BroadcastMed | Grand Rounds: Breast Cancer Genome Guided Therapy Study: next generation sequencing for the management of women with high risk breast cancer

SPEAKER 1: All right, today is October 3rd. Welcome, everyone, to our weekly grand rounds for CCATS, translational science. It is my pleasure today, as the Director for the Education Program for the Center for Individualized Medicine here at Mayo Clinic, to introduce Matt Goetz and Judy Boughey for our grand rounds today. Judy Boughey is a consultant and professor here at Mayo Clinic. She's a surgical consultant and a physician, chair of the Division of Surgery Research, vice chair of the research department research for the Department of Surgery, Medical Director of the Surgical Clinical Research office at Mayo Clinic here in Rochester. She's got international and national roles in many multi-center clinical trial development efforts and is widely published in the highest regarded journals in her field. Her research is funded numerous times, too many times to count, by NIH, NCI, and other organizations.

Matt Goetz, he's a physician, a consultant in the Division of Medical Oncology and a professor of oncology, associate professor of pharmacology here at Mayo Clinic. Chair of the Mayo Clinic Breast Cancer Disease oriented group, co-leader of the Mayo Clinic Women's Cancer program, and deputy director of the Mayo Clinic Breast Cancer spore. He's a leader in translational research in pharmacogenomics and clinical oncology, and again, is also funded by NCI, NIH and others. Together Dr. Boughey and Goetz are the co-principal investigators on the Breast Cancer Genome Guided Therapy or the BEAUTY study, which you'll be hearing about today.

It's a major initiative of the Mayo Clinic Center for Individualized Medicine and the Mayo Clinic Cancer Center, all of which work with CCATS and the other centers to really try to integrate translational medicine research into our Mayo Clinic practice. And it's really just an amazing organization and really amazing opportunity here at Mayo Clinic to see all of these centers work together so well to really promote translational research here. The overarching goal of this study is to identify novel markers of resistance and identify new drug targets for patients with breast cancer who are at the highest risk of treatment failure. It's my pleasure to introduce Dr. Goetz and Dr. Boughey to talk about the progress that they have in the BEAUTY study.

MATTHEW GOETZ: Well thank you, and we're really excited to be here. On behalf of, really the whole team of people that you can see up here that have really contributed to make this possible, we're pleased to make this presentation today and would like to thank the organizers for the opportunity. So we're going to take the opportunity just to present some of the interim results. These are actually not the final results. But I think as you hear a little bit about we're going to talk about, you can see that there's actually really been a lot of very interesting findings that have resulted from this particular study.

I'm going to start first with disclosures. We do not have any disclosures, and obviously that's important to show you. The learning objectives-- we want to first begin by telling you a little bit about the role of neoadjuvant chemotherapy. And this is important because understanding why we are essentially embarking upon this program in a particular clinical setting-- and that is high risk women who are receiving neoadjuvant chemotherapy-- is important. We are going to additionally spend some time talking a little bit about the importance of germline sequencing in breast cancer in patients with high risk tumor biology. One of the major focuses or parts of this study is the patient derived xenografts. And we're going to talk to you a little bit about that and why we think that's going to be an important part in the future for developing new drugs. And then finally, how are we using this information to help guide and really direct the next generation of studies that's going to come out from this BEAUTY study.

So I included this particular slide here, and I included a definition from Wikipedia. I thought it was pretty good. Wikipedia says that precision medicine is the application of omic analysis and system biology to analyze the cause of the patient's disease, one, and to utilize targeted treatments to address the disease process. So in the case of breast cancer-- by the way, fill in your favorite disease, but really we're focusing on breast cancer here-- the idea is to understand everything you can about omics. In this case, we're talking today about germ line sequencing as well as tumor sequencing and then to use that information to apply a specific therapy for the patient. So this idea, really is something that some people would consider relatively far-fetched. But as we're going to show you, we think it actually is very close to coming to fruition in the next few years.

Well, excuse me, to understand precision medicine and understand this, you really have to understand sort of where things have come from in the past. And the application of precision medicine to breast cancer is this context. And that is, a woman presents to her physician with a lump in the breast or a lesion that's identified by a mammogram. And she goes to surgery. And the surgeon, in many situations, renders that patient not only free of disease, but also renders that patient cured of therapy. And that patient then goes on and has no additional problems the rest of her life from breast cancer. However, we know that there is a subset of patients that can develop recurrence, and of course, in that situation, metastatic disease is a very serious situation for breast cancer because invariably, metastatic disease cannot be cured. And so the treatment is to number one, identify these patients who will develop metastatic disease, and then come up with additional therapies to prevent that is very key.

Well, we can't talk about precision medicine in 2014 without understanding really what's happened, especially in the last decade. And that is, that we know that there is-- through a series of studies, first by Chuck Perou, published in Nature in 2000. And I'm referencing this study here in PNAS from 2003 by Sorlie. But that is essentially that there were the identification of specific molecular subsets of breast cancer. And this was a so-called luminal A, luminal B, HER2, and triple negative breast cancer, sometimes referred to as basal-- that when you use a standard microarray-- that was developed, of course, back in the 1990s and applied-- that you could identify the specific outcomes of patients and that not only was there specific and different genes that were regulated, but that gene regulation led to substantial differences in clinical outcomes.

And this here just shows you from two different large data sets the identification of these different subtypes could allow the stratification of outcome. Now the one thing I'll show you on this is that in the pink here is the so-called HER2 positive. And when I was first starting my training back in oncology in 1999, there were no targeted therapies for HER2 positive breast cancer. And we used to tell patients, gee, it's bad if you have HER2 positive breast cancer because of this particular observation and that these patients do very poorly. But with the advent of targeted therapies, actually, the survival of patients with HER2 positive breast cancer now is better than many of these other subtypes. So that tells you a little bit how things can change over the course of-- in this case, a decade-- if we understand the biology and that biology then can allow us to develop new drugs. So the takeaway here is that we know that there is heterogeneity and this has informed us in terms of our treatments.

What about chemotherapy? Well this is a study that was first started back in the 1970s. And this is the use of multi-agent chemotherapy when delivered after surgery. I told you that we know that in patients that have breast cancer, that you have this heterogeneity. You have a subset of patients that develop metastatic disease. Oftentimes, this is not even clinically apparent. And that metastatic disease has spread even long before the surgeon walks in the room to say hello. And so in this situation, after this tumor has been removed, administration of-- in this case, multi-agent chemotherapy-- substantially clinically, significantly improved the outcomes of patients. And you can see that this is a study first started in 1975, and the 20 year outcome was reported in the New England Journal of Medicine. And notice that 386 women were randomized, and 40% were alive and free of disease.

Let's fast forward down to 2014 and there are better drugs that have been developed. These drugs are actually now considered a standard for the treatment of breast -- Adriamycin, cyclophosphamide, and docetaxel. And you can see that, number one, that the outcomes are substantially better. So here at five years, you actually see 80% of patients alive and free of disease. But I want you to note that it took nearly 5,000 patients in this particular clinical trial to be able to-- in this case, unfortunately show that there was no difference between these two different or these three different chemotherapy regimens. So things have improved, but because things have improved, we need to identify new ways and better ways to develop drugs and simply to identify those high risk patients.

JUDY BOUGHEY: So what we're using in BEAUTY, and what we're using increasingly in our clinical practice, is delivering the chemotherapy prior to surgery and the so-called neoadjuvant setting. And so if we look at the top half of this slide, that shows what I'm referring to in many ways, kind of like the old way that we used to treat these patients with high aggressive breast cancers. That was, they presented-- often the first person that saw them was the breast surgeon. They would undergo surgical resection. We would be happy that we thought we had rendered the patient disease-free and essentially cured. And then in the post-operative setting, they would see our medical oncology colleagues and be considered for adjuvant systemic therapy. And as Matt showed us on the last slide, in that era, it would take us thousands of patients followed for five to 10 to 15 years to really show whether the systemic therapy agents we were delivering were having any improvement in the patient's overall outcome.

Increasingly now, what we're using is the scheme on the bottom half of this slide, which is giving the chemotherapy first. And so what we're doing in this era is-- from learning how that tumor responds to the arrangement chemotherapy, it kind of renders us, as the surgeons, kind of as the trash collectors. But what it means is that at the surgical resection, when we get that pathology report from that surgical resection, we actually now have an idea of how that patient is going to do in the long run. And so if that chemotherapy has completely eradicated the disease from the breast and from the lymph nodes, we know that those patients are likely to do very well in the long run. Whereas if those patients have residual disease left at surgery, then those patients have a poorer outcome.

So whereas the top half of the slide takes us 10 to 15 years to know how a patient really does, in the bottom half of that slide, we are now getting an insight into that patient's likely outcome just six months from their diagnosis. And this has actually been validated and seen in many studies across the country and the world. And this graph here on the left really just shows us this green line as those patients that achieve a pathological complete response, essentially, eradication of all invasive disease from the breast and the lymph nodes. And their survival is actually very good. Whereas those patients that have residual disease post chemotherapy, do not achieve complete eradication of the tumor, have a poorer survival.

This has been underscored in 2012 by the FDA actually acknowledging that it would consider granting accelerated approval of novel drugs in the neoadjuvant setting based on studies performed in the neoadjuvant setting showing an improvement in pathological complete response. And pertuzumab was the first drug to achieve approval in HER2 positive breast cancer in 2013 and is now part of the clinical practice.

So how do we move forward in 2013 and 2014 to really take the next step forward towards individualizing a drug therapy for these patients? Really, in order to apply precision medicine to breast cancer, there are three key features that we need. We need to be able to fully characterize the mutational landscape of the patient's tumor. And with more advances in genetic sequencing and decreasing in the cost, that is far more available to us now than it is in the past. We need to be evaluating new drugs or potentially applications of other drugs that are used in other diseases to test these in tumors that we can show have been resistant to the current cutting-edge standard chemotherapy of 2014. And then we also need some new tumor models, really, to evaluate these drugs in. And for this in this study, you'll see we're using these patient derived xenografts, which allow us to take the patient's tumor, inject them in the mice, grow up the tumor in the mice, and then use this patient derived xenograft as a model to test drugs, to see whether those drugs would be effective on the patient's tumor.

So really, in a very basic level, what we think is really novel about the BEAUTY study is it's bringing together three pretty cutting-edge opportunities. It's bringing together the treatment with neoadjuvant chemotherapy, which is providing for us a response phenotype within that first four to six months of diagnosis. We're using genomic sequencing information to drill down and identify potential drug targets. And then we are using the patient derived xenografts to give us the ability to test these drugs in mouse models.

Now as you can imagine, this degree of work takes a huge team. And the first slide gave you an idea of the names of the people involved in this team. And this really just shows you currently all the specialties that have come together to make this study work. And I think, really, that was kind of one of my big aha moments when we started the study together when I said, this is why I'm at the Mayo Clinic. There are not many other institutions in the world where you can have cutting-edge basic scientists, clinicians, c the whole team coming together for a single project. And I think this is a real true example of team science, where everyone is coming together for a huge study. And then you can only imagine, when we have our BEAUTY team meetings, I mean, there isn't a conference room large enough to have all the members of the team in. But we're really thankful to the clinical side, the research side, the labs, the collaborations, and it's also really important to note that this has been a three site venture-- so Mayo Clinic Florida, Mayo Clinic Arizona-- have all been involved with this.

So this is the schema of BEAUTY, and we activated BEAUTY in March of 2012. And we actually closed it to a crawl in May of 2014. So we were open for just over two years. Actually, from the time we had our first meeting to talk about it to activating it was just shy of about eight months. So it was probably one of the quickest clinical trials I've ever opened in my life. Our goal was to identify patients with biopsy-proven invasive breast cancer who were recommended by their clinical team to be treated with chemotherapy in the neoadjuvant setting. So essentially, that for us, is a way of identifying patients that the oncological team is viewing them as being a high risk tumor biology.

We enrolled 140 of these patients, and they underwent intensive imaging with molecular breast imaging, as well as MRI. These patients had additional research biopsies of their primary tumor. And that was critical. And we took those research biopsies, both for the genome sequencing and also for the development of the mouse avatars, and then we also had blood samples from these patients. They were all treated with what was standard of care chemotherapy throughout the study. And that did evolve a little bit throughout the timeline of this study. So when carboplatin was approved, we allowed it to be utilized in the trial. Same with when pertuzumab was approved, we amended it to allow it to be used in the trial. So patients were receiving the cutting-edge for the time standard chemotherapy.

One of the very novel aspects of the way we designed this was we gave the taxane therapy first. And after they completed 12 weeks of taxane therapy, patients had repeat imaging and a repeat biopsy. And this is a tremendous resource now that we have the repeat biopsy with sequencing information post-taxane therapy. And there's not many other studies around the country that have that kind of resource, because historically, the taxanes were usually the second drug that was given. And you'll hear some of the data related to taxane response that we'll report here later. Then the patients completed the anthracycline portion of that chemotherapy, had repeat imaging, and then surgery was always performed at Mayo sites. And the important aspect here was any residual disease at surgery. You have to think about this. This is residual disease despite 20 weeks of chemotherapy. If there's still residual viable disease there, we've been gathering that tissue and taking that also for sequencing and also developing mouse avatars from that tissue. And obviously, these patients are being observed. And at the time of any recurrences, we're also biopsying and obtaining tissue from the recurrences.

MATTHEW GOETZ: This slide just goes through what we do with the biopsies. I say that when we consent these women to this particular study and they sign on the dotted line, these women are obviously very generous. Because what they are doing is not only consenting to undergo a study, but this, to start out with, was six to eight cores from the breast, from the breast tumor. Now as it turns out, we have a fantastic group of radiologists here that have done this in such a way that when our patients have gone through, they said, you know it really wasn't bad. I was well anesthetized. And I would say that amazingly, we had 140 patients, we had no complications as it relates to biopsies.

So what do we do with these biopsies? Well the biopsies immediately went for sequencing. So we did tumor exome sequencing, RNA sequencing. The germline was also sequenced as well. We did a SNP array. And we're just in the process of evaluating the proteomics from this particular study as well. But the other thing that we did was to take these mouse cores. And the mouse studies in here were run by Dr. Liewei Wang and her laboratory, which is on Gonda 19. But-- by the way, the mouse lab is actually over in Guggenheim, so we had people from her laboratory. The technicians would come over to the operating room, would collect those tumor samples, and they were injected oftentimes within 30 minutes to an hour into a mouse in usually two to three cores per patient. So ultimately, we had about, as I mentioned, six to eight cores. We did actually take one core for each patient and also set that aside for future studies, especially as a needs for validation.

So we have enrolled, as I mentioned, 140 patients. I'm giving you the clinical summary on the first 134, because the last six were just recently enrolled. So we had a mean age of 51. So this is a young group of women. You can see that most of these patients have a larger tumor. T2 is a two centimeter or larger tumor, so this is, in and of itself, a high risk. So you can see that over 83% or excuse me, close to 90% of patients had T2 or greater. The great majority of patients had node positive disease. The actual size of these tumors, the mean tumor size was five centimeters. And the Ki67 which is a marker of proliferation was high at 40%. So this is a high risk group of patients. This tells you the molecular subtype. So I started out this talk by showing you the importance of using what we know already. And that is that we know already, of course, that molecular subtypes are important. Luminal B, HER2, as well as basal are the really, I would call, the most aggressive tumors. And you can see that those comprised about 90% of patients in this particular study. And you see that most of these patients had high grade tumors.

This next particular slide shows you a number of things. So first of all, what is RCB? RCB stands for residual sorry excuse me, residual cancer burden. So what is RCB? So RCB is a measure of the residual disease at the time of surgery. It's been classified. It was first developed by a pathologist named Fraser Symmans at MD Anderson. It's now been validated in multiple different cohorts, that is, in the neoadjuvant setting. And essentially, it's a patient who has a 0, is a patient who's had a complete pathological response. So this is the ideal scenario. And you can see that in this study, approximately 33% or 34% of patients had a complete pathological response. These patients have an excellent prognosis. Whereas we can see that about a quarter of patients had RCB-3. So these patients here would be considered at the highest risk of developing future recurrence of their breast cancer. And some of the best studies would suggest that patients with an RCB-3 could have a risk of distant recurrence within a matter of two to three years of 50%.

So what we've done by simply administering chemotherapy in this particular setting is we've identified who are the high risk patients. Now that's important because now we're going to show you a little bit of data about how we would use this information. So our goals in this particular study can be looked at a number of different ways. One of would be the n of one. And of course, this is the idea that many people are thinking about in cancer. It's the idea that is being used in the Sim clinics right now. So if a patient comes in, has metastatic disease, is failing standard therapy, and they want to have their tumor evaluated, you can sequence the exome-- obtain RNA sequencing-- and try to come up with the identification of a specific drug target, and then provide a prescription for the patient.

This is clearly brand new. We wouldn't have thought of doing this even two or three years ago, but it is beginning to make its way towards the clinic. And in fact, here at Mayo Clinic, we have a sim, what's called service line one, that's specifically dedicated towards this. I think what was interesting, though, is we wanted to see, well what does this look like that is doing this type of sequencing. What does it look like in the context of a standard chemotherapy, what we know, what we have evidence that works for patients. As Judy already mentioned, we have an opportunity to look at paclitaxel response, and I'll talk to you a little bit about that. And then finally, looking at a response at the time of surgery.

So this is a very busy slide. But this was looking at only one subtype. So this is the ER negative HER2 positive breast cancer. And what I'm focusing on here, in this particular slide, is those most frequently mutated genes where we would consider today that we either have a target, meaning a drug that could target, or there are drugs that are in development. Some would argue that P53 is not a targetable gene and or mutation. But there actually are drugs that are in development for P53. But you can see some of the common players, such as PIK3CA, EGFR, and others here.

Well what we've done in this particular slide-- and you can see across, in this case, the x-axis here-- either are based on purple, which is those patients that had a complete response, versus black, those patients that did not have a response. And then you can look at the various genetic alterations. And they're organized as regards to splice site missense, frameshift, nonsense, and then, of course, RNA-seq date, which is overlying here. And you can see whether a gene is up and down by the arrows. So this gives you an idea of the complexity of the data, and then, of course, it begs the question, is this useful for our patients that we see in our clinic. And we think, eventually yes, but it's going to take some time to actually get to that point where we can apply this routinely for our patients.

JUDY BOUGHEY: So to bring us back to the clinical setting, I'm just going to highlight a clinical example of one of the patients that was enrolled in the BEAUTY study. This was a 40-year-old lady who presented, actually, with a palpable mass. It was a six centimeter mass on physical examination, and she underwent a biopsy. And you can see already from her percutaneous corneal biopsy at diagnosis, that this was a relatively aggressive disease. It was six centimeters in size. It was a grade three invasive ductal. It was ER, PR, and HER2 negative. So that's the triple negative, or the basal type. And the Ki67 was extremely high. 88% is a very, very high proliferation rate. Luckily, she was clinically lymph node negative, and she enrolled in the BEAUTY study. When we look, this is her MRI when she initially presented. And you can see here in the upper outer aspect of the left breast, she had about a 6.1 by 3.5 centimeter tumor, so very much in fitting with what we were seeing in the clinical examination.

She was treated on BEAUTY. She received her 12 weeks of taxane therapy. And then when it came to the time point for her interval biopsy and interval MRI, you can see she's had a little bit of response from her tumor, but really not a great response. She still got about 4.7 centimeters of disease in that same area. She then went on to receive the anthracycline portion of her chemotherapy, had a little bit more response to that, and you can see here her MRI prior to surgery showing about a much smaller focus, just measuring about 2.2 centimeters.

So I think when we see these patients in the clinic, there are multiple different ways to look at this. I would say maybe about five years ago, we would have looked at this lady and said, well, you know, your tumor shrank from six centimeters to two centimeters and it's lymph node negative, so that's actually a good response. Well, she elected to undergo a mastectomy, and her pathology from surgery showed that she had residual viable invasive disease. It was still a grade three tumor. It was still triple negative. Her viable focus was a 2.5 centimeter mass, and that mass had a Ki67 of 93%. So yes, no longer six centimeters of tumor, but still 2.5 centimeters, a very viable active proliferating disease. Her overall tumor cellularity in that focus of disease was 100%. Her lymph nodes were negative. But again, this-- when you calculate it out with the RCB-- would be an example of a patient with an RCB-3. So a much smaller mass, but still pretty significant disease left.

When we look at what we have learned about these patients from other publications in the breast cancer literature, more and more studies have shown that the Ki67, this measure of proliferation post-chemotherapy is actually a very important factor to look at. And a little bit different in the hormone receptor positive patients-- if they still have a very high Ki67, that's a bad sign. But the lower Ki67s don't quite separate out so much. But for the hormone receptor negative patients, as this patient was, you see just a stepwise decrease in overall survival based on increase in the proliferation rate of the viable disease post-chemotherapy.

So this patient would have fallen here on this purple line, Ki67 of greater than 35%. And at five years, she would have had only about a 35% chance of being disease free. So if we know that-- unfortunately for this patient, just four months after she completed her surgical resection, she came in with some diffuse nonspecific complaints of pain and not feeling so well and fatigue. And her PET scan shown here, unfortunately showed widely metastatic disease. And we biopsied her liver metastasis, and that was consistent with a triple negative metastatic breast cancer. So these are the patients that we need to be using this individualized therapy on and intervening earlier to change the outcome.

MATTHEW GOETZ: So as we think about the type of sequencing that's going on, we'll talk a little bit about the germline sequencing here. So there's a paper that actually should be going out fairly soon. We've completed sequencing in 124 patients. And using an algorithm to identify the set of genes that we know are associated with her hereditary cancer syndromes, not just breast cancer, we identified 27 variants in 24 patients. So if you think about this study, we are enrolling a high risk population, but 19% of the patients enrolled in the study were identified to have a deleterious mutation in a gene that's been previously associated with the risk of developing cancer and specifically breast cancer. Now some of these genes, such as BRCA1 and BRCA2, there's a very high lifetime risk of developing breast cancer. For some of these others, the risk might be a bit smaller, but in each situation, they've been documented.

As you might know, that was a recent publication just a week or two ago in the New England Journal of Medicine, really, again, cementing the fact that PALB2 is a very important gene. And mutations in this particular gene confer a very high lifetime risk of developing breast cancer, actually similar to BRCA2. This table here just gives you a summary. This is a busy table. But again, just identifying or just going through the different genes, their nucleotide change, their protein effect, and how they were classified.

One of the things that we did in this particular study was we actually allowed patients to obtain their results. So when patients were consented to this study, they were asked specifically, would you like to have the results of our germline testing returned to you. And interestingly, about 97% or 98% of patients said yes, they would. And so we actually had 12 patients in the study that met the criteria for return of results. That is, we identified an alteration. We felt it was in a gene that was clearly known to be associated with the risk of developing cancer, and that also that alteration was deleterious.

There were 12 patients in this study that met that criteria. As it turns out, eight of them had actually already gone through clinical testing, but there were four patients that had not undergone clinical testing. They were approached, and they were asked one more time, would you like to get these results. And in all situations, they said yes. As it turns out, three of these patients had a BRCA2 mutation, and one of them actually had an FH mutation, which is known to be causative for hereditary leiomyomatosis. So it's important to note that in this study that-- I think overall the take home from the germline sequencing is that number one, this is much more common than I think that we've thought about. When we think about BRCA1 and BRCA2, we usually think about something in that range of 2%, 3%, 4%. But you can see that in a very high risk population of breast cancer patients that are young, that their frequency of these mutations are relatively common.

JUDY BOUGHEY: So how about the mice? The patients always want to know, how is my mouse doing and what do we using our mice for. We try not to tell our patients how their mice are doing. They don't want to know the answer to that. But the patient derived xenografts has really been a huge value add to this study. As Matt already alluded to, we have a really fine-tuned system here, where the study coordinator is there at the time of the breast biopsy, and we really minimize the time between the biopsy being removed from the breast and being injected in the mice.

So they literally run from Gonda Two over to Guggenheim, and they have the mice ready so that we minimize the time between tumor and injection. The samples are injected into the flanks of between two and three mice, depending on the amount of tissue we have. And then these mice are fed with estrogen supplementation for their water. We define take rate, which is the metric that we're looking at here, as a mouse where the tumor grew in the mouse and then we were able to pass through that tumor from the first mouse into a second generation of mice, and then it grew in the second generation of mice. So obviously, not old tumors are going to take in mice.

In the published literature, the take rates for xenografts in breast cancer is probably around about 30% to 40%. And so we were closely watching what our take rates were doing in the study. As we look at our take rates, this is probably one of the few studies around the country where xenografts have been established just from needle biopsies and not from big samples. Like with ovarian cancer surgical samples, you have a lot of tissue. Here we're dealing with needle biopsies, and we've been very pleased with the take rate. The average is around about 40% across the study. The take rates have been highest in the triple negative tumors and in the estrogen receptor positive, HER2 positive tumors. 52% and 42%, respectively, so we've been really pleased with that.

It's not surprising, but it's obviously interesting to note, that the luminal A tumors-- these are the tumors that probably do relatively well even in the absence of chemotherapy, and we didn't have many of them on the study, but some with larger tumors did enroll-- these are not really growing in the mice. We've also been trying to grow patient derived xenografts from the residual tumor post 20 weeks of neoadjuvant chemotherapy. And obviously, this is a much more challenging tumor to grow. It's a lot harder to identify at surgery, necessarily. But those cases where we've been able to identify residual disease and get that from the surgical lab into the mice-- we now have six established patient derived xenografts of breast cancer post-treated chemotherapy. And these are three triple negative tumors and three luminal B tumors. And we've done the validation that the histology of what we're seeing growing in the mice correlates with what was seen on the clinical side from the patient slides.

One of the exciting things when you take what Matt just presented, in terms of the baseline germline mutations of the patients, is a very interesting and a very rich resource to note that we actually have tumors growing in mice from a variety of patients with germline mutations. And I think this is a very unique resource that probably is not available very widely around the US and definitely something that will lead to further ongoing work and opportunities.

So what about this tumor that's growing in the mouse? Is it going to act the same way as the tumor does in the woman's breast? I mean, really, that's what we need it to do in order for it to be a faithful model for us to use for drug development. So most of these patients in the study or all of the patients in this study were treated with their taxane therapy first. And post-taxane, we had MRIs. And so we looked at their MRI post-taxane therapy, and defined these patients as either being a responder, meaning that their tumor disappeared on the MRI to the 12 weeks of taxane, or non-responder, meaning that they still had significant disease on the MRI post 12 weeks of taxane therapy. And then we took the established patient derived xenografts in the mice and treated those grafts either with nothing or with taxane.

And lo and behold, here are some great examples in That this is just such exciting work. What you can see here on the bottom is the patient example. This was a patient who presented with a disease, again, on the left breast. Post-taxane, before her anthracycline, she had essentially a complete response and imaging response in the breast. When you look at her xenograft, if we did nothing to the xenograft it grew. And if we had an established xenograft here and we treated it with taxane, it shrank away. Did the same thing in the mice as what it did in the patient.

What about the flip side? Well, this is the patient that I presented earlier. You'll recognize these MRI images. She didn't have a great response to taxane therapy. If you left the xenograft alone, it grew pretty rapidly. And if you treated that xenograft with taxane, it still grew pretty much at the same rate. So what we're seeing happening in the xenografts in the mice is the same as what we're seeing happening in the patients. And this provides for us a tremendous platform for testing novel agents in the mice.

MATTHEW GOETZ: So we'd like to get back to this particular patient, because when you see something like this, this makes you take pause. Because what you've done in this situation, is you've sort of given it your best shot, that is, with multimodality therapy. And this particular patient received 20 weeks of chemotherapy. She was treated with surgery, the optimal surgery. And then she went on to receive radiation therapy. And then despite that, four months later, we're at this point.

And of course that, as I mentioned, it does make you think. And that is, what can we do additionally for this particular patient. Is there something that could be done? And I think it doesn't take, really, if you will, much thought to say that we should be treating this patient not at this stage with novel therapies. Because when the patient presents with this stage, no matter if you have some of the best therapies, it's very difficult to actually overcome what's already happening. For example, at this point, the patient's performance status was already rapidly diminishing.

And so with this particular patient, a couple of things. First of all, it was mentioned that her xenograft grew both at the time of initial diagnosis as well as the time of surgery. Now you can see from what Judy showed you that there were actually were not very many tumors that grew at the time of surgery. But you could also expect that it's a bad prognostic sign if a tumor is able to take in the mouse at the time of surgery, after being exposed in a human to 20 weeks of chemotherapy. And indeed that was the case.

But I think the other thing that was really important and educational about this was based on some data that were pointed out to us by our bioinformatics team. We actually saw an alteration in a protein called DNMT3A, which is a DNA methyl transferase, specific isoform. And the one thing that you can see here-- this is actually looking specifically at the xenograft comparing V1 and V3-- is that there's dramatic effects over these two samples that are separated only by 20 weeks of therapy. So what we're seeing in this situation is the evolution of this particular tumor.

And as you might imagine, when we go to treat this particular patient and I were to see this particular patient in the clinic, guess what we've done in the past to try to identify targets. We've actually gone and used the original biopsy and said, well, based on that let me think of another therapy. But the reality is, these tumors are changing rapidly. And we actually should not be making our recommendations based on the tumor that was present before chemotherapy, but that is, the tumor that evolved after treatment.

In this situation, and this is from multiple different xenografts, you can see that this DNA methyl transferase was markedly upregulated. Even for someone like me, who was a nonscientist, right-- I do a little bit of science-- but you can see that this is a markedly increased. So what would we think about using?

Well, each one of us that saw this, we immediately thought about the drug decitabine, which targets these DNA methyl transferases. And this was a simple experiment that was done where mice were randomized to either control. And by the way, what happens is you inject these mice with tumor, and after about anywhere from about one to three weeks, they get to a large enough tumor size-- in this case, about 200 millimeters cubed-- where you could then begin to treat them. So these mice were randomized to either control and that is, no treatment versus the drug decitabine. So this was a very exciting finding. This work was all done in the laboratory of Dr. Liewei Wang. And this has actually subsequently led to the development of a grant that's actually going in led by Dr. Wang and Dr. Weinshilboum with regard to a P50 that was literally just submitted a few days ago.

So this concept here that I think we're trying to show you is that number one, is that there can be substantial evolution over a short period of time. Number two, we need these new models to be able to understand how to treat patients. And number three, by doing that, we may be able to impact patients. But finally, I would argue, that we need to be doing this, not at this point here. We cannot be waiting until this happens, but we actually need to be proactive.

So I'll leave with one other important point here. And that is, that there has been some very interesting tumor biology that's come out of the studies here by-- and this again, has been done in the laboratory of Liewei Wang and Dr. Weinshilboum. And it really goes to show you that having a team that actually can immediately take some of these findings and then go on and do the functional genomics can really lead to some exciting things. So we did an early analysis, again, looking specifically at taxanes and using that 12 week time point. Remember, these patients had a biopsy right after the 12 weeks of taxane. And we used proliferation as a response phenotype.

So you saw the data from Judy showing how important Ki67 is. If you shut down proliferation, it actually is associated with better outcomes. But if you don't, those patients have a much higher risk of developing future problems. So we used actually Ki67 as the response phenotype. And in this particular analysis, looked across the genome-- and this is RNA sequencing-- and Dr. Kalari identified a snip. And this is a snip in a particular gene that we actually had no idea what was involved in the area in breast cancer. It's a gene that's associated with chronic kidney disease. And it, again, certainly there's no sense in the literature that really anybody's ever reported this.

So what was found was that this snip number one, alters the protein levels of the protein that if you knock it down, that it substantially sensitizes tumor cells to taxanes or it excuse me, substantially sensitizes tumor cells to as it relates to the tumor viability. But more importantly is that we know that taxanes inhibit mytosis, that in the setting of knocking down of this gene, that you see substantial alterations in cell cycle. And this actually has gone on. So Dr. Liewei Wang's laboratory has gone to show that this gene actually leads to regulation of an incredibly important pathway, the PI 3-kinase AKT pathway. And you can see that knocked down and without the presence of drug leads to a substantial reduction in the protein of both AKT as well as increase in Caspase-3, a marker of apoptosis. And that in the presence of these different drugs that you see-- for example, in the presence of etoposide, you see actually very little effects, but in the presence of docetaxel, you see marked effects. So what we have identified here is a potential biomarker of taxane response. And then secondly, we actually have identified that it potentially involves a pathway that, of course, all of us are very familiar with, even those that are not dealing with cancer. And it's very exciting, and we're taking this forward, again, in a project in the P50 grant that's being led by Dr. Weinshilboum and Dr. Wang.

So what about the n of one analysis? And we're going to leave on this note. So we have about eight patients that have developed distant recurrence. And in every situation where patients developed a distant recurrence, we were able to identify a novel target. For example, you already showed you the data with DNMT3A. We had a patient that we identified a HER2 mutation. Now this is actually interesting, because this is a patient that did not have a HER2 amplify tumor. You know how important HER2 is, but just recently, there was identified in a cancer discovery paper out of the Wash U University group, Matt Ellis demonstrated that there are activating mutations in HER2. And these activating mutations actually confer a more aggressive phenotype. For example, the gene is not amplified, but this activation in HER2, much like we think about in EGFR or some of the other kinases, can actually activate PI 3-kinase AKT pathway.

This particular patient had this mutation, and interestingly, she was referred to a study-- and we believe that she's actually on the study. She's actually from the east coast right now-- where we're administering a drug called neratinib. And so interesting that some of the preclinical data demonstrated that neratinib, which is a pan HER inhibitor, so it inhibits HER1, HER2, HER3, and HER4 is active in these tumors that contain this activating mutation. But Herceptin or trastuzumab, the drug that you're all familiar with, that we treat for tumors that have HER2 amplification, does not work in HER2 mutated breast cancer.

So there was a number of other mutations that were seen here, but I think in each situation, you can see that we believe that we identified a novel target that could be used either with a drug that was available or one that was potentially available in the clinical trial.

JUDY BOUGHEY: So where do we go from here, and what's going to be the focus of the next steps? Obviously, we still have ongoing analysis of the data from BEAUTY1, but we're also avidly working to move forward with BEAUTY2. And so our focus is, we're working to develop BEAUTY2, is going to be to focus on these high risk patients that have persistent disease post-chemotherapy, so chemotherapy-resistant disease. So residual disease and the triple negative HER2 positive patients or luminal Bs that have elevated Ki67 post-treatment.

Our goal really is going to be to take the sequence data from what we've learned from BEAUTY1 and what we gather from the biopsies on the patients on BEAUTY2, both from baseline and post-exposure to taxane and some anthracycline, and use that data to match them with some druggable targets. What we want to do is, actually, then give these patients the opportunity to be treated with a short term, kind of a window therapy trial with a targeted sequence-based approach to their therapy. This will then allow us then to evaluate their response at surgery post this window trial, comparing from their pre-novel agent biopsy to their surgical specimen, whether the novel agent has been effective in shutting down their KI67 and eradicating that tumor.

And then obviously, in sequence to this, we're going to use the xenografts to identify biomarkers. So this is kind of our preliminary picture of where we see BEAUTY2 taking off. It'd be the similar high risk patients with an invasive breast cancer being treated with neoadjuvant chemotherapy. It's really impossible in this day and age to remove standard chemotherapy from any of these patients. So they need to be treated with the standard taxane therapies, with or without pertuzumab, trastuzumab, and carboplatin, depending on their tumor type.

We then plan to give them the first two cycles of their anthracycline cyclophosphamide chemotherapy, and then image them here. So rather than giving them the whole four cycles, we're going to image post-two cycles. We're hoping from the MRI and imaging, maybe MBI and also with the percutaneous biopsy to identify those patients where they have had eradication of disease and they don't necessarily need it novel agents, in which case they could just complete the standard third and fourth cycle of AC. Or those patients that have residual disease visible on the imaging and on the biopsy, still has an elevated Ki67, then we're going to take a targeted approach, based on the information from their genome sequencing and expose them to a novel agent in a window study, based on the target that we identify. And then look at that response at surgery.

And so obviously, this is an ongoing huge effort. We've been working very closely with pharmaceutical companies in order to obtain drugs that would match the potential targets that we've identified and obviously, looking to raise the funds to move this study forward. And we hope that this will be opening up, probably in the first part of 2015.

So obviously, none of any of this work that we've presented up to this point would have been possible without the huge input from all of our scientists and our clinician teams. It's also critical to note all the benefactor dollars and the Center for Individualized Medicine funding and SPORE and Cancer Center and the PGRN support infrastructure and funding that has come together to make this huge project possible. And Matt and I would like to take the opportunity to thank you all.