Yale CANCER CENTER answers

WNPR Connecticut Public Radio

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Genetic Heterogeneity in Breast Cancer

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Gore   I always get a little nervous when the topic of the show is more complicated than I can understand. Tell me what genetic heterogeneity in breast cancer is? That is just a mouthful.

Hatzis   Cancer, especially breast cancer, is a very diverse disease. There are many different types of cells that make up the tumor and these cells are genetically diverse. They just have different sets of mutations. They might originate from different initial cells, so when we try to understand cancer, one important aspect of it is to have a good understanding of how heterogenous cancer is because it might affect how well that particular tumor could respond to chemotherapy.

Gore   How do you figure that out? How does one discover whether a particular breast cancer has these kind of differences, genetic differences between the cells, in the same breast cancer, is that what you are saying?

Hatzis   Yes.

Gore   How do you figure that out?

Hatzis   Maybe we should take it back a bit, pathologists have known that cancer and breast cancer is very heterogeneous because they can actually see individual cells and they can see that the cells are morphologically different, they just look different.

Gore   Different shapes and sizes.

Hatzis   Different shapes and sizes, so that was the first clue and then they have been trying to stain or attach different colors to stain different parts of that cell so they can see that there are different molecules that are present in these different cells.

Gore   Under the microscope.

Hatzis   Yes, and our tools have become much more refined now where we can actually take individual cells, if we take it to the extreme and actually analyze the DNA of those individual cells, we can

compare the DNA from two different cells from the same tumor, we can see many differences, and they look like they probably have originated from different cells.

Gore   So are you telling me, you can take the DNA from one cell at a time and get all this information?

Hatzis   Yes, that is possible, that is more cutting-edge, and right now it is not part of what we do every day. But this is within our capabilities and that is really what gives the more definitive evidence or information about the heterogeneity of tumors.

Gore   What would be a more common approach, would it be different parts of the tumor?

Hatzis   Yeah, I mean really, when we see heterogeneity usually we mean heterogeneity between tumors of the same type. For example, there are three different broad categories that oncologists usually use to classify breast cancers, whether they are ER positive or if they have certain molecules on their surface, which indicates certain treatments, whether they are HER2 positive or whether they are triple negatives, and usually these three different categories were considered as a relatively homogeneous groups.

Gore   And just to clarify for our audience, you are talking about ER, that is the estrogen receptor, so those are cancers that have the estrogen receptor, is that right?

Hatzis   That is correct.

Gore   And the other one is this HER2 oncogene protein.

Hatzis   Yes.

Gore   And the third does not have either, is that correct?

Hatzis   The third one does not have either the estrogen or the HER2, or a third receptor which is called progesterone receptor, so it is negative.

Gore   So it is no receptors and no HER2.

Hatzis   Correct.

Gore   Got it, go ahead please.

Hatzis   Clinicians have been treating this disease as relatively homogeneous, so if you are ER positive, estrogen receptor positive, you will be given a certain treatment. Now, we have looked at enough of these cancers and we understand that all ER positive tumors are not the same. Similarly all HER2 positive tumors are not the same and triple negative tumors are not the same, so there is

heterogeneity between tumors of the same clinical type which has profound implications as to how to treat these patients. Maybe all ER positive tumors should not be given the same therapy and how we characterize the heterogeneity and how we link to it to potential treatment courses for the different subtypes of the diseases is a very important issue that we are actively evaluating in our group.

Gore   How far along are we in this endeavor? Is it time for women with breast cancer to ask their doctor about heterogeneity and whether they are ER positive or standard or type A, B, C, or D?

Hatzis   For some of the diseases we are there. For ER positive disease, there are several tests available including one test that is called Oncotype DX, that is commercially available where it can use some of the molecular characteristics of the tumor to determine whether this particular tumor is high risk if they are treated with only endocrine therapy or whether this particular tumor is so aggressive that it will need to be treated with chemotherapy and endocrine therapy together. That is an example of a differentiation within the ER positive disease that really dictates different treatment options.

Gore   And this is something we are using right now?

Hatzis   This is something that is being used right now, correct. And for triple negative disease, it is a little more difficult because the tumors within that group are genetically even more heterogeneous and the problem with those tumors is that they are not very many treatment options because they do not have any of the receptors that we have discussed. The only option for them is chemotherapy, and although there are a few options as to different types of chemotherapies, there are not many markers yet that can tell us whether one type of chemotherapy will be more effective than another kind of chemotherapy for this particular disease, so we are actively researching and trying to find different biomarkers, different mutations at the DNA level that will tell us how likely one of these tumors might be to respond to a given standard chemotherapy, say if they carry specific mutations and specific genes, and hopefully within maybe a few years, two or three years or five years down the line, it could lead to tests that can be used for that disease which is very challenging to treat.

Gore   In practical terms, in terms of what you do research wise, are you taking these various patients' tumors and looking for mutations and collecting the data on how they respond? What is actually involved?

Hatzis   When we try to discover biomarkers that might tell us whether that particular tumor is more likely to respond to treatment or not, usually we have to collect samples from groups of patients that have been treated, so we collect biopsies upfront and then the patients are treated and then we know how well that patient did on that particular chemotherapy, so we compare the DNA or the molecular constituents of the tumors from patients who really did well on the chemotherapy and we contrast those to tumors from patients that did not respond and that were resistant to

chemotherapy and then we can see from that comparison, specific mutations or specific biomarkers that might tell us how likely someone may respond to the chemotherapy or maybe on the other side, do not respond to the chemotherapy, which is important to know because obviously you do not want to treat a patient with chemotherapy that you know is not going to be effective for that patient.

Gore   It seems like you have to study a whole lot of patients DNA to make these associations.

Hatzis   That is true and that is part of the challenge. Because of the heterogeneity that we have talked about, you really need to see enough tumors from each type so that you can find biomarkers that seem to be working for most of the tumors. You do not want to find a biomarker that will be effective for maybe one or two of these tumors, so yes, you have to look at a large enough number of tumors which needs to be done in a systematic way. It needs to be done in a large place like Yale where you might have some clinical trials where patients may be able to enroll and allow study of these kind of issues in a systematic way.

Gore   Let's say a patient is being treated at Yale or has a biopsy, a breast biopsy at Yale, a tumor biopsy, do they automatically get included in this analysis?

Hatzis   Typically, they are not automatically included. There are what are called clinical trials where treating physicians, the doctor, the oncologist that treats the particular patient when they show up at Yale will inform them that there are certain clinical studies that might be available to them for the particular type of cancer that they may have and if the patient consents, they might be able to donate part of their biopsy to our lab so that we can run the sequencing, run the analysis to determine the mutations in the DNA and eventually after a large enough number of patients have been analyzed, be able to really try to see if there is any connection between specific mutations and how well they did on the treatment.

Gore   So it is voluntary on the patient's part.

Hatzis   It is entirely voluntary on the patient's part.

Gore   Let's say there are patients who do not particularly want to have experimental treatment but are interested in having their tumor studies, can people sign up just for that piece without being on a treatment clinical trial?

Hatzis   Yes, actually most of these patients are not really being asked to be take part in an experimental treatment necessarily. They will be given the standard course of treatment that is indicated based on the disease that they have, but the fact that they will enroll in the study just gives us the benefit of studying their DNA and maybe try to link the particular aspects of the DNA as to how well they

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did on the standard treatment, so they do not necessarily have to get on the new treatment. Even just getting their regular treatment, that will help treating future patients, just matching the treatment with the specific tumor characteristics for future patients.

Gore   For patients, I would think, it is bad enough to unfortunately have developed cancer, but this is some way I would think of contributing and feeling like they are helping future patients and maybe it makes more sense out of it, I do not know, but if I were a patient, it seems like I would be making a positive thing out of something that is inherently kind of negative.

Hatzis   I do think so, although I am not directly involved.

Gore   I understand.

Hatzis   My understanding from my colleagues is that most of the patients really see it in a positive way and they are willing to participate in such studies.

Gore   So what happens next, then the DNA comes to your lab or the tumor comes to your lab?

Hatzis   DNA is usually put in a liquid in a preservative to just make sure that the tissue stays, the tumor cells do not disintegrate. Then we take the biopsy in our lab and we extract the molecules that we need, DNA and RNA usually, and we send those molecules to the sequencing facility at Yale and they perform all the sequencing. We get back the data and we actually determine based on the sequence of the tumor, what specific mutations this particular tumor has.

Gore   This is such a fascinating topic and we are going to want to pick up on it right after the break. We are going to take a break for a medical minute. Stay tuned for more information about breast cancer and genetics and bioinformatics with my guest, Dr. Christos Hatzis.

Medical
Minute   *Smoking can be a very strong habit that involves the potent drug nicotine and there are many obstacles to face when quitting smoking, but smoking cessation is a very important lifestyle change especially if a patient is undergoing cancer treatment. Quitting smoking has been shown to positively impact response to treatments and decrease the likelihood that the patient would develop second malignancies. Smoking cessation programs are currently being offered at federally designated comprehensive cancer centers such as Yale Cance Center and at Smilow Cancer Hospital at Yale-New Haven. The smoking cessation service at Smilow operates on the principles of the US Public Health Service Clinic Practice Guidelines. All treatment components are evidence based and therefore all patients are treated with FDA approved first line medications and smoking cessation counseling. This has been a medical minute brought to you as a public service by Yale Cancer Center and Smilow Cancer Hospital at Yale New Haven. More*

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Gore   Welcome back to Yale Cancer Center Answers. This is Dr. Steven Gore and I am joined tonight by my guest, Dr. Christos Hatzis and we have been discussing genetic heterogeneity in breast cancer which incidentally I am still having trouble saying, Christos, so please forgive me about that. Before the break you were telling me about getting the tumor specimens from different patients and isolating DNA and RNA, you said you send it off to the sequencing lab. What is the sequencing lab?

Hatzis   I think the name is the Yale Center for Genomic Analysis.

Gore   And what do they do, I have no idea?

Hatzis   It is a complicated process, but I will try to simplify it a little bit. They take the DNA, which is one of the biggest molecules in our body and they break it down to smaller pieces and they make multiple copies of those pieces so that they can be sequenced, so when we say sequence, we determine the individual basis of our present individual letters.

Gore   Those are the four letters that make up the DNA.

Hatzis   Yes.

Gore   A, T, C and G.

Hatzis   Yes, that is correct. We have millions of small pieces of DNA, usually around 120 million such pieces.

Gore   Wow.

Hatzis   And we determine very quickly, they have the machines that can determine those letters very quickly, and they give us back a big file that has 120 million sets of small sentences and each one has four letters.

Gore   I guess you do not send that by e-mail, probably.

Hatzis   No that is a very big file. I like to describe it is as, you go to a magazine stand to buy a magazine and instead of the person giving you the magazine, they just run it through the shredder and they give you a bunch of small shreds of paper and your task is to go home and put those shreds together, to put the magazine together.

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Gore   I would want my money back.

Hatzis   Yes, but that is what we do, we just try to put the DNA sequence together from those small shreds of DNA that we get from the sequencing facility.

Gore   How do you do that? I would not know how to start doing that.

Hatzis   There are tools that have been developed. We do not have to do the whole thing ourselves. There is a lot of help from large institutions like the Broad Institute up in Boston and they have been working on these problems for many years, so we just have to figure out what the right tools are to use for these tasks and then use them effectively, but once we put it together, then we have to line up the DNA from the particular tumor to the reference DNA which is the DNA of an average person and then go letter by letter through all the 3 million letters that we have to find potential differences which are called mutations and then we know that these mutations are assigned to this particular tumor or maybe small pieces of DNA might be missing which are called deletions or you might have additional pieces of DNA inserted, which are called insertions, so we have to determine all those things on individual tumors and typically it is a list of maybe 20 to 30, 20 to 50 differences for each tumor and then we prioritize those and find mutations on genes that are more important in cancer which might tell us what might be the best way to treat this individual tumor based on those specific mutations that these tumors might have.

Gore   We have this big data file that has all these letters, these different pieces and you feed it into your machine or your database, how long does it take the computer to figure this out or for you to interact with it? Does it go into some algorithm that spits out answers?

Hatzis   That is a very good point. It usually takes about a day per sample to do that and it usually does not have to be interactive, so we do all the work to put all the patients together in what is called a pipeline, so we put different types of programs together that analyze, that solves, a different part of the problem until we get to the mutations and then we just let this whole program run on the computer and again it usually takes about a day but at the end, because these are very important, you do not really want to make a mistake, so we have to go back to the individual data and check every single mutation to make sure that it was not a mistake at the center when they did the sequencing or it was not a mistake on our side so that this mutation is a real mutation, which is very time consuming, but you have to do it, you have to check every individual mutation manually to make sure it is correct.

Gore   Sounds very tedious.

Hatzis   Yes, it is. But I do not think there is any automated way to do this validation.

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Gore	Quality control. What kind of computers are you using, are these giant mainframe things or PCs or do you do it under MAC book?
Hatzis	You could probably do it on a MAC if it had enough memory but it would take a long time. What Yale has is several computational clusters, they are called clusters because they consist of 100s of computers linked together so that they can all work in a coordinated way to do this way, and I was talking about 120 million different pieces of DNA and the way this program works, they might send a thousand of these pieces to one of the computers in the cluster, and another 1000 in another computer and then all these computers can do their own little work and at the end the results are combined, so you can get this parallel processing of the information which we cannot really do on a MAC.
Gore	Very cool, are all these computers working just on that one problem at a time or are they working on different patient samples simultaneously?
Hatzis	They can work on many different patient samples at the same time, so if the computer has 1000 individual nodes, you can maybe get access to 50 of those and an another researcher is able to get access to another 50, so many people work at the same on these systems, but we also have our own system which consists of about 50 computers that we just use for own only purpose, only our group uses that to improve efficiency in some of this calculations.
Gore	That is fascinating. Is your background mainly in the informatics piece or the biology piece? How did you get involved with this?
Hatzis	That is a very good point. When I did my PhD, bioinformatics did not really exist.
Gore	Right, I was going to say.
Hatzis	So my formal training is in biochemical engineering. We were involved in genetic engineering some of the early work there. We had to deal with a lot of data when flow cytometry came along, so that really got us into the multivariate statistics and some of the aspects that need to do the bioinformatics work and this was kind of a natural progression into this field. Then we worked in microarrays back in 2000 and actually as part of my path, I founded two companies that developed diagnostic tests for breast cancer utilizing a lot of those bioinformatics skills that we developed over time. And I have been with Yale for two years now, continuing work on breast cancers that I have been working on over the last 15 years.
Gore	Wow and do your companies still exist?

Hatzis   The company, as far as I know I am not part of it, but as far as I know, it was sold off and it was licensed to another diagnostic company that took over to commercialize it.

Gore   So I see you still have to work, you cannot just retire at an early age.

Hatzis   Unfortunately, but it is fine.

Gore   I see sometimes at what we call our Precision Medicine Tumor Board, and for the audience, the idea here is that we try to use or we are learning to use this kind of mutational information and genetic information to actually choose therapies, especially when patients have not been cured by the standards. Is this something your group in breast cancer is participating in or you are just kind of there as an audience like me?

Hatzis   We actually are involved in a clinical study, a clinical trial which is called MAP-IT where we take tumors from patients who consent and agree to participate in this trial and then we evaluate all the mutations in those patients and then we try to see what would be the best treatment that matches the characteristics of the tumor and this is an ongoing study, but the challenge with that study like you have seen in those Tumor Boards, because typically tumors do not have a single mutation, so maybe a tumor might be presented with say 10 mutations which might all be somewhat actionable.

Gore   Right.

Hatzis   So there might be drugs that you can direct against some of these targets, but the challenge is how to get inside, which targets to go after and how do you pick among the options that you may have and to do that you really need to have an understanding of the biology. You need to be able to go back and see how the genes fit together in doing their work in the tumor cell and using that understanding to say, for example, if a particular mutation makes a gene work harder, so it just makes a gene that is supposed to not do anything actually work harder, and as a result, it will make the cell grow or spread to other parts of the body. If you understand the relationship between that particular gene and other genes in the pathway, you may be able to go after some of the genes that regulate, that affect the parts of the pathway, parts of the biological system that are connected to that particular gene. So just understanding is not enough to really know a particular mutation and a particular gene. You have to figure out how these different pieces fit together. It is not easy for a physician to do on the fly or a clinician when they have to treat this patient, so we try to develop tools, a database with some biological logics, so some of this information as to how these genes are connected together to help understand the effects of targeting a specific gene. We are actually working with, I do not know if you know, the Watson Group from IBM, the people who have developed the super computer that beat Jeopardy.

Gore   Yeah, I have heard something about that.

Hatzis   So we are working with that group to try to put tools together that may use that smart computer system to try to make some of these decisions.

Gore   So are thinking this is harder than winning Jeopardy?

Hatzis   Definitely, much harder.

Gore   Can you say that as a question instead of an answer? Fascinating, so is it your expectation that at some point when we look back 10 years from now or whatever, this is going to seem so Mickey Mouse, that it was so complicated, that we are just going to do our biopsies and put them through and it is going to spit something out and tell me what to do as a clinician, or is this really complicated stuff that is always going to require this very high level of interaction with bioinformaticians like yourself, what is you expectation?

Hatzis   Cancer is so complex, so complicated, it is like peeling an onion, right. The more we learn I think the more we realize we do not know enough and I think that we have just started uncovering and maybe to some extent appreciating how complex this disease is, so on the positive side and I truly believe that, I think whatever we do now will really have an impact for some of the patients. I do not think it is going to solve the problem of cancer, but it will incrementally in maybe finding some tumors that we cannot recognize right now, but maybe using this technology be able to sort of identify those and maybe find the best way to treat them, so it will help maybe another you know 5 to 10 or 20% of the cancer patients and it is going to lead to the continued progress that we have been seeing over the last 10 to 20 years.

Dr. Christos Hatzis is Assistant Professor of Medical Oncology and Director of Bioinformatics for Breast Medical Oncology at Yale School of Medicine. We invite you to share your questions and comments, you can send them to canceranswers@yale.edu or you can leave a voicemail message at 888-234-4YCC and as an additional resource, archived programs are available in both audio and written format at yalecancercenter.org. I am Bruce Barber hoping you will join us again next Sunday evening at 6:00 for another edition of Yale Cancer Center Answers here on WNPR, Connecticut's Public Media Source for news and ideas.