Lecture 4

Biomarkers: Harnessing the immune system for early detection of disease-II

Applications of Interactomics using

Genomics and Proteomics technologies

Welcome to MU codes on applications of **Interactomics,** using genomics and proteomics technologies. We have started discussion about, how to make contents for high-throughput assays, in one of the

technology platform, which we are discussing is, protein microarrays, protein microarrays without need to purify the protein of interest, that's what you heard from in the last lecture from Dr. Joshua LeBaer, about the technology development aspect of, nucleic acid programmable protein arrays or Nappa, in the same line let's continue, our lecture with our distinguished invited faculty Dr. Joshua LeBaer, to talk about some applications of these technologies. Biomarkers are, very valuable for variety of applications which we want to decipher for example? You want to monitor a drug response, you want to detect a disease at early stage, you want to follow the disease progression, or you want to follow, how long a patient can survive? You want to see that a disease might reoccur, right?

So there are a variety of ways different type of bimolecules, is starting from the proteins, another bimolecules could be used as biomarker, to indicate variety of physiological states, and therefore biomarkers could be diagnostic biomarkers, prognostic biomarkers, recurrent biomarkers and you can name, main biomarkers for, different type of applications, but exactly what these biomarkers are? and how they can be used especially? In the clinical settings in the clinical scenario, Dr. Joshua LeBaer is going to talk to you today in much more detail about, biomarker based assays and of course protein microarrays, based applications will follow that how you can use protein microarrays, for the biomarker discovery programs. So, in this light Dr. Josh is going to talk to you about what two biomarkers and what are your considerations? When you are thinking about a biomarker discovery program or you want to discover some biomarker. What should be your criteria's, to determine the sensitivity and the Specificity? The biomarkers it's going to talk to you about some of these basics in today's lecture.

Today what I thought I'd do is take a moment and talk a little bit more about biomarkers, both in the context of what they're useful for and also how do you evaluate the quality of a biomarker? So, here's the definition that I put down Okay? So, it's a measurement of some kind, with the intention of providing information about a clinical state, or a biological state of the organism.

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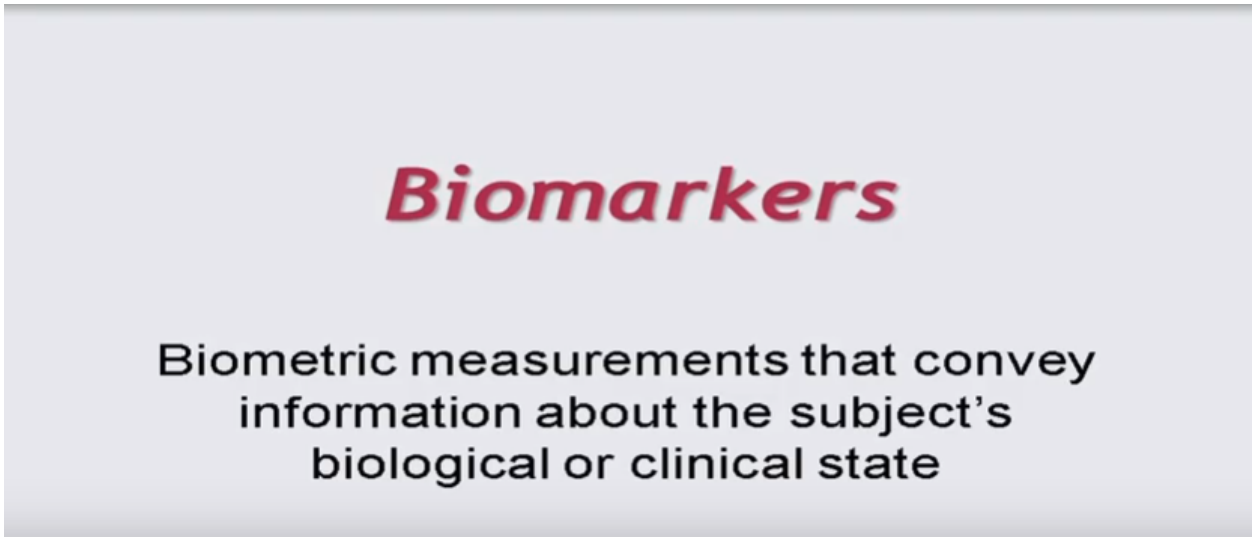

Right so, if I if I if you go to a doctor's office and they measure your blood Pressure. That's a biomarker they're using the blood pressure to get a sense of your Health, and they're gonna use that to predict the likelihood of getting, hypertension or cardiovascular disease Right? If you go to the doctor and they measure your cholesterol that is a biomarker. it doesn't exactly measure the health of your heart. But it's a

predictor of the health of your heart Right? So, anytime that you measure something, you're the goal is to predict the outcome and that's important to remember because you're not exactly measuring the outcome, you're measuring a predictor of the outcome and that predictor may not be perfect, some predictors make mistakes, right? Okay? So, the types of biomarkers that I work on are proteomics biomarkers. I'm not going to ask you what proteomics is. Because, I know all of you know that. But let me mention that there are two general approaches to proteomics,

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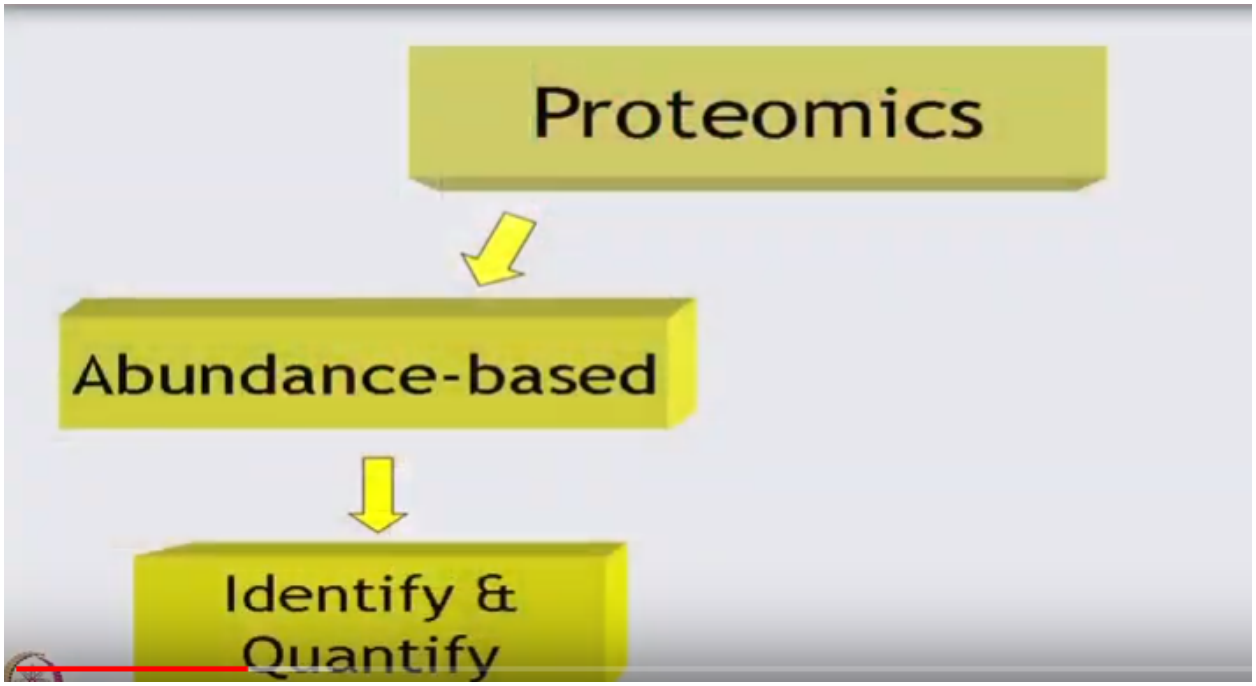

one is this abundance based approach and, and we talked about that a couple days ago, that is essentially measuring the amount of particular proteins in the blood and or in any other in tissue or in any other, setting and typically on the abundance based approach you measure the difference, in the abundance of specific molecules, in the healthy, state and the disease state and you look for differences that are biomarkers. That they're predictive of the outcome right? Some proteins will be different but it has no predictive value, it's just, random variation and the job of a scientist is to figure out. When those differences are predictive and when those differences are just random variation or sometimes they're a little bit in between maybe they're mildly predictive but not predictive enough to do you'd be useful.

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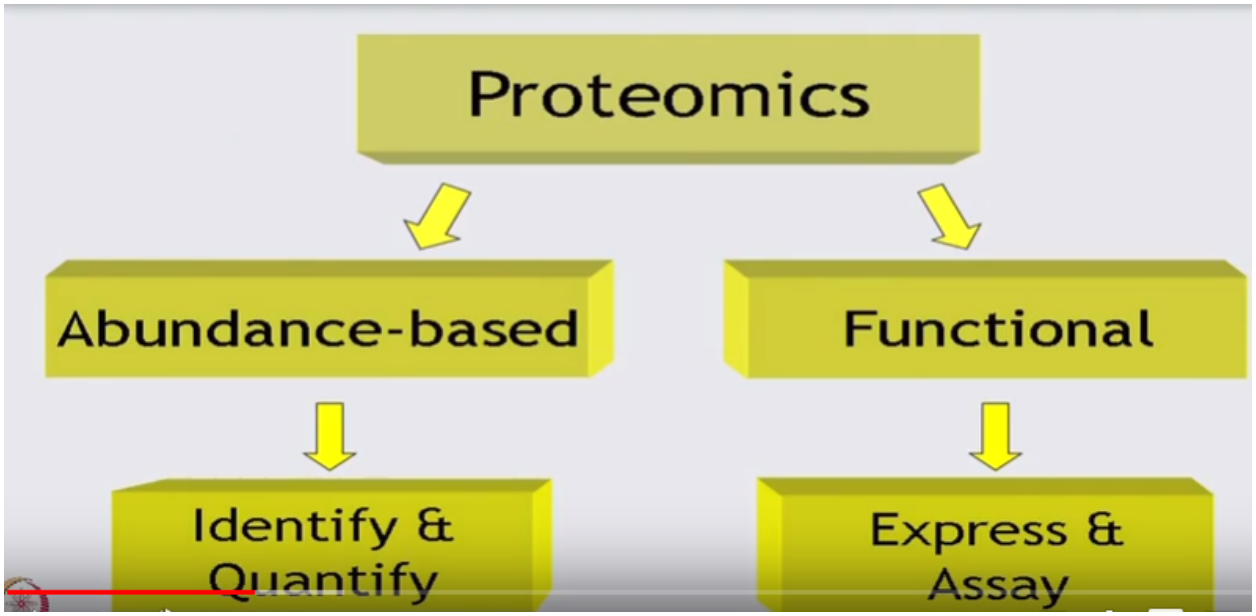

The other, type of Marker, that the other type of proteomics that we'll talk about is functional proteomics and that's the kind that I do, that's producing proteins and then studying their their function and once again we use that to look for biomarkers okay?
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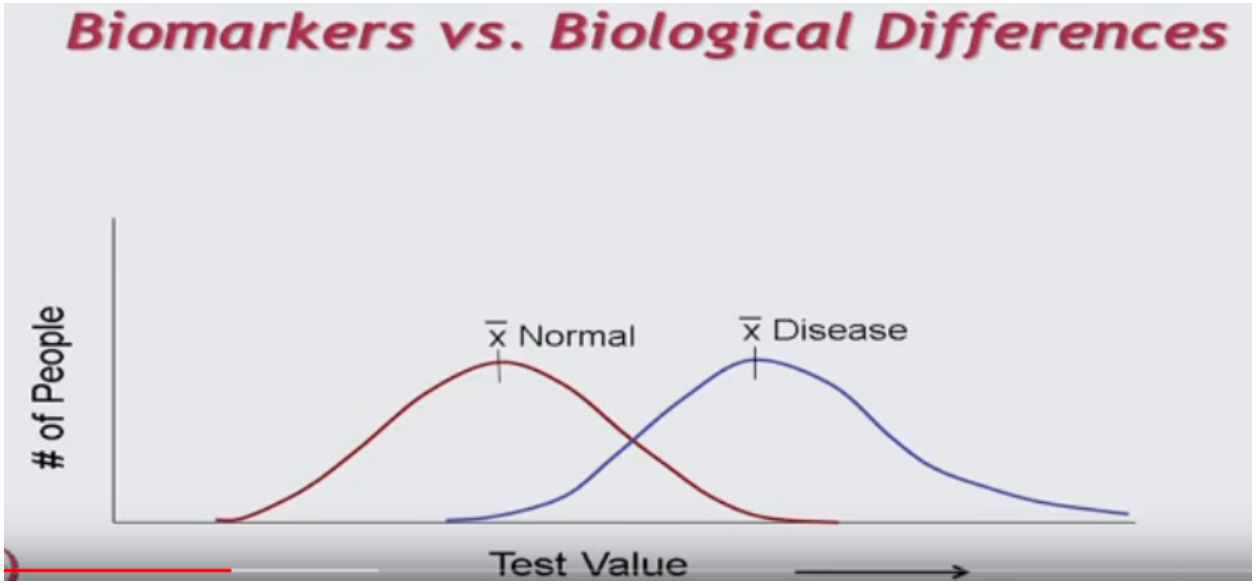

So, this is a common, a common issue that comes up a lot in biology as some of you may know I'm also the editor of, the Journal of, proteome of, Journal of, proteome research. I'm one of the editors there so; I get papers all the time from scientists who want to publish. Biomarkers and this is one of the most common mistakes. I see all the time in the marker field so, imagine that you're you're measuring some value of some molecule let's say it's a protein that you discovered when you did your mass spectrometry or an antibody marker. That you discovered on a protein array and you see that in the case of the disease. It's much higher than in the case of the normal and you say wow look at that difference it's the, the mean

value over. Here is much better than the mean value over here and therefore I've got a biomarker okay? So, does anybody see any problem with that what the overlap that is the problem right? The overlap. This Right? Here this is this particular measurement. While probably significant in terms of the biology is not a good biomarker

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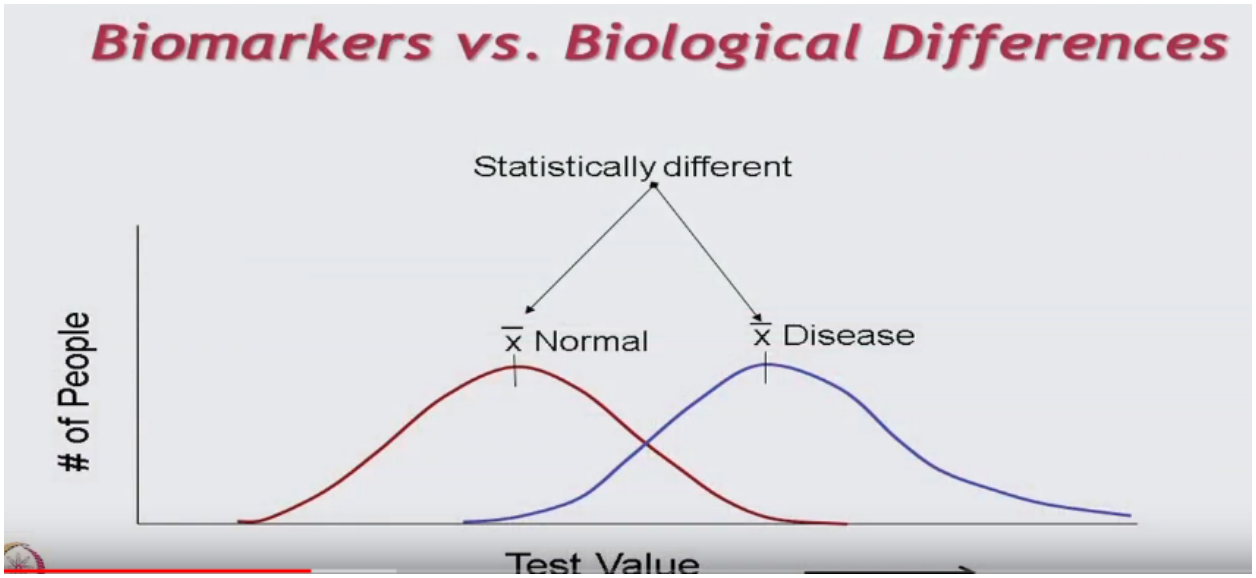

So, those two values are statistically significant there's no question they will have a very good p-value. So, m you'll be you know you'll be tempted to say it's a good biomarker.

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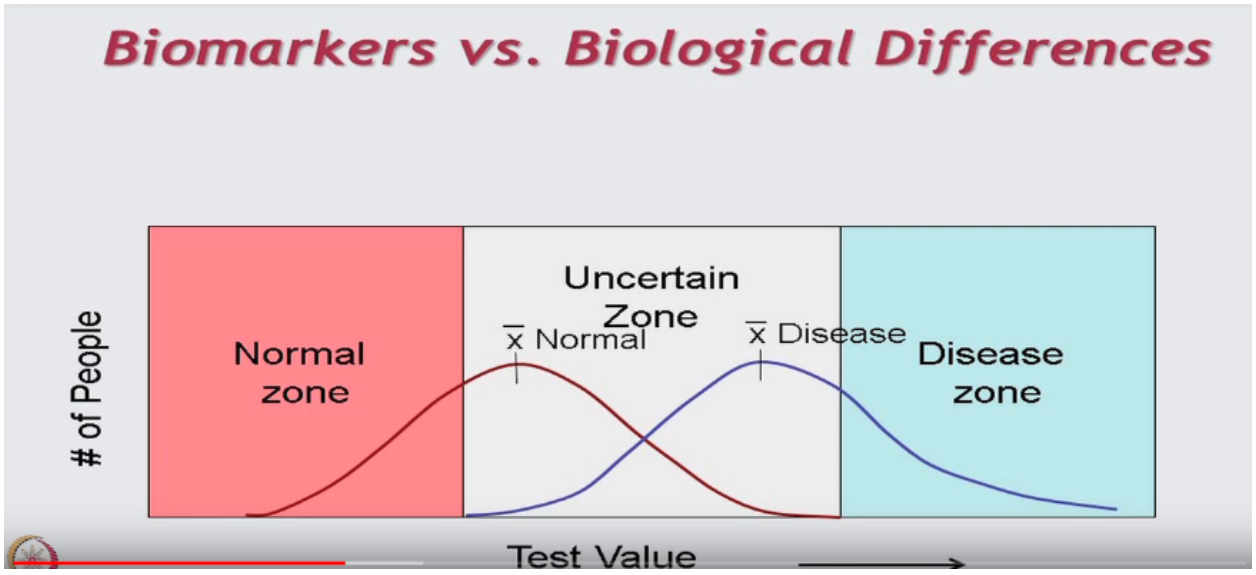

But let's consider the use of the biomarker. If the value is down here you could very safely say that that's normal you could. Say if you if you measure that in a person no problem that person is healthy. If you if you measure the value up here you can say very clearly that person has the disease but, but as she pointed out look at how much there's overlap here if you if you measure anything in this range maybe it's disease maybe it's not disease it's, it's the, the, the separation is not clean enough to make this a good biomarker and so that's and that's why we're gonna talk a little bit today about how do we define good biomarkers okay? So, there are lots of different ways to classify biomarkers. So, you could definitely talk about the uses. What other what other types of ways to classify biomarkers? What yeah what you're measuring what, what the material is you're measuring so what type of molecule absolutely? What other so that's the type of Molecule? What any other?

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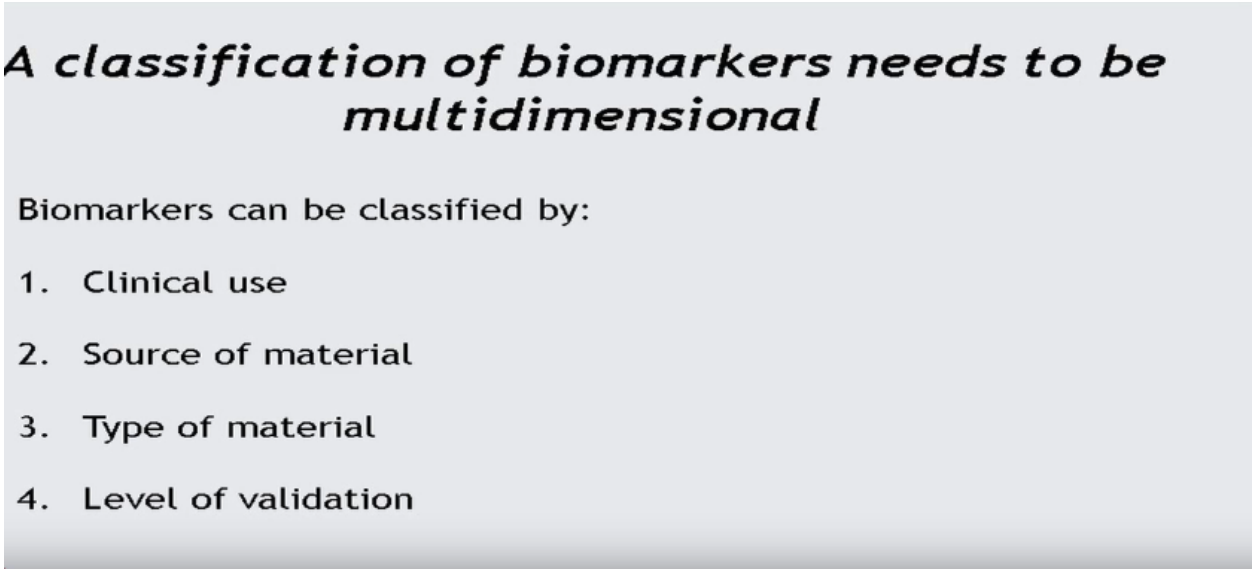

What about the source of the molecule is it blood is it cerebral spinal fluid is it urine. So, Where you get the molecules also you can you could be looking at DNA protein or, or, or lipids so that's the type of molecule but you could also be looking at blood markers or urine markers and then the last thing. I would say in terms of classifying markers is the level of validation so that's what's that's what's shown here so you can classify? Them by what you're going to use them for are they prognosis, prognosis diagnostic that sort of thing you can talk about where you're going to get. What you know where you're going to get? The material what the type of material is and then how well validated it is there's also a level of validation for the biomarker itself and we'll come back. To that so here are some of the clinical uses that we have for biomarkers okay? So, let me let me remind you what they are so, one of the one of the applications of biomarkers. Is what's called disease stratification?

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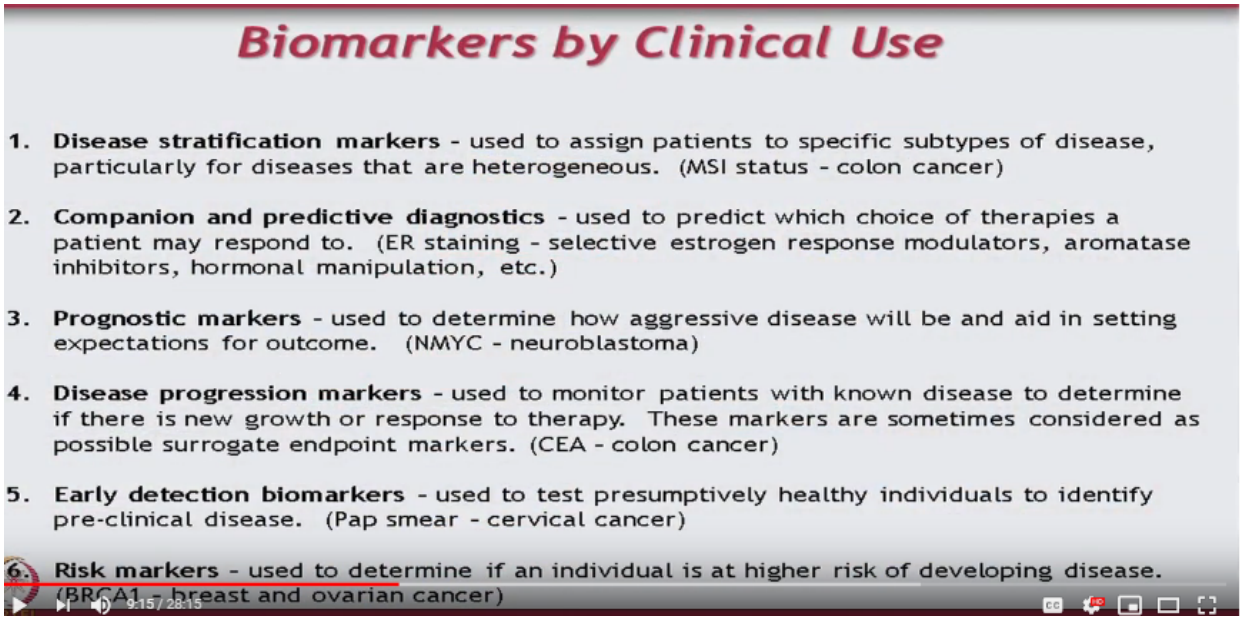

And, and what that means is there are times? When you have a particular disease and you, you know that the population of individuals. Who have this disease are not all alike Right? Some of them have one subtype of disease and others have another you know any no examples of cancers. That has different subtype's breast cancers the classic Right? So, breast cancer has five or six known different subtypes there they're classified based on the molecular classification looking at different surface markers like the estrogen receptor progesterone receptor. The her2 new receptor but also looking at other genes that are expressed or not, expressed in the tumor and based on that gene pattern the different subtypes of breast cancer will have different, prognosis it will those different subtypes will respond to different therapies and so, the markers the, the gene expression that you look at those are biomarkers that help you stratify. Patients and that turns out to be very important because by stratifying patients in two different groups. Now you know better how to treat the and what to tell them in terms of expectations for their disease okay? What's a companion biomarker anybody hears that term before? So, anybody familiar with the drug Herceptin, Herceptin is a drug that we use to treat people with breasts women with breast cancer. Who have her2 new positive disease so why is it important that they have her2 new positive disease that's right? So, Herceptin specifically targets her2 so, if there's no her2 o he cells Herceptin will probably not be very effective so when the drug. Herceptin first came on the market it was a revolution it was the first drug in the modern era that was based on specifically targeting a biochemical Pathway. That we knew was related to cancer all the drugs before that were basically toxic chemicals that kills. Dividing cells cytoxan Adriamycin all those drugs they kill cells because, they're dividing they were not selective for cancer pathways. Herceptin was the first molecule that was developed to target a particular pathway and yet. When they gave it to women with breast cancer the the response rate was probably 15% so, that means. That when they gave it to a hundred women 15 of them would have some response 85 of them would have nothing and of course this was a drug. That was going to cost in the u.s. Between 50,000 and $100,000 So, they're taking a very expensive drug and 85 out of 100 women would get no benefit at all and probably the bigger issue is those women would be waiting and waiting to get a good therapy while they were on a therapy that was doing them no good Right? So, what did they do well they someone

reasoned maybe we should only give this drug to women who have her2 new positive cells because if they don't have her2 new positive cells they probably won't respond so if you now do a diagnostic test and look only at women. Who have her to do positive cells? What do you think the response rate is there? Well over 60% so you went from almost nobody responding to well more than half of women responding so all of a sudden the drug companies and the pharmaceutical. Before you give Herceptin you need to first test for her2/neu positivity and only women. Who have her2/neu positive are eligible to get the drug and that way they could. Have a much better chance that this drug would be effective these days with Herceptin and other ages that specifically target the her2 pathway women who have that disease who at one time had one of the worst outcomes in cancer. Now have one of the better outcomes in cancer because those drugs are so selective for their specific. Subtype of disease so a companion marker is a blood test that you give together. With the plan of giving a drug to determine if that patient will respond to that drug so it's specifically designed in these days at least in the US. If you're going to develop a new therapeutic for any type of cancer that targets a pathway the FDA usually requires that you have a companion diagnostic you have to have a test that will specifically tell that patient that they're likely to respond okay? What's a prognostic marker It, it, it tell it tells you the likely. Outcome of how aggressive the disease is and whether or not it’s Gonne you know how what you can expect from the future. So, that's that's basically right? So, generally speaking the the prognostic markers are not related specifically the therapy although sometimes they are but the idea of a prognostic marker is you know when you see a patient who has disease oftentimes one of the main question. That they're gonna ask you is what can I expect what's my outcome how am I gonna do and the prognostic markers intended to tell you that okay dizzy what's a disease progression marker it's a little bit related to this but it's not quite. The same so, once you have the disease doctors will order that test every time. They see you and they will look at that test and say how are you doing how is that disease coming along are you responding? To the therapy so in the case of cancer which is what I know best if we have a patient who has a liver cancer for example we might every time we see that patient order a CEA test carcinoembryonic antigen this is a protein that's produced by by liver cancer cells or, or, mitat or metastatic cells in the liver and when the tumor is growing the CEA level goes up when the tumor shrinking. The CEA level comes down and so the doctor will monitor that level maybe give drug monitoring again and use that as a as a biomarker to tell. The doctor how the patient is doing over time to track the disease so, that we call that disease progression marker and they are very useful I mean the clinic. We use them for diabetes a hemoglobin A1C if you ever heard of that that's a disease progression marker. It monitors how the disease is doing how well the insulin therapy is doing CEA in the case of Kent in case of cancer lots of diseases have these markers. That let you know how the patient's doing okay. early detection marker that's kind of obvious from the name so, an early detection marker the goal is to find the disease very early usually because, you believe that by catching the disease early you have a better chance of having. A good outcome so the argument is if I can catch the disease at its early stages then I can treat it early and have and reduce the likelihood of you know does he spread or bad disease outcome now the, the tricky thing about early detection biomarkers if you think. About it is who is the population you're going to use the bar early detection. Markers on who are you going to give it to so you could give so, that would be one way to do it people have a family. History that would be a great idea in implicit in what you're saying is that you're using early detection markers on healthy people or apparently healthy people Right? So, all these other markers I've talked about before right? disease stratification companion markers. Prognostic markers disease progression markers those are all people. Who are already in your clinic and they're already sick and your job is to use these markers to tell you something about the state of their illness but an early detection

marker that's something. That you give to a healthy person to see if they're sick so it's a very different kind of marker um it also has a lot of implications in terms of cost and usage because if you're going to give a marker to healthy people right you need to know that it's a pretty good marker otherwise you can cause all kinds of trouble and we'll come back to that in a little bit Okay? And then risk marker what's a risk marker all right? someone back there I didn't get it so you could predict the risk o The disease so that the difference between both five and six hour markers that you would do on healthy people the difference being that this marker . Doesn't really find the disease it just tells you the likelihood that you might have it whereas this marker is really intended to find the disease you have it Right? Now if you do a pap smear on a woman you're looking for the presence of neoplasia right? Now and asking does she have you know the possibility of cervical cancer if you do a risk marker. Like a brca1 test you can't say. That the person has breast cancer all you can say is that she has an increased likelihood of getting it okay? so, a genome getting your genome sequenced that's a risk file that's a risk marker that's not a disease. Detection marker so, certainly you know I'm a big believer in this early detection biomarker mostly because, I think it will have

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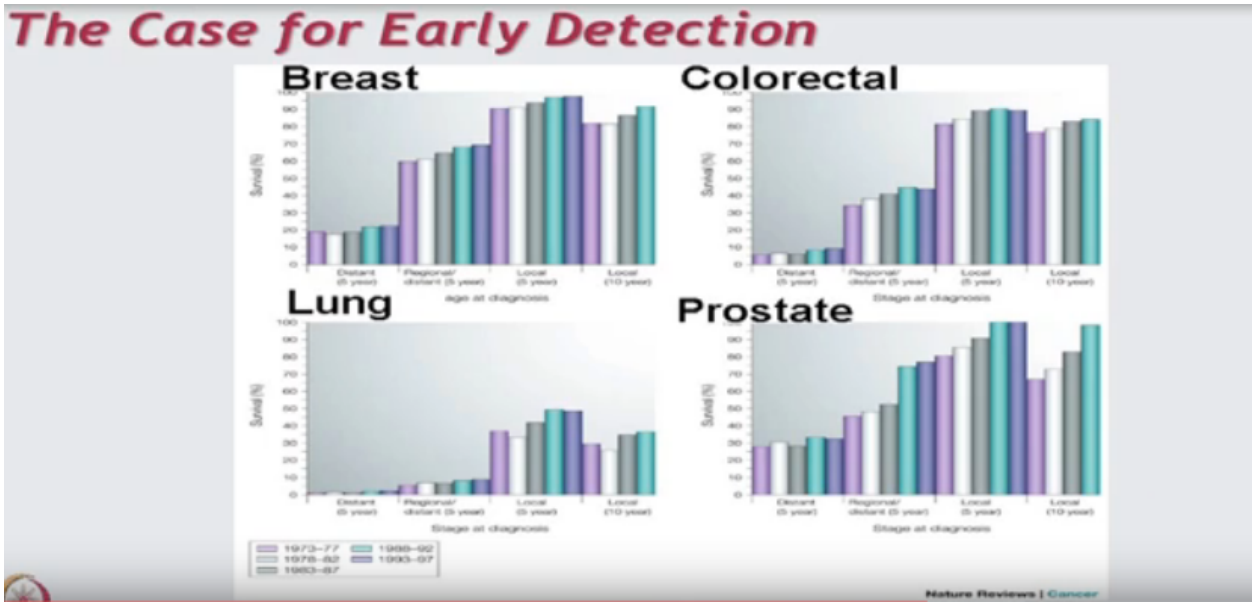

Important outcomes and I think I've I've shown you. This already just to remind you what we're looking at here is, is these are the four most common diseases cancers in, in the u.s. At least they're all epithelial based cancers they are the big, big killers of people in our country. With cancer these are survival plots so the percent of people surviving based on stage of disease stage of disease basically, tells you how early you caught it and it starts with the latest stage disease and moves to the earliest. Stage disease and what you can see is if you catch the disease early you have very good. Survival rates except maybe lung cancer. Which never has a good survival rate but nonetheless the survival of early stage. Disease is always better than certain for survival with late stage disease so, this is an argument for why we want to catch the disease early.

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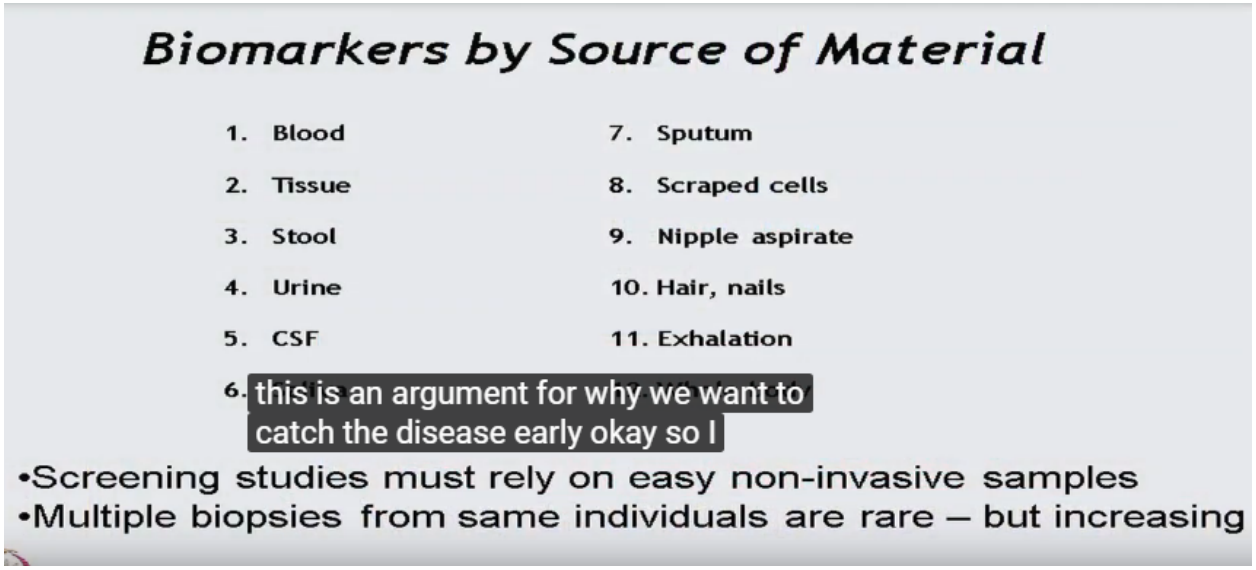

Okay? So, I mentioned that, that you can look for biomarkers based on the source of material and these are just some the types of materials that you could use to get biomarkers from certainly blood is a popular one tissues a popular. One well I should say blood isn't popular sputum is good urine is good tissue really only in the case of cancer you don't often do biopsies there are a few other diseases where you might do biopsies but you know most patients are not thrilled about having parts of them cut out. So, don't do that often but all of these in one way or the other have been used as one form of biomarker for people.

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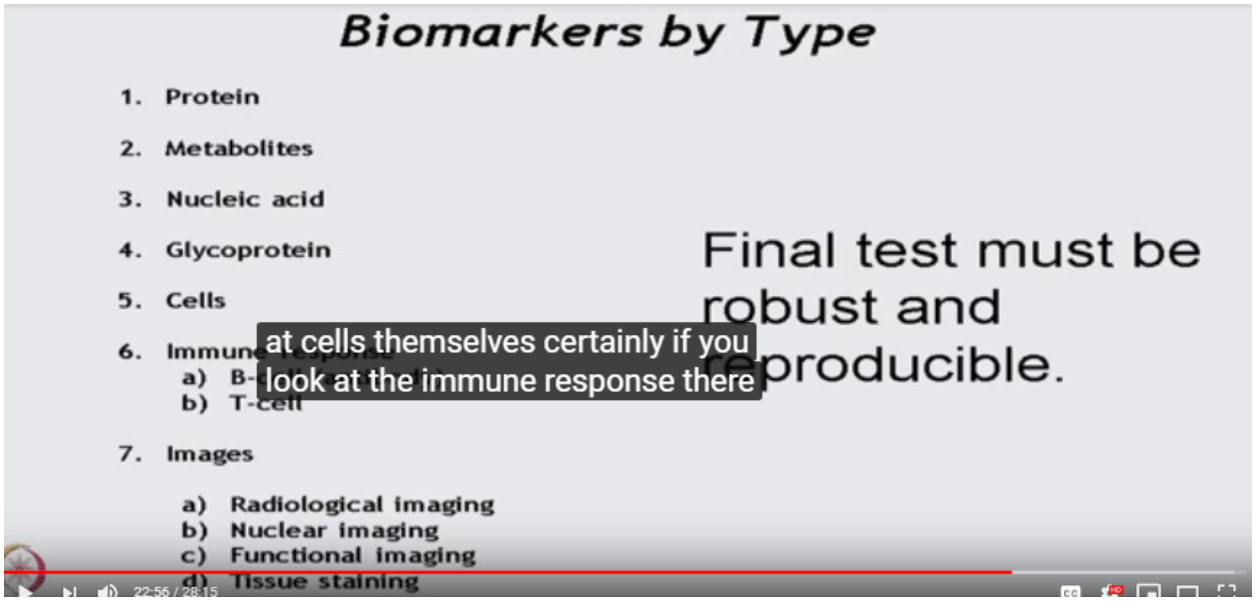

Okay? And then we mentioned earlier that you can look at so that was a source of material now we're looking. At biomarkers by type oh yes, yes. So interesting that you ask that if you ever in fact some of the most famous historical poisonings rights have been determined by looking at hair and nail. Because arsenic shows up in hair and nail and it's dated where it occurs. Along the nail or along the hair can tell you how long ago the person had arsenic and so, I forget which European monarch was murdered by arsenic. Poisoning but they went back to the corpse and measured it and you can actually determine. Arsenic in the hair and nails there are probably other things you can measure there too but, that's the most famous one yeah okay? And then so, these are these are types of things that you can measure in sources. From all those places so, obviously you know all of us are proteomics people. So, we're gonna be interested in number one which is why it's listed number one? But, you could look at metabolites you could look at DNA, or RNA, or other nucleic acids long-chain RNAs or whatever you could look at glycoprotein's you can look at cells themselves certainly if you look at the immune response there are two things you can measure here. So, in the case of b-cells you would look at antibodies that are in the bloodstream and that's what Nappa does and Apple loops at antibodies in the blood stream you could also look at T cells, T cells are much harder to look at in a high-throughput to Tisa the classic t-cell assays called. The le spot assay and what you do is you have to present the t-cell with presenting cell and an antigen and then you have to measure the secretion of gamma of gamma interferon or something like that it's a complicated. Assay and you can't really do it at the scale of omics and then of course images are also a type of biomarker right? So, x-ray CT scans PET scans all those various technologies in a sense they are also measuring things and and you can imagine that some of those imaging studies. Themselves could be early detection biomarkers in the case of breast cancer mammography is an early detection, Biomarker right nowadays in the US. There is a recommendation to do spiral CT scans on very heavy smokers as an early detection biomarker for lung cancer okay? But no matter what test you do this has to be true it has to be robust and reproducible all right? So, now I know I the last thing I want that mention we talked about the use of biomarkers we talked about the source of biomarkers we talked about the type of biomarkers and remember. I said the fourth way to classify markers was their level of validation so let's talk a little bit about, what? I mean by validation okay? So, the first thing you have to do if you want to validate a biomarker is you need to define how you're going to use it .So, the key is understanding the quality of the marker and what it needs to do determines is determined by, what you're going to do with it if you're going to use a biomarker for early detection then you have to remember. I'm gonna use this biomarker on healthy people so these people are walking around living their lives and everything is fine I'm gonna do a marker on them and suddenly. I'm going to tell them that they may have a disease and now they've got to go to a doctor they've got to get a biopsy they've got to do a test they've got a scan whatever yeah and they're all of a sudden you're gonna cause them to do a whole bunch of stuff so you, you need to know that that marker is really robust, or else you're gonna cause a lot of trouble on the other hand if you already know. The patient has cancer and you're all you're doing is trying to monitor how it's doing relative to the drug maybe that marker? Doesn't have to be quite as, as specific because you already know that they have cancer that's not a question all you need to know. Is the level of the cancer so the level of specificity for that is not as high as for early detection markers and the first thing you have to do is define the clinical usage and everything else? Will follow from that and it's it's the number one most common mistake made by people who go for biomarkers is they ,they decide they're going to get a biomarker but they never stop to think. About what they're gonna use it for I've seen people come up with markers for things that have no value because, no one would ever look for that so, you have to you have to think about that.

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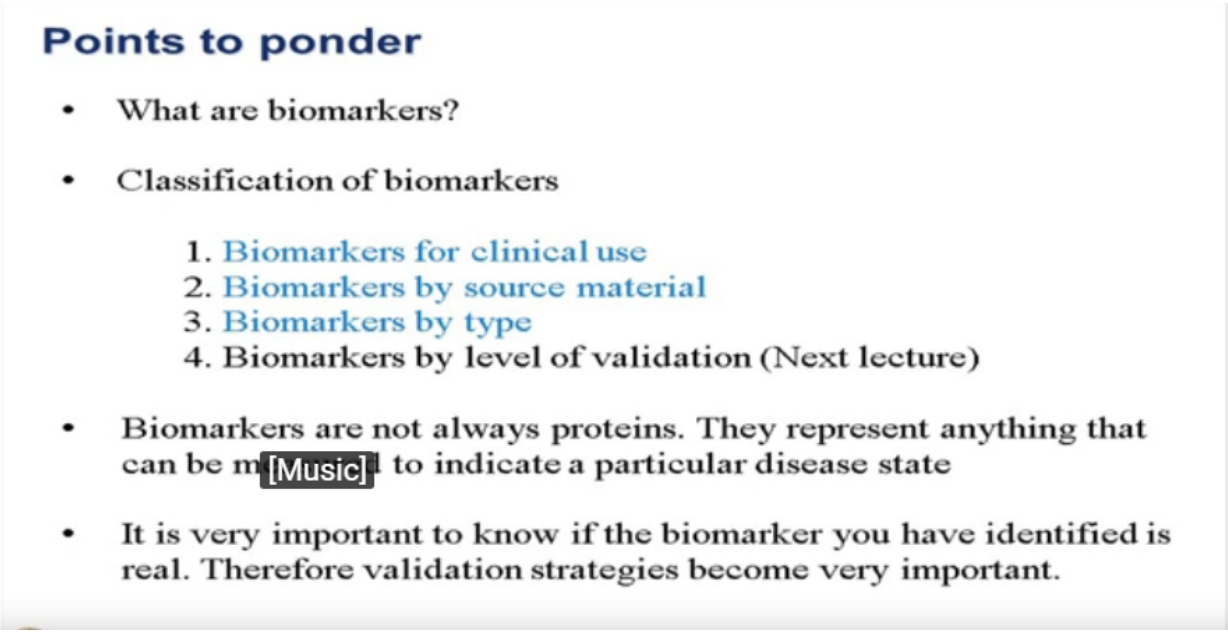

So, in conclusion I hope you have learned now some basics of biomarker of course you know there are a lot of changes. Happens in the physiological states of any individual and then technology platforms are very robust but this still if you think about the you know measuring. The slight perturbation slight changes the technologies can have some noise which may appear to us you know these are some changes. Which are happening from the Bello G induced you know from the samples so how to really determine that what small changes? We are measuring they are real and these are real biomarker candidates determining them is you know really challenging and that's why I think there's lot of investors globally to discover biomarkers and bring new biomolecules for the clinical assays however is still our success has been limited. But if you follow the basic which is discussed today in the lecture by Dr. Josh Labelle I am sure our efforts of following up and making success of a biomarker program will be very valuable.
Thank you very much you.