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HER2 Testing: The Evolving Role of Immunohistochemistry (IHC)

Announcer:
Welcome to ReachMD. This activity, titled “HER2 Testing: The Evolving Role of Immunohistochemistry (IHC)” is brought to you by COR2ED and is supported by an Independent Educational Grant from AstraZeneca.

Chapter 1

Prof. Fernando Lopez-Rios:
Hello, everyone. I am really happy to be here today with Christian Rolfo and Charlie Gourley to talk about the challenges related to HER2 immunohistochemistry. So thank you very much for the invitation.

So a challenge is really a call to prove something. So in the next few minutes, I will try to improve the perspective that we have of HER2 immunohistochemistry when we try to use immunohistochemistry in a pan-tumor fashion, particularly for patients with lung cancer and ovarian cancer.

Because I don't know about you, but I'm a bit confused sometimes, so that's why I thought that it would be nice to start with a polling question. There's no right or wrong here; I'm just trying to understand what you think about this topic so we can have an interesting discussion afterwards. So when would you order HER2 immunohistochemistry as a predictive biomarker in this new pan-tumor perspective? At diagnosis simultaneously to diagnostic immunohistochemistry; after discussion at the clinical or molecular tumor board; or after I receive the NGS report; or maybe you leave this decision to the pathologist. So please vote. Let's see what you think.

We are currently trying to implement this at a diagnosis. So I'm happy to see that 50% of the audience are doing exactly that. But again, there's no correct answer. So I think we’ll have time to talk about this later.

So I'm going to start by looking at the challenge, and then I will talk about the workflow for the pre-analytics and analytical phase, and also the interpretation of HER2 immunohistochemistry.

So I used to say that this is a HER2 revolution, but it's more of an evolution from the typical idea of testing patients with breast cancer and gastric cancer. As you can see there on the slide with those dark blue dots, we can find HER2 overexpression in patients with ovarian cancer and lung cancer as well, but also in many other tumor types. For example, we are finding quite a few patients with HER2 overexpression that have cholangiocarcinomas, bladder carcinomas, or endometrial carcinomas. So we have to get used to this idea of using HER2 immunohistochemistry across many different tumor types.

The tool that we're going to be using is HER2 immunohistochemistry. So if you are curious, and you want to scan that QR code, it will take you to a whole slide image of a lung adenocarcinoma stain with HER2 IHC, particularly with the 4B5 clone. And as you can see there, the whole tumor has an intense membrane staining that is very easy to score. So this would qualify as a 3+ case.

So let's try to understand the workflow. So when trying to organize a HER2 IHC workflow, I think those are the two questions that we need to try to answer. First, how can I implement a high-quality HER2 IHC assay? And the second question would be, how long can my patients wait for the results? That's why I brought up these goals low, which essentially is just to try to keep it simple, because if you work in the lab, you know that many times going from the requisition form to the result looks more like that tangled line at the bottom of the slide.

So in terms of where to position this new perspective, this is the vision that we had for cancer biomarker testing. It was initially for patients with lung carcinomas, but now I think it can be easily adapted for other tumor types as well. And the idea is to organize and run predictive immunohistochemistry at the same time that you organize your diagnostic immunohistochemistry. And then you're ready to

send the paraffin block to the molecular lab for comprehensive genomic profiling.

In terms of the preanalytical phase, I think we all know these. There are many factors that can influence the results. My main comment here is probably that we have to involve clinicians and technicians. It's very important that they help in trying to make sure that all these parameters are within the expected range. So we now know that we need to fix our samples quickly in buffered formalin, avoiding decalcification. Going back to this idea of involving technicians, we also need to understand that all the tissue processor variables are very important, and it's probably a good idea to document all these to improve over time, or in case something goes wrong.

Also, I think it's best practice to select the best available paraffin block. We are currently trying to record the best and worst paraffin blocks for predictive biomarker testing in all of our path reports, and that saves us a lot of time, a lot of troubles along the way.

And finally, in the preanalytical phase, I think it's good to use as little diagnostic immunohistochemistry as possible. I know this is sometimes not very popular among pathologists, but the whole idea is to try to present the sample for further testing. This is something that we drafted for lung carcinomas, but I think the same holds true for other tumor types as well.

In the analytical phase, I have to admit that there is a bit of confusion, because different assays were used in the different clinical trials, as you can see there, so probably it's just best to follow the manufacturer's protocols. If we're looking at the pan-tumor indication, the scoring algorithm that was selected, it's the same scoring algorithm that we were using in gastric carcinoma in the past, so we need to identify patients at the 3+ intensity with that 10% cutoff.

And I was also happy too when I reviewed the recently released Principles of Analytical Validation of IHC Assays that was published by the College of American Pathologists, because there is some flexibility in the way we need to validate these multiple assay scoring systems within different tumor types. So essentially, as I said before, we need to score only definitive linear membrane staining, looking at those patients that have 3+ intensity at that 10% cutoff. I think the cutoff is not difficult, but the intensity sometimes it's less reproducible. So I think it's a good idea to revisit this magnification rule that was proposed a number of years ago in gastric carcinoma patients when scoring HER2 immunohistochemistry. So essentially, the idea is that HER2 scores correlate with the size of the precipitates, and that's their link to the magnification that you need to look at those precipitates. So if you need a 5x magnification, then that's probably a 3+ case. If you need a 40x that's probably a 1+ case.

We are also starting to implement artificial intelligence algorithm. This is still a work in progress because most of these algorithms have been used in the breast carcinoma space, but I think they are going to be very important if we try to evolve in this pan-tumor HER2 IHC scoring.

And finally, in interpretation, I'm really fond of the use of this checklist. So we have a checklist for ovarian carcinomas from the College of American Pathologists. So essentially, try to include as much raw data as possible. There's also a general checklist for predictive immunohistochemistry. I think it's the same concept: it's probably not a good idea to only say this is a 1+, 2+, or 3+, but also include the percentage of cells with this membrane staining. And finally, I was also relieved to see that you should also include if you have been using artificial intelligence for your interpretation.

If you want to read more about this topic, those two papers from the College of American Pathologists addressing the preanalytics and the analytical validation are a good place to start. And that *Nature* review paper at the bottom looking at HER2-targeted therapies beyond breast cancer is also a very good manuscript if you want to understand how the field is evolving from breast carcinoma HER2-targeted therapies.

Chapter 2

So I think now it's time for Christian Rolfo's presentation. So let's look at HER2 from the lung carcinoma point of view. So thank you, Christian, whenever you're ready.

Prof. Christian Rolfo:
Thank you. Thank you, Fernando. And we will discuss specifically in lung cancer this part on the on the topics of testing. But starting from the families, a big family will have actually some of the alterations that are there in the extracellular domain, and some of the alterations that we can target in the intracellular domain. So if we are looking, for example, in the ADCs that incorporate the HER2-targeted actions in the trastuzumab with this extracellular compounds, we have two different compounds there: the T-DX and the T-DM1. And we also have intracellular in case of a small molecule, tyrosine kinase, that you have there, a bunch of drugs that were more in the phosphorylation of the tyrosine kinase residuals. We will see the difference of these two different specific alterations on T-DM1 and T-DX. But if we are looking the mutations in lung cancer, some of the ones that are, for example, in the extracellular domain, is the 5.1% and this the S310F, and we have the most common ones in the intracellular domains that are around 33.9%, this is the most common one, 5.7% is the other one, and 3.4 is all in the exon 20, these mutations, and we are targeting these specific alterations with

the new drugs.

The recommendation of NCC guidelines and ESMO guidelines is to do molecular profiling, also including the HER2 mutation testing. And that is because we have the approval of these drugs that we will discuss. Here, if you are looking at the alterations in terms of the amplification, we can go for, we say NGS or PCR for mutations. But if you are looking for amplifications or overexpression, we can also use technologies that are readily available in our labs, which could be the FISH or the immunohistochemistry, and that's what we are discussing today.

So if we are looking, for example, at exon 20 insertion in HER2, we can go from digital PCR to next-generation sequencing. We prefer, obviously, next-generation sequencing, because we will have an important number of alterations or co-mutations that could interfere, like we know is happening in other targets in lung cancer. So having this co-mutation information is very important. In terms of the FISH, it's very depending on the pathologist that is doing this. So we need to have a pathologist that has a big volume and an expertise on that. And then the immunohistochemistry—we have differences in the immunohistochemistry also, and I will go in depth on that, because there are differences also in the antibodies that we are using.

This is the typical FISH. And then you see here on lung cancer in hematoxylin how we can extrapolate later on when we are doing the FISH, the areas that are positive for the HER2 amplification.

In the area of the immunohistochemistry, we have this overexpression. Using the immunohistochemistry, we can have different scenarios from negative to full positive or the HER2 3+. And you see that there are variabilities, but there is not actually a definition for overexpression in lung cancer. There is not a consensus like we have in breast cancer. The one that you are seeing here is an algorithm from breast cancer, and we would love to have the same for lung cancer. If we are looking, for example, at the immunohistochemistry scoring that we are using in breast cancer compared with the gastric cancer, there are also differences in the interpretation guidelines. So these differences are important because when we are going to see—and this is a slide that is coming from Dr. Bruna Pellini—that they did an analysis in an important number of patients looking for in the difference between the guidelines in breast cancer and gastroesophageal cancer. And you see that around 13% of the patients were having differences of the scoring using the different classifications. And this is a case that is coming from her upcoming publication, where you will see here that in an adrenal gland—this is a case of adrenal gland metastatic lung cancer—there are differences in the scoring when we're using the gas, first of a gastroesophageal classification or interpretation, and the other with the guidelines for breast cancer.

So we need to have here a better understanding what is happening. And for that reason, having a companion diagnosis that was mentioned before is very important, because we can have an opportunity to distinguish the specific characteristics of lung cancer.

These are xenograft models where there are also some differences in the staining when we're using lung cancer. This is in patients specifically in this study of xenograft model was using for KRAS-positive patients. But there are also differences using this when we are using the guidelines for gastric cancer and breast cancer.

So if you are looking for the treatments specifically for each alterations, I would like to start before with a polling question, which of the statement of about HER2 alterations in lung cancer is correct? Only HER2 mutations are relevant for lung cancer; only overexpression is relevant in this scenario; the HER2 mutations in lung cancer are most commonly found in the squamous cell carcinoma; or HER2-targeted therapies have shown clinical activity in patients with HER2 mutant and HER2 immunohistochemistry 3+ in lung cancer. So please vote. And we see that the majority of the people was doing the correct one, that is the D, and we have one of the answers that was for all limitations are relevant, but we will show that overexpression is also relevant with the study.

So the HER2-positive lung cancer with HER2 is important too, and that is something that Fernando was discussing before. In contrary, with breast cancer, the HER2 overexpression often doesn't occur like in breast cancer, with the amplification, the co-occurrence is less consistent in lung cancer, and also the mutations are less associated with the increased level of amplification. So this is important, because when we approach the HER2, we need to consider that we are not treating breast cancer, we are treating lung cancer, and seems to be a lot of difference there.

And also there are differences in the drug between the T-DX and the T-DM1 from the payload that we have in these drugs are different. The drug antibody ratio also, and the tumor selected cleavable linker, and also the evidence of the bystander anti-tumor effect. So we see that there are more opportunities in terms of the capacity of the drug, with this new generation, and this is the trastuzumab deruxtecan compared with trastuzumab/bendamustine. That is the first-generation ADCs.

So this is the DESTINY-Lung01, which is for HER2 mutant. In the trial design, you see that in the design there are two cohorts. The Cohort 1 is the overexpression, and I will go back to that later on, but specifically for HER-mutant patients that were including patients with stage IV or unresectable, non-small cell lung cancer, non-squamous with measurable disease, asymptomatic metastasis, and

ECOG PS 0 to 1, and locally reported HER2 mutant for this cohort, the primary endpoint was overall response rate by investigator committee and different secondary endpoints. We have also an exploratory biomarkers analysis that was tried to confirm the biomarker of response.

And I think here is a very interesting slide. So you have some difference there in the part below of the graphic where it's mutation. So majority of the mutations that was in the kinase domain, but also some of the patients that were responding, and you have there in orange, were also in the extracellular domain of the mutation. As you see there, the protein expression and the gene amplification are completely different. There is not a big correlation between the mutation, the presence of the mutation. So coming back to the question, do we need to do the testing or not? Yeah, it could be interesting, but we don't need it for the treatment specifically.

And some of the patients that were responding also received prior TKI against HER2. The overall response rate was 55% in these 91 patients included. And if we are looking at progression-free survival and overall survival it's also impacting in all 8.2 months and 17.8 months. We need to remember that HER2 mutations are the characteristics of these alterations and also are important. So these alterations are giving not a behavior of the disease a little bit more complex than our normal mutations that we are used like EGFR or ALK. So the responses are also important.

Safety toxicities of these drugs also include interstitial lung disease which was fatal, unfortunately, in two cases. And you can go for two, because what we are showing here is the second part of this trial is the overexpression. So you have here in the first cohort of the patients that they were, including patients with immunohistochemistry 3+ or 2+. The primary endpoint in this cohort was overall response rate. And the overall response rate in the two different cohort was Cohort 1 and 1A, amd the difference here was 6.4 mg/kg compared with 5.4 mg/kg of trastuzumab deruxtecan. And the overall response rate were 26 and 34. But if we are looking specifically in the HER2 immunohistochemistry, the 3+ component of the population was 52.9% compared with 20.8% in terms of overall response rate. The results for progression-free survival, 5.8 months, with the drug was at 6.4 versus 6.7 months, with the drug was 5.4 mg/kg.

And in this trial, that is also this is the phase 2, but in this case, we are looking also for the same dose in patients who have HER2 mutations, and this is a good talk that we have in the analysis of 3. And in these patients, you see here, in patients who are demonstrating an overall response rate of 50% and 56%, and in second line HER2 mutated, as you see, their progression-free survival in the 6.4 mg was 12.9 months, and the overall survival of the same cohort was 17.3 months. So this is an important activity of the drug.

And side effects, as I say, are important. The importance of interstitial lung disease, we need to remember that here, is that when we are talking about ADC, the ILD is completely different on the immunotherapy that we are accustomed to. So it's important that we have in our institution a good radiologist that is involved in this discovery of this initial interstitial lung disease, because seems to be a little bit more difficult to treat than the ones that we have with immunotherapy. There are also some other toxicity like diarrhea, decrease of appetite, leukopenia was also a grade 3 in some cases now, some fatigue, and there were some differences, minimal, in grade 3 in the two doses, 5.4 and 6.4 mg/kg.

This is the phase 1 multicenter, open-label, and dose escalation in the case of the HER2 immunohistochemistry; that is for the patients with HER2 3+ and 2+. And the part that we are analyzing here is the part 1 that is T-DXd, the trastuzumab deruxtecan monotherapy. And that was the enrolled complete 36 patients, and they received 5.4 mg/kg intravenous every 3 weeks. Overall response rate was the K endpoints, together with duration of response, disease control rate, progression-free survival, and overall survival. And that were investigator assessment. This is in April 2024, which was the cutoff. And what we see here, overall response rate is important, 44.4%. And you see here that there are some important response rates in patients with HER2 immunohistochemistry plus, 2+, or 3+. And also the duration of response was 11 months. And also difference in the overexpression of 2 or 3+, and also the PD-L1 status. That is important also to remember that some patients will have this as a certification in order that we know which kind of treatment we need to go but also to see if there is any correlation here. And some of the patients have PD-L1 less than 1% even more than 50%.

In this, if we are looking in total, the overall response rate by expression, by overexpression, in total, the overall population was 44.4%. But you see here that immunohistochemistry 3+ is an increase in the overall response, 56.3% compared with 35% in the immunohistochemistry 2+. And also patients who received prior EGFR TKI were having an overall response rate higher than the EGFR TKI-naïve. Duration of response, also difference between 81.3 in the median duration of response, that is disease control rates, or duration of response 12.5 months, compared with 6.6 months. So very important activity in the 3+.

And there is also an exploratory pooled brain metastasis analysis that is very important, because several of these patients will have brain metastasis. In this analysis, in this pooled analysis, we combined the data of Lung01 and Lung02, and you see here the endpoints were in patients with and without brain metastasis, systemic, and also systemic overall response rate, systemic duration of response. And in patients with measurable disease, it was looking for similar intracranial activity. It's a small number, obviously, because if we took together this population is in the 5.4 is only 14 patients, and in the pooled of 6.4 is 27 patients. But there are an important number

of response, so 50% of intracranial overall response in the 5.4 and 30% in the pooled analysis of 6.4 with an intracranial duration of disease control of 92.9 versus 73.3, and the intracranial duration of response was not reached, and the median was 9.5, and 4.4 in the pooled analysis of the 6.4. So it seems to be an important activity. Obviously, we will need to have here better data and specific trials and see what happened in this population where we have untreated, and also in serious areas like in leptomeningeal disease where we would like to have this data as well.

But with all this data, the approval of the upcoming trial, so we have the approvals in August 2022, the FDA approved for trastuzumab deruxtecan for unresectable or metastatic, non-small cell lung cancer with HER2 mutations, and EMA was approved in September 2023 also in these HER2 mutations ERBB2 alterations.

And the DESTINY-Lung04 is the one that we are awaiting because it's first-line advanced non-small cell lung cancer with the exon-19 or exon-20 insertions and mutations. And that is a big trial, including 450 patients, and randomization will be trastuzumab deruxtecan versus the standard of care that is pembrolizumab, pemetrexed, and platinum. And the primary endpoint of this trial will be progression-free survival.

So in conclusion, we can say that the HER2 alterations are different compared with other cancer, in non-small cell lung cancer. There is a variability on the scoring, and this is important to have a standardization. Maybe we will need to go for a companion diagnostic for this specific alteration. There is a challenge also for the target in the area of the driver of non-small cell lung cancer with therapeutic options for our patients. But it is also important to understand the difference in the alterations requiring diagnostic techniques available in our community and different predictive effect. And we need to know very well about the safety profiles, and specifically ILD could be in some of these patients, an important side effect, so we need to be able to have an opportunity to treat the patients in time and have enough flow in our institution to detect these kind of alterations.

Chapter 3

Prof. Fernando Lopez-Rios:
It is my pleasure to introduce Charlie Gourley, who is going to give us the ovarian cancer perspective. So whenever you're ready. Thank you very much.

Prof. Charlie Gourley:
So the key challenges in terms of HER2 testing in ovarian cancer, in terms of the lack of consistency around the HER2 storing systems, the staining issues, and inadequate consideration of histological subtype to date—well, certainly the first two of those have been dealt with by Fernando. He's talked about difficulties around about lack of consistency around HER2 scoring and so did Christian with regards to lung cancer, and the same thing applies in ovarian cancer. There hasn't really been much consideration of histological subtype in a lot of the analyses done. I'm going to show you some slides that look at it to some extent.

But what I'm going to focus on is some of the other challenges in terms of getting this delivered in our hospitals and through our multidisciplinary teams. One of the key issues is the lack of reimbursement for downstream therapies. So, you know, certainly within the UK, we sometimes have some doubts in our routine pathology lab about the medicine testing. Do we have to make that case? There's also issues about intratumoral heterogeneity of HER2 expression which are underexplored, and also an inadequate understanding the stability of the HER2 expression throughout the patient journey. So I'm going to look at some of these, and I'm going to talk to you a bit about the trials have been done so far in ovarian cancer. They aren't as mature as the trials that have been done in lung cancer. And I'm also going to present a case to you.

So let's talk about the reimbursement. So in the US, ovarian cancer patients can get hold of antibody drug conjugates—they are targeting HER2, trastuzumab deruxtecan through the agnostic and site agnostic approval. There is no such site agnostic approval in Europe, but certainly in the US, it would be possible to access through that FDA approval on a site agnostic basis.

So in terms of the studies that have been done, I'm going to refer first of all to the DESTINY-PanTumor02 trial of trastuzumab deruxtecan. And the patients in this study had 2+ or 3+ of HER2 expression by IHC. These patients were required to have had at least one prior therapy. Actually, as it turned out, most of them were very heavily pretreated. The most frequent group were patients who did five prior lines of therapy. And really across gynecological cancer, excellent response rates were seen, given that this is quite a kind of resistant patient group. Across the cohort and ovarian cancer, the response rate was 45%, with a 64% response rate if the tumor was IHC 3+, and a 37% response rate if it's IHC 2+. And this difference in response rate was also reflected in a slight difference in progression-free survival, with the 3+ patients having a median progression-free survival of 12.5 months, which really is very good in this patient group. So that's quite encouraging.

When you look at some of the other plots—ere's the waterfall plot—again, this is what we like to see. And this is the swimmer plot with a

number of the individuals have censored or having had a complete response. So some patients clearly are doing very well.

So that's not the only study. This is a study using a different antibody drug conjugate. It still had trastuzumab, and it was still conjugated to a topoisomerase-I inhibitor. And this was an early phase study that looked at a number of dose levels in a number of different cancers, but then in the variant endometrial and cervical cancer, it expanded out three of those dose levels. The patients were required to have 1+, 2+, or 3+ of HER2 alteration, and over 65% of them only had 1+. Of course, in these early phase studies were very interested in toxicity. And determining the toxicity from this agent—it's quite similar to chemotherapy, really.

I'll get you to look, first of all, to the figure on the right-hand side, and this shows in the red, the orange are the grade 1 and 2 toxicities, and in the blue are the grade 3 or higher toxicities. As you can see, there weren't a huge number of grade 3 or higher toxicities, although in total, they did occur in about 23% of patients. But there's a fairly high instance of grade 1 or 2 anemia, nausea, and other forms of myelosuppression, which is what we expect with chemotherapy. Thankfully, there was a low instance of interstitial lung disease, which was obviously really important, because that's one of our main fears with these drugs.

And then in terms of the response to these drugs, as you can see on the waterfall plot, there was a high response rate, and really a lot of patients seeing at least some shrinkage in their tumors. And in fact, in the top dose group, so these were the patients with brain cancer, 12 mg/kg dose, and the confirmed overall response rate was 53% with a disease control rate of 90% which is very good. And then this is the median progression-free survival, which is 6.8 months, which again, in this patient group, is really exceptionally good. So the HER2-targeted therapies, they appear safe, they're effective, and it seems very likely that they will be soon with us in HER2-expressing ovarian cancer, although we don't have specific approvals as yet.

So let's talk a bit about intratumoral heterogeneity of HER2 expression. And so this a study, this isn't in high-grade serous ovarian cancer, it's actually in serous endometrial cancer, but biologically, it has a lot of similarity to high-grade serous ovarian cancer. And in this study, they took paired endometrial curettings or biopsies with hysterectomy samples, and they ended up with 40 pairs. And when they did immunohistochemistry, they found that in 65% of cases, the curetting or the biopsy was identical to hysterectomy, but there was discordance in 35% of cases. So this suggests some heterogeneity from sample to sample. When they added in FISH, then the concordance for HER2 status was much better.

And if we turn now to what happens to the HER2 expression through the patient journey, this is quite an important thing in ovarian cancer, because these agents are going to be primarily used initially in relapse disease, but most of the samples that we look at will be from really any treatment. You know, there'll be the primary resected specimen or the initial biopsies taken before neoadjuvant chemotherapy. So a lot of the patients will get a lot of treatment between the sample you use to say that they're HER2-positive and the therapy that you subsequently give because of that.

So this was a study that looked at HER2 immunohistochemistry in 200 individuals with ovarian cancer, and it used the Ventana platform and a DAKO antibody. And found that 28% of the patients had 2+ using this scoring system outlined here, and 6% had 3+.

What was good about this study was they looked according to the histological subtype, and that's actually critical, because, as you probably know, ovarian cancer has at least five separate subtypes, which really need to be treated as five different diseases. And in recent ovarian cancer, 23% of patients had 3+ IHC per HER2, endometrioid development plus clear cell had 9+, and actually high-grade serous, the most common, only had 5% that were 3+.

Now, 90 of these patients underwent multiple biopsies, and of those, 11 showed increased HER2 expression in later biopsies. So that's quite interesting. And this figure here shows the change. And so there were three patients whose HER2 status decreased, a number of patients in whom it increased, and two patients in who stayed the same. So, I guess the fact that it included all histologies is good because we get an idea about the expression in the different histologies. But then when it comes to look at what happens, and you're looking at these numbers, you probably need to look in the individual subtypes separately.

And then what about if you give neoadjuvant chemotherapy? Well, you know, I'm not aware of any ovarian cancer studies that have looked at this. And this shows how far behind we are in this. But I turn to some of the breast cancer studies, because obviously neoadjuvant chemotherapy is often given in breast cancer. And in the studies that did immunohistochemistry before and after the neoadjuvant chemotherapy, it seemed that there was quite often a change in the HER2 staining status, with between 1 and 16% going from positive to negative, and between 4 and 20% going from negative to positive. It seemed that this changing of status was less if you used FISH.

And of course, there are a number of potential reasons why the HER2 expression could change. Biological reasons in terms of increased protein internalization, increased protein degradation, maybe loss in what's happening genomically through selection for particular clones. But basically, this needs to be repeated in ovarian cancer.

Alright. So I'm going to turn now to a patient case that we treated in Edinburgh. So this patient was actually diagnosed in a different country in the UK, but ended up coming to us. So it was a 47-year-old patient who presented with tightness around her bra and a change in her breathing, and she was found to have a right pleural effusion and a CA-125 of 496. She had pleural drains, and cytology actually showed no malignant cells. So she had a CT scan that showed, as well to right pleural effusion, left complex ovarian mass with ascites, peritoneal enhancements, and omental stranding. This is the big complex mass here, and there's the pleural effusion. So the big red line on the bottom of the CT scan shows the pelvic mass. Anyway, the pathology revealed a grade 1 stage 1c/II mucinous carcinoma of the left ovary, and it had the expansile pattern of invasion, which is important.

So we've got a polling question here. So what other molecular tests would you routinely request in this situation? So it's interesting, obviously, that in other forms of ovarian cancer, we tend to do the nine gene germ-line test, and we do homologous recombination deficiency testing. But actually, you know, there's no routine testing indicated at this time, although what I often ask my pathologist for is KRAS decreasing in HER2 immunohistochemistry. So actually, it's really C and D, is what I would sort of say. But then yeah, and not exactly straightforward. But because it's mucinous, most of the guidelines say you should do, you know, BRCA testing and HRD testing in non-mucinous ovarian cancer, but because this is mucinous, it's a bit different.

And I just want to clarify the point on expansile mucinous ovarian cancer, because the risk of relapse in that form of mucinous ovarian cancer is low. The other form is called infiltrative mucinous ovarian cancer, and that has a much worse outcome, both in terms of recurrence-free survival and overall survival. And indeed, the expansion invasion type has a very good outcome.

So what therapy would be recommended in this situation? I know we have a lot of pathologists, so just have a go at this if you know, just to see what you think. Yeah, so it's really any of the above is the right answer. And obviously most ovarian cancer, we give carboplatin and paclitaxel, but because this is the Ic/II, and expansile ovarian cancer, you might not necessarily give anything, and you could just follow it up. And some centers give more gastrointestinal type regimes rather than gynecological regimes. So they would give capecitabine and oxaliplatin.

And these are the ESMO/ASCO guidelines. And as you can see, so this patient had expansile grade 1 to 2, so the adjuvant chemotherapy would be optional, although, you know, having had the pleural effusion, even though the cytology was negative, might make me a bit anxious, and maybe might push me more to giving adjuvant chemotherapy.

The center that she was treated decided not to give adjuvant chemotherapy, and she was regularly followed up. Her CA-125 level started to rise 2 years after diagnosis. CT scan this time showed no recurrent disease. And then she moved to Edinburgh, and her care was transferred to us. And pretty much immediately, we found that her CA-125 had gone up even further, and that the CT scan showed relapse disease with some nodules adjacent to the cecum on the bottom image of the CT scan, shown by the green arrows, and also some nodularity and thickening of her right pleura. So we set her up for video-assisted thoracoscopic biopsy. Suspicious nodules were seen throughout the parietal pleura, the talc pleurodesis performed, and the pathology showed metastatic mucinous ovarian adenocarcinoma.

So which of these molecular abnormalities is not associated with mucinous ovarian cancer? Is it BRCA1? Is it p53? Is it KRAS mutations? Or is it HER2 amplification? Yeah. So it's actually BRCA mutations that are not associated with mucinous ovarian cancer because number of p53 mutations, a decent number of KRAS mutations, and HER2 amplification also occurs.

Okay, so what did we do in this case? So we did a talc pleurodesis. We did KRAS testing, which revealed no activating mutations. We did HER2 staining which suggested overexpression, and there's the image there. And we decided to give her capecitabine and oxaliplatin chemotherapy. So this is the more GI type regime. After three cycles, her CT scan showed stable disease, but her CA-125 was falling, and that's shown on the right-hand side here. Unfortunately, she developed chemotherapy-induced angina or coronary artery spasm, so we stopped giving the chemo at that point. And unfortunately, just 2 months later, it's a progression of her right-sided pleural disease.

So we decided to enter her onto a study that we were doing at the time, which was a platform study for rare ovarian cancers, including mucinous ovarian cancer. And in this study, which was called BOUQUET, patients had Foundation Medicine testing, and on the basis of what came through the Foundation Medicine testing, they were then entered onto the appropriate arm of the study. And as you might expect, because she had such strong HER2 expression in the tumor, she was found to have HER2 amplification. And so she drew the cohort, and she entered the cohort that was treated with trastuzumab emtansine, which is an antibody drug conjugate.

She actually received 17 cycles of three weekly trastuzumab and did very well. Her only toxicity was grade 1 nausea. Her ejection factor was satisfactory throughout, and she actually had a partial response in her CT scan with normalization of her tumor markers. So this seemed great. Unfortunately, shortly after the scan that suggested a partial response, she presented with acute right-sided weakness and was found to have multiple bilateral cerebral and cerebellar metastases. Unfortunately, in both scans, you can see the

metastases. So we ended up treating her with whole-brain radiotherapy, and she returned to her home country for end-of-life care, unfortunately. But it seemed that she had derived some benefit below the neck, certainly, from this therapy.

So in conclusion, there is no question that HER2-targeted therapies for ovarian cancer are on the horizon. They produce response rates that we cannot see with standard cytotoxic therapy. So along with other antibody drug conjugates, these things are definitely coming along, which is great. And initially they'll be used in relapse disease in the palliative setting. But there's also a high chance that they'll be moved earlier in the patient journey, in particular for certain ovarian cancer histological subtypes that are not particularly responsive to conventional chemotherapy and also specific molecular subgroups. And so uncertainty remains regarding whether sensitivity to these agents will differ between the histological subtypes for a given level of HER2 expression, because it seems that for some antibody drug conjugates, the level of HER2 expression doesn't seem to matter, and for others, it matters a lot. So this still seems to need to be worked out. And of course, as we've said, uncertainty remains regarding the intratumoral heterogeneity of HER2 expression, how that expression changes during the patient journey, and the extent to which this is impacted by previous therapy. So thanks very much.

Chapter 4

Prof. Fernando Lopez-Rios:
In the last few minutes, I wanted to provide you with some future perspective and some key clinical takeaways. The future, I think, we would all probably agree that we need comprehensive genomic profiling and HER2 immunohistochemistry for all patients with lung and ovarian carcinomas. That would be, I think, the perfect situation. And in order to do that, probably the best possibility is to try to integrate high-quality next-generation sequencing ultra-fast if possible, now with robotics, and maybe also start using those AI algorithms for virtual scoring, or, as we discussed also, even the question that we received in the chat, the possibility of trying to guess the alteration directly from the hematoxylin and eosin slide.

So we might be looking at a new member of the clinical or molecular tumor board, which is the AI algorithm. So I think this is still work in progress, but probably my personal opinion is that we're going to see more of those algorithm with clinical use in the very near future.

So the clinical takeaways are those shown in the slide. We need to make sure that we standardize HER2 immunohistochemistry for lung and ovarian carcinoma patients. So in order to have a high-quality HER2 immunohistochemistry result, we need to be more engaged in the preanalytical processes, because there are no in situ positive controls in lung carcinomas or ovarian carcinomas. So we need to involve everyone in the team, from clinicians all the way to the technicians. We need to also make sure that we are using validated assays and I think there is some flexibility here on how to validate these new scoring systems and cutoffs. And it's probably, I think, best practice if we try to report as much raw data as possible using checklists and remember that the cutoff that we need to make sure that we don't miss is that 10% 3+, because those patients are the ones that are going to be considered for treatment. In lung carcinoma, the HER2 alterations include not only overexpression, but also amplification and mutations. And in the case of ovarian carcinoma patients, like we've heard, it is probably a good idea to start testing earlier, and particularly in that mucinous subtype, because of the higher likelihood of finding a 3+ positive patient. And everything we are doing in this space is because we now have HER2-targeted therapies, including antibody drug conjugates in the space. So these are new therapeutic opportunities for our patients with lung cancer or ovarian cancer.

So thank you very much for attending and for your input during this session. Thank you very much everyone. Bye-bye.

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