Lecture 8
NAPPA and its application in study of antibody immune response in disease and in drug screening -11

Welcome to more codes, on applications of Interactomics, using genomics and proteomics technologies. In the last lecture token, Dr. Josh La Baer provided, his expert opinion and comments, not only the researchers, but also as a clinician, who works in the areas of, breast cancer. In today's lecture procedures, Dr. Josh La Baer will continue his lecture, about studying, Auto antibody signatures, using NAPPA technologies. Which could be used for, early detection of breast cancer. So, let me welcome, Josh again for his lecture on Auto antibody detection, for breast cancer, using NAPPA arrays. Okay? So, I talked to you a little bit before about good study design and so, when we went to do our breast cancer study, that's what we did.

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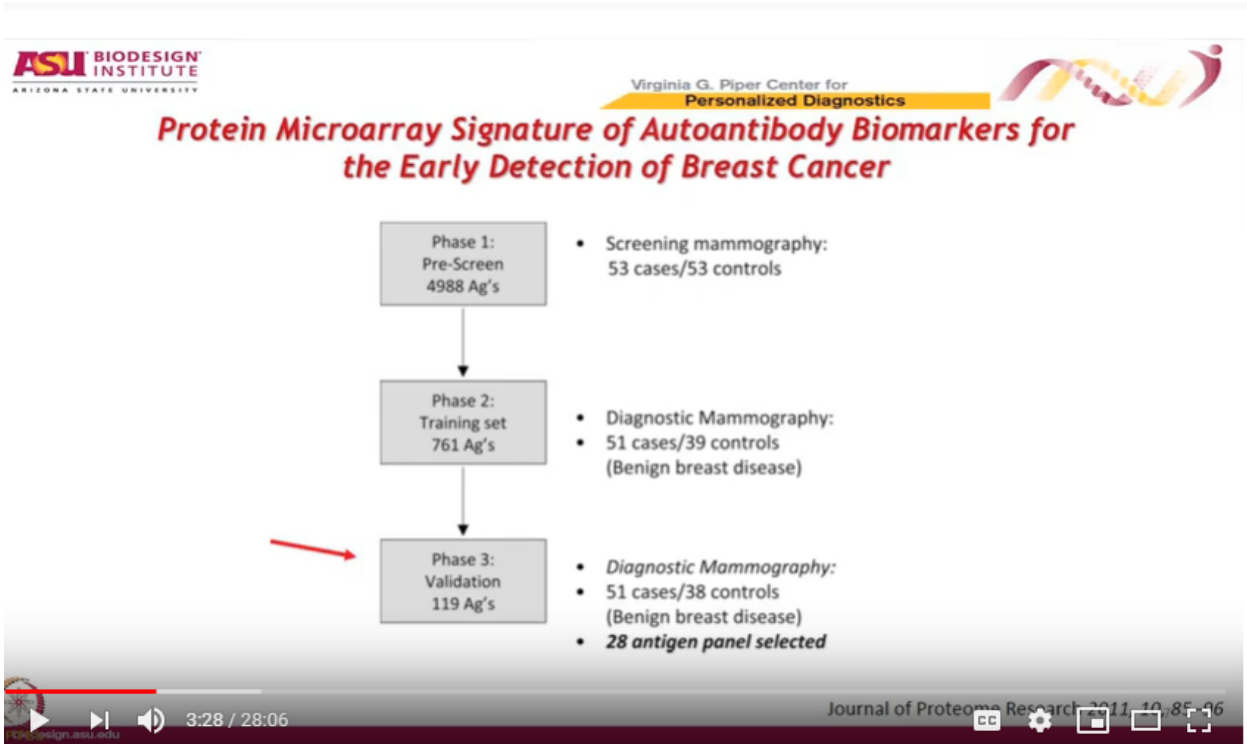

I think I may have mentioned this already but we had around five thousand antigens that we studied, we sent in 50 cases and 50 controls these, these, these are women with breast cancer and these were women who were going to a clinic, for routine mammography, who did not have breast cancer. Okay? so, these were healthy women in the population we got from there we identified around 760 antigens ,possibly different between the cases and controls. So, at this stage of the game from here to here we did we did not want to be overly selective, any possibility alive we will just want to exclude everything that was not even possible. So, even though we know we knew that there would never be any where near 760 we had already eliminated well more than you know 4,000 and that was already good for us, then for this set we printed a new array, duplicates on the array and we can compare 250 cases to 39 controls, in this case where the controls had benign breast disease. So, they had they had cysts and other things in their breasts but no cancer. And so, that allows us to distinguish being cancer and controls I will tell you that it's a little odd when you look at it that we had 50 and 39 you would have thought that we would had 50 and 50. Right? It turns out that that we did originally have 50, but about 11 of them turned out to have cancer after all and so, we had to take them out of the study. So, that kind of thing can happen you have to be prepared for that and then we did a third said these women were different from these women and these women were different from both of these women and this was the final validation. So, three phases of validation, in this case we did it blinded. So, we didn't know who was what and then we identified 28 antigens that even blinded were predictive of breast cancer. Okay? And I think in the end what worked for us was the fact that we were so, careful and going through all of these phases that what we got in the end really did make a difference and that's, that's why it was licensed by this company to make this Vanessa which is a blood test that's based on our biomarkers and a couple, of other markers, that they added to, to detect breast cancer.

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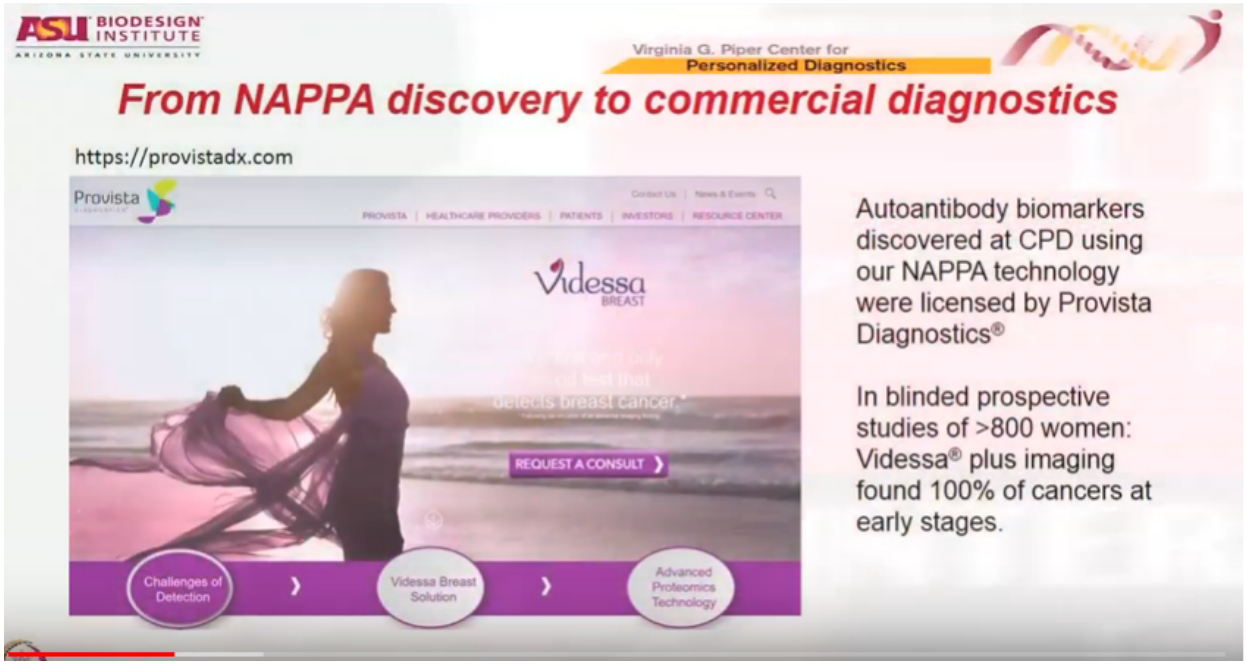

And this, this has now been through a couple of clinical trials I think

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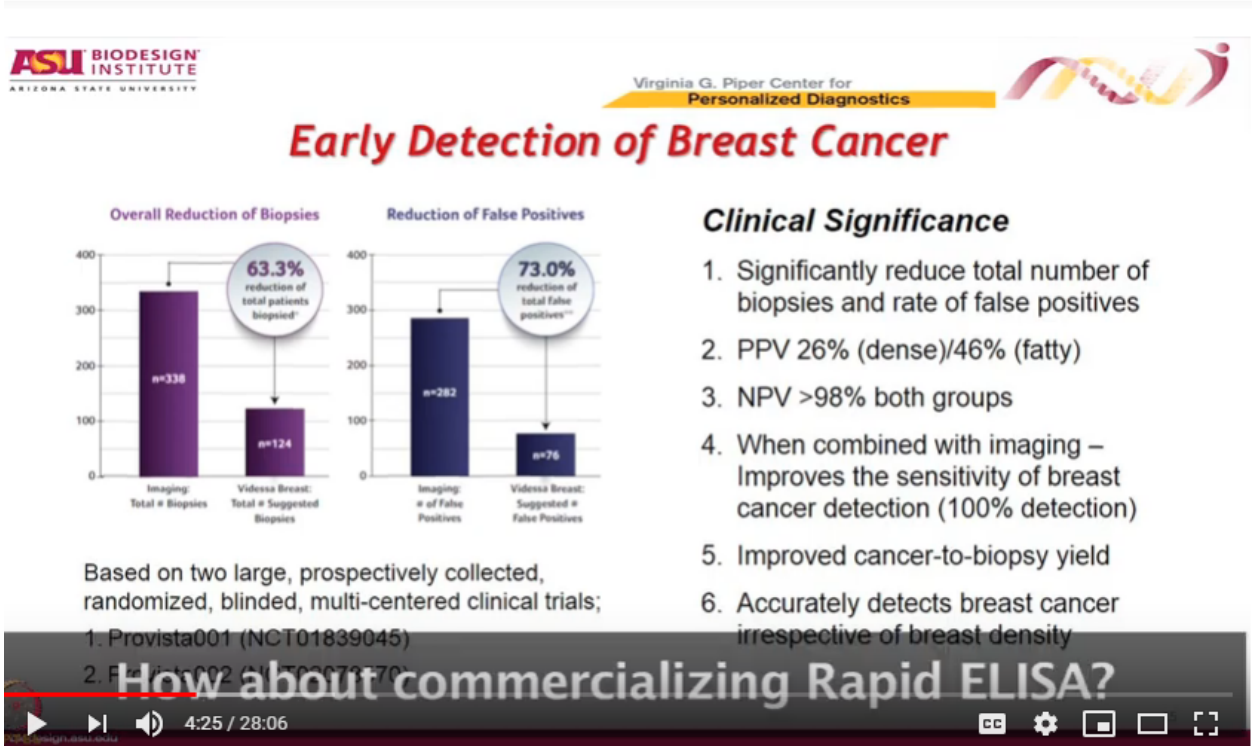

I mentioned that earlier you can see that in, in this clinical trial and this clinical trial they've reduced false positives dramatically while at the same time detecting all of the cancers. So, the combination of the marker, plus mammography found every single cancer and, and at the same time reduced, a lot of biopsies and women who didn't need it .Okay? so, that I think I told you a little bit of that story before, but any questions of this part so, far before I go on no we haven't commercialized the rapid Eliza, we have a core facility that will do it we'll help you do it if you want yeah it's not hard to do an academic lab can do it and we've written up the protocols, you know I think they're, they're kind of anywhere between certainly anywhere up to I would say 100 nanomolar maybe if you use SPR you can get up to micro molar affinities, but anything below that any, any tighter affinity than micro molar you can, detect on the arrays what you can yes yeah. So, micro molar is a little iffy certainly anything sub micro molar you can detect, yeah all. Right? So, we, we moved on and I mentioned to you before that we know that breast cancer is a heterogeneous disease that means that there's multiple subtypes.

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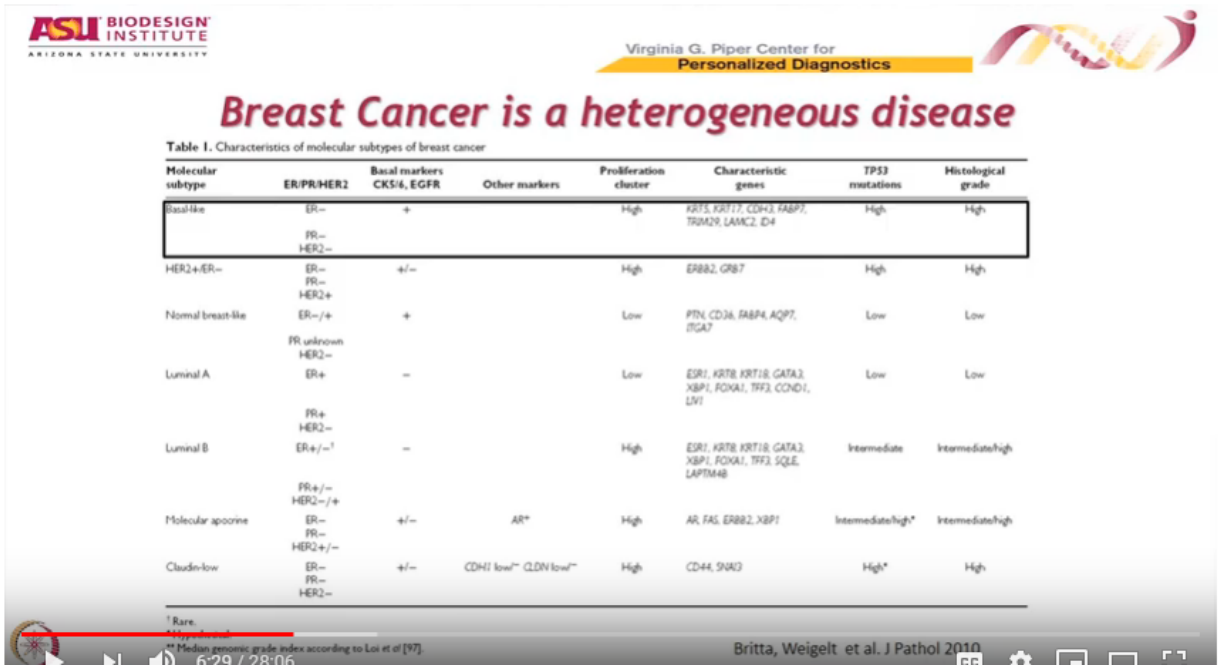

But there's the basal-like subtype the her2/neu subtype luminal a luminal b claudin like low. So, these are all various subtypes of breast cancer and we were especially interested in this one here. The basal-like breast cancer because, it's estrogen receptor negative progesterone receptor, of negative and her2 negative it also tends to have either EGF 4 or cytokeratin positive disease and this particular type of breast cancer, is very lethal it tends to occur in younger women, tends to occur in women of color and it is often not detected well by mammography. And so, all those features make it a disease that would benefit from early detection, because it doesn't have estrogen receptor and it doesn't have her 2 receptor, a lot of the drugs that we have today like Herceptin and the different inhibitors will not like tamoxifen and that sort of thing will not work on that disease because, it’s it the companion diagnostic is negative. Right? So, we, we thought wouldn't it be good if we could get some markers for that disease. So, to do that we collaborated with joining Figueroa she was at NIH at that time and she was running

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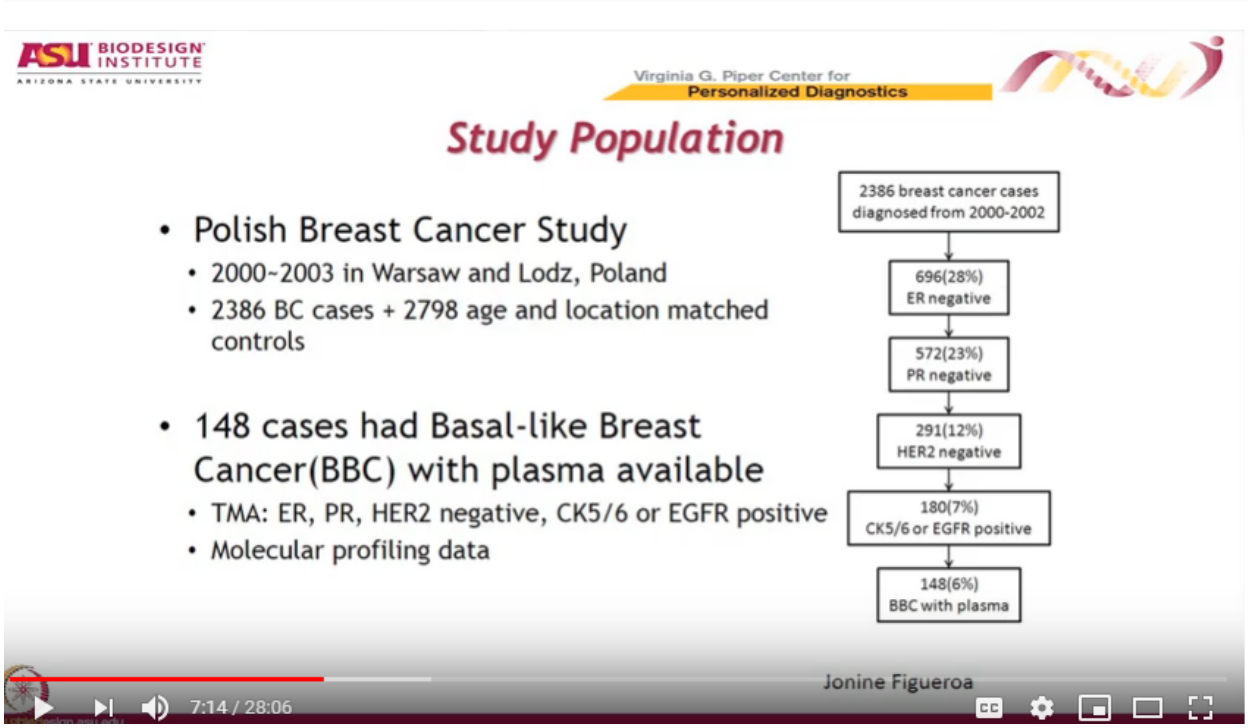

This large Polish health study, this study had roughly 2,400 breast cancer cases in Poland along, with around 2800 age and location matched controls. So, we took her study and we sorted it for women who are estrogen receptor negative, progesterone receptor negative, her2 negative and who had either seek a positive or EGFR positive. And so, from all of these we got down to around 148 cases of true basal-like breast cancer and then she was able to get us 150 age and age-matched controls. Okay? So, I won't go through the details of the study design here but I will point out that we did very much the same that we did last time.

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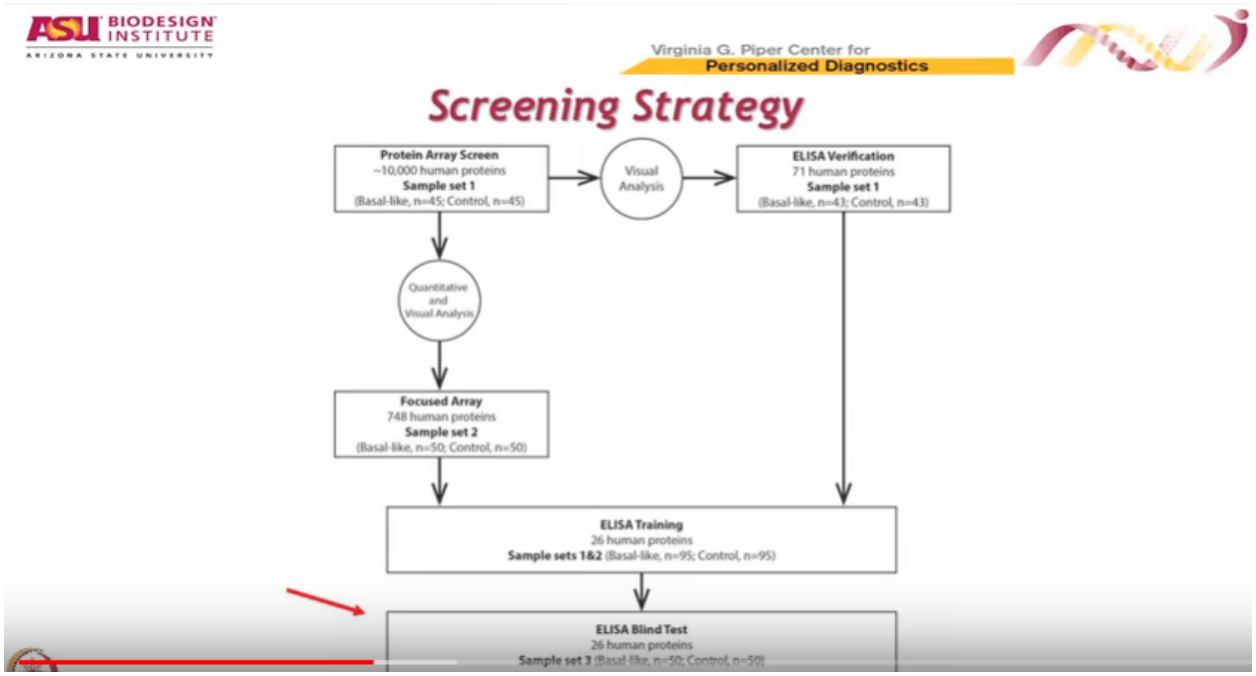

So, we have this sort of multi-phase study, where we you know did, did basically did two stages of ,of discovery and then verification and then did a third level of validation, where we tested a set of 26 proteins, on a sample set that had never been seen before, completely independent sample set and we did that blinded. And it turned out that we did not get a lot of markers that held up all the way through but we did get a couple.

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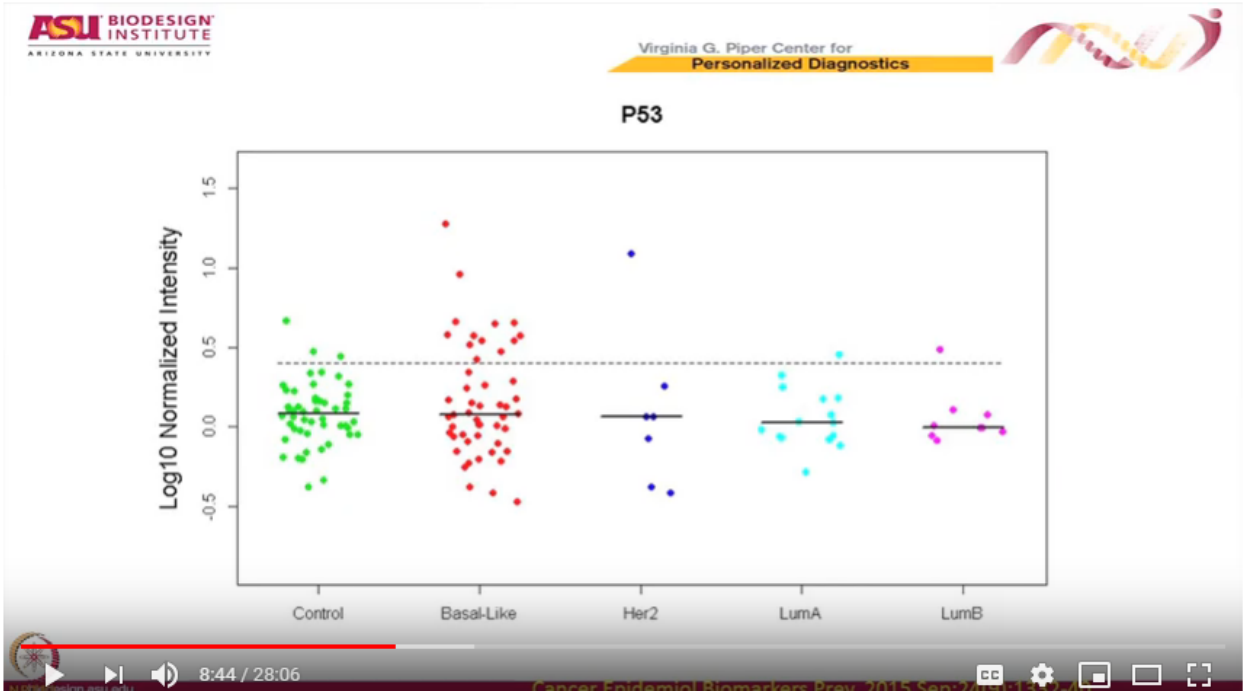

This is C tagged 1a and you can see these are different types of breast cancer, luminal B luminal a her two these are control women who are healthy, this is the cutoff value for the test and you can see that that at least for the basal like there are definitely a number of responders here now not everybody is a responder but all of these were responders. Right? Here's another protein you may have heard of p53 before, it turns out that it's a good auto antibody protein and again it was particularly positive in these basal-like breast cancer cases. Okay?

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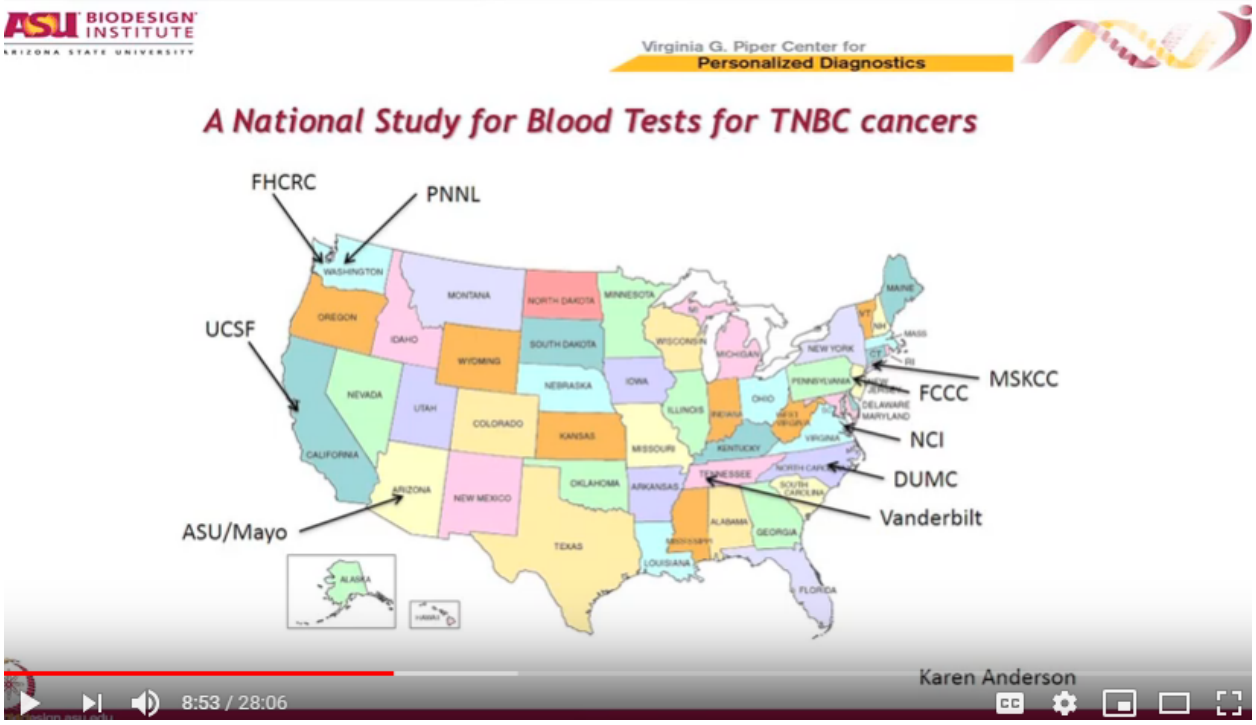

So, we actually participated in a national study, to see how these markers held up against other markers for triple negative breast cancer, this is through the early detection research network at the National Cancer Institute in the US, there were I think 14 different laboratories that participated in this study, it was a fully blinded study so, that all of the participants, were given samples that were unmarked and asked to test them and predict cancer, for them there were over 80 markers tested and after all of that and all the studies ,that were done the only markers that held up were our markers the antibody markers I just showed you.

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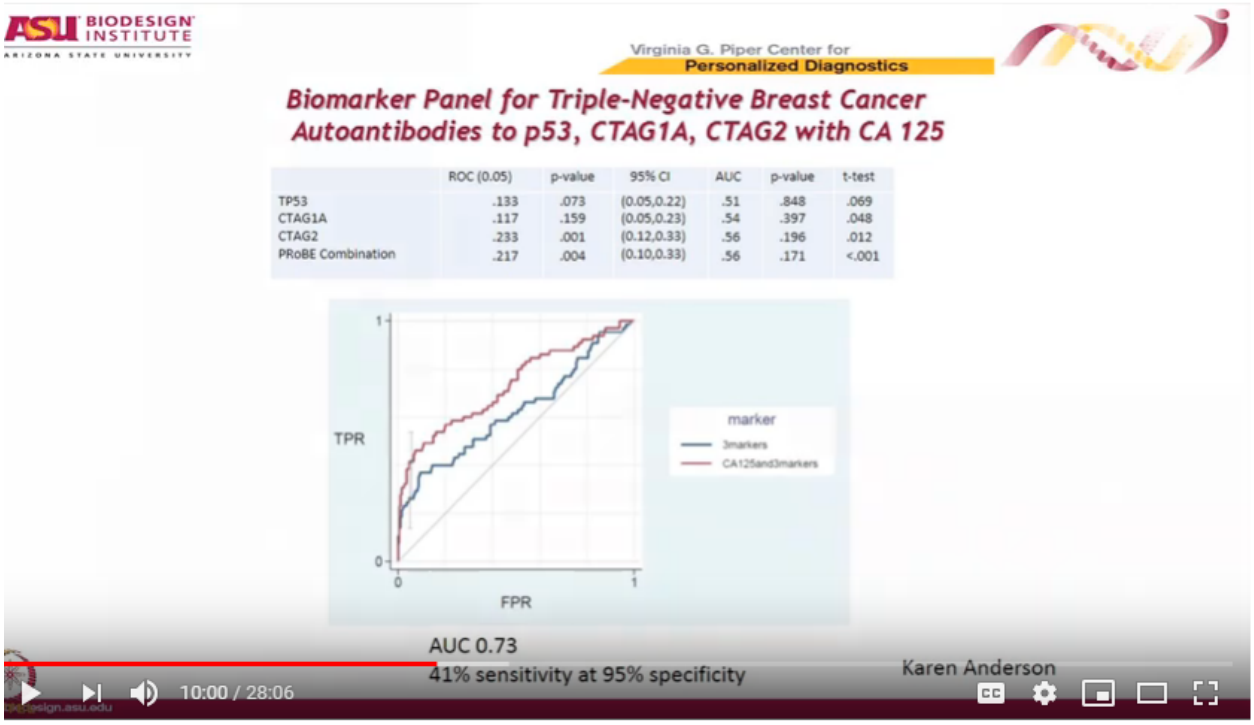

And those markers well there they are so, p53 CTA and B, here's the AUC curve we didn't really talk a Oh speakers I think you're going to get that soon when you get your statistics lecture, but this gives you some flavor of that and then we added that together with ca-125 began a slightly better performance, here in red and so this. Right? Now I think is the best blood test for, for basal for basal cancer. Okay? I'm going any more questions on that. Okay? So, I'm going to enter the last section of the talk.

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I'm going to talk a little bit about where we're headed with breast cancer studies and we think that the future of biomarkers is looking at post translational modification.

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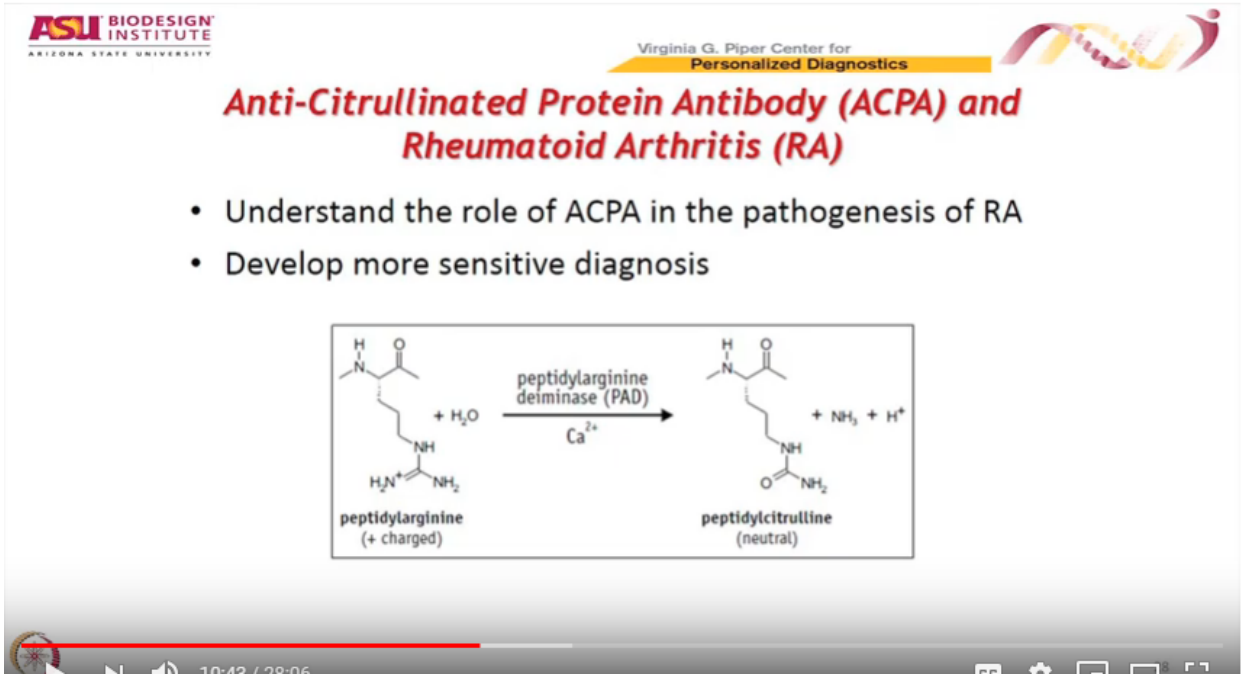

So, here's one form of post translational modification what's going to come back later, this is called,' Citralination', it's when you take an arginine and you treat it with this enzyme pep to deal arginine deaminase and it converts the, the arginine into this thing called,' Citrullinated', and that this is, this is the sitting molecule here so, you get this ketone here. Okay?

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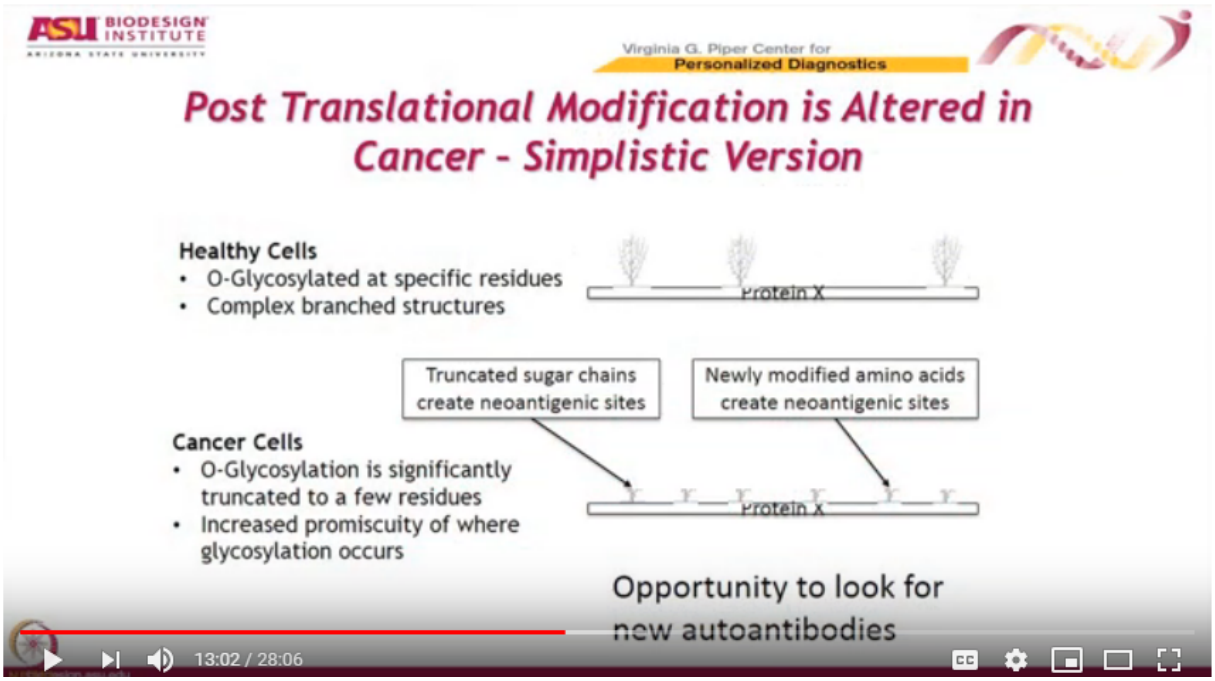

So, what we mostly have been interested in is like oscillation. So, you guys are all familiar with like oscillation. Right? So, what fraction of proteins, in the human do you think are glycosylated I heard someone say 70 certainly more than 50 anyway, I don't know if it's as high as 70 it could be, but certainly more than 50 to 60 percent of proteins have sugars on them. So, it's very common and you know that like oscillation occurs and linked and old linked and today I'm only going to be talking about old link like oscillation mostly because, most of the studies done in cancer have been done on Oh link like oscillation. So, typically what happens with like oscillation is you have these sugars attached to specific amino acids and they form these very complicated branch structures. Right? So, they have this you know many, many, many sugars stacked upon one another that's, that's pretty typical in normal cells in cancer two things happen the first thing happens, is that you get truncated sugar structures, they get truncated because there's an enzyme called cosmic that's missing and that is the one that only adds the sort of fourth level sugar. So, you get three levels and then it sort of stops so, it's like getting a crew-cut it gets very, very short hair. Right? The other thing that happens in cancer is, is it gets more promiscuous and so, instead of only a couple of places on the protein you start adding sugar at multiple places on the protein. So, to someone like me that looks like an opportunity. Right? Because, there are two things now that are different about these modifications, they create two new shapes, that could induce an immune response the first is that you get these short sugars and so, the immune system might see that as different from normal and the second is that you now have amino acids that are modified they didn't use to be modified so both of these are potentially inducers of an immune response and that would mean that for me they're an opportunity to look for new auto antibodies. so, to get our platform to work well with looking at post translational modification, we had to clean up the platform a little bit and so, consequently

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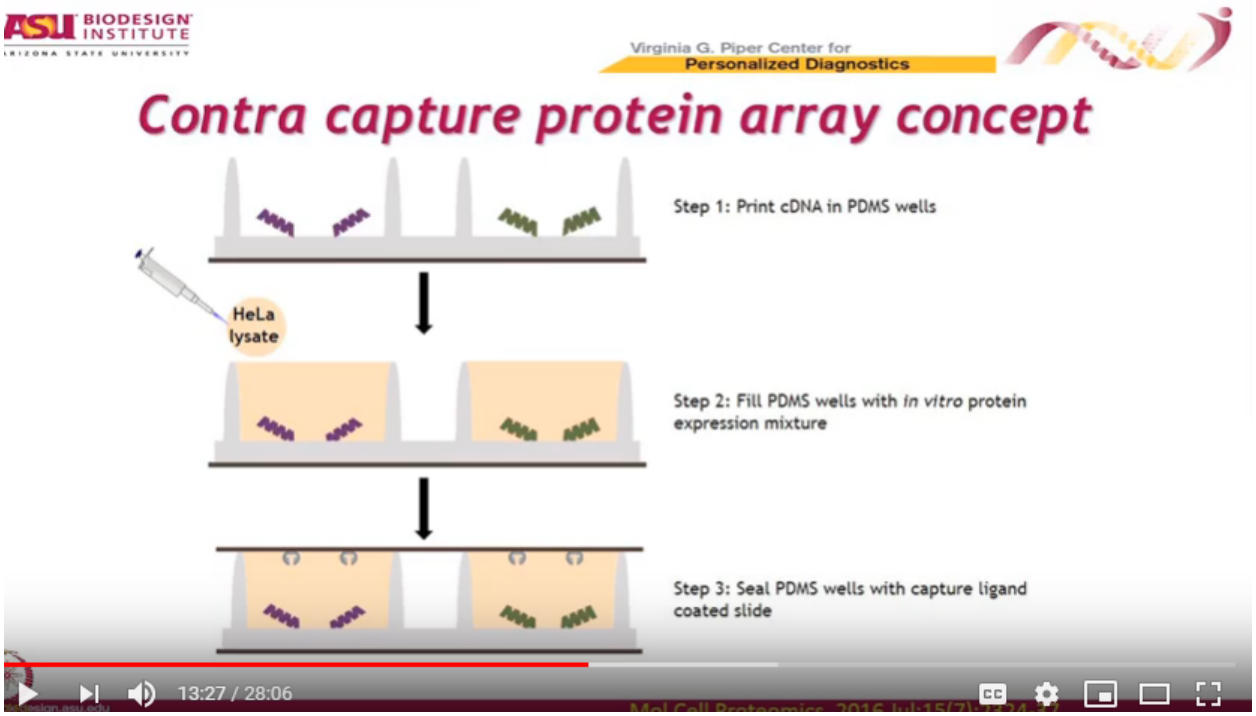

We developed this method here which is called,' Contra Capture what we do is instead of printing on our DNA on a glass slide like the way you're doing it. Right? we print our DNA in these soft wells, of a PDX membrane and then we put the DNA in these wells, we add the HeLa the lysate to make the protein and then we cover the well with a glass slide, that has the capture agent on it. So, we separate where the, the capture agent is from where the DNA, is you know you normally at NAPPA you print the capture agent and the DNA at the same place, here they're on opposite ends of the of the sandwich the other thing that we're doing here is we're not using anti GST anymore we're using the halo tag are you guys familiar with halo tag how many people know what halo tag is not so, many.

So, halo tag is it's in it's an enzymatic tag, that it's an enzyme that normally is supposed to bind to halo alkanes that is you know alkanes are structured of halo alkanes have a chloride, at the end or a bromide, at the end and, and the enzyme in, in its normal function binds to that and removes the bromine or the chlorine, in the case of the halo tag the enzyme has been mutated. So, that it binds to the chloride but it can't remove, it and because it binds to the chloride in a covalent attachment it now becomes, stuck permanently stuck to the chloride. So, an essentially way of causing a protein, to form a covalent linkage with a specific tag the reaction is very specific and so, you can selectively pull one protein out of a mixture of millions, by having a halo alkane on the end of it. So, so we put the halo the halo ligand on this glass slide and the proteins that we're producing have the halo tag and so, when they see the glass slide they form a covalent attachment to the glass slide, what we like about a covalent attachment of course is it's much more permanent, you can watch it really hard and it'll stay stuck all. Right?

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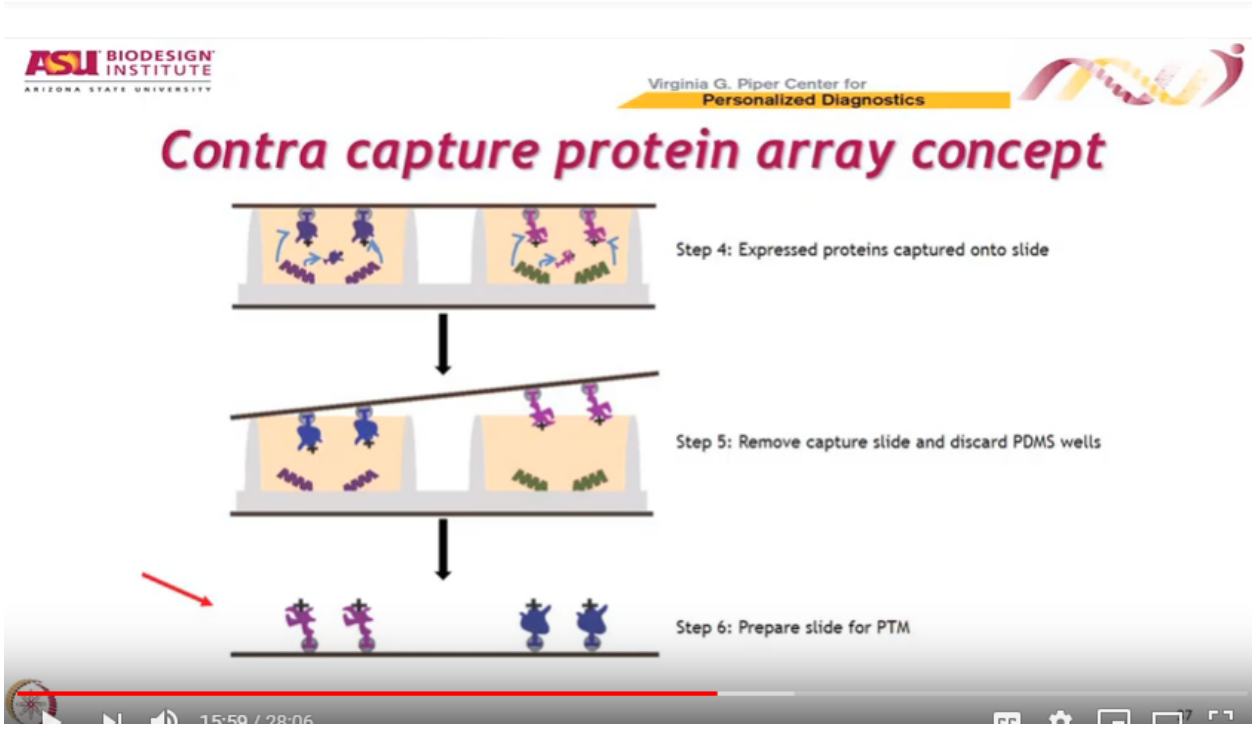

So, then we expressed the protein, the protein, goes up it links up into the halo tag up there where it forms a covalent linkage we lift the cover slip off the off the well. Right? And now we can throw this stuff away and we're left with a glass slide that has these proteins covalently attached to them and they're absolutely clean there's nothing on this slide but the, the tag and the protein.

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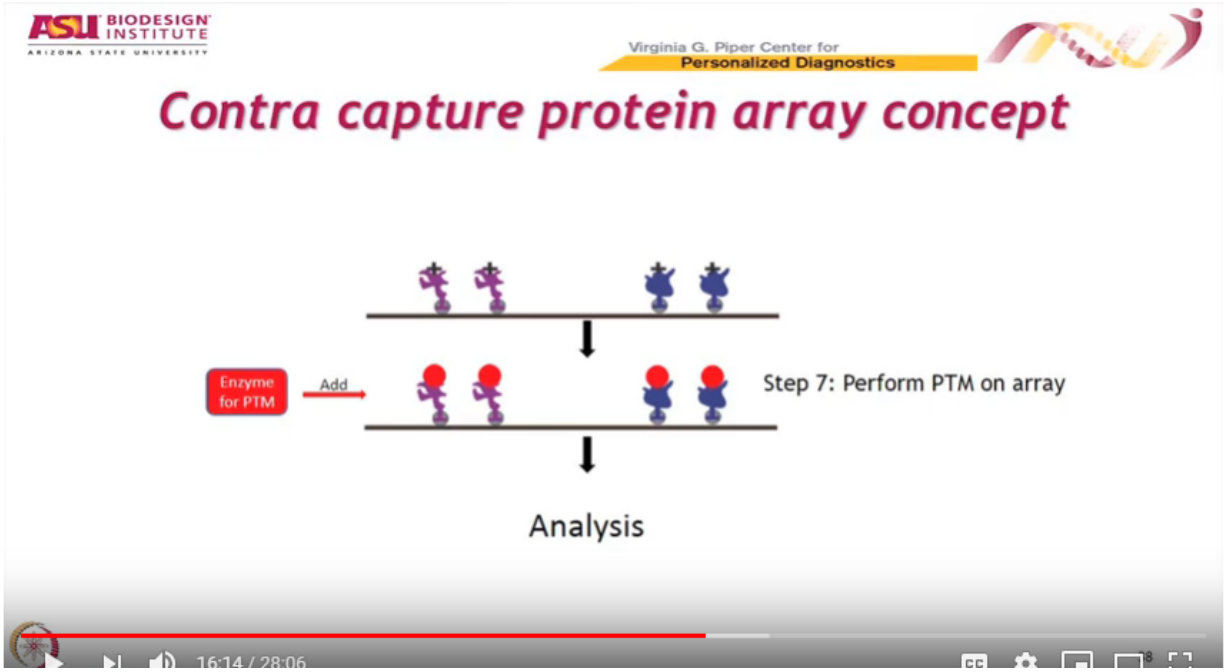

So, now we can come in with, an enzyme and we can add a phosphate group, sugar group, whatever we want to add and we can modify the proteins and then study them.

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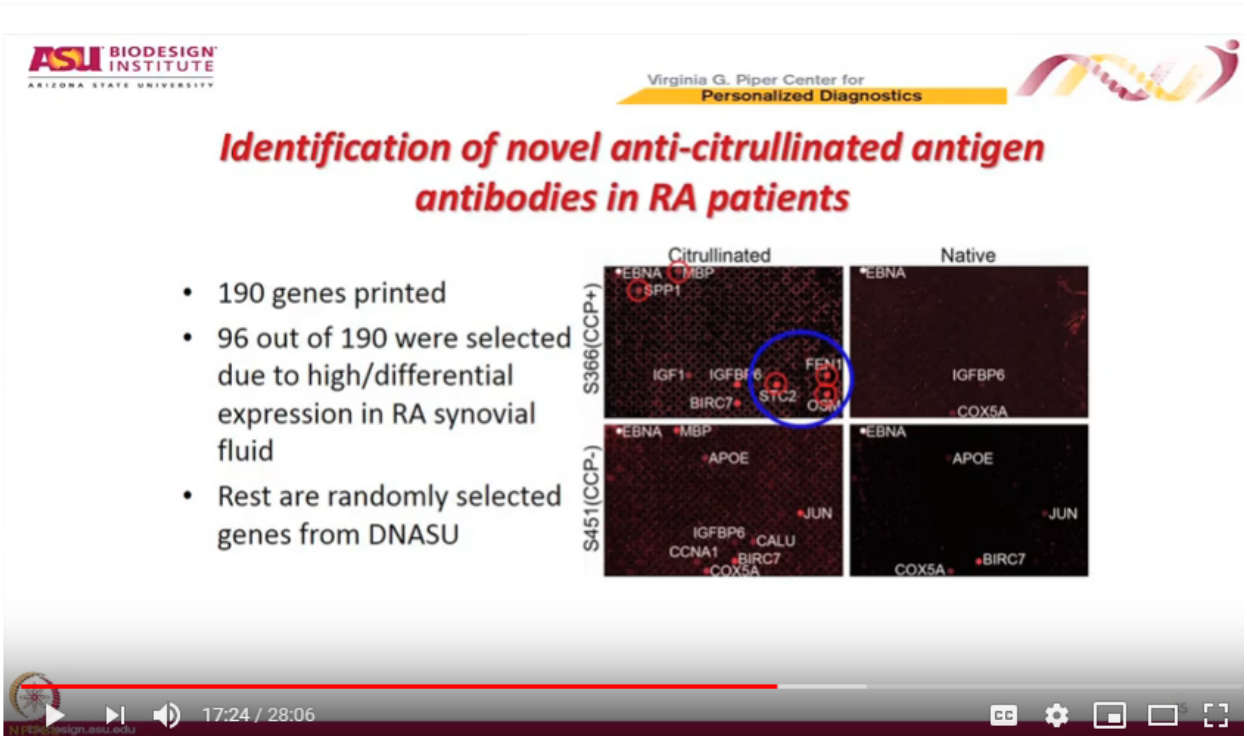

And I'll just show you a couple of examples, here's some NAPPA arrays, here remember I mentioned the situation, here we've either situated the array or we did not situate the array and here's a patient blood this is a patient who happens to have, rheumatoid arthritis in the case of rheumatoid arthritis patients make antibodies against citrulline and you can see that this patient has all these antibodies, to situated proteins but, but does not recognize the proteins when they're not situated you'll notice that we have this Edman of protein that's our positive control. So, that, that that's, that's a different antibody and not socially need rated .Right? related and yet it shows us that the this the assay was working but not taking all these other proteins, here's a bunch of others another patient same idea and again only signal are present in the in the sit related array identifying new interactors based on the situation here you can see some responses here, they were not previously known and again their situation positive or specific this is the same general approach but now we're doing with glycosylation. So, here we've added some sugars to the protein.

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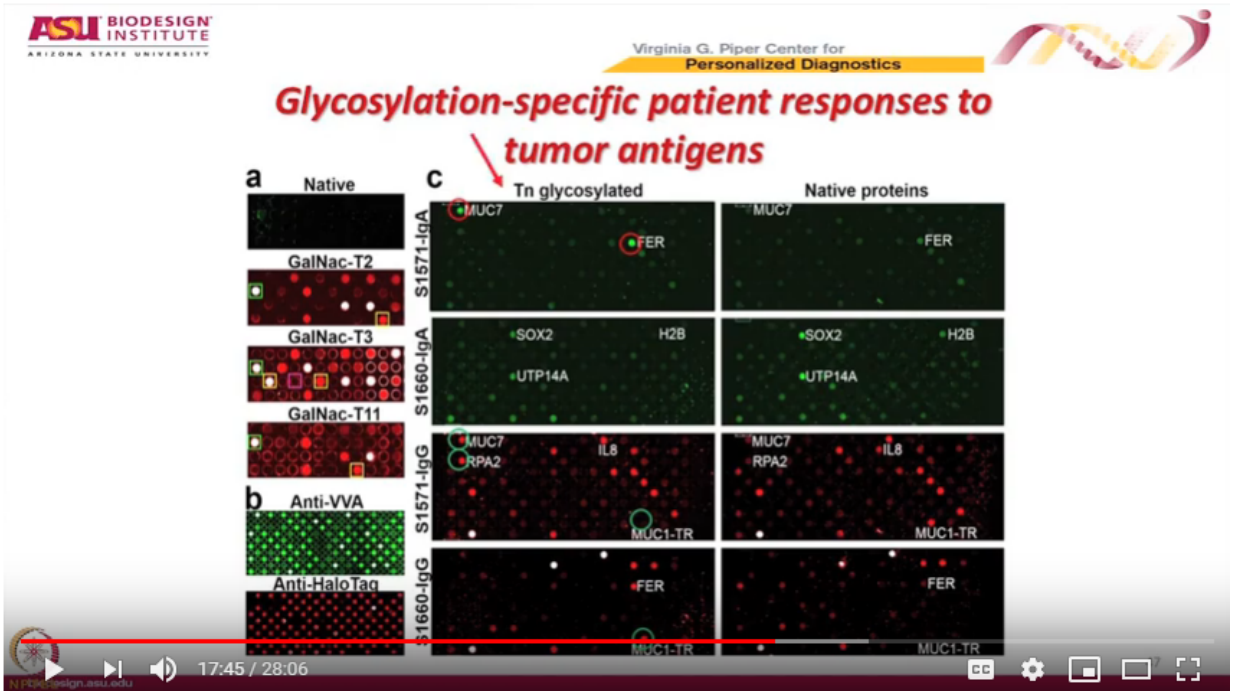

And again these are different patients, Sarah and if you look carefully you see a strong response, when it's like oscillated but not when it's not here's a couple of responses that you see on their unlike oscillation the are not present when they're not and so, and here's another one down here. so, the hope is that these responses, will give us a new opportunity to find biomarkers or specific disease ,because of the profound differences in glycosylation that occur in cancer. Okay? So, these are some of the diseases that we've studied using the NAPPA

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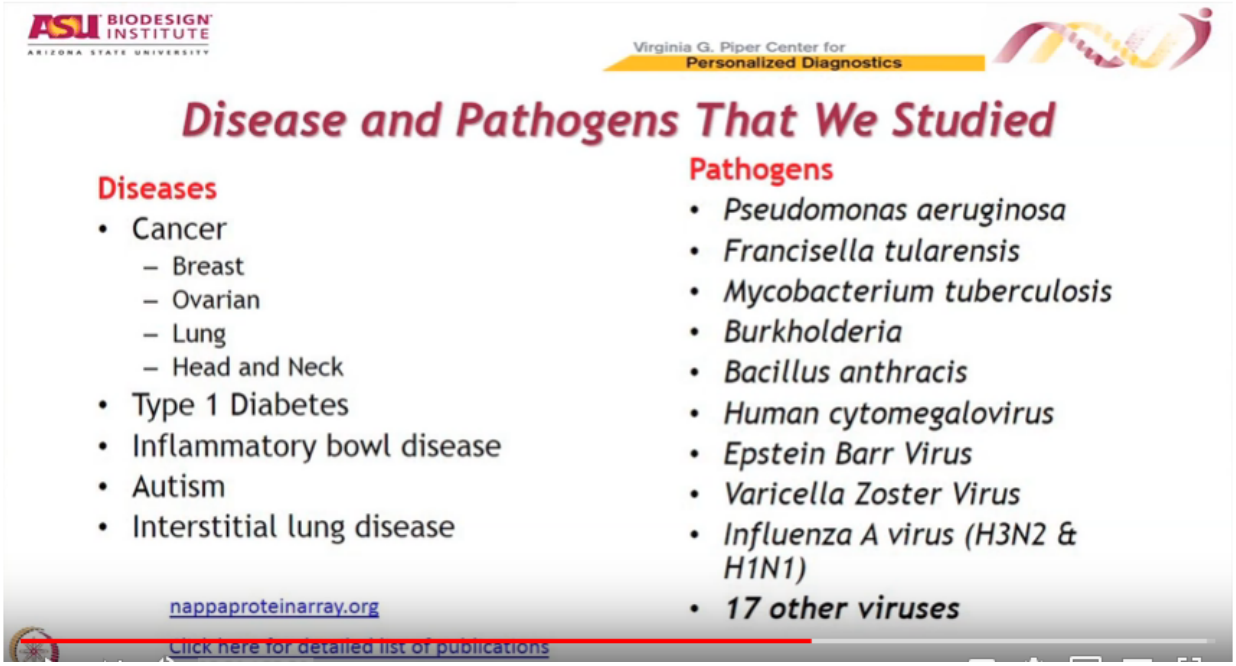

I've talked a lot about breast we've also done ovarian and long and also head and neck these entire have we've identified some markers, that have been published over the years type one diabetes is an autoimmune disease. And so, we've looked at that we've looked at inflammatory bowel disease we recently published a paper there we're looking at autism that's early stage and also interstitial lung disease. And then we've looked at a series of different pathogens over the years, this one we just published earlier this year and actually now ,we're increasing the list of viruses dramatically so, with that I'm going to stop for today I can take questions. Right? Right? So, how do we avoid diffusion so, I my last lecture I'll probably talk about that a little bit more, you do get some diffusion, at the spacing that we do on the arrays and the size of the features that we create, the amount of diffusion is pretty limited it's about we actually measured it it's about a couple percent, compared to the spot itself so, that immediate neighbors will have around two percent of what you would have the signal so, we felt that that was low enough that we could get away with it where it becomes, an issue is if you try to make the arrays at a much higher density and move the spots closer together, then you start to see more, more diffusion to neighboring spots and so, when we get to that stage we have we've induced a different technology, that involves creating Nano wells.

That's a good question, well first of all I don't know the numbers here, certainly, there's there could be a number of factors certainly there could be risk factors that are different in that population than this population, it's also possible that it's detected more often in the US possibly because, of more aggressive screening programs, which may mean that that like I don't know what the overall per capita mortality is from breast cancer in India versus the US does anybody know that, well it's different from mortality or just the disease because, one of the issues that people raise a lot with breast cancer not unlike prostate cancer is are we detecting disease that doesn't need treatment. So, as you know in prostate cancer that's a big issue, because of the use of the prostate specific antigen, were detecting a lot of men with prostate

cancer, who will die with prostate cancer but not because of prostate cancer. And so, they end up getting treated when they probably didn't need to be treated and so, that may be true people have argued that's true with breast cancer as well that we may be over diagnosing it in the US and we don't really know yet. So, I yeah I'd have to look at the statistics but I don't know offhand that any major differences but I could imagine a number, of them I don't think the markers would help us in that particularly mostly in part because, these markers were developed in the US. Right? So, they were based on a population in the US. I don't know if, if that if that would necessarily tell us about breast cancer here, it might but we just don't know yeah with what yeah Oh, Oh interesting question well yeah. so, so I there first of all I should just make the point that we don't use wheat germ we use human, cell-free we do that because we're looking to make human proteins and we think better chance of getting good folding, that said all of the, the cell free lysate tend to be a little finicky they definitely are you they if when you think about what they're doing they're very complex within that tube, you have everything to do both transcription and translation.

So, you're asking a lot first you're making you have a promoter binding, transcription factor to produce, RNA then you have to have ribosome's bind to the RNA, tRNA is recruited amino acids added and then you have to have an energy generating system, because as you know translation, requires ATP usage and then and then you have to have chaperone proteins present, to fold the proteins in the natural folding. So, you're asking a lot and to get all those components in a single concentrate that works it doesn't entirely surprise me that it's that it is temperature sensitive and that it's fragile that said believe it or not you can, lie awful eyes the cell free lysate from bacteria. So, you can make it a powder you can add water and make protein, from it so, that's pretty stable efficiencies low and, and it's not good for large proteins so, wouldn't be my favorite choice by far but it I think it doesn't surprise 6me that such a lysate would be a little bit sensitive, it would be cool to get a file to do that I don't know anyone's tried. Okay? Oh yeah I don't know, do you vote for CML, yeah that's interesting about I don't know I mean usually when you get cancers in young people, that's a sign of either translocation, genetic translocation ,yeah chromosomal, abnormalities because, that's a pretty young age to have just sporadic mutation. Okay? I think I think we're done

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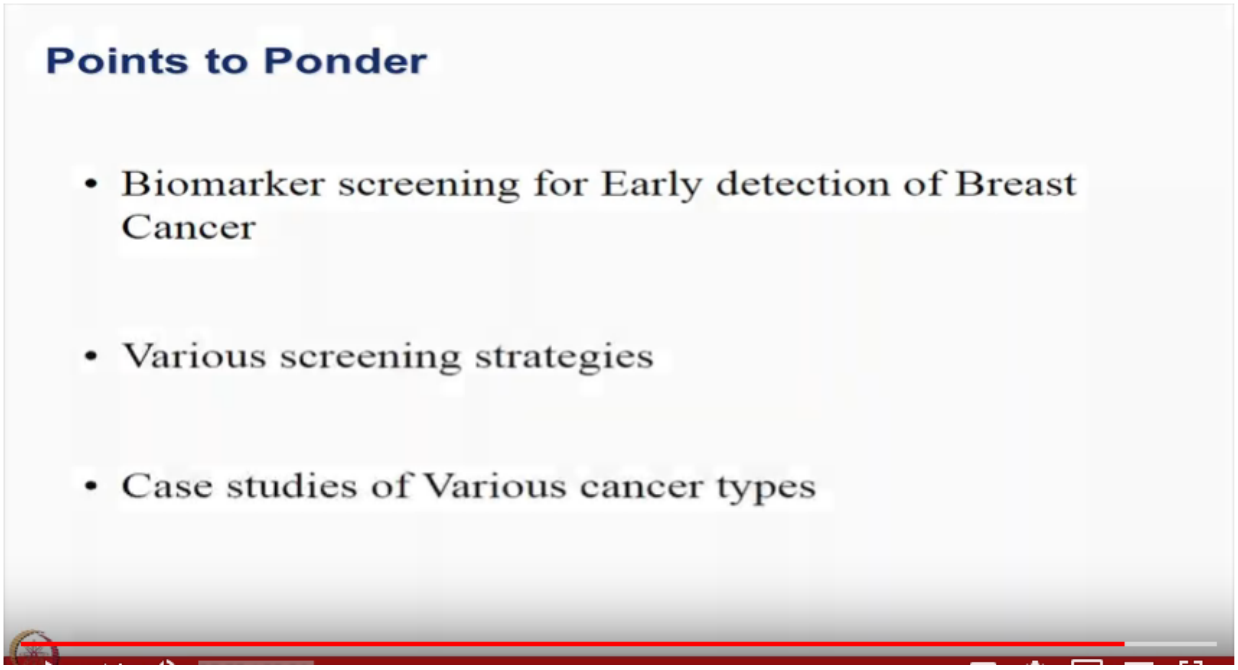

I'm sure you have enjoyed, both these lectures delivered, by Dr Josh La Baer talking to you about, utility of a technology especially NAPPA technology platforms, the insights which are required while performing these are says, your details for testing the reproducibility thinking about extramental design and then finally, the outcome which one could obtain from these experiments, can be so remarkable which could be utilized for the patient care, you must have understood, the clinical significance of a rejection of biomarkers, you also studied about the biology of cancer, in some detail and the tests that are now being used in the clinical trials you are introduced to the concepts of contra capture protein arrays, which could be utilized for studying post translational modifications and you also got a glimpse of how various diseases could be studied using NAPPA technologies. In the next lecture we'll continue our discussion about, use of novel technology platforms for various biological applications and you will have series of interesting examples and illustrations, to convey the utility of these technologies at the same time what entails to obtain the success from these experiments which is your careful experiment your quality control checks your data analysis and your insight and understanding about how to make a meaningful medical experiment. We'll continue these in the next lecture till then. Thank you