Interactomics Basics and Applications

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Lecture – 08

Biomarkers: Harnessing the Immune System for Early Detection of Disease – I

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 is what you heard from the last lecture from Dr. Joshua LaBaer, about the technology development aspect of nucleic acid programmable protein arrays or NAPPA.

In the same line, let us continue our lecture with our distinguished invited faculty Dr. Joshua LaBaer 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 the patient can survive, you want to see that a disease might recur, right.

So, there are variety of ways, different type of biomolecules starting from the proteins and other biomolecules 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 you know many 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 LaBaer 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. Joshua is going to talk to you about what are 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 to determine the sensitivity

and they specificity of biomarkers. He is going to talk to you about some of these basics in today's lecture.

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

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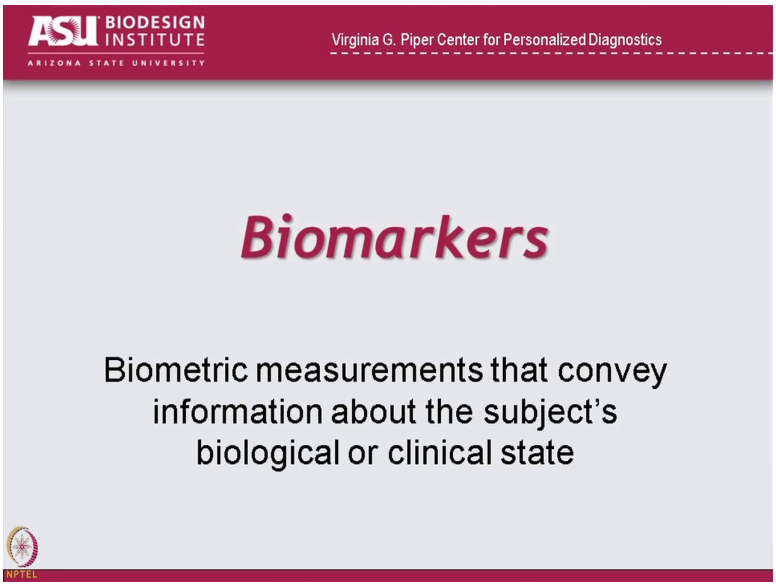

So, if I if you go to a doctor's office and they measure your blood pressure that is a biomarker. They are using the blood pressure to get a sense of your health and they are going to use that to predict the likelihood of getting hypertension or cardiovascular disease, right. If

you go to the doctor and they measure your cholesterol that is a biomarker, it does not exactly measure the health of your heart, but it is a predictor of the health of your heart, right.

So, anytime that you measure something, you the goal is to predict the outcome and that is important to remember because you are not exactly measuring the outcome, you are measuring a predictor of the outcome and that predictor may not be perfect. Some predictors make mistakes, right.

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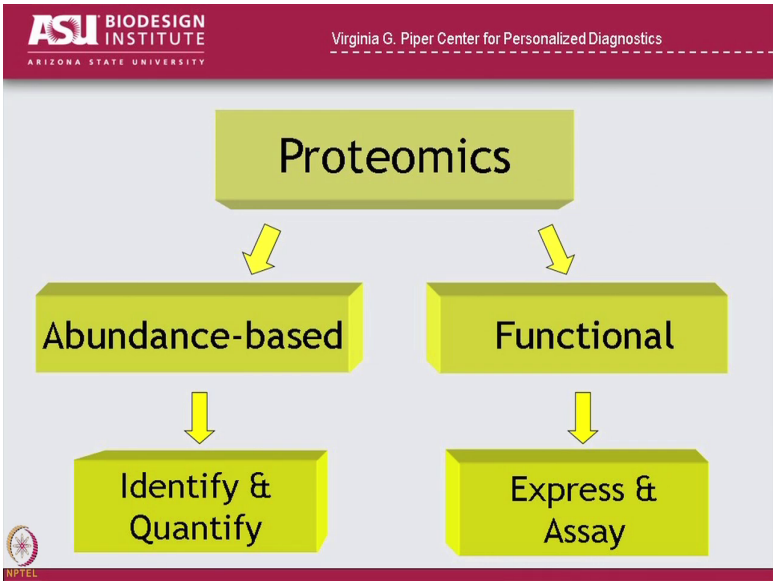

So, the type of biomarkers that I work on are proteomics biomarkers. I am not going to ask you what proteomics is because I know all of you know that. But let me mention that there are two general approaches to proteomics one is this abundance based approach, and we talked about that a couple days ago. 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 are predictive of the outcome, right.

Some proteins will be different, but it has no predictive value, it is 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 are a little bit in between maybe they are mildly predictive, but not predictive enough to do you would be useful.

The other type of marker that the other type of proteomics that we will talk about is functional proteomics and that is the kind that I do that is producing proteins and then studying their function and once again we use that to look for biomarkers, ok.

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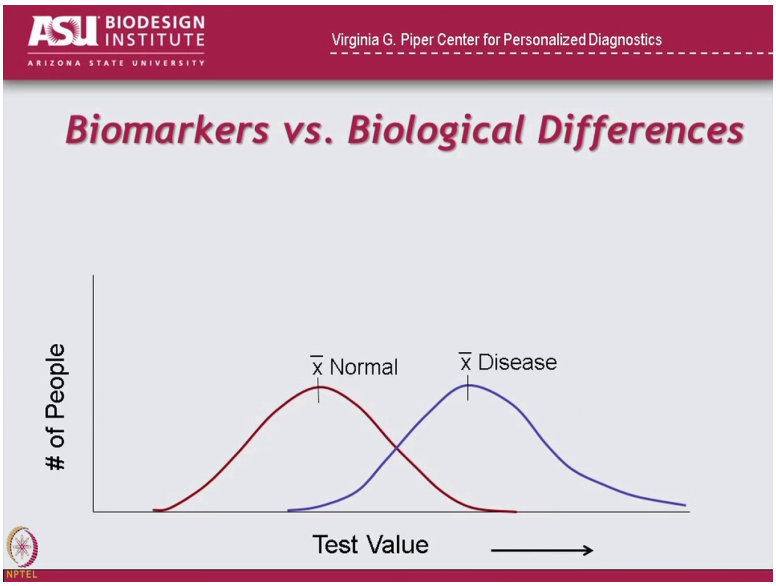

So, this is a common a common issue that comes up a lot in biology. As some of you may know I am also the editor of the journal of proteome of journal of proteome research I am one of the editors there. So, I get papers all the time from scientists, who want to publish biomarkers. And this is one of the most common mistakes I see all the time in the market field. So, imagine that you are you are measuring some value of some molecule let us say it is 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 its much higher than in the case of the normal. And you say wow, look at that difference it is the mean value over here is much better than the mean value over here and therefore I have got a biomarker, ok. So, does anybody see any problem with that?

Student: Overlap.

What?

Student: Overlap.

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. So, those two values are statistically significant, there is no question. They will have a very good p value. So, you will be you know you will be tempted to say it is a good biomarker.

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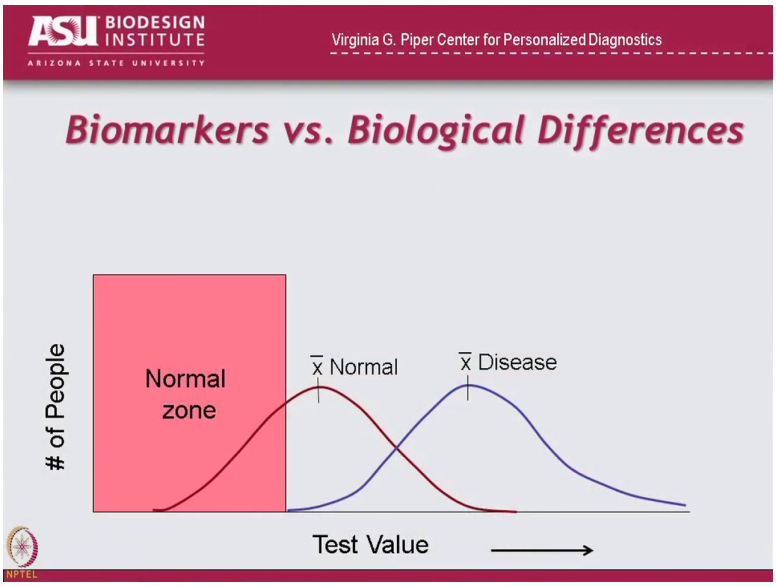

But, let us consider the use of the biomarker. If the value is down here you could very safely say that that is normal you could say if you if you measure that in a person no problem that person is healthy.

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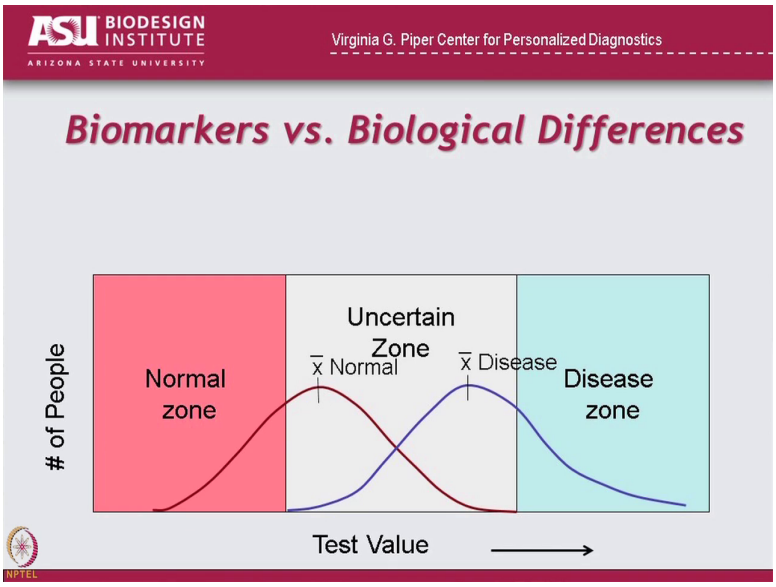

If you if you measure the value up here you can say very clearly that person has the disease, but as she pointed out look at how much there is overlap here. If you if you measure anything in this range maybe it is disease, maybe it is not disease. It is it is the separation is not clean enough to make this a good biomarker and so that is and that is what we are going to talk a little bit today about how do we define good biomarkers, ok.

So, there are lots of different ways to classify biomarkers. So, you could definitely talk about the uses. What are the what are the types of ways to classify biomarkers?

Student: Type of molecule.

What?

Student: (Refer Time: 07:42).

Yeah. What is your measuring? What the material is you are measuring? So, what type of molecule? Absolutely. What are the. So, that is the type of molecule.

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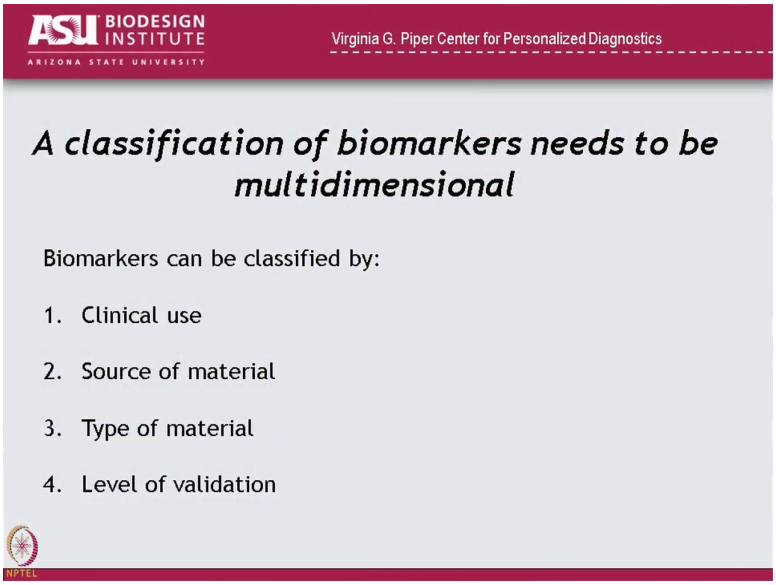

Any other? What about the source of the molecule? Is it blood? Is it cerebral spinal fluid? Is it urine? So, where you get the molecules also you can you could be looking at DNA, protein or lipids. So, that is the type of molecule, but you could also be looking at blood markers or urine markers, and then the last thing I would say in terms of classifying markers is the level of validation. So, that is what is, that is what is shown here. So, you can classify them by what you are going to use them for are they prognosis, diagnostic, that sort of thing you can talk about where you are going to get what you know where you are going to get the material, what the type of material is and then how well validated it is. There is also a level of

validation for the biomarker itself and we will come back to that. So, here are some of the clinical uses that we have for biomarkers, ok. So, let me let me remind you what they are.

So, one of the one of the applications of biomarkers is what is called disease stratification.

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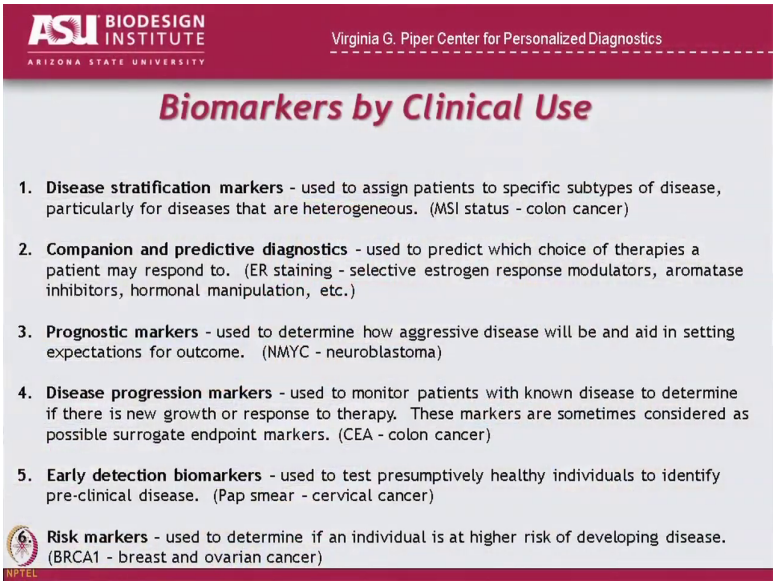

And what that means is, there are times when you have a particular disease and you know that the population of individuals who have this disease are not all alike, right. Some of them have one subtype of disease and others have another subtype. Does anyone know any no examples of cancers that have different subtypes?

Student: Breast cancers.

Breast cancers, the classic, right. So, breast cancer has 5 or 6 known different subtypes. They are they are classified based on the molecular classification looking at different surface markers like the estrogen receptor, progesterone receptor, the (Refer Time: 09:34) 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 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 to different groups, now you know better how to treat them and what to tell them in terms of expectations for their disease, ok.

What is a companion biomarker? Anybody hear 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-neu positive disease. So, why is it important that they have HER2-neu positive disease?

Student: Because certain molecule (Refer Time: 10:42) bind to the Herceptin.

That is, right. So, Herceptin specifically targets HER2, so if there is no HER2 on the 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 are dividing. They were not selective for cancer pathways.

Herceptin was the first molecule that was developed just to target a particular pathway and yet when they gave it to women with breast cancer, this the response rate was probably 15 percent so that means, that when they gave it to a 100 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 US between 15000 and 100000 dollars. So, they are 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-neu positive cells because if they do not have HER2-neu positive cells, they probably will not respond. So, if you now do a diagnostic test and look only at women who have HER2-neu positive cells, what do you think the response rate is there? Well over 60 percent. 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 and the insurance companies said look 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 agencies that specifically target the HER2 pathway, women who have that disease who have 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, its specifically designed in these days at least in the US, if you are 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 are likely to respond, ok. What is a prognostic marker?

Student: Which actually released about the progression of disease and that is rigorous to the (Refer Time: 14:17), it is not like (Refer Time: 14:19).

It tell it tells you the likely outcome of how aggressive the disease is and whether or not it is going to you know how what you can expect from the future. So, that is that is basically, right.

So, generally speaking 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 a disease, oftentimes one of the main question that they are going to ask you is; what can I expect? What is my outcome? How am I going to do? And the prognostic markers intended to tell you that,.

What is a disease progression marker? It is a little bit related to this, but it is 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 is produced by liver cancer cells 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 monitored 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 in the clinic we use them for diabetes a hemoglobin A1c, if you ever heard of that. That is a disease progression marker it monitors how the disease is doing, how well is the insulin therapy doing. CEA in the case of in case of cancer, lots of diseases have these markers that let you know how the patient is doing, ok.

Early detection marker, that is 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 disease spread or bad disease outcome. Now, the tricky thing about early detection biomarkers if you think about it is who is the population you are going to use the early detection markers on. Who are you going to give it to?

Student: Genetically (Refer Time: 17:15) people have the family history or family.

So, you could give, so that would be one way to do it, people have a family history that would be a great idea. Implicit what you are saying is that you are using early detection markers on healthy people or apparently healthy people, right. So, all these other markers I have 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 are 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 is something that you give to a healthy person to see if they are sick. So, it is a very different kind of marker.

It also has a lot of implications in terms of cost and use it because if you are going to give a marker to healthy people, right, you need to know that it is a pretty good marker otherwise you can cause all kinds of trouble. And we will come back to that in a little bit, ok. And then risk marker. What is a risk marker?

Student: (Refer Time: 18:24).

All right, someone back there, I did not get it.

Student: Predict the risk of others (Refer Time: 18:29).

So, you could predict the risk of the disease. So, that the difference between both 5 and 6 our markers that you would do on healthy people. The difference being that this marker does not 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 are 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 BRCA one test you cannot say that the person has breast cancer, all you can say is that she has an increased likelihood of getting it, ok.

So, a genome, getting your genome sequenced that is a risk file, that is a risk marker that is not a disease detection marker. So, certainly you know I am a big believer in this early detection biomarker mostly because I think it will have important outcomes.

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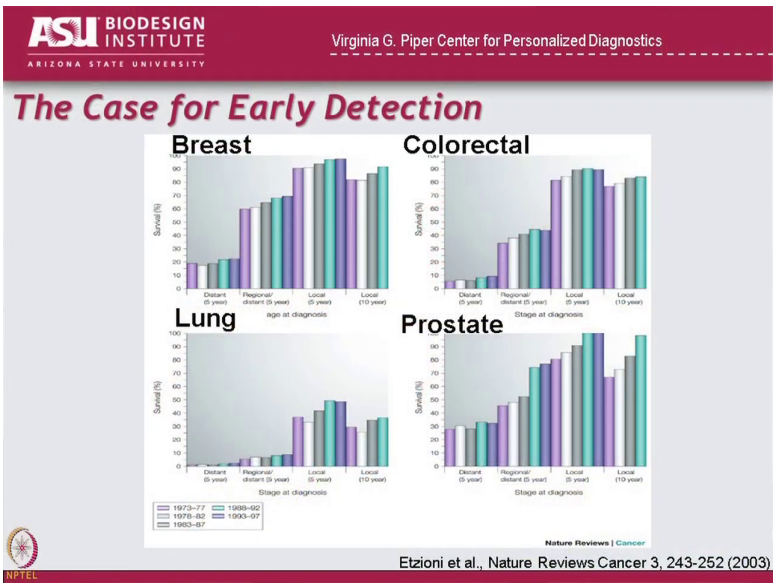

And I think I have I have I have shown you this already. Just to remind you, what we are looking at here is these are the four most common diseases, cancers in the US at least. They are all epithelial based cancers; they are the big killers of people in our country with cancer. These are survival plots, 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 for survival with late stage disease. So, this is an argument for why we want to catch the disease early, ok.

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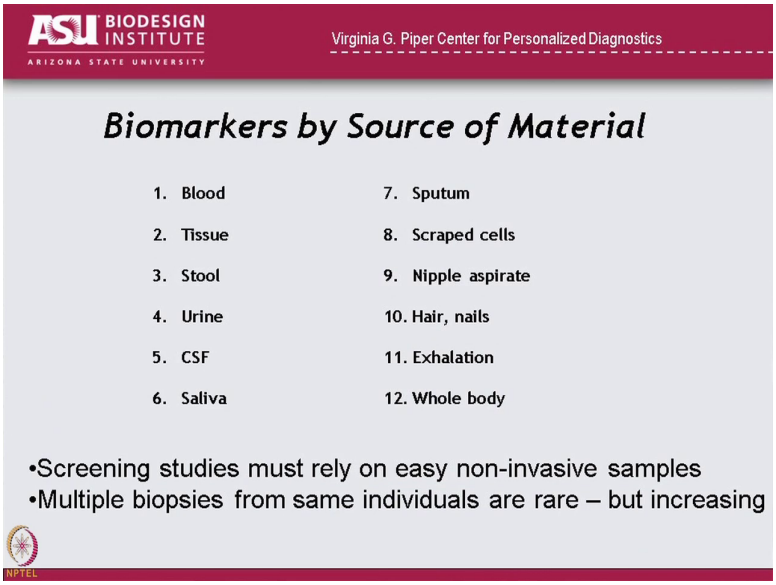

So, I mentioned that that you can look for biomarkers based on the source of material and these are just some of the types of materials that you could use to get biomarkers from. Certainly, blood is a popular one, tissue is a popular one. Well, I should say blood is not popular sputum is good urine is good tissue really only in the case of cancer, you do not 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 do not do that often. But all of these in one way or the other have been used as one form of biomarker for people, ok.

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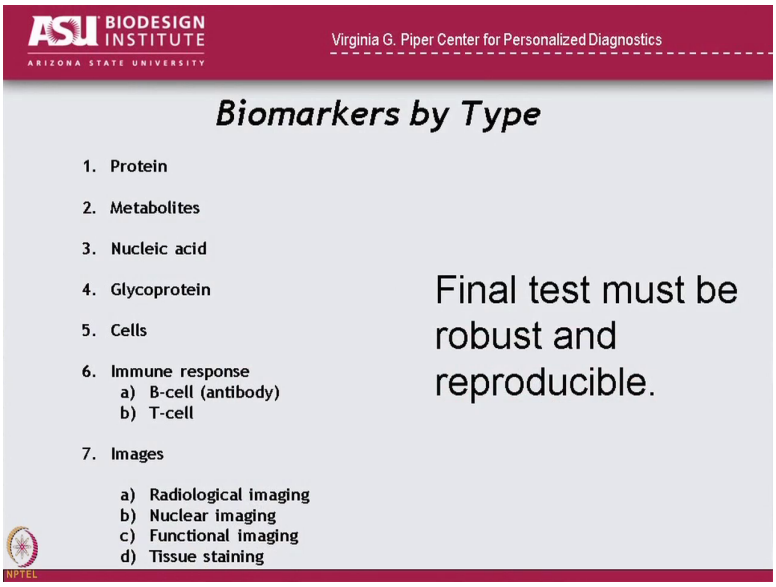

And then we mentioned earlier that you can look at, so that was a source of material now we are looking at biomarkers by type. Oh yes.

Student: Hair and Nail.

Yes.

Student: So, what would be fun?

So, interesting that you asked that. If you ever, in fact, some of the most famous historical poisonings, right have been determined by looking at hair and nail because arsenic shows up in hair and nail, and its (Refer Time: 21:41), 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 is the most famous one.

Yeah, ok, 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 are going to be interested in number 1, which is why its listed number 1. 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 glycoproteins, 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 is what NAPPA does. NAPPA 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. T-cells the classic T-cell assays called the le spot assay and what you do is you have to present the T-cell with a presenting cell and an antigen and then you have to measure the secretion of gamma of gamma interferon or something like that, it is a complicated assay and you cannot 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 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, ok. 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 to 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 us talk a little bit about what I mean by validation, ok.

So, the first thing you have to do if you want to validate a biomarker is you need to define how you are 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 are going to do with it. If you are going to use a biomarker for early detection then you have to remember I am going to use this biomarker on healthy people. So, these people are walking around living their lives and everything is fine, I am going to do a marker on them and suddenly I am going to tell them that they may have a disease and now, they have got to go to a Doctor, they have got to get a biopsy, they have got to do a test, they have got a scan whatever and they are all of a sudden you are going to cause them to do a whole bunch of stuff. So, you need to know that that marker is really robust or else you are going to cause a lot of trouble.

On the other hand, if you already know the patient has cancer and you are all you are doing is trying to monitor how it is doing relative to the drug maybe that marker does not have to be quite as specific because you already know that they have cancer. That is 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 the early detection markers. So, the first thing you have to do is find the clinical usage and everything else will follow from that and it is it is the number one most common mistake made by people who go for biomarkers is they decide they are going to get a biomarker, but they never stop to think about what they are going to use it for. I have 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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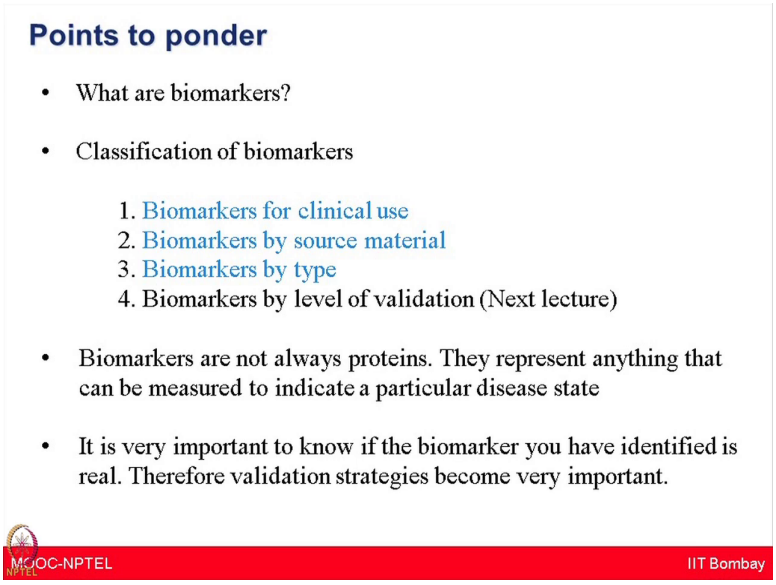

So, in conclusion, I hope you have learned now some basics of biomarker. Of course, you know there are a lot of changes happens in the physiological states of any individual and then technology platforms are very robust, but this still if you think about the you know measuring the slight perturbation, slight changes the technologies can have some noise which may appear to us you know these are some changes which are happening from the biology induced you know from the samples. So, how to really determine that what a small changes we are measuring they are real and these are real biomarker candidates. Determining them is you know really challenging and that is why I think there is 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 basics which is discussed today in the lecture by Dr. Joshua LaBaer, I am sure our efforts of following up and making success of a biomarker program will be very valuable.

Thank you very much.