Video Podcast Transcript

Pre-analytical Phase Challenges and Biomarker Testing in HER2+ Metastatic Breast Cancer

Brought to you by:
Dr Carlos Barrios, Hospital São Lucas-PUCRS, Brazil.
Prof. Gary Tse, Chinese University of Hong Kong, Hong Kong.
Moderated by the Oncology Brothers (Dr Rahul Gosain and Dr Rohit Gosain).

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This video podcast is an initiative of COR2ED and developed PRESCISION ONCOLOGY and BREST CANCER CONNECT, a group of international experts working in the field of precision oncology and breast cancer. The podcast is supported by an independent educational grant from Daiichi Sankyo.

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Dr Rohit Gosain
Hello and welcome back to the Oncology Brothers Podcast. I am Rohit Gosain, a practicing Community Medical Oncologist, here with my brother and co-host, Rahul Gosain, another Community Medical Oncologist. Well, with our mission of Oncology Brothers, it has truly been to bridge the gap between academic research and community practice. And today, the topic at hand we'll be tackling is HER2 testing in the breast cancer space. What used to be a rather binary classification that is positive or negative has rather evolved into something more profound. That is what we are using HER2 status being reported as positive or negative, now we are also reporting that as positive or low or even ultralow.

Dr Rahul Gosain
It is important to appreciate the shift and these nuances in testing because HER2 has now become not just a classic oncogenic driver, but also a target for antibody drug conjugates such as trastuzumab deruxtecan. To help us unpack all this, we are joined by two excellent guests. Professor Gary Tse, a Pathologist from Chinese University of Hong Kong, and Dr Carlos Barrios, a Medical Oncologist from Brazil. Gary, Carlos, welcome. Thank you. Thank you.

Dr Rohit Gosain
Before we talk about the testing itself, Carlos, could you start us off here? Why biomarker testing is rather important, particularly for HER2? So, how does this play in metastatic breast cancer space today?

Dr Carlos Barrios
I think that we need to recognise that over the last 20, 25 years, oncology has changed significantly. We have come to recognise that the biology of the disease is much more complex than we usually recognise before. It is obviously heterogeneous, very complex. Besides the complexity of a single tumour, it is very important also to recognise at the same time that disease changes as it evolves. A number of different new biomarkers have emerged. *PI3-kinase* mutations, *ESR1* mutations, *BRCA*, *PD-L1*. Besides the very important HER2 modifications that you have mentioned in the introduction. So, essentially the importance of these biomarkers is that they do have a significant impact in the way we manage patients and we select treatment.

Dr Rahul Gosain
Thank you so much for getting us started. Coming back to that clinical implication we do need to appreciate that HER2 status as we have treatment options based on this. Gary, with that background, can we start off with some basics? How do we first define HER2 in our clinical practice today?

Prof. Gary Tse
HER2 or human epidermal growth factor receptor 2 is expressed in 15 to 20% of breast cancers and they are very aggressive, but they respond well to targeted therapies. So it is crucial to test for HER2 expression in breast cancer. We can look for the gene amplification by doing in situ hybridization. This is very accurate, but it is more expensive and takes longer. In a clinical setting it is not very easy or quick to do for a lot of patients.
The other way to do is to look at HER2 protein expression by immunohistochemistry. So, by looking at this, we score the tumour cells expression from 0 to 3+. 0 is no staining or very, very weak staining. 1+ is weak, 2+ is moderate and 3+ is strong. Only 3+ staining correlates with gene amplification and thus considered positive. The rest of the cases are considered HER2 negative, even though they do have some staining. So this includes score 1+ or 2+, and now they are called HER-low. And for cases that are scored 0, but they show very, very weak staining, we call this HER-ultralow.
Previously, there were only drugs that were effective on HER2 positive cancers. Recent development of powerful new drugs, for example HER2 antibody drug conjugates, has shown efficacies in tumours that are HER-low or

even ultralow. So, this is very crucial for us to be able to accurately evaluate HER2 for allowing optimal patient treatment options. However, there are a lot of practical challenges in HER2 evaluation, particularly at this low end of the spectrum. Heterogeneity, as Carlos just alluded to in HER2 expressions, either within the tumour itself or between multifocal or different tumour foci in a metastatic setting is a real issue and that happens very frequently. So, this is very significant and it adds a lot of problems for us in testing HER2 for our patients.

Dr Rahul Gosain
Carlos, given this testing with the HER-low, ultralow in a hormone receptor positive, this is essentially making 85% of our patients. In terms of our available therapies, you have been involved closely in these clinical trials. Can you briefly touch on what we have learned from DESTINY-Breast06? Because that is what today is approved.

Dr Carlos Barrios
As an introduction to that question. And thank you for that. I think for the community oncologists, it is extremely important to recognise that traditional anti HER2 therapy works for HER2 3+, the HER2 positive disease. And the major change in HER2 evaluation of this biomarker evaluation that we have seen is that instead of a positive to a negative result for the test, we have a continuous expression, which changes significantly the way we need to approach the results. Okay?
Because some of the new therapies, as you mentioned, for example in the DB-06 (DESTINY-Breast06) trial, also work in patients that are not HER2 3+. They also work in 1+, 2+ and in the ultralow, as Gary mentioned. So, this concept of moving from a binary result, positive or negative, is extremely important for the community oncology to learn that we are changing. There is therapy that works also in patients that have lower expressions of HER2.

Dr Rohit Gosain
Thanks so much for covering that, Carlos. But where we stand today is, which is again exciting times, where we have TDXd now approved for that low and ultralow metastatic hormone receptor positive disease.
But coming back to the testing and some challenges that we tackle here, we run into this in our clinical practice often. Gary, you talked a bit about the heterogeneity, but where do you see most commonly some of the pre-analytical challenges that arise in the metastatic breast cancer space?

Prof. Gary Tse
Well, there are multiple issues in this setting. The first problem is metastatic diseases Biopsies are always taken from distant sites, multiple sites, bone, liver, lymph nodes, and many of these are very difficult to assess, unlike the primary tumour which is much easier to assess. Therefore, only limited materials can only be found or can be taken from the patient. So, this limits the materials that we were able to perform our tests.
Secondly, very often at this juncture our oncologists would like to do, as Carlos has mentioned, not only HER2, but a whole bunch of other tests that would be targetable by different types of therapies. So, for example PD-L1, PIK3CA mutations, ESR1, etc. So every test will be fighting for specimens for these tests to be performed. So,

we need to have a very clear mind and clear pathway to how allocate our precious sample for prioritising the test to be done.
Thirdly, very often in ER+ cancers, when they metastasise or recur many years afterwards, these metastatic sites very commonly are in the bone. And we know biopsying bone samples are very difficult because bone is very hard and it is not amenable to normal histological processing. We need to decalcify the bone. So, we would have to use acid or materials or chemicals to dissolve the calcium and with this we may partially or partly destroy the antigens in the tumour cells thus that would also jeopardise the accuracy of the testing for immunohistochemistry.

Dr Rahul Gosain
Can you touch on some clinical pearls or best practices, recommendations to overcome these pre-analytical challenges, especially around specimen selection, fixation, decalcification and handling that tissue in metastatic settings?

Prof. Gary Tse
In clinical practice, of course, we always try to obtain tissue from the most accessible or adequately preserved specimens. Once the specimen is obtained, there are specific things that we need to do or have to observe. The first is to avoid delays in fixation. Very often as soon as we get the specimen, we should fix them so that the protein would not be denatured. And this is called cold ischemic time. And we would like to keep the cold ischemic time as short as possible and certainly less than an hour. This may help to preserve the HER2 antigen so that they can be detected even at low level.
Secondly, the use of fixative and the duration for the fixation. In most pathology labs, the recommended or standard choices are 10% neutral buffered formalin and the recommended fixation time is between 6 to 72 hours and a maximum of 96 hours is recommended. So, ideally HER2 should be assessed within this framework. Thirdly, when we prepare slides for staining, we should use freshly or recently cut slides from a paraffin block. It has been shown that sections that have been cut for more than a few weeks, which shows reduced antigenicity, so the sensitivity will be lower. And our low HER-low would become HER2 negative probably.
And for bone specimens, one of the better way to deal with bone specimens when we have to obtain a metastatic biopsy would be to separate the soft tissue adjacent to the bone from the bone itself. So we can process the soft tissue in a routine manner. Hopefully there is good preservation of the antigenicity. And in a bone specimen we try to use EDTA rather than acid to better preserve the proteins within the tumour cells. Overall pre-analytical errors usually would lead to reduction of the antigen expression and thus it would lead to false negative results rather than false positive results. This is particularly important in HER-low, ultralow setting because when they are already very low, if you lost your sensitivity, then it will become decreased from ultralow to negative. And again that that would change the treatment protocol for our patients.

Dr Rohit Gosain
If you have multiple metastatic disease site, that is larger bone lesion and even lung findings, where you did go for a larger bone lesion because it was rather easily accessible, but you got completely HER2 0 or no expression at

all. In that particular case, do you want to chase for that lung lesion that you may find that less than 10% staining here, where T-DXd might be applicable?

Dr Carlos Barrios
Not necessarily. It is obviously important to review that biopsy to make sure the pathologist actually when they said it was HER2 0 to make sure that they are using the correct assessment. Okay? Because this has changed not only for the clinician, but also for the way the pathologist is evaluating the biopsy. So, it needs an adjustment both from the clinical and from the pathology point of view in order to make a correct diagnosis for a correct treatment selection.

Prof. Gary Tse
I agree with Carlos. I remember years ago the criteria for HER2 positivity has been switched multiple times over the past 10 years, from 10% to 30% to 10%, etc. So I think with the change of the treatment protocol and paradigm, it is always good to review, particularly using the old definition when we diagnose or score the HER2. Carlos, very good point.

Dr Rahul Gosain
And again here, retesting and chasing that right tissue is ideal. Gary, can you touch a little more on the testing aspect? I know we talked about IHC and ISH. What about NGS? What about liquid biopsy? Because now we also potentially have ERBB2 mutation. Can you touch a little on this comparing IHC, ISH and the role of NGS here?

Prof. Gary Tse
ISH and IHC are the routine pillars that we are doing day in, day out in most laboratories of pathology there are emerging tools such as, for example, liquid biopsy or Next-Gen sequencing. NGS is very powerful and is looking at the entire genome of the tumours. So, it is not only looking for HER2 amplifications, but other changes like HER2 mutations or other the genomic changes in a broader context. And it is also more sensitive because it amplifies all the genomes so it can help us to detect more changes and hopefully many of these would be targetable. The other thing that I have just mentioned is liquid biopsy. You are only taking blood from the patient and it is convenient, it is non-invasive, and it also has a very good advantage over the other tissue biopsies because in the patients with multiple sites of tumour metastasis, each individual metastatic focus may have different genetic changes. So, there is no way that we can assess all the individual foci by doing tissue assessment. And rather it is much easier to look for the ctDNA in the blood because that would represent basically an amalgamation of all these tumour metastatic sites. So, we would have a very clear overall picture of the genomic landscape of the metastatic disease the patient had. However, because these tests are very sensitive and very dependent on good sample quality. So this is very important that we would be very, very careful when we take the samples and prevent contamination.

Dr Rohit Gosain
Wel, testing is one thing or one puzzle, but making sense of the reporting is another

aspect. Carlos, how challenging it is to interpret these results in clinical practice, and how do you handle that particular scenario, borderline or equivocal cases?

Dr Carlos Barrios
One of the questions I ask myself whenever I receive a pathology report is, does this pathology report actually speak to the clinical picture this patient is presenting or not? Because from that moment on, everything I do, it is going to be based on that piece of paper. Because this will have an absolutely fundamental influence in whatever happens afterwards. So, I think that these questions from the clinical point of view for a community oncologist are absolutely critical to consider.

Dr Rahul Gosain
Thank you for touching on this, because getting that right diagnosis, communication, all this essentially in the bigger scheme of things, is part of MDT. Gary, today, how can pathologists take a little more proactive role with multidisciplinary settings to ensure that testing, interpretation and communications are optimised for these metastatic disease cases?

Prof. Gary Tse
We play a very important and active role increasingly in MDTs in the setting of patient management. As we all know now, managing oncological patients is a very, very complex multidisciplinary collaboration.
We can highlight sample limitations and potential errors. All tests would have some inherent errors and it is very important to recognise there are things that is unavoidable, elements of uncertainties that it is better to be made known to the management team. We could also bring to our colleagues, the possibility of issues related to HER2 heterogeneity and the subtleties of low level expression. So, this would explain how the report/the results come about and how to interpret the results. And it is probably best for us to be there for equivocal or difficult or results that does not really tie in with the clinical scenarios.

Dr Rohit Gosain
Well, thanks so much for touching on that, Gary. With regards to multidisciplinary approach, Carlos, from your perspective as a treating medical oncologist, how does that multidisciplinary tumour board collaboration look like when you are managing HER2 positive metastatic breast cancer patients across multiple lines of therapy and also repeatedly reassessing the biomarker itself?

Dr Carlos Barrios
Those concepts are extremely important. Multidisciplinary care does not necessarily mean a meeting on a Friday afternoon on the fourth floor where everybody goes. This can be feasible in an institution, a tertiary institution where everybody works at the same place.
Community oncologists in the US, community oncologists in other parts of the world are completely different. Probably the best multidisciplinary approach is a phone call where you are going to discuss this with the pathologist, with the radiation therapist, with the surgeon. It is not necessarily the same thing as a multidisciplinary meeting where everybody gets together in the same room. It does not help to tell a

community oncologist that you need to discuss every single case in a multidisciplinary meeting where everybody is together.
So, answering your question specifically, I think that discussing these things with another colleague, trying to build the best possible recommendation for a single case, identifying the biomarkers or the tumour characteristics that could be important, influencing treatment, selection, prognosis, I think that those are the important aspects. Oncology is becoming so complex that we cannot manage this kind of disease without help and without discussing this with other people.

Dr Rahul Gosain
Carlos, that is very, very, very important again, because the change that we are seeing in the world of cancer is fast, it is complex, MDT is important, but this looks different in every setting.
Before we wrap up, we would love to leave our listeners with some clinical key takeaways. Gary, starting with you, what are your clinical takeaways from today's discussion?

Prof. Gary Tse
From the pathological perspective, accurate assessment of biomarkers is very important and it is increasingly difficult because we are getting closer and closer to the threshold. There are many variables that would affect the outcome or the results of biomarker testing. High-quality pre-analytical handling of all specimens and nuanced interpretation are essential. So, talk to your pathologist.

Dr Rohit Gosain
We could not agree more. Carlos, your clinical key takeaways from the discussion today?

Dr Carlos Barrios
I think that the critical key message is that we need to recognise this complexity, the understanding of the disease, the resistance mechanisms, actually leads to one very important concept. We improve patient outcomes. We need to embrace the the way of discussing this with different subspecialties is not only the pathologist, it is the radiologist, the interventional radiologist, the surgeon, the radiation therapist, et cetera, et cetera. I think that cancer requires obviously a multidisciplinary and it is a multidisciplinary effort.

Dr Rohit Gosain
Why we are doing this is all because there is meaning to it, because there are clinical implications tied to it. Gary and Carlos, thank you so much for both of your insights today and this discussion, where we are highlighting how far HER2 testing has come along from binary readout to more nuanced decision. Today that is HER-low, HER2 ultralow and how all of it translates into our clinical practice to give our patients more treatment options.
Well thanks for tuning in. Make sure to follow us along for more conference highlights, approvals and treatment algorithms. We are the Oncology Brothers.

Tonke (COR2ED)
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