it clearly was a new branch in the [phylogenetic] tree. A human virus. And our virus wasn't exactly the same as the prostate cancer virus. It's still XMRV, because it's 99% similar. But that's enough to show it's not a contaminant.

One of the things you have to use to get a good PCR is at least 750 nanograms of DNA. They have no idea how much DNA was there. And they quantitated 3-9 out of 186? Sure they found a band of globins, but globins are in every single cell, so again you're making an unfair comparison of what you're saying you see. And then you amplify it for less cycles than what would really push the envelope.

We've also done to show no mouse contamination with the CDC, and Bill Switzer - after he saw the results in the paper before it became published. He said, "I have an assay that'll show it's a mouse contamination. It's a very sensitive, very specific PCR. Will you do it?" I said, "Sure, send it to me". We did it on all 100 and not a one. Not one cell line in our lab. He's found it in a couple in his lab, but we didn't find any. Perfectly controlled. He said, "Congratulations, it's not a mouse contaminant."

So there's little else we can do except wait for the rest of the community. It's there.

And the prostate cancer people didn't say: "oh you didn't find it in 500 people, it must not have anything to do with prostate cancer". Because it just didn't have anything to do with that population.

And again, Norbert Bannert, is a high quality scientist. As soon as he saw the paper he called me and asked for the reagents. Because he's gonna go back and look to see if it really is there, and help us find some answers.

He's also looking at a CFS group. So it just depends on what you really want to find. We weren't biased in our study. You know, I'm a cancer cell biologist and we aren't biased.

I had to work really hard to get most of the people – not Frank and company. The NCI didn't know what CFS was. You know, fortunately, our scientists can to some level do what they want as long as it's along with the mission. I remember one gentleman, high level NCI official, said "tell 'em to get over it." You know if you can't, and again that's the credit to Annette and the formation of the WPI. We got a grant where we literally named XMRV within my first six months in 2007, because of the juxtaposition of seeing that paper in prostate cancer, right when we met.

And thinking about the possible mechanisms and the grant got rejected three times, you know, because scientifically, retroviruses aren't in CFS. If you went to Wikipedia in August, it said retroviruses aren't in CFS. It doesn't say that anymore. So we made progress. [Audience: Wow]

So will there be a variant? Maybe England is the variant. Maybe there's XMRV2, and one causes more. Maybe we found the one that causes the least severe disease, and maybe there's XMRV2.

A group emailed me from China, and the guy said "please", you know in very broken English, "please don't leave me, please write me back. Please help me". And so Sam Chow was going over there, and I said will you look up this group? And Sam Chow has found a virus that looks like it might not necessarily be the same virus, might be a lot more aggressive virus over there in disease, but that's just anecdotal.

Question:[Inaudible, something about SARS?].

Judy: I have no idea, I'm not familiar with this literature.

So we don't know. The good news is we have something to work with that is a very testable hypothesis. This has been very rigorous. You don't get more rigorous than Science and certainly not Frank and Sandy Ruscetti. They're highly regarded. That entire team are world experts. So the CFS population had the opportunity to have them look at us and they are good enough scientists that they didn't show any bias. They looked. You know, I asked Frank to come out to Reno before I took the job, because I said, you know, I hear a lot of things about this. So he came out and stayed a

few days and talked to some patients and spent some time with them. And I was on an East coast trip working with my drug-company. So I came back a few days later and I said, "Well, what do I do?" And he said, "Take the damn job!"

So if you're really looking unbiased and you look at this, it could be only 10 million people in America, it could be only endemic right here, it could be like Japan. And HTLV is pretty-well innocuous. 5% of people get ATL and 20% get that tropical spastic paraparesis or HTLV1-associated myelopathy. I was glad when they changed that thing by the way, because I can't say that and I couldn't spell it either! So those patients, they actually go on steroids to dampen the inflammation, the immune response and they are fine for decades but you know in the Caribbean and in Japan it was a health problem so maybe we only have a health problem and the distribution is not…but we do have at least 10 million Americans and maybe a majority of the CFS population here in American that we have a lot of work to just to treat and that will be the focus of the Institute in the coming years. Certainly we will treat everybody else but obviously we don't see anyone else. Dan was funny, he was like "Well, Judy I have to take care of everybody else" and I said "of course, there's not anybody else!" You know, so it's an interesting and an exciting time for sure.

Question: I just wanted to say, you know, it is really exciting to hear you say we need to do this, we're gonna do this. The virus you are studying and it's our virus, and I think it is really important to note everyone that for you to do what you're talking about and for other researches to do it, we patients need to get behind funding the research in a way we've never done before or it is not going to happen.

Judy: But have your government fund the research. HIV incidence in this country is about 800,000 people, I just quoted 10 million.

Questioner: What it probably needs here to stimulate [the research] is the most brain dead CFS patient in the Federal Government.
[laughter]

Judy: In the early days they took condoms with red stuff in that we didn't know weren't blood and threw them I think. I shouldn't say this but when I saw it was a prostate cancer virus I said "man those men can't possibly ignore this now!" [laughter]. I never said that publicly.

Question: Would you talk about what is coming up in the next years, what are the next steps, where are possibilities with your treatments, with the replication studies?

Judy: Yeah, so all of those people I showed you internationally are working to replicate the study as is the Blood Working Group. We have been intensely working with them we have another conference call on Monday, these things are happening, we will put probably 20% effort in our lab into that study. We are very serious about transmission studies in our lab and in the Institute and we have one starting where we're simply comparing infected people, people who we isolated from the blood with…, and just taking DNA in their saliva for example, to see if there is any evidence in saliva or of that kind of transmission just again, that is just because of anecdotal, you know, stories, where people say, well, you know a bunch of kids on the playground with a water fountain or at school I don't know what the anecdotes are, but just thinking about ruling those kinds of things out is a study we are doing. We are also actively looking at the incidence in other Neuro-Immune Diseases so we are looking at that study I told you about, we are looking at cancer and CFS, we are looking at Fibromyalgia, Atypical MS. We've got a study going with Vanderbilt in POTS, which is Postural Tachycardia, because of the overlapping symptoms. So he is simply just sending me a bunch of samples both serra and DNA and we are just going to take a look to see if we see it there.

Autism, we do have families with some autism and there are some immune defects characterized by Judy Van de Water at the Mind Institute in Sacramento and she sees some NK cell dysfunction, some inflammation, some of the things I told you about there, so in that group of autism we are looking at to see if maybe there is not an underlying pathogen or XMRV infection. Those are just the priorities just in the coming year. The NCI, the National Cancer Institute has already put $1 million into the development of the reagents and the assays, so very soon the best of tests and all the reagents that can be distributed so the AIDS reference program (there is an AIDS Reagent Reference Program that if you just google that you will find them) has agreed to set up an XMRV and send reagents around the world. We're spending considerable time and resources just shipping … Katie feels like the shipping department these days and… [inaudible comment from the audience]

We are continuing the studies of the immune system so I had an entire program set up from the beginning where we are looking at the genetics

with Mary Carrington. We are looking at the type 1 so Vinny Lombardi will continue his studies. Just because that single nucleotide variant we didn't find having any correlation with XMRV infection doesn't mean there's still not something wrong with RNase-L and that might be a therapeutic target and so Vinny is actively studying research, the type 1 interferon pathway and RNase-L. Isabel Barao-Silvestre is a faculty member that's just joined us, she is a professor and she is at UNR as well and she is an expert in natural killer cells and killer cell function so she is doing a lot of the innate immune response and understanding how XMRV infection in NK cells might contribute to disease, as I hypothesize, we don't know how yet, so those are the internal programs going on. Because of my background I'm actively perusing all the drug development efforts in our laboratory by working with at least three companies right now, to look at that.

Prohealth Organizer: Judy has answered many, many questions from all over the world and we are going to make that available on line for everyone.

Judy: Yes sometime in the next week, that will be fun, yes so we got a lot of questions so those of you in the audience and around the world if you email a question we try to answer every one of them and so we will post them up at the Prohealth website we'll post them and we will probably put them on our website so that you can get some direct answers, there were some more specific questions there.

The only one that came up which I think is good to address is a lot of people wanted to know is if XMRV somehow "piggybacked" on EBV or other pathogens to get into an affected individual and I didn't know what that term meant. It's not a scientific term. But if it meant that you couldn't get XMRV unless you had have come in with another infection, there is just no evidence of that in any retrovirus. So yes people think that people got infected with HHV8 and HIV at the same time because of the Africa thing, you can get infected with 2 pathogens at once but there is no need for any to piggyback. You don't need another pathogen in order to be infected with XMRV or any other retrovirus. So I wanted to just clear that up that term because that came up probably at least 4 or 5 times.

And any other question if you have written them all down and I am happy to answer them and answer direct questions. If I don't get … some people are like I emailed you yesterday and you didn't answer and I said "oh I slept yesterday ". If I don't answer you within a week then write me back

because sometimes I miss it and sometimes our emails are so full these days that they are throwing things into spam and sometimes it is the bills and I have to pay them, so there are things that are getting sent so if you haven't heard from me you will usually hear from me within a week because I really do try and answer essentially every one that I get which is probably foolish but I like to actually, I like to work with the patients.

Prohealth Organizer: That was wonderful thank you.