Eve Kleinerman:

Right. Thank you so much. We are going to get started. I first want to thank everyone for joining us this evening for this important discussion with Dr. Frederick Howard on biomarker testing and how it impacts treatment decisions after a cancer diagnosis. To introduce myself, I'm Eve Kleinerman, Sharsheret's Illinois Regional Director. And, as we are getting started this evening, before we start with the content of our program, I do want to ask that you participate in a quick poll that is going to be popping up right now. Feel free to answer as we get started.

And I also want to begin by thanking our sponsors for this evening's program who their generosity has allowed us to continue to provide this type of support and education to you. I would like to thank Bayer, Daiichi Sankyo, GE Healthcare, Merck, Novartis Oncology, [Semonics 00:01:05] Pharmaceuticals, and the Sigmund and Edith Blumenthal Memorial Fund. Thank you to all of our sponsors. Your generosity has made tonight possible.

Before we begin, I want to cover a few housekeeping items. Tonight's webinar is being recorded and will be posted on Sharsheret's website along with a transcript. Participants faces and names will not be in the recording. And if you would like to remain private without your name on the screen this evening, you can turn off your video and rename yourself or you can call into the webinar and instructions for both of those are in the chatbox right now. You also may have noticed that you are muted upon entering the Zoom room. Please stay muted during the call and if you have any questions, please feel free to send them into the chatbox. We do recommend that you keep your screen on speaker view. This will allow you to see the presentation more clearly and you can find that option in the upper right hand corner of your screen.

As we move into the webinar itself, I do want to remind you that Sharsheret is a national not-for-profit cancer support and education organization and we do not provide any medical advice or perform any medical procedures. The information provided for you this evening by Sharsheret is not a substitute for medical advice or treatment or for any specific medical conditions. You should not use this information this evening to diagnose or treat a health problem. If you do have any questions that are specific to your medical care, you may be advised that you speak with your medical provider. Always seek the advice of your physician or qualified healthcare provider with any questions you may have regarding to a medical condition.

Now I am going to end the poll. So thank you all for participating. And before we begin with Dr. Howard, we are going to share one of our callers, Janis' pre-recorded remark sharing her personal account of the way biomarker testing impacted her treatment decisions.

Speaker 2:

Hello everybody.

Speaker 3:

Sharon, we can't hear.

Eve Kleinerman:

Sorry for this brief technical delay.

Speaker 3:

And if someone's unmuted themselves, can you please make sure you're on mute?

Eve Kleinerman:

Okay. I think we're actually going to just move forward without Janis's video. My apologies to Janis for not being able to share her video, but due to technical difficulties, we are going to continue on with Dr. Howard's portion of the presentation. We are all looking forward to hearing from Dr. Howard.

Dr. Frederick Howard is an instructor of medicine and the Elwood V. Jensen Scholar at the University of Chicago. He specializes in the treatment of all types of breast cancer. He is interested in identifying better predictors of response to chemotherapy, immunotherapy and endocrine therapy in order to match the right treatment to the right patient. Dr. Howard's research focuses on answering several important questions at the intersection of digital health and breast medical oncology.

Such questions are, can artificial intelligence be used to identify good responders to standard neoadjuvant chemotherapy to better personalized treatment decisions? Can deep learning use readily available pathogenic and imaging data to improve upon supplemental existing genomic biomarkers in breast cancer in order to reduce costs, prevent unnecessary treatment delays and improve access to biomarkers and can rapid growth of big data and artificial intelligence tools and oncology? What safeguards need to be in place to ensure that these tools do not recapitulate healthcare disparities that are current and prevalent in healthcare. Dr. Howard, thank you. The screen is all yours.

Dr. Frederick Howard:

Great. Thank you so much for having me. Really excited to give this talk here today. So I'm a breast oncologist at University of Chicago and I have a lot of interest in kind of biomarkers and I hope this will serve as kind of a good overview of the currently kind of clinically relevant biomarkers for breast cancer.

I have no real financial relationships to disclose. So I think the first thing is biomarker gets thrown around a lot and it's actually a rather vague term that can mean a lot of different things. And so you might hear a biomarker be referred to as a biomarker of someone's risk of disease, or you might hear about a biomarker for detecting a cancer recurrence early or for the most part I think we talk about biomarkers as identifying features of cancer to select the most appropriate treatment for a cancer.

But really it's kind of a more broad term. And so I just wanted to highlight that all of these definitions are kind of true and I will touch on all of the relevant biomarkers for breast cancer. But most of it is going to be kind of talking about biomarkers that are relevant to treatment decisions.

So first, as far as predicting risk of disease, we can think about susceptibility risk biomarkers or just measurements for things that predict someone is at an increased risk for disease. So in breast cancer, obviously there are a number of classic examples, including single gene mutations such as the mutations in BRCA 1 or BRCA 2 that lead to an increased risk of breast and ovarian as well as other cancers. There are a number of other single gene mutations that increase the risk for breast cancer.

And so who should really be getting evaluations for having these single gene mutations? There's a number of criteria that are proposed by the NCCN, including women who are diagnosed at a young age, less or equal to 45. Anyone who has a diagnosis of triple negative breast cancer as rates of triple negative breast cancer are higher among women who carry mutations in breast cancer predisposition genes. Anyone with a diagnosis of breast cancer in a male. People with family history of young onset breast cancer or ovarian pancreatic or prostate cancer, or anyone actually with a diagnosis of ovarian pancreatic or prostate cancer should have genetic testing. Those of Ashkenazi Jewish ancestry with breast cancer diagnosis because of a high rate of BRCA mutation.

So those are kind of some of the canonical risk factors that tell us who should be referred for genetic testing upon diagnosis. And then cascade testing could be done to family members to then better customize their own screening plans to detect cancers earlier. However, we are coming to an increasing

realization that we can detect these mutations in single genes that confer high risks of breast cancer, but oftentimes the single gene doesn't tell the entire story. And a lot of women are diagnosed with breast cancer who don't have a clear cause even at a young age.

And so there's kind of an increasing recognition about the contribution of multiple genes kind of put together can have increasing additive effects about risk of breast cancer. And so something that's kind of coming to the forefront now is what's called a polygenic risk score, which looks at basically a single base pair change within DNA across multiple many different points throughout the entire genome of patients. And using kind of associating which of how each of those points interplay with risk of breast cancer, you can really stratify patient risk even in those who don't have mutations and kind of these classical breast cancer risk genes.

This work has been ongoing for several years now and it's not yet something that I think is approved for the routine use for patients to try to customize their screening, but there are ongoing studies to try to customize screening based on your own risk for using polygenic risk score testing. So it's kind of something that's right now just in the trial setting, but there might be a future where you can use these kind of genetic risks aside from some of the more common single gene mutations to have a customized plan for breast cancer screening. There's not really as many breast cancer biomarkers for the early diagnosis of breast cancer. There's some that are kind of emerging-

Speaker 5:

I didn't get to.

Dr. Frederick Howard:

So there's some that are emerging, but nothing that's yet clinically approved. So next I'm going to talk about just kind of biomarkers for breast cancer at the time of diagnosis. And so the first biomarker that I think about is really looking at the classic breast cancer subtypes that many of you may be familiarized with. But essentially when we first see a case of breast cancer, we divide them into whether they are hormone receptor positive, whether the cancers have expression of the estrogen or progesterone receptors, and then whether or not they are HER2 positive and they have amplification or overexpression of the HER2 gene.

And cancers that have neither expression of the hormone receptors or amplification of HER2 are term triple negative. So most cancers fall into the group of hormone receptor positive and HER2 negative. So that's the most common type of breast cancer, but there are significant proportion of cancers that are either HER2 positive or triple negative.

But although we classify cancers by these subtypes, there's kind of a gradation. So a cancer may be hormone receptor positive and HER2 negative, but not all hormone receptor positivity is the same. And so typically if you have your breast cancer pathology report, you're going to see percentage expression. You might see an ER percentage and a PR... that's estrogen receptor and a PR or progesterone receptor percentage ranging typically from one to a hundred or zero to a hundred. And that actually has a strong impact on outcomes as well as sensitivity to hormonal therapy.

This is what pathologists are actually looking at when they are saying that a cancer is estrogen receptor positive. It's basically a stain that marks the estrogen receptor on the cell surface and you have no staining here. You can see a little bit of the brown staining in some of the cells here and you can see strong staining of a lot of the cells here. And essentially, why this is important is because you might have a cancer that's technically hormone receptor positive, but when the hormone receptor positive percentages are very low, the outcomes are and the behaviors of cancer much more similar to triple

negative. And so we actually treat those cancers similar to triple negative cancers and that does alter kind of the therapies that might be effective for a specific cancer.

Similarly, we assess whether a cancer is HER2 positive through two different modalities. So one of them is what's called immunohistochemistry. So that's that same kind of staining that we were talking about with estrogen and progesterone receptors. And so you can have a cancer that is negative or positive by immunohistochemistry, but we also use a supplemental test in many cases, especially when the immunohistochemistry is kind of indeterminate. And so when you're going through a cancer diagnosis, you might find that additional testing is needed with this what's called fluorescent insight tissue hybridization or FISH testing to really determine whether the cancer is HER2 positive or negative. And so that generally takes less than maybe a week of time, but you might be waiting a little bit for those results to further clarify.

And really, HER2 is another important biomarker. Essentially cancers that are HER2 positive benefit from a drug called trastuzumab, as well as other drugs that target overexpression of HER2, whereas cancers that are defined as HER2 negative by the immunohistochemistry and FISH testing don't benefit. So it's kind of a good biomarker for a benefit for a number of drugs that target HER2. But we're going to talk a little bit more about this for a specific new drug that's been approved later.

You might have also heard, the clinical breast cancer subtypes that we talk about; triple negative, hormone receptor positive are one way to characterize breast cancer. But for a number of years now, scientists have used essentially the profiles of genes that are turned on and off in these types of cancers to further subdivide the cancers into these different categories based on gene expression patterns. Most of the triple negative cancers end up being categorized by gene expression patterns to what we call basal like. And most of the hormone receptor positive cancers get categorized as what we call either luminal or luminal A or B. And then the HER2 positive cancers tend to be categorized as HER2 enriched.

But sometimes you might have a triple negative cancer that actually looks kind of a little luminal when you do the gene expression testing on it. And so this might be mentioned in pathology reports or reports for certain testing that can be done on a cancer. And that's kind of just, if you hear the words luminal or basal, oftentimes that essentially just means hormone receptor positive or triple negative. But it's a test that's being derived by looking at the gene profile of the cancer rather than the staining of these markers, the hormone receptor, her two markers on the surface of the cells.

Another way to subdivide breast cancer at the time of diagnosis is the histologic subtype. So there's about maybe 10 or so different well defined histologic subtypes of breast cancer, but the most common subtypes are ductal and lobular. Essentially, these are cancers that are thought to derive from the lactiferous ducts that deliver milk through to the nipple. And the lobules are the actual glands that generate the milk. And so those generate cancers that have different appearances under the microscope. Lobular cancers kind of have this infiltrative single file cell appearance, whereas the ductal cancers will often have kind of an appearance of a duct under the microscope.

And these cancers, the different subtypes of breast cancers behave differently. And for example, lobular cancers oftentimes or many times we can see a lobular cancer that ends up being larger at the time that it's taken out than it appears on initial imaging like ultrasound or mammogram. Lobular cancers tend to be more hormone receptor positive and so they do tend to respond better to hormonal therapy. But there are these differences based on the histologic subtypes. So if you see your cancer classified as ductal or lobular, there's not a lot of differences as far as the therapies that are used, but there might be some differences in terms of how your doctor is thinking about your cancer and how that cancer might behave.

Other kind of basic biomarkers that we have at the time of a breast cancer diagnosis, and you might see on a pathology report that you get back at the time of breast cancer diagnosis includes grade. Grade is essentially a measure of aggressiveness of cancer ranging from 1 to 3, with 1 being the least aggressive and 3 being more highly aggressive. And it's a reflection of how similar the cells appear in relation to normal breast tissue. And for one, we look at what we call differentiated the cancer is, and by that, does a ductal cancer form normal ducts or does it not? We also look at how rapidly proliferative the cancer appears and basically how regular the shapes of the cells are. And so cancers that are less differentiated and more proliferative are marked with a higher grade in the pathology report.

Another marker for grade that you might see is this marker called Ki-67 or Ki-67, which is similar to the estrogen progesterone receptors is measured and reported as kind of a percentage from zero to a hundred, which basically tells the percentage of cells that are undergoing active proliferation. So low is kind of generally less than or equal to maybe 10%, but you can see anything up to even up to 100% or 60%, 80% in highly proliferative cancers.

For those low kind of less than 10% cancers as well as for cancers that are lower grade, it does correlate with likelihood of recurrence. And so those cancers that are higher grade or with higher Ki-67 made, deserve more aggressive treatment.

So there's a lot of these different biomarkers that we have at the time of breast cancer diagnosis. We have the subtype, we have how much estrogen is being expressed, we have the Ki-67, we have the grade. And it makes it challenging to integrate all of these different biomarkers to make treatment decisions for patients. And so, one of the more recent advances in terms of biomarkers for treatment decisions for hormone receptor positive HER2 negative breast cancers are what we call a genomic recurrence score testing. And what these tests do is, instead of just looking at what that percentage estrogen receptor is, what that Ki-67 percentage on the cell is, what the grade is, is it looks at actually the gene expression of the cancer. So which kind of genes are turned on and off in the cancer and puts it all together into a score for breast cancer predict risk of recurrence.

And so currently in hormone receptor positive HER2 negative breast cancer, we have two assays that look at this that are validated to predict a risk of recurrence. One of them is called Oncotype, which is probably the one you'd be most familiar with. The other one is called Mammoprint. There's work being done to develop kind of similar tests to predict risk of recurrence in HER2 positive breast cancer. There's actually a new test called HER2 DX that was recently approved. And similar work is being done in triple negative breast cancer.

But essentially, and this is the given that there's been I think more studies in the hormone receptor positive HER2 negative breast cancer patients, we have a lot more kind of experience and confidence in using these genomic tests to identify patients at higher risk of recurrence. And studies have since been done to show that not only are patients with a high Oncotype score at a higher risk of recurrence, they actually show that those patients benefit more from chemotherapy.

The cutoff for when chemotherapy needs to be delivered for hormone receptor positive HER2 negative breast cancer has kind of varied over time and a recent large study has shown that when you use this Oncotype score for women with a score that is less than or equal to 25 who don't have lymph nodes involved, there's really no difference in recurrence with or without chemotherapy. For women with a score of greater than 25, there is a benefit, but for those with a score less than 25, there doesn't seem to be a chemotherapy benefit except in the population of women who are under 50. And those who have breast cancer prior to menopause or younger women with breast cancer, this is kind of an area with a lot of debate, but when they looked at the subgroup of women under 50, especially when the scores were getting in that 21 to 25 range, they found that the difference in recurrence with or without chemotherapy was as high as 6 to 8%.

And so a lot of times we will recommend chemotherapy for premenopausal women who have kind of these intermediate risk Oncotype recurrence scores. There's a lot of debate as to why the risk of recurrence or why this test might identify postmenopausal women as not needing chemotherapy and then premenopausal women then end up needing chemotherapy. Some of this benefit might be due to the fact that chemotherapy and premenopausal women actually will oftentimes induce premature menopause. And by inducing premature menopause and reducing the amount of hormones that are potentially driving breast cancer growth, you might end up reducing the risk of recurrence through the effective chemotherapy solely on the ovaries.

Now, obviously it would be nice to not use chemotherapy to induce menopause if the benefit is really causing premature menopause because we also have drugs that can essentially temporarily shut down the ovaries to reduce the hormone production and then allow that same benefit. But those studies need to be done in the meantime and we can't really rule out that there's a benefit directly to the biology of the cancer in younger women who have these kind of intermediate risk recurrence scores. So this is the kind of discussions that might be had if you fall into this range as a premenopausal woman about whether or not you need to just have suppression of the ovaries with medications to reduce the hormone exposure of the breast cancer or if chemotherapy is also needed.

So this initial study was done in women who had no lymph nodes involved with their cancer. And subsequent studies have looked at women who also have lymph node involvement. For up to one to three lymph nodes, patients who are postmenopausal again, who have an Oncotype score that's less than or equal to 25 don't get any additional benefit from chemotherapy. So we can kind of comfortably exclude chemotherapy for those patients. Whereas again, in the premenopausal patients there did seem to be a benefit of chemotherapy. And so we'll oftentimes, again discuss chemotherapy when the score is for premenopausal patients who have lymph node involvement of their breast cancer.

So another kind of emerging biomarker for hormone receptor positive HER2 negative breast cancers is Ki-67. So we kind of talked about this a little bit earlier and it's a marker of proliferation. So there was a new therapy that was recently approved for the use after surgery to further reduce the breast cancer recurrence risk for hormone receptor positive, HER2 negative breast cancer patients. And that's a drug called abemaciclib or Verzenio. And essentially that drug is indicated for patients who have a high number of lymph nodes involved or who have some lymph node involvement with a high grade, a large tumor or who have a high Ki-67 score.

However, if you look at the benefit based on the patients who had high versus low Ki-67, and this is basically a curve showing the risk of having the disease recur and you can see that in the red curves are the patients who received the Verzenio or abecmaciclib in the blue curve are those who just received hormonal therapy without abemaciclib.

And you can see that there's a much bigger difference in this bottom cohort which had a high Ki-67, which in this case is defined as over 20% compared to this top cohort that had a lower Ki-67. And so the FDA currently only approved this therapy of abemaciclib for women who have had their cancer removed and who have had risk factors for recurrence, but also have a high Ki-67. And so this is a test your oncologist might use to determine if you're a candidate for this therapy.

I will say there's a lot of debate in the community about the use of it. There is still some benefit in the patients with a lower Ki-67. And so for those who are at really high risk of recurrence, sometimes we can get approval off label of the drug. And so there's still a lot of debate and work that needs to be done. But for those who do have a high Ki-67, this is another treatment option that reduces the risk of recurrence.

And then I think one of the last few biomarkers I'm going to talk about for hormone receptor positive early breast cancer is the Breast Cancer Index. And so as I've been talking about, one common way to treat hormone receptor positive breast cancers after surgery is hormonal therapy, which is usually continued for at least five years after a breast cancer diagnosis. But there are studies now that have shown that extending therapy beyond five years, especially for women at higher risk, may further reduce the risk of cancer recurrence.

And so for patients at the highest risk, I'll sometimes recommend continuing hormonal therapy for up to even 10 years. But there is this biomarker test called the Breast Cancer Index that can further identify which patients need that extended duration of hormonal therapy and women after five years of their hormonal therapy, this test can essentially identify those who need additional extended therapy versus those who probably don't. And so this is something that can be helpful to better customize how much hormonal therapy is needed for a breast cancer diagnosis.

So there are a number of other biomarkers that are applicable either for multiple cancer subtypes, for early breast cancer. So one of them is whether or not someone has a germline BRCA mutation. And this is actually a biomarker for both early breast cancer as well as for metastatic breast cancer. So there are a number of drugs what we call PARP inhibitors. And so those drugs include olaparib and talazoparib and they've been approved now for many years for metastatic breast cancer in patients who basically have a mutation that they were born with in the BRCA gene. And essentially these inhibitors work on an enzyme that is not necessary for DNA repair.

When you have a BRCA mutation, DNA repair is inhibited. And so those cancer cells, when that additional DNA repair function is suppressed, those cancer cells die essentially are unable to kind of survive. And so for a long time, these PARP inhibitors, olaparib, talazoparib have been approved for advanced breast cancer. So cancer does kind of spread beyond the breast through the lymph nodes. However, in the last year or so, olaparib also showed benefit as a potential treatment to be added after surgery in the same population for women who are born with a mutation in the BRCA gene and who either have hormone receptor positive HER2 negative breast cancer or who have triple negative breast cancer. So that's been another promising option that can be used after surgery.

In hormone receptor positive HER2 negative breast cancer, we'll oftentimes sequence therapy such that patients will receive surgery first and then chemotherapy potentially after surgery and then radiation and then hormonal therapy. Whereas in patients who have HER2 positive breast cancer or triple negative breast cancer, usually we'll be giving some amount of chemotherapy and targeted therapies prior to surgery. In these patients, the most important biomarker is response to chemotherapy prior to surgery. And so the best thing that you can see if you receive chemotherapy prior to surgery is that there's no residual cancer cells in the breast or the lymph node at the time of surgery.

And we have a number of therapies that are approved for patients who have some reduction of their cancer, but they didn't reach that point where all the cancer was completely eradicated in both HER2 positive as well as in triple negative breast cancer. So in HER2 positive patients, those who don't have that complete response at the time of surgery, generally we use a drug called trastuzumab emtansine or Kadcyla, which is essentially an antibody towards the HER2 protein on the cell surface with some chemotherapy attached to it. So it kind of directs the chemotherapy directly towards that, any of those cancer cells that are remaining.

And so that is a treatment that has shown effectiveness. Here's kind of the risk of recurrence in patients who had residual disease who received that drug versus didn't. And it's shown effectiveness in HER2 positive breast cancer patients who didn't have a complete response to chemotherapy. Similarly, in triple negative breast cancer patients, those who don't have a complete response to their chemotherapy, there is a benefit to a drug called Xeloda or capecitabine when administered after

surgery. Now recently immunotherapy was approved for early triple negative breast cancer. And so in those who didn't have a complete response, we would also include immunotherapy as part of that treatment.

So moving on from kind of early stage breast cancer, I want to talk a little bit about some of the biomarker we use for more advanced stage breast cancer. So one of the common kind of biomarkers we talk about is looking at the genetic profile of the cancer. Now, we already talked a lot about looking at the genetic profile that you are born with, but we oftentimes will find that cancers will acquire mutations in their own genes and that allows those cancers to become more aggressive and more rapidly proliferative. And that's actually, those mutations are kind of how those cancers grow from your normal tissue into something that requires treatment and can spread and cause issues in the rest of your body.

So cancers have changes in the DNA or the genetic code that result in the formation of abnormal proteins. And those abnormal proteins are then what causes cancers to grow uncontrolled. And we can detect these mutations and by doing what we call sequencing, again on the tumor and not on... this is looking at the tumor and not on the genes that you were born with that predispose your risk for the cancer. And we can do this actually through a specimen, either a biopsy or the cancer that was removed at the time of surgery, or we can actually do this from looking at tumor cells that are circulating or tumor DNA that is circulating in blood in patients who have advanced cancer that has spread, again beyond the breast and the lymph nodes.

And so that's testing that we generally recommend to perform for anybody with advanced breast cancer because there are therapies that we can use based on the presence of specific mutations that we find that were driving that cancer growth. And so when you have this kind of sequencing done on your cancer, oftentimes... there's a number of companies that will do it, academic institutions may be able to do this within their own institution. At University of Chicago, we do it at our own institution, but the results are generally the same across whatever platform you get it done with. This is what a result might look like if you get the testing done with a platform that's called FoundationOne. And essentially you'll get results with mutations and specific genes as well as whether a gene is amplified or kind of turned on excessively. And for certain of those mutations, we have specific therapies that we can then use to customize the treatment of cancer.

Currently, all of these kind of genetic mutations within the cancer itself are only used to select therapies for advanced breast cancer as in cancer that is again spread beyond the breast and the lymph nodes to other parts of the body. Obviously, as studies are ongoing and some of these therapies are tested in earlier breast cancer, there may be relevance to getting the genetic testing done on your tumor even for earlier breast cancer. But currently this is kind of only really relevant for cancers that are kind of more advanced.

And so again, there are a number of mutations that we can use to direct therapy. One of them is what's called a PI3 kinase or PIK3CA mutation. So this is the common mutation in hormone receptor positive breast cancer. And we have a drug that's approved for the treatment of advanced or metastatic hormone receptor positive HER2 negative breast cancers that have a PIK3CA mutation and that's a drug called Alpelisib or Piqray. And so that's a drug that we'll often use as one of the treatment lines in women who have advanced breast cancer.

I will say that although this is a common mutation in hormone receptor positive breast cancer, the patients who can actually benefit from this drug is a smaller proportion of patients because it does have a lot of side effects and particularly it causes a lot of issues with high blood sugars and diabetes. And so we have to use it in a lot of caution in patients who have issues at baseline with their blood sugars. But it

does have a benefit as far as improving the duration of time when patients can go without progression of their cancer.

Another mutation that we can kind of see that currently we don't have a therapy for but or we have therapies for, but whether or not it should impact therapy is kind of unclear is what's called ESR1 mutation status. So ESR1 is the gene that codes for the estrogen receptor on the surface of cells. And so we don't see a lot of ESR1 mutations in patients at the time they're first diagnosed with breast cancer, but when they're on drugs that lower the estrogen in their body like aromatase inhibitors or other antiestrogen or hormonal therapy, sometimes cancers will develop a mutation in that estrogen receptor that allows that receptor to act like there's estrogen there even when there isn't estrogen. And so you can imagine that would allow the cancer to continue to grow even if you are suppressing all of the estrogen in the body with drugs with hormonal therapy.

Now, many of our hormonal therapies work by reducing estrogen in the body, but actually one of the hormonal therapies, Fulvestrant is a drug that degrades the estrogen receptor. And so it actually is more effective in women who have this ESR1 mutation. However, it's not yet clear if we should switch patients to Fulvestrant when they have this mutation present. You can see this is a trial where they detected patients were being treated with aromatase inhibitors. And at the time, when they detected this ESR1 mutation, they either switched them to Fulvestrant or they continued the aromatase inhibitor.

And clearly patients who then were switched to Fulvestrant had a longer time before the cancer started to grow again. However, I will note that there were a number of patients, maybe 20% that were doing okay when they were just being treated with the aromatase inhibitor. And so although it does tell you that the cancer is likely to be more resistant, it's not necessarily the whole story. Although if you do find this mutation, your doctor may still say, if you're on this aromatase inhibitor or other hormonal therapies that are not Fulvestrant, you may still want to continue those therapies as long as they remain effective because if you're one of those patients that get a long benefit on the aromatase inhibitors, you might as well maximize that benefit and then you could use Fulvestrant later on down the line.

So I want to next talk a little bit about tumor markers because there are different ways that we can detect cancer throughout the body. I mentioned that we can kind of detect genetic mutations in the tumors through looking at the sequencing of the tumors. And so that's actually kind of a way that we are moving towards to follow patients with breast cancer after the time of a breast cancer diagnosis.

Prior to using these kind of genetic profiles of cancer, the older way of monitoring cancers were through what we call tumor markers. And so these are arbitrary names that are essentially different carbohydrate antigens that are present on cancer cell surfaces. And the ones that are relevant for breast cancer are the three that I have listed here. But the problem is that after a breast cancer diagnosis, that doing these tumor marker tests, many patients who have recurrent cancer will not have positivity of these tumor markers. And then many patients, actually about 6% of patients will have positive levels of these tumor markers, but actually not to develop recurrence of their cancers. And so we generally don't use these tumor markers to diagnose cancers that have occurred early, but they can sometimes be useful to follow patients with advanced disease where we know that they have cancer that needs treatment, but we can't measure it well on CT scans or bone scans, sometimes we will use tumor markers to follow their disease.

However, unlike the tumor markers, there's been increasing use of what's called circulating tumor DNA, which is essentially like I was talking about before, using a sample of your blood to look for mutations that are being shed from tumor cells that are present in the body. So we can do that very easily to detect mutations in patients who have metastatic disease. And so there was this thought that maybe we can also detect those mutations if a cancer at diagnosis had some mutations that define the cancer,

maybe we can use that same kind of technology to identify if there are levels of tumor DNA that are present in the body after the time of a surgery for an early breast cancer.

And you can see that there is a big difference in the rate of recurrence of breast cancer in patients who have detectable circulating DNA versus those who don't have detectable DNA. And so this is kind of becoming a very promising way to detect cancer recurrence early. However, the challenge that we're then left with as an oncology kind of community is, what's the best way to treat patients who have a positive result in CT DNA testing and can we actually eradicate cancers and basically prevent metastatic disease from recurring or are we just giving therapy early and not actually changing the time course of disease?

If you see here, when these patients were having recurrence, there are patients who are here five or six years after the time of surgery and they had a positive result for this ctDNA test and they hadn't yet had recurrence. Now yes, many of the patients, about 50% of them had recurrences within two years in this study. But there are many patients who are still alive five years from their disease without cancer recurrence even being positive for this ctDNA or circulating tumor DNA biomarker.

And so really generally in the community, we'd recommend that patients are kind of interested in these kind of technology to detect cancer recurrence early should really be thinking about enrolling in clinical trials where the test is done and then patients are randomized to receive a treatment that could potentially reduce the risk of recurrence. But since we don't yet know for sure that it's going to actually make an impact on those actual rates of recurrence, it's hard to use this study to then treat all of these patients, some of whom might not have cancers come back for five years with some chemotherapy with no defined endpoint.

So those are kind of the big biomarkers that come from genetic testing of the tumor. Another biomarker that's kind of relevant for metastatic disease that's emerging is the use of novel radioisotopes for PET scans. So many of you may know, PET scans are used to detect areas of active tumor throughout the body. And the classic PET scan that we've used for a long time is called a FDG or it's basically a glucose PET. And what that PET does is it uses a labeled sugar molecule that's injected through an IV and goes throughout the body and then anywhere in the body, which is uptaking a lot of sugar, in other words, areas of the body that are kind of more metabolically active is going to show up right on a classic FDG PET.

And normally, we see activity in the kidneys and the bladder because the sugar is actually peed out through the kidneys and the bladder. The brain of everybody is very active and so that actually uses up a lot of sugar. But then you can sometimes see spots of cancer that light up bright on these FDG pets. And so that's been used to monitor cancer for a long time. However, there's actually been an advance with a novel radio tracer that instead of using a labeled sugar molecule, actually uses a labeled estrogen molecule. And what that can do is, instead of identifying areas that are very metabolically active, it will identify areas in the body that have a lot of estrogen receptor. And so this can be used to identify cancers that are throughout the body that are positive for the estrogen receptor.

And so you can see this is actually two images taken from the same patient, one with this new [siriana 00:46:10] or the FES PES, which targets the estrogen receptor and one with this old glucose PET. And you can see that a lot of the cancer was actually better visualized with this estrogen receptor PET because it was not very metabolically active, but it had a strong expression of the estrogen receptor.

So this is very useful for patients who have disease that may not be easy to biopsy, to look under the microscope for the estrogen receptor because you can then use the PET scan to do this noninvasively and tell whether the cancer's positive for the estrogen receptor. And there are ongoing studies to look to see if we can use how bright the cancer is on this scan to determine whether someone should get

additional estrogen directed or hormonal therapy or whether someone should get chemotherapy if it doesn't have a lot of expression of the estrogen receptor and is not going to benefit from hormonal therapy.

Another new area that's kind of been a really big breakthrough for metastatic breast cancer is this concept of HER2-Low. And so I showed you before some images of breast cancer slides that were stained for this HER2 protein. And classically we defined only those that have really the highest staining for that HER2 protein as being HER2 positive. And that's because a lot of the therapies that we have that target HER2 only work in those that have a lot of expression of this HER2 protein like the Trastuzumab drug or Herceptin, which is kind of the first drug that was developed to target HER2.

However, I talked with you a little bit before about one HER2 antibody drug conjugate and this is another one called Enhertu. This is a picture of an antibody and that antibody kind of binds onto HER2 and it has actually eight chemotherapy molecules attached to the base of that antibody. And what we've found is that because of the high amount of those chemotherapy molecules, that even if you have only a few of those cells or very scattered amounts of those cells that are positive for this HER2 protein, enough of this antibody will then still target the cancer and then can be effective to treat cancers that have low levels of HER2 expression but are not HER2 positive.

So typically when we do this HER2 test, HER2 is graded on a score from 0, 1, 2 or 3 positive. And basically those that are 1 or 2, but are HER2 negative are the cancers that have benefited from this new antibody drug conjugate. So this drug was compared to using chemotherapy for patients who have had HER2-Low breast cancer. And there was a really big difference in the length of time where someone would go where their cancer was under control when they received this Enhertu drug versus standard chemotherapies that the physician was choosing. Almost a doubling in time that the cancer's remained under control on this treatment. And so, in the last year, this is probably the biggest breakthrough as far as new therapies for metastatic breast cancer.

One of the problems, however with this HER2-Low, there's a lot of challenges with HER2-Low classification because that test I showed you where those cancer cells are being stained for HER2 was really optimized to try to identify which of the cancers that are high for the HER2 and which of those that are not high. It's not very good at identifying which of those have just a little bit of HER2 and which of those have none. And so although that test was used, and that's currently what we used to identify patients for this drug, it's probably still not the best test. And this graph here kind of illustrates that. This was a study done where they had 170 patients which were classified by a number of different pathologists when they were looking at that image, those kind of images that I showed you before and the pathologists were tasked at grading the cancer as either being her HER2 0 or HER2 1, 2 or 3 or some amount of positive.

And you can see that for a number of cases here, there was discrepancies based on who was looking at that pathology sample. And so some pathologists might have called it positive and some of them, they were split 50/50 and some of them would've called it 1+ and some of them would've called it 0. And that makes a big difference when we're thinking about using this drug because if it's 1+, we can use this a potentially effective therapy, whereas if it's 0, it's not an option. So there's a lot of interest in developing better tests to identify HER2-Low breast cancer so we can better use this drug.

The last topic that I'm going to talk about is immunotherapy for breast cancer. And I guess I'm running a little short on time, so I'll go through this kind of quickly. Immunotherapy is revolutionized a lot of different cancer subtypes and it's essentially cancer cells develop this camouflage protein called PD-L1. And what that camouflage protein does is when you have your immune cells come and find the cancer cells, if they see that camouflage protein, it's kind of telling the immune cells that it's a normal healthy cell and the immune cells will then not attack it. Immunotherapy actually blocks this PD-L1 and so then

the immune cells will recognize the cancer cell for what it is and your body's own immune system can fight the cancer.

Immunotherapy is currently approved for both advanced as well as early stage triple negative breast cancer. In advance breast cancer, we actually have to measure the amount of PD-L1 on the surface of the breast cancer cells and only those that have what we call a combined positive score of greater than equal 10 actually benefit from immunotherapy.

And so this is another biomarker that we use. Unfortunately not all patients with advanced triple negative breast cancer benefit from immunotherapy. It's really kind of only those that have enough expression of this PD-L1 protein on the tumor cells that is telling the immune cells to ignore the tumor cells. And so in those that had a high PD-L1 testing, the addition of immunotherapy to chemotherapy for advanced triple negative breast cancer did better than chemotherapy alone.

In early stage triple negative breast cancer immunotherapy is also approved as a treatment. However, doing this PD-L1 test actually regardless of the result, patients had a similar improvement in the amount of patients who had no residual cancer at the time of surgery. And so there's not really currently a biomarker for earlier triple negative breast cancer.

The last two things I'll say is that there are other biomarkers for response to immunotherapy that are true for not only triple negative but also hormone receptor positive and HER2 positive breast cancers. And one of those is called tumor mutational burden. Essentially if cancer cells have a lot of mutations, they will generate a lot of abnormal proteins on their surface and the immune cells in your body can then recognize those abnormal proteins more readily. Cancers that are traditionally very responsive to immunotherapy like melanoma and lung cancer tend to have a lot of mutations, whereas breast cancer has fewer mutations. But there are definitely patients where you may have a high tumor mutational burden and immunotherapy is actually approved as a single drug without chemotherapy for metastatic breast cancer with a high tumor mutational burden.

And then there's also another test called microsatellite instability that is also another test that identifies advanced cancers that benefit from immunotherapy. This is probably only present in about 1% of breast cancer, so it's very rare, but it's another test that can be performed to identify if you would benefit from immunotherapy.

So just in general, we have a lot of different and very complicated tests that we can use to best personalize therapy for breast cancer. And this is allowing us to get better and better results and longer, greater number of patients cured from their cancer and patients who are living longer with metastatic disease thanks to that. So, I think I've covered most of the relevant biomarkers that I use in clinic today, but hopefully you've had some answers about biomarkers that you may have heard of and happy to take any questions.

Eve Kleinerman:

Thank you so much, Dr. Howard. This is such an important topic and I know that I feel much more equipped to understand it and I'm sure that everybody on screen does as well. We do have many questions and because we are running a little bit short on time, hopefully we'll be able to answer a few of those and then we will send out some of those questions and answers in a follow-up email. So if your question does not get answered tonight, it doesn't mean that it's not going to get answered, it just may need to get answered after this evening.

I want to start with one of the most popular questions that we've gotten both before the webinar tonight and in the chatbox is, is there a space for biomarker testing for those who have already

completed their breast cancer treatment and they are now years out and are wondering whether there's any benefit to that testing?

Dr. Frederick Howard:

So it depends on how far out you are from your treatment for breast cancer. So for example, treatments like the abecmaciclib drug for those who have hormone receptor positive breast cancer who have a high Ki-67, it may be reasonable to consider that. The trial, I think allowed up to something... I think it was around nine months or so from after completion of all the surgery and radiation and chemotherapy. And so there was a time lag for which patients were allowed to be screened and enrolled in that trial. And so I would consider that drug in patients who are relatively early after the completion of their therapy for that.

Similarly, thinking about using olaparib in patients who have residual disease or who have other high risk factors for their breast cancer if you're in that maybe around a year timeframe, it might still be relevant. If you're many years out, the problem is, especially I think we'll be seeing a lot more of the circulating tumor DNA being used in earlier breast cancer... but if you're many years out from your breast cancer, it's less likely that doing some additional new therapy is going to alter the ultimate course of disease.

And so in other words, the goal would be to do some testing that is going to impact what kind of therapy that you should receive. But the therapies are really going to be most beneficial close to the time when you've had the cancer removed and had your original treatment because if there is any cancer cell that it escaped from the breast and the lymph nodes and set up shop elsewhere in the body, it's going to be the smallest amount of cancer. Whereas if you do this testing years and years down the line, I think it's either going to be that there's enough of a cancer established that unfortunately you might be at risk for metastatic disease or you're going to be in the clear.

And it will eventually make itself clear, but I don't think doing the additional testing is going to be of high yield for patients who are many years out from their diagnosis.

Eve Kleinerman:

That definitely makes sense. And a related question, which you may have already answered in what you've been saying, is there a benefit to any repeat biomarker testing? So somebody who's had testing, but is that possible that the answers would change over time?

Dr. Frederick Howard:

Yes. So especially in metastatic disease, actually, there's a lot of heterogeneity in a lot of these tests. So in early stage disease, sometimes we'll see a lot of differences from that initial biopsy as far as the estrogen receptor percentage, the progesterone receptor percentage from what that biopsy showed versus what's in the cancer itself. Now, we won't always repeat those tests on both the biopsy and on the cancer that's removed at the time of surgery, but if something kind of doesn't make sense, sometimes we want to see what the biopsy showed, but the biopsy might just be a small fraction of the cancer.

And so sometimes you might see some lower estrogen receptor on the biopsy, but maybe more of the cancer is triple negative when it's taken out. And that's going to really alter kind of treatment decisions. In advanced breast cancer, some of these mutations that can occur will change over time and so we will oftentimes repeat looking for mutations that a cancer may have developed at multiple time points.

Eve Kleinerman:

Great, thank you. And then one last question and I do want to say we will follow up with Dr. Howard and get your questions answered because we are running out of time, but I do want to ask Dr. Howard that we also at Sharsheret serve women who are affected by ovarian cancer. Are there similar biomarker considerations for women who are going through ovarian cancer that they can ask their doctors about?

Dr. Frederick Howard:

Yeah, there are similar biomarker testing. I saw that as I was scrolling through some of these questions here, I saw that question in the chat, but I'm a breast medical oncologist and so I really speak most confidently about the biomarkers that we use for the treatment of breast cancer. But there are similar biomarkers in a lot of these different domains. So for example, the use the mutations in germline BRCA1 and 2 can be used to identify patients who benefit from PARP inhibitors in ovarian cancer. And so there's a number of the tumor mutational burden and the microsatellite instability status that I talked about at the end that show benefit for immunotherapy across different cancer types are similarly useful in ovarian cancer to select patients for immunotherapy.

And the same kind of testing that we're doing for circulating tumor DNA and looking for mutations for cancer, that's also relevant to ovarian cancer. But the specific drugs that can be used, a lot of these things like the HER2-Low for example, is currently really only a thing in breast cancer and there's kind of some data in lung cancer, but it's going to probably be explored in other cancers, but a lot of these other customized kind of biomarkers or specific treatments are really relevant to breast cancer.

Eve Kleinerman:

Great. Thank you. And I do want to again mention we will be sending out questions and answers in our follow-up email. But Dr. Howard, I really want to thank you for educating us this evening on biomarker testing and treatment decisions. You've answered so many of all of our questions and I'm sure that we all feel more knowledgeable after hearing your presentation.

And I again, want to thank our generous sponsors for this evening's webinar, Bayer, Daiichi Sankyo, GE Healthcare, Merck, Novartis Oncology, Semonics Pharmaceuticals, and the Sigmund Edith Blumenthal Memorial Fund. I do want to remind everybody that there is a link to the evaluation survey that's being placed in the chatbox. Please click on it now. You will be able to still hear what I'm saying while you're clicking on there. But our evaluations really do inform future programming, so if you can please participate, we would greatly appreciate it.

And please never forget that Sharsheret is here for you and your loved ones during this time. Sharsheret provides emotional support, mental health counseling, and other programs designed to help you navigate through the cancer experience. All of our programs are completely free and private and one-on-one and contact formation is in the chatbox now. Our social workers and our genetic counselor are available to each and every one of you. You're our priority, so please do not hesitate to reach out. Thank you and have a great night.