# What Pathologists Need to Know About HER2 Testing Guidelines

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**Dr. Jason Scapa:**

Hello everyone and welcome to today's episode of the CAPcast. I'm Dr. Jason Scapa and I'm a general surgical pathologist at a large hospital system in Southern California. Today I am joined by two guests of the immunohistochemistry committee of the CAP. The first is Dr. Andrew Bellizi. He is the clinical professor of pathology at the Carver College of Medicine at the University of Iowa. There he serves as the co-director of the Immunopathology Lab, and has served as the immediate past chair and current advisor of the CAP Immunohistochemical Committee. He is joined by Dr. Gregory Bean, who is assistant professor of pathology and associate Director of Breast Pathology at the Stanford School of Medicine. He's also a member of the CAP Immunohistochemical Committee. Thank you both for joining me today. So today's topic has to do with this buzz that's been going on at the American Society of Clinical Oncology Conference last year with regards to some new data with regards to HER2.

And so I saw an article recently in the Archives of Pathology and Laboratory Medicine in January 2023 by Dr. David Rim entitled The Pathologist Conundrum in which he was talking about new companion diagnostic tests pushing the limit of pathology interpretation of IHC stains and that might require assessment of subjective parameters in a largely non-reproducible assay. And so he referenced some markers such as PDL1, KI67, and HER2. And so with regards to this pushing the limit of detection by pathologists for their interpretation, it seems like HER2 has gotten some pretty big buzz with regards to the low HER2 mainly negative, which is zero and one plus. So I wanted to introduce this topic because you two being from the immunochemistry committee are kind of experts in this field, and what should us general surgical pathologist do with this information? So my first question to both of you is can you give us some background on HER2 immunohistochemistry as a companion diagnostic tests in breast cancer?

**Dr. Gregory Bean:**

So between 15 and 20% of all breast cancers are known to be amplified at the gene level for HER2, and that's typically shown at the protein level by HER2 protein at the cell surface, which we can detect using immunohistochemistry. We can also detect the gene amplification by techniques like [inaudible] hybridization, mostly FISH. And traditionally that has been the marker of what we call HER2 positive breast cancer, so HER2 amplification and overexpression of protein. And for the last more than a decade, that's the marker that we've been always testing for. We do this with IHC and we do this with FISH. And when we're talking about HER2 as a prognostic and predictive marker, we're just talking about that positivity and that context, and that pathologists are really good at identifying that positive state and patients are therefore treated with anti-HER2 therapy as a result of that being positive. That's the traditional sense of what HER2 positivity is.

**Dr. Andrew Bellizi:**

Great. And I'm going to talk a little bit about HER2 low. I'm also going to riff on one of the points that you brought up in David's, his editorial, the pathologist conundrum. And I'm going to introduce a couple concepts. So one concept is fit for purpose. Everybody needs to know about fit for purpose in terms of immunohistochemistry, and that is an immunohistochemical assay should be conceived, optimized and validated according to purpose. And for the sake of the HER2 discussion, contemporary current, HER2 immunohistochemistry was conceived, optimized and validated for the historical purpose of separating negative from positive. It also has, as Greg mentioned, an orthogonal gold standard, which is HER2 copy number and HER2 step 17 ratio by FISH or FISH. So fit for purpose is really important. The other things to know about are there's the analytic phase of testing includes the on instrument IHC protocol and the pathologist readout.

And both of these come together to form the analytic portion of the test. And for the IHC readout, I call that brown or not brown, how brown, where brown. And then our interpretation of that, HER2 negative equivocal positive or now HER2 negative low equivocal positive or now HER2 negative, ultra low, low, equivocal, positive. That's the interpretation, that's the post analytic phase. Another thing I want to mention is the readout for some tests is much harder than for other tests. And that is you can think about what is IHC really the signal, the brown, it actually reflects some underlying concentration of analyte and for some readouts, the populations that we're trying to separate, their analyte concentrations and thus the signal is so far apart that the readouts pretty straightforward. So three versus zero one is really easy. And then we even have this equivocal gray zone in between that we can adjudicate with FISH. Other IHC assays, and it depends on the assay and it depends on the cut point are intrinsically more difficult, are less reproducible.

And that's because we're trying to distinguish populations where the signal is much closer together and these populations are much more overlapping. And then for PDL1, for example, there are PDL1 readouts that are pretty easy and then there's a readout that's medium difficult, and then there's one that's actually impossible. And the one that's pretty easy is TPS at the 50% threshold. So if you stain a bunch of lung cancers and give them to a bunch of pathologists and ask them to assign PDL1 positive or negative at A TPS threshold of 50%, they do pretty good with that. There's less reproducibility around TPS assessment at the 1% threshold, but it's still moderately good. And then CPS in squamous cancers or gastroesophageal cancers, the readout at that, not 1%, it's a score, at that score of one is poor. And actually there are two papers that are in press in modern pathology just published online this week that shows the overall percent agreement for a CPS of one in GI cancers is 30%.

The test is poor. And then we can bring this back to low HER2, we have fit for purpose and we have ease of pathologists readout. And the problem with low HER2 is the tests that were deployed in the clinical trial, in the clinical trials, the DESTINY trials were not fit for purpose. The second problem is, although this is a new problem, so groups are generating data around this, but the second problem is that right now the clinically actionable IHC readout, namely zero versus one, is probably not as easy and reproducible as TPS 50 or even TPS one. It is probably in between the ease likelihood of agreement between pathologists for a TPS1 and a CPS1. And so we have, I would say mainly a biochemistry, an IHC biochemistry problem, but we also have an IHC readout problem.

I can tell you right now that there's a lot of attention that's being placed on the IHC readout. And so a lot of the materials that I've seen online or the webinars courses, tweets have been around, hey pathologists, there's low HER2 and we should talk about why. We'll talk about trastuzumab deruxtecan. Greg can tell us about trastuzumab deruxtecan and the DESTINY trials, and maybe we could talk about the DAISY trials a little bit. All this attention and effort on hey pathologists pay attention to zero versus one and we can train you to distinguish zero versus one. But the intrinsic problem that's not addressed is the assay that's been deployed in the clinical trials is not one that was conceived, optimized or validated around selecting patients for response to trastuzumab deruxtecan.

**Dr. Gregory Bean:**

And just to add one other thing to what you just said, Andrew, in breast, triple negative breast cancer, the CPS threshold is 10, which at least in the experience of me and my group is even harder to estimate than when using one. So even more difficulty.

**Dr. Andrew Bellizi:**

Yeah. Do you want to talk a little bit about, and I say this consistently, so I'll talk about PDL1 then I'll pitch to Greg and he can talk about trastuzumab deruxtecan and it's efficacy. People are always, Andrew, you're all up in arms about this stuff, but don't you think that checkpoint inhibitor therapy is amazing? And I say yes. It's not the drugs, it's the tests. And so pharma is so good and knowledgeable and sophisticated around drug design and development and we have wonderful clinical trialists, but what appears to be lacking is any sophistication in technical knowledge around the science of immunohistochemistry that would enable folks to intelligently design fit for purpose assays that pathologists can read successfully. So Greg, what's trastuzumab deruxtecan? How were patients qualified for in clinical trials? And then-

**Dr. Gregory Bean:**

Yeah, again what you're saying, it's a huge breakthrough in terms of the drug therapy and the number of patients that could potentially be helped by it. Trastuzumab is a drug we've had for a long time. It's a monoclonal antibody that's directed against HER2. More recently they had developed a antibody drug conjugate that we're talking about, trastuzumab deruxtecan. It's been approved for a few years in setting of metastatic HER2 positive, traditionally positive breast cancer. But what changed back in August was it became approved for, based on this DESTINY-04 trial, use in patients that had tumors that showed either by IHC1 plus or two plus with negative FISH results. So not only the three pluses but also these what they termed as low positives. They did not include patients that had tumors with HER2 zero in that trial. So it was an eligibility issue rather than anyone ever showing any kind of predictive value for the drug.

And so now we are faced with where we are in terms of pathologists being asked to distinguish between zero and one plus, which is something that we had never done, we traditionally have not done before. And there's a lot of data that's been coming out recently, and Andrew's discussed it already that this is really not a reproducible distinction that we can make. It's not just us, it's actually mostly the test was never designed. IHC was not meant to be testing that linear range of distinguishing zero and one plus. So the unfortunate joke is that you can test the same sample one day, get a zero, test it another day and get one plus.

So this has caused a lot of questions from our oncologists about, we've sometimes gotten calls about are you sure it's zero? Are you sure it's one plus? And people are asking questions like, do we have to modify our reporting and not use the term negative for one plus or two plus with negative FISH? And right now we're in this sort of state of flux right now. But the good news is that in terms of patient eligibility for patients with metastatic carcinoma, these patients often have multiple tissues that have been sampled. The biopsy, the excision, confirmation of the metastasis, perhaps the recurrence. And really the drug is eligible for any of those being non-zero. So you might not have to necessarily quibble over one specific case. Usually these patients have a variety of different tissues that can be looked at.

**Dr. Andrew Bellizi:**

And so this is the HER2 ultra low, which is HER2 greater than zero and less than one plus. So it had been demonstrated in other smaller trials I think called DAISY, that there were patients that were called HER2 zero that actually responded, which has just highlighted that the amount of HER2 that a person's breast cancer needs to express to derive benefit from this drug is less than one, and that's low HER2. But then ideally we would design and implement a separate assay where instead of zero versus one being at the sort of background noise negative versus weekly positive of an assay, that it would actually be within the fat part of the linear range of the assay, such that these zeros were dead negative. And the ones we wouldn't have to struggle with, that they would be fairly strongly positive to make these populations that are really actually different to really dichotomize them with the assay to create an assay that can be read reliably so that all the patients that could potentially benefit from the therapy get the therapy that they can potentially benefit from.

But then also importantly, unfortunately, trastuzumab deruxtecan isn't as benign as trastuzumab. Trastuzumab, I don't know, you might have an allergic reaction. Trastuzumabis deruxtecan's associated with about 10% risk of pretty severe interstitial pneumonitis. So we can't just give the drug to everybody. We do need to be able to identify these true HER2 zero or HER2 null patients. And that's fingers crossed that that's what's on the horizon, that in the next year or two there will be a new fit for purpose assay developed probably by Roche and Agilent to help select patients for this very important and highly impactful therapy.

**Dr. Jason Scapa:**

Dr. Bean, can you talk a little bit about HER2, the basics of how it's tested and this new drug trastuzumab deruxtecan, and how it's used?

**Dr. Gregory Bean:**

Typically HER2 is tested using immunohistochemistry and or in situ you hybridization. The immunohistochemistry is graded on a semi-quantitative scale of zero and one plus, which are both considered negative, two plus, which is considered equivocal, and three plus, which is considered positive. And if you get a two plus result, then you are obligated to do [inaudible] hybridization to resolve that equivocal status. I've said that zero and one plus are considered negative, and one of the issues that has arisen during this news is that because one plus and FISH negative two plus cases were used as inclusion criteria for this DESTINY trial, we are now being asked as pathologists to score and make sure that we accurately score between zero and one plus, which is not something that we've ever been asked to do, nor was it what the assay, when first developed, intended to do. So that's why we're in this situation now.

**Dr. Jason Scapa:**

I think it's important that we figure out how to test for this in this new way, but as we've talked about, we don't really have a test at least commercially available or readily available for us to answer this question. And can you remind me again why it's important in the DESTINY trial that we figure out between the zero one and two plus?

**Dr. Gregory Bean:**

So the DESTINY trial included cases that were one plus or two plus and FISH negative and found that patients that were included in that trial and got the drug responded, but importantly they did not include patients that had tumors with HER2s of zero. So we don't actually know if tumors that have HER2 of zero would or would not respond, they were not included in that trial. So we don't know if in using HER2 in this way it really is a predictive factor for response to this drug, or because it was simply just an inclusion criteria.

**Dr. Jason Scapa:**

So I'm out in the community, just a general surgical pathologist, not an academic center. Do you envision me if I score a HER2 as zero one having to send it to a reference lab to get this more defined answer between zero and one, or do you think eventually this will be a commonplace as a second test at most sites?

**Dr. Gregory Bean:**

Well, luckily for you and for all of us, breast cancer patients usually end up having more than one pathology specimen. They got a biopsy, they get an excision, depending on their recurrence or metastasis status, they might have other things. And really the drug is approved for any specimen to be one plus or two plus with FISH negative. So an alternative to sending it somewhere else is to test another sample if your clinician really wants you to try to get a different answer. We haven't been asked to send anything out anywhere or get a second opinion at most. We've been asked to do it on a second sample and people seem to accept that answer whether if it's reproducibly zero on both samples. So that's what I would say in terms of that issue.

**Dr. Andrew Bellizi:**

I have a couple comments and follow up. One is history repeats itself, especially folks who aren't students of history tend to bump into the same mistake. So what Greg's saying like, oh, and what you're suggesting, oh, I call this zero, send it out to another lab and see if somebody will call it one plus or keep testing specimens until one is one plus, entirely recapitulates the situation at the turn of the century with the initial approval of trastuzumab in HER2 positive breast cancers, especially at that time, HER2 assays, they weren't really locked down. People were really inventing the wheel. And so it was really easy to send a case that was zero in your lab to another lab and get a three plus, which seems crazy now, but that's where we are really with the low HER2.

So my hope is, and it goes back to this point of discussion from the beginning of the recording around the lack of a fit for purpose assay. My optimism is for the deployment of a fit for purpose assay. Immunohistochemistry is all about signal-to-noise. And the reason that this assay is not fit for purpose is the signal for one plus is within the noise of a zero. And so it's theoretically possible and not even all that challenging to develop an assay where there's more separation of negative and frankly negative and positive and the assays, they just need to be stronger. So either more concentrated primary antibody or longer primary antibody incubation or stronger retrieval or longer, stronger detection. And I'm optimistic that that will come.

One of our colleagues on the immunohistochemistry, David Rim, has developed a fit for purpose low HER2 assay with the limitation that it uses fluorescence instead of being a chromogenic assay, and also requires image analysis to read. So best case scenario, we get a chromogenic assay that's easy to read, that's fit for purpose. And then sort of medium case scenario is that we require an assay like David made that's fluorescent requires digital pathology, and then we're just not going to be able to, you can make it in your lab if you have those resources, or this'll be a test that can be done in a handful of reference labs.

And then maybe best case scenario is just everybody responds to this drug. And again, the reason that we have to be careful, even though this is an amazing therapy that can be deployed in a huge number of breast cancer patients, it's not without cost. I mean, cost cost, but mainly 10% of patients develop interstitial pneumonitis. That's sort of a limiting toxicity. So that's sort of maybe a peak ahead. That's the range of possibilities. I hope we end up on the rosy side.

**Dr. Jason Scapa:**

So as we wrap up, it sounds like the immunochemistry committee is really abreast at this topic and is coming out with some new ways to test at least the image-based way of looking at this. And I think the science is also still being worked out on the clinical trial side, like you said, with whether the drug could be given more broadly in which maybe this test isn't necessary. But for us in the community who are maybe fielding questions from oncologists about this topic right now, maybe they heard it at one of these ASCO or one of their conferences and are now coming to pathologists to say, "Hey, do you know about this and how would you test for this?" What should be our message to our community clinicians and our oncologists throughout the country?

**Dr. Andrew Bellizi:**

I've got some comment. I'd say, well, everybody that's listening to this podcast is doing the right thing. So it's always be informed about the issues. And this is a really hot topic, and this is just another one. These predictive markers are, this is the wave of the future. Every oncology drug that's not chemotherapy, everything that's a monoclonal antibody to a target, I suspect that there's going to be a challenging immunohistochemical assay that's tied to it, and then go to where you go to get your news. I'd say pay attention to what's in archives, but all the major professional pathology society, so USCAP and ASCP and NCAP all have so much, they have such deep benches, there's so much content expertise among their membership that those professional organizations are going to be huge sources of information. And then you'll also have your experts local regionally.

So if you're in the community, talk to the folks at your local academic medical center. Here we are on the national scene, we're recording a podcast for everybody. But I say I'm also sort of the lend an ear for all the labs in Iowa. So I'd say hit up your local academic medical center. I mean, ASCO CAP of course is, there's sort of the bellwether for topics like this. And then there are other big educational things for immunohistochemistry in general. One thing, and this is what I tell fellow IC lab directors, always look to Nordic QC, which is the Scandinavian EQA external quality assessment program. So it's like the CAPIHC committee's Scandinavian cousin.

Also, there's a organization called ISIMM, the International Society for Immunohistochemistry and Molecular Morphology. So that's the Immunohistochemistry Professional Society. And we have monthly webinars. Actually that's where I came from before I hopped on the line with you guys. And so any biomarker topic is a hot topic for ISIMM, and we've already had several webinars on HER2 and low HER2 and are planning more on low HER2. And all the ISIMM webinars are, they're all recorded and they're all published online. So there are actually a couple of hours of educational content on low HER2 that are available on the ISIMM website.

**Dr. Jason Scapa:**

Alrighty, I think that wraps up today. So thank you so much for joining us on the CAPcast and we look forward to seeing you on the next one.