



Genetic Sequencing of Tumors for Personalized Medicine

Hosted by: Steven Gore, MD

Guest: Janina Longtine, MD

January 7, 2018

Support comes from AstraZeneca, committed to pioneering the next generation of innovative lung cancer treatments. Learn more at astrazeneca-us.com.

Welcome to Yale Cancer Answers with doctors Howard Hochster, Anees Hochster and Steven Gore. I am Bruce Barber. Yale Cancer Answers is our way of providing you with the most up-to-date information on cancer care by welcoming oncologists and specialists, who are on the forefront of the battle to fight cancer. This week it is a conversation about genetic sequencing of tumors for personalized medicine with Dr. Janina Longtine. Dr. Longtine is a Professor of Pathology and of Laboratory Medicine at Yale School of Medicine and Director of the tumor profiling Laboratory at Smilow Cancer Hospital. Dr. Hochster is a Professor of Medicine and Medical Oncology at Yale and the Clinical Program Leader of the Gastrointestinal Cancers Program at Smilow Cancer Hospital.

Hochster So, we talk a little bit about like what is a gene and why we are interested in them?

Longtine Absolutely, genes are wonderful. The genes we have over 20,000 genes in our bodies and they are responsible for all of the diversity between people and also the changes between normal tissues and cancer, and we have to try to understand them and although we do not completely understand them now, we are learning more and more about how if we can sequence these genes, that is to read them, we can better understand the diversity between people, what causes diseases and what causes cancer.

Hochster I see, and we are using that approach now as part of what we call personalized or precision medicine, so what does that mean exactly?

Longtine Shall we talk about cancer specifically?

Hochster Sure.

Longtine Okay. So traditionally, I am a pathologist, traditionally cancers have always been classified by the organs that they start in, lung cancer or breast cancer and the way they look under the microscope and often may be the proteins that they express and we all have a tools and pathology departments to do that, but with the advent of our understanding of genes, we now have another layer of ways to examine cancers to be able to sub-classify them into distinct groups and by doing that, we may be able to find and often have now found therapies that are specific to different changes within the genes of the cancer. So it is important for us now to add the special tool to sequence genes of cancers and help patients with their diseases.

Hochster So if somebody has cancer today, we can take the tumor biopsy tissue, pull out the DNA, pieces of DNA and then run it through the sequencing process to tell us what is abnormal in the tumor?

Longtine That is exactly right.

00:03:05 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

Hochster And sometimes that helps us make treatment decisions?

Longtine Absolutely or maybe prognostic decisions about which cancers would be more aggressive and may need some more aggressive therapy even if it is not "precision therapy."

Hochster But so all that can help the oncologist direct therapy for this specific patient instead of treating all lung cancers the same way?

Longtine Right, I think it is that sub classification of diseases, there is no one lung cancer anymore and there are many types of lung cancers and we can discern that at the genetic level.

Hochster I see. So, you started by saying there are 20,000 genes in the body, these are little pieces of DNA that can tell us to make certain proteins and things, but we do not look at 20,000 genes when we look at the tumors right?

Longtine No, we tend to focus on the genes that we know that are most important in cancer. There are many genes we do not really understand their role and so it would be wasteful to examine them at the genetic level, so there is an algorithm that we often use for cancers and often maybe specific type sub cancers, we only look at subsets of genes and might look at a different type of group of genes for leukemia, that is a blood disease versus a lung cancer that is tumor of the lung.

Hochster And so, what is it exactly mean to sequence the gene and how do you do it?

Longtine So sequencing a gene is actually reading the DNA within the gene and just a little bit I think I have to talk a little bit about the underlying biology so there is clarity to it. Basic structural unit of the DNA is called a nucleotide and this still staggers me, our DNA only has 4 different nucleotides and we refer them to by their first letters, C, G, A, and T.

Hochster It is pretty amazing.

Longtine And those 4 nucleotides are clustered into groups of 3 and that group of 3 determines which components of a protein are going to be made. For example, if you have T, A, C as a code, then amino acid which is the building block of the protein will be tyrosine, but if instead of T, A, C, you have T, C, C, instead of a tyrosine, you have a serine and those little building blocks changes by this very subtle changes in the DNA code and as a result, cancer can change just 1 nucleotide. Instead of a C if you have an A, you will get a completely different amino acid, completely different function of the protein and it can really be the driver that makes the cancer cells multiply and be aggressive within the body versus normal cells and that is what we are looking for when we are sequencing DNA in the clinical laboratory.

00:05:55 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

Hochster So the DNA is like having the whole encyclopedia with 4 letters?

Longtine Correct.

Hochster And what you are saying is that if there is a misspelling of one point of this long sequence of 4 letters of one of the letter that substitute, you might land up with a bad protein or a protein that causes cancer?

Longtine Yes or a protein that does not function and if it is one of the important tools within the normal metabolism, then yes. So it is like looking for needles in a haystack, but once we understand the cancer genes better, we actually know what areas to look at because they often are commonly acquired in similar cancer types and we can focus on those and that is what makes a little bit more efficient in the laboratory than looking at every gene that is possibly sequenced.

Hochster Before we go little further with the tumor DNA sequencing, I wanted you to just make the point or discuss like the difference between looking at the tumor DNA and the person's normal DNA?

Longtine It is often important for us in laboratory to look at both of them, because as I mentioned earlier in our discussion, there is a lot of diversity between individuals and some of that is just normal diversity and when we are trying to sequence the cancer, we only not focus on the mutations that are specific to the tumor, so we often will sequence the patient's normal tissue and the cancer and then compare them and pull out all of the variants that seem to be just specific to that person versus another person and only focus on the changes that are specific to the cancer type, was that clear?

Hochster Yes. So you know everybody has got a little variation in their DNA from another person, whatever, but the tumor DNA is not inherited, like if they find a mutation in the tumor DNA, that is something your family can have.

Longtine That is right, that is called somatics, so it is only present in the tissue that you are examining, it is not present in every cell of your body, like the ones that you inherit from your parents.

Hochster So these are not the kind of things that are protected by the privacy and that goes back to your actual what we call germline DNA, the DNA that is in your normal cells.

Longtine Right.

Hochster So there are 2 kinds, you look at both when you do the tumor profiling, you are saying.

00:08:29 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

Longtine We do when we look at lots of genes, so if we are just looking at a few targeted panels, we do not really need the germline because we are looking on very specific regions and that unnecessary. The reason to look at the germline is because of the complexity of actually reading the DNA, it is just simpler if you read many genes of the patients normal, so that you can pull out and not bother with those and just look at the ones that are specific to the cancer.

Hochster Okay, but you can do many places do just the tumor and look for known databases there, catalogues there, thousands and thousands of known mutations, so people rely on those to certain extent.

Longtine Right and there is a number of databases that we are reliant to help with that decision tree.

Hochster Okay, alright, so you know, I treat people with GI cancers. If I order DNA tumor profiling test on a patient, what is it you are going to do with my request and what information can you give me back?

Longtine So usually, we have already had your diagnosis because the tumor has been sampled on the patient either by surgery or by a biopsy and so we work in concert with the surgical pathology department at Yale who has the patient's tissue in their archives, usually it has been embedded in paraffin tissue and so there is a sample we can receive and then we can isolate the DNA from that specific tumor that you requested us to study.

Hochster So it does not require new biopsy?

Longtine Generally not, no.

Hochster And there are several levels of testing, you were kind of alluding to?

Longtine Yeah, so there is different approaches, laboratories have for example in colon cancer, colon cancer is one of the tumors where we know very specific mutations that can really influence your choice of therapies and so for often for the first pass, we will use one of our smaller panels, which is 50 gene panel that can highlight some of the mutations that we know that you are interested for your particular patient to determine whether he candidates for one therapy or another and the advantage of using a small panel is the turnaround time is relatively quick and is less expensive for us to perform.

Hochster And so we have specific panels for colon cancer and for example, lung cancer, a number of different of these smaller panels.

Longtine Yeah, but one of the advantages of some of the smaller panels is that with R50 gene panel, we can cover the national guideline suggestions for mutations for lung cancer and melanoma cancer and colon cancer and so that allows us to bundle specimens together and do them in an efficient manner. When we go to a more complicated disorder, when patients have developed metastatic disease and tumor is evolved, then we might go to a larger panel to find more unusual mutations that are known in cancer that would helpful to your care.

00:11:43 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

Hochster I see and can you tell us a little bit more about those more advanced panels?

Longtine Yeah sure, so in the tumor profiling lab at Yale, our more advanced panel is called the Oncomind panel, the current version has 148 genes, so we look for changes in the nucleotides in the cancer, but now instead of 50 genes, we are looking at 144, so over those in addition, we can look for changes in the level of the gene. Some cancers are driven because the gene amplifies, and so it makes lots and lots of protein that drive the tumor cells and so we are able to look at copy number variation in this particular panel and we can also look at changes in structures. Sometime cancers are driven because 2 different chromosomes join up abnormally and create a novel protein and so we can look for those in this broader oncamind panel.

Hochster So the copy number variants, as you were saying, will tell us if a gene is overexpressed and that can be important for example for breast cancer with certain proteins like HER2 and/or what we call translocations where the chromosomes have rearranged themselves in an pathologic way and so your laboratory does these tests routinely and how long does it usually take to process that?

Longtine So our turnaround time for the 50 gene panel now is 9 days from when we receive the tissue in the laboratory and the more complicated oncomind takes us 18 days from when we receive the tissue.

Hochster So then you get the results, you give them back to the physician to help with treatment decisions and that is how helpful to the patients then?

Longtine So what we do in the laboratory is we analyze the DNA, we read the DNA and we also look at the literature to help write an annotation or an interpretation to guide the physician because not all physicians are as sophisticated and so we provide some information, and the oncologist can use this information to either decide to use standard chemotherapy because there is not an actual mutation or perhaps change therapy to some other personalized medicine drugs that will allow the patients some better opportunities.

Hochster Well thank you very much. We are going to take a short break now for a medical minute. Please stay tuned to learn more about tumor profiling and genetic sequencing with Dr. Janina Longtine. We will talk more about how the doctors are using this information for personalized medicine when we come back.

00:14:39 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

Medical Minute Support comes from AstraZeneca, committed to pioneering the next generation of innovative lung cancer treatments. Learn more at [AstraZeneca.us.com](https://www.astrazeneca.us.com).

This is a medical minute about lung cancer. More than 85% of lung cancer diagnosis are related to smoking and quitting even after decades of use can significantly reduce your risk of developing lung cancer. For lung cancer patients, clinical trials are currently underway to test innovative new treatments, advances are being made by utilizing targeted therapies and immunotherapies, the battle II trial at Yale aims to learn if a drug or combinations of drugs based on personal biomarkers can help to control nonsmall cell lung cancer. More information is available at [YaleCancerCenter.org](https://www.yalecancercenter.org). You are listening to WNPR, Connecticut's public media source for news and ideas.

Hochster Welcome back to Yale Cancer Answers. This is Dr. Howard Hochster and I am joined tonight by my guest, Dr. Janina Longtine, director of the tumor profiling laboratory at Smilow Cancer Hospital. So Nina, we have been talking about if somebody has cancer today, there is additional information available beyond this old pathology kind of diagnostics by looking at the DNA in the tumor and that is what you do in the tumor profiling lab and you were just saying that when you do the larger test, you give information back to the physician about specific treatments and so forth. So how does that work and why this is helpful to patients?

Longtine So let me give you a specific example, for lung cancer, the microscopic description is subsets them into the way they look under the microscope, which I'm sorry is redundant, and there is a group of tumors called adenocarcinoma and they all look alike under the microscope, but in the past 10 years of my life, I have learned that instead of 1 type of lung adenocarcinoma there are 4 or 5 different types and it is really based on sequencing the DNA and identifying the mutations. For example, a common mutation in lung cancer is mutations in EGFR gene, epidermal growth factor receptor. Mutations in this gene will allow the tumor cells to grow much more rapidly than normal cells and we can sequence them and identify a very, just one change in one amino acid can actually drive the tumor cells and there is a drug that is available, they are called tyrosine kinase inhibitors, put the brakes on that protein and actually have the tumor regress just with this 1 drug and it is that that is really the thrilling part of what we can do because this paradigm has been repeated over and over again as the drug industry has been able to identify more drugs that are used for targetable therapy.

Hochster Yeah, that is a very interesting story. It is only about 10 or 12 years since people discovered that these mutations could give people lung cancer and it kind of came up because they were looking at lung cancer, particularly in young women who were not smokers and it turned out that they tended to have these mutations, so then we have the tools to look at the DNA more, we could see that this particular protein was stuck in the on position and that was which was causing the cancer, so fortunately there were some drugs around that could turn it off.

00:18:16 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

- Longtine Right and then the corollary as you know in the field, so the same EGFR pathway can be shut down in metastatic colon cancer by using drugs that are anti-EGFR antibodies, but we have discovered that if you have mutations in the RAS genes in colon cancer that they do not respond to this treatment, and the treatment is very expensive, and so it is much better for you to understand that your patients would not be candidates for those and the more we studied them, more we just found more RAS genes, so now originally we only looked for 2 different mutations and now we look for 5 or 6 or 7 to help even that therapeutic planning.
- Hochster Right, so that is another great example in that case instead of finding the driver protein that you could have a specific treatment for, we found out that there were these mutations that made the drug ineffective so we are able to personalize the treatment by not giving people ineffective and toxic treatments based on their DNA profile. So that has been very useful and today in treating colon cancer, we kind of break up colon cancer into those 2 groups the ones that have the mutations and the ones that do not. So those are 2 really good examples of how DNA sequencing can be helpful. There are a lot more things that are happening in this area though right?
- Longtine For example?
- Hochster Well, I mean the thing that I am excited about is that we had a couple of drugs that were approved for treatment today in immunotherapy that are not based at all on a diagnosis, they are only based on the molecular characteristics and biology of the tumor where I am talking about people who have something called microsatellite instability or you know, kind of a problem with DNA repair, so that they have a lot of DNA breakages, those anybody who has that kind of DNA biology whether it would in colon cancer or a brain tumor, they can get these drugs now and due to FDA approval that was not based on a specific kind of cancer.
- Longtine Yes, I agree, these is an exciting era where we do not have to basket tumors based on what organ they came in, but we can understand there is some common underlying abnormalities which you just mentioned as increased mutation burden within the tumor broadly, the tumor will make proteins that are unusual and the immune system recognizes that and starts to attack and then we can help enhance that attacking so that the immune system can help control the cancer. The methodology that you just mentioned is often a immunohistochemistry test where we look for abnormalities in these mismatch repair and also, now people are starting to look at broad mutation burden across DNA, so that if you have tumors like from smoking or other environmental drivers, you can see that there are many, many, many more mutations in melanoma or smoking lung cancers and there are some other types of tumor and you can use immunotherapy in those cases.
- Hochster So I think just to kind of restate what you said is that there are some ways that we can look more simply by staining proteins on slides of your tumor, but there is a lot more sophisticated ways once we extract the DNA to look at how many mutations there are in a big piece of DNA and that may be reflective of the susceptibility to immunotherapy.

00:22:06 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

- Longtine Exactly, that's in its infancy yet so we do not really have the criteria to make sure for everyone but I think it is a new emerging field that will add immunotherapy more broadly to all our patients.
- Hochster So I mean things are changing pretty quickly in this area, I mean what kind of things in the last few years have changed in the area of DNA tumor profiling?
- Longtine I think it is just the breadth of our understanding and it is across many many different tumor types and there are many different types of genes and from profession, it is hard to know exactly how fast to bring things on to bring as to clinical testing because the field moves so quickly and there are many laboratories now that are moving towards whole exome sequencing, so they are looking at all of those 20,000 genes and just think of how difficult it would be able to manage that both intellectually and in a cost effective manner.
- Hochster Right. So you were talking about the 150-ish gene panel today which is kind of broadly accepted one, we helped develop it here at Yale along with the National Cancer Institute for some of our clinical trials, but that does include most of the mutations that exist in cancer today right?
- Longtine Yes, I think most of the ones that are involved in clear clinical indications for therapy.
- Hochster So when we do broader sequencing like looking at the whole DNA and this whole exome sequencing, we are actually kind of going on a fishing expedition that is a big- pretty big expedition?
- Longtine Yes, but I think that in the university settings, often this is incorporated into translational research where you are looking more broadly and then you will be able to have a clinical information about these patients and retrospectively perhaps understand more about the biology of cancer, so that we can then incorporate other genes into our routine.
- Hochster Yeah and I think that is very important that we are always trying to learn more even if today there are 150 genes on our panel, you know, it could be 200 by next year. If you do not look for the other ones that we do not know about yet, we are not going to be able to really continue to deliver the best information and best treatments.
- Longtine Right then, I have just returned from my professional meeting – the Association for Molecular Pathology and so there is parallel opportunities going on across the country and we are building practice guidelines just as you have in your field to make sure that is everyone is trying to look for the proper genes and right number of genes in clinical care.
- Hochster And so that kind of subset of all the pathologists or these molecular pathologists, so how does the group define itself or how do you as a molecular pathologist, how you guys define yourself?

00:25:05 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

- Longtine I think in original days it was by just self-proclamation because those of us who were interested in understanding the molecular biology of disease and in particularly cancer, but it is also relevant to germline disease and infectious disease, but now with the practice of medicine, there is an urge to have people come together with consensus and then association for molecular pathology works on practice guidelines and also works on us better understanding all the new instruments that are available and how we could incorporate those into our laboratory.
- Hochster I see, well that is pretty interesting and what do you kind of anticipate in the next couple of years, what changes are coming along in your field?
- Longtine Actually, there is always excitement about how we are learning more about cancer and how to bring it into the field, but the corollary of that is these tests are not well reimbursed by insurances and they are quite costly to do, so from the director of laboratory it is always trying to balance the wishes and the realities to make sure that we provide what we need for our patients in a cost effective manner.
- Hochster Well that is a really important point and everybody is focused on cost effectiveness today, but some of these things are amazingly cost effective if we find the right treatment for the right patient at the right time.
- Longtine Right, globally we are, but from the laboratory's perspective, we have some times we are working with pairs very closely to make sure they understand the value of the test so that they can be reimbursed.
- Hochster And finally, could say a couple of words about something that is called a liquid biopsy? I mean to me it is just drawing blood, but I know it is been called this liquid biopsy thing because I guess that is how the billing works.
- Longtine Yeah, actually what is, as you said, the molecular field is always fast growing, so once you feel like you have conquered like 150 genes, there is something new coming down the pike and so what everyone is interested now is actually rather than going to just depending on getting a biopsy of tissue that you can actually look at cell-free DNA which I will explain for a moment, when tumor cells are growing in your body, they grow very rapidly and they often release their DNA into the blood stream, so you can take a sample of blood and get DNA that is relevant to the tumor and so you can sequence that in your laboratory and get a hint whether the patient has an actual mutation or as you and I know, after you give patients a drug, sometimes they develop resistant to that drug and there may be hallmark in their blood of developing resistance and so this is all in the experimental phase right now, but it may be a way to help, analyze patients after they started their therapy to find more effective therapies.

00:27:55 into MP3: https://ysm-websites-live-prod.azureedge.net/cancer/2018-yca-0107-podcast-longtine_324079_5_v1.mp3

Hochster Yeah I think there is a lot of potential looking at the circulating tumor DNA. I mean the challenge for years as everybody knew there was DNA out there, but to get it and measure it because it is in such sample quantity and amplify it, but we are getting to point and I was on a call yesterday with the National Cancer Institute where we are trying to start an effort to look at people who have had their colon cancers taken out and to see if they have any DNA circulating in their blood still, those are people who might need more aggressive treatment. So there are areas like that and besides what you said is on treatment you could get mutations that you can detect that will help us decide on the next therapy. So this is really exciting for many of us in the therapeutic area and I think it is kind of going to help us give a lot more personalized or precision treatments to these patients across the whole spectrum of therapy. We are coming pretty close to the end. Anything else that you would like to add in your last thoughts?

Longtine I think it is an exciting field and something that is really impacting clinical care and it has been a real pleasure for me and my 25 years of practice to see the evolution of this field and the promise that it hopes for bringing personalized care to our cancer patients.

Dr. Janina Longtine is a Professor of Pathology and of Laboratory Medicine at Yale School of Medicine and Director of the Tumor Profiling Laboratory at Smilow Cancer Hospital. If you have questions, the address is canceranswers@yale.edu and past editions of the program are available in audio and written form at YaleCancerCenter.org. I am Bruce Barber reminding you to tune in each week to learn more about the fight against the cancer. You are on WNPR, Connecticut's public media source for news and ideas.