Second act for HER2, in gastric cancers

Karen Titus

May 2014—If one were to map out a "family tree" of tumors, breast and gastric cancers might end up looking like second cousins. One is common, the other is not, but it's rapidly becoming known that they share a kinship of sorts with HER2 testing and the targeted therapy trastuzumab.

The breast-HER2-Herceptin connection needs no introduction. Now the GI version has emerged.

GI and breast pathologists at URMC are working together on gastric HER2 testing. "It's helpful to have both groups provide reference and be open to help with interpretation," says Dr. Aram Hezel (left), with Dr. David Hicks and Dr. Christa Whitney-Miller.

Fifteen to 20 percent of patients with adenocarcinomas of the stomach and gastroesophageal junction overexpress HER2, a subset that has been shown to benefit from trastuzumab. More broadly, patients with intestinal types of gastric cancer are known to have a better prognosis, as opposed to those with diffuse type, and it appears that patients with intestinal types are more likely to overexpress HER2.

The first reports ignited plenty of excitement among medical oncologists, says Cathy Streutker, MD, director, surgical pathology, Department of Laboratory Medicine, St. Michael's Hospital, Toronto. Now pathologists are catching up. "The oncologists were ahead of us on this," she says.

The impulse came from the ToGA (Trastuzumab for Gastric Cancer) trial, a large, multicenter study that showed overall survival benefit in HER2-positive patients with advanced gastric cancer. (The trial observed a 22 percent positivity rate among patients enrolled.) The study (Bang Y-J, et al. Lancet. 2010;376:687–697) was initially reported at the 2009 ASCO meeting as an interim presentation, with more in-depth discussion at the meeting the following year, says Eugene Hsieh, MDCM, pathologist, Sunnybrook Health Science Centre, Toronto. With that, medical oncologists began making their HER2 requests.

"They started asking us right away," says David Hicks, MD. Likewise, as he traveled the country giving presentations on HER2 testing in breast cancer, "More and more I would get questions about gastric cancer as well," says Dr. Hicks, professor of pathology and laboratory medicine, and director, surgical pathology, University

of Rochester (NY) Medical Center.

In fact, the growing interest led to his working with a GI pathologist colleague, Christa Whitney-Miller, MD, to develop a joint presentation on the topic, which was given at CAP ’13.

For those who aren’t medical oncologists, ToGA’s hopeful message may have seemed overstated at first. Trastuzumab was shown to extend median survival to 13.8 months for patients who received trastuzumab plus chemotherapy versus 11.1 months for the chemotherapy-alone group. Progression-free survival was 6.7 versus 5.5 months. “But to us, we look at the numbers on the ToGA trial and say, ‘Oh, well, hmmm, um, is that really significant?’” says Dr. Streutker. In fact, she recalls one colleague at a meeting questioning the value of using Herceptin in patients who are likely to die soon anyway. “Which I thought was rather harsh,” she says. “But to the oncologists, this is huge. This is the biggest increase in survival they’ve seen in many years in gastric cancer. They were so excited to finally have something.” Gastric cancer, in their view, no longer means a speedy death sentence for their patients, and they were eager to start HER2 testing.

Aram Hezel, MD, calls those extra months a significant advantage. Some patients have seen a much longer benefit. “It can have a big impact. We began testing as soon as it was apparent at ASCO that the use of Herceptin was useful in this class of tumors,” says Dr. Hezel, vice chief, hematology/oncology, and associate professor of medicine and oncology, University of Rochester. “We knew we could change our treatment.”

For many pathologists, that has meant dusting off information they may not have used since their early training. Breast pathologists are helping GI pathologists learn how to do HER2 testing, but they may not be well versed in the intricacies of GI pathology. And most GI pathologists have had little reason to understand HER2 interpretation beyond the most basic level.

Dr. Hicks and Dr. Whitney-Miller, director of GI/liver pathology services at Rochester, have already been approached by the CAP to participate in developing a guideline for GI HER2 testing—similar to the ASCO/CAP HER2 guideline for breast cancer. But that lies in the future.

First, they faced a learning curve of their own at Rochester. Two papers provided important guidance and serve as a good starting point for labs considering GI HER2 testing.

Fig.1 Comparison of breast and gastric cancer HER2 interpretive guidelines

IHC score	Breast (biopsy or resection)	Gastric resection	Gastric biopsy
0	No staining; incomplete or faint/barely perceptible staining in ≤10% of invasive tumor cells	No staining; non-membranous staining; staining in <10% of cells	No staining; non-membranous staining; staining in <10% of cells
1+	Incomplete or faint/barely perceptible in >10% of invasive tumor cells	Complete/basolateral/lateral staining in ≥10% of cells evident only at 40x	Complete/basolateral/lateral staining in ≥10% of cells evident only at 40x
2+	Incomplete or weak/moderate circumferential membranous staining in >10% of invasive tumor cells, or complete intense circumferential membranous staining in ≤10% of invasive tumor cells	Complete/basolateral/lateral staining in ≥10% of cells evident at 10–20x	Complete/basolateral/lateral staining in ≥10% of cells evident at 10–20x
3+	Complete intense circumferential membranous staining in >10% of invasive tumor cells	Complete/basolateral/lateral staining in ≥10% of cells evident at 4x	Cluster of at least 5 cells with complete/basolateral/lateral staining evident at 4x

The criteria for interpreting HER2 immunohistochemistry in gastric cancer are similar to, but differ slightly from, those for breast cancer. In addition, there are also different criteria for biopsy and resection specimens in gastric cancer. Sources: Hofmann M, et al. *Histopathology.* 2008;52:797–805. Rüschoff J, et al. *Virchows Arch.* 2010;457:299–307. Wolff AC, et al. *Arch Pathol Lab Med.* 2014;138(2):241–256.

The earlier one, published in *Histopathology* (Hofmann M, et al. 2008;52:797–805), reported on an effort to establish a HER2 scoring system for gastric cancer as part of the ToGA trial. The authors found that the method used for scoring breast HER2 IHC could accurately and reproducibly be used for gastric cancer cases, though it would need to be refined. While the lab techniques are essentially the same, says Dr. Hsieh, "the interpretation is fairly different." (See Fig.1.)

A later paper (Rüschoff J, et al. *Virchows Arch.* 2010 ;457:299–307) came on the heels of European approval of trastuzumab for HER2-positive metastatic gastric and GEJ cancer. The authors looked to validate the HER2 testing procedure, both interlaboratory (looking at IHC HER2 scoring issues among eight German and French laboratories) and interobserver (looking at IHC HER2 intensity and score concordance between six German pathologists). Just as importantly, says Dr. Whitney-Miller, the paper clarified the staining criteria—what was a weak stain? what was moderate?—used in ToGA.

Rüschoff and colleagues found the IHC immunoscoring method used in the ToGA trial to be reproducible between the different pathologists, provided they adopted certain precautions. The paper also noted that the training and ASCO/CAP guideline used for breast HER2 translates somewhat to gastric cases—but not completely. In short, pathologists and their clinical colleagues need to see that while breast and gastric tumors may have a family resemblance, they're hardly identical twins.

Dr. Hicks drives home the point when he talks about validating HER2 testing. "I get asked all the time, 'If we have a validated test for breast HER2, immuno, or FISH, does that mean we have to validate for gastric? Or can we just throw gastric cases into the mix?'" Dr. Hicks is unequivocal: "I think the answer is yes—you need to validate."

Despite the differences between breast and gastric HER2 cases, there is common ground, and comparing the two is a reasonable place for pathologists to start looking at how to do GI HER2. "It makes sense," says Dr. Hsieh. "A lot of papers out there now talk about this in the context of breast." It may be impossible to separate the two completely, just like it can be difficult to reference Liza Minnelli without eventually invoking Judy Garland.

As interest in GI HER2 testing grew, Dr. Hicks turned to Dr. Whitney-Miller, who had recently been recruited to lead the GI pathology subspecialty group at Rochester. The institution has a large GI oncology unit, says Dr. Hicks, so it made sense to start looking at GI cases retrospectively. Dr. Whitney-Miller oversaw these efforts as both a research project and a clinical initiative. Knowing that the Hofmann paper showed that the interpretation criteria were somewhat different for gastric and breast, Dr. Hicks says there was no doubt in his mind "that pathologists are going to struggle with this. They're not going to know how to interpret this."

Some pathologists may find that HER2 IHC is a trip down memory lane. "I haven't interpreted it since I was a resident or fellow," says Dr. Whitney-Miller. To bring themselves up to speed, the GI pathologists sat down with the breast pathologists, in addition to reviewing the literature, including the Rüschoff and Hofmann papers as well as the ASCO/CAP HER2 guideline. Next, they took current and archival cases at their institution, tested them, and then sent them out to ensure they had a high level of agreement. (They also subscribe to the College's proficiency test for HER2 and gastric cancer.) There were a lot of double reads, says Dr. Hicks, as well as sharing cases on a multiheaded scope to ensure good interpathologist agreement on results.

Dr. Streutker

In Toronto, Drs. Streutker and Hsieh were part of a broad effort to introduce GI HER2 testing. Cancer Care Ontario, a government agency that oversees cancer services in the province, set up an advisory board to look at the issue; one member, Wedad Hanna, MBBCh, a pathologist at Sunnybrook who has close ties to the ToGA pathologists, felt strongly that GI pathologists needed to be involved in addition to breast HER2 experts, and as a result, Drs. Streutker and Hsieh (among others), both of whom are primarily GI pathologists, joined the board and began looking at gastric cancer cases and validating studies. Sunnybrook and St. Michael's were the first two centers in the province to start HER2 testing for GI cases; six more Ontario centers do testing now as well, but the two original labs serve as reference centers for the province. (Sunnybrook does the testing for sites in a couple of the Maritime provinces as well.)

As these efforts unfolded, breast and GI pathologists have resembled a relay team. The breast experts have plenty of HER2 knowledge to hand off—but the GI pathologists need to run with it on their own.

That point became clear when Dr. Streutker and Dr. Hsieh went to Germany to train with the group that interpreted the cases for the ToGA trial, as part of a contingent of both GI and breast pathologists from multiple institutions. Those with lots of breast experience "had a hard time wrapping their heads around some of the differences between gastric and breast interpretation," says Dr. Hsieh. The GI pathologists, given their relative inexperience with HER2, might have been more open-minded in terms of how to interpret HER2 from the gastric side, he suggests.

Dr. Hsieh is being exceptionally politic. Dr. Streutker's observation is more succinct: "Clearly the breast people thought the gastric people were nuts."

By the end of the training session, that was no longer the case, Dr. Hsieh hastens to add. "But it took longer for those with extensive breast training to get used to the gastric side." He recalls being a bit surprised. "For me it was just learning a new technique and adding to our repertoire. For those in breast, it was more unlearning what they'd learned before." This may not be a one-off experience, given the possibility that the use of targeted therapies could expand to other types of tumors that are already known to overexpress HER2. "Pathologists are going to have to stay very flexible," says Dr. Streutker, especially as personalized medicine gains more traction.

In GI cancer specifically, there's strong interest in amplifications or translocations of the FGFR2 gene, says Dr. Hezel. It's only a matter of time before drug development catches up with knowledge of tumors' genetic landscapes. "The importance of these tests is only going to grow," he predicts.

As the Toronto and Rochester pathologists began testing GI HER2 at their respective institutions, they encountered similar curiosities and challenges.

At Rochester, HER2 testing of gastric cancer is not routine. When clinicians do request it, which they do for patients with metastatic disease, both IHC and FISH are ordered upfront, and the lab runs the IHC first. In the case of early-stage disease, select patients with potentially curable disease may also be tested and offered access to national

clinical trials, says Dr. Hezel.

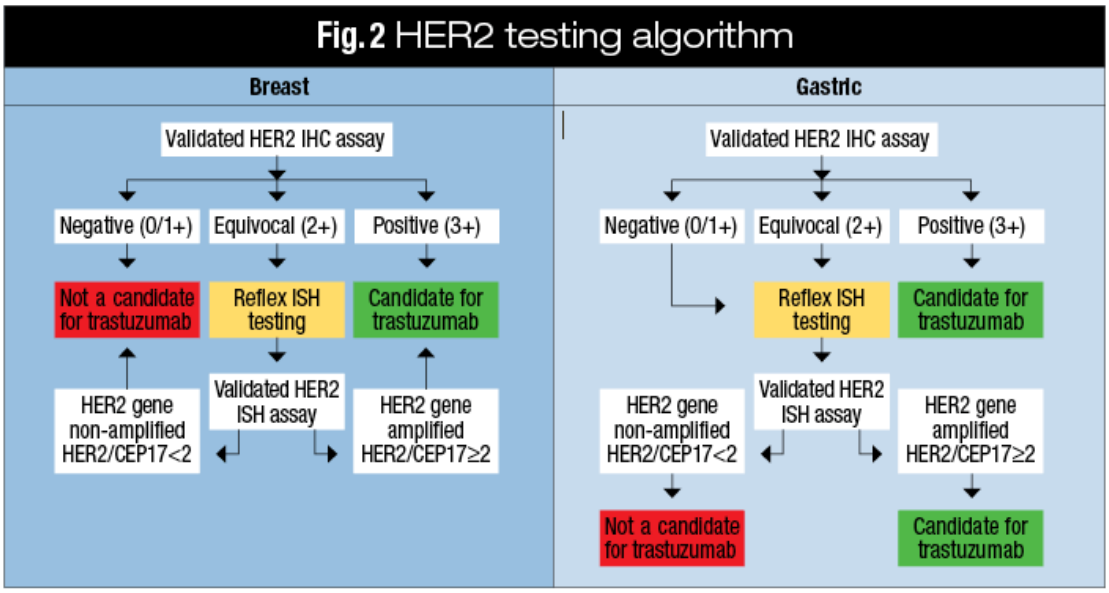
Data from Dr. Whitney-Miller's studies suggest that the percentage of cases that are IHC negative and FISH amplified is quite a bit higher in gastric cancer than in breast cancer. The Rochester pathologists decided they wouldn't perform FISH on cases where IHC was positive (3+), since those patients would be treated regardless of FISH results. "But if the IHC was anything less than positive [0/1+ or 2+], we were going to FISH all those cases," says Dr. Hicks, recalling early discussions. "Because we were seeing a significant number of zeros and one-pluses that turned out to be FISH-amplified." Not everyone agrees this is the best approach—these are still early days of gastric HER2 testing, after all. Dr. Hicks notes that in Europe, cases that are 0 or 1+ do not undergo ISH testing. Those that are 2+ do, however, based on data from a ToGA subset analysis that suggest IHC does better than FISH in identifying patients who will benefit from a HER2-targeted therapy. (See Fig.2, page 38.)

That approach is not without its controversies, says Dr. Whitney-Miller. The National Comprehensive Cancer Network recommends upfront testing of all specimens. In fact, she says, some institutions might be treating these cases like breast cancer specimens and doing the testing reflexively, which is what happens under the Cancer Care Ontario protocol.

Moreover, Dr. Whitney-Miller notes, not everyone agrees on the implications of IHC-negative cases. In the ToGA trial, all patients had their tumors tested by both IHC and FISH. If either result was positive, the patient qualified for the trial. However, some people advocate for a HER2 testing algorithm similar to breast cancer's: A patient who is IHC negative is not eligible for trastuzumab. Dr. Whitney-Miller argues that for now, it makes sense to follow ToGA's inclusion criteria, given that it's the only trial that has shown benefit from using Herceptin in gastric cancers. "Dr. Hicks and I don't feel there's strong evidence to do otherwise."

Interestingly, a post-hoc analysis of ToGA data showed that patients who benefited the most were those who were IHC positive, regardless of FISH status. Those who were IHC negative/FISH positive did not benefit.

"It's fascinating," says Dr. Whitney-Miller.



The gastric cancer HER2 testing algorithm advocated for by University of Rochester (NY) pathologists (right) differs from the CAP/ASCO HER2 testing algorithm for breast cancer (left).

In fact, says Dr. Streutker, that group actually did worse in the ToGA trial. "But it's such a small group," the implications are hard to assess. (Researchers are now revisiting the data from breast cancer studies, Dr. Whitney-Miller says, and early reports suggest this might be the case for breast cancer as well.)

Some clinicians may look at discrepant cases and suggest the IHC failed in some way. “We don’t have a lot of those cases, but I see no reason we should doubt ourselves,” assuming the test was done carefully, says Dr. Streutker. “Why can’t there be a genetic problem where there’s something wrong with the protein, so that it doesn’t get to the membrane?” Or, she says perhaps rare cases that are IHC 3+ and ISH negative might be caused by increased gene transcription—the result of a promoter that’s been turned on, say—rather than by an increase in copy numbers. And that doesn’t even take into account issues associated with chromosome 17 polysomy, which in breast has emerged as a controversial and important area of discussion, she says. In short, this dissonance may not be a lab problem, but rather an unanswered question, at least for the time being.

Not that there aren’t other lab challenges.

One of the biggest ones, says Dr. Whitney-Miller, is that in breast cancer, the emphasis is on complete membrane staining and obvious circumferential distribution. Gastric cancer, on the other hand, frequently has an intestinal phenotype, as noted. Such cases may show only basolateral or even lateral staining. “When you FISH those cases, they’re amplified.”

Heterogeneity also rears its head in GI cases, which seem to have more heterogeneous HER2 overexpression than in breast cases (though Dr. Hicks suggests “it’s probably more common than we thought” in breast cancer). That can make things tricky. As Rüschoff, et al., found, cell clusters of ≥ 5 cohesive stained tumor cells must have a moderate-strong complete, basolateral, or lateral membrane reactivity, says Dr. Whitney-Miller. But those cells may not account for 10 percent of the total cellularity.

While in Germany, Dr. Streutker noticed that the breast pathologists struggled with the lower thresholds used for GI cancers. They appeared to have an easier time accepting that GI samples didn’t require complete membrane staining or circumferential distribution. “But the patchiness, I think, was very unnerving to people who focused on breast.” (See Fig.3, page 40.)

Adequate sampling has posed issues for pathologists doing GI HER2 reads. Typically they work with tiny endoscopic mucosal biopsies, which have sufficient tissue to identify presence of a malignancy. But is it enough to rule out HER2-positive disease? Given the issue of intertumoral heterogeneity, it probably isn’t. “If the mucosal biopsy is negative, I don’t think you’ve excluded a HER2-driven gastric cancer,” says Dr. Hicks.

The Canadians have emphasized the need for adequate sampling. Dr. Streutker says she tells her clinical colleagues, “The more the better. I’ve had a few cases sent in for testing where the tumor’s gone.” While she concedes that many of her colleagues are trying valiantly and supplying her with multiple biopsies, there may be tumor in only one specimen. “Honestly, they need to biopsy the heck out of these things.”

The message is clear: “If you’re suspicious of gastric cancer, get as much tissue as possible,” says Dr. Hsieh. Ideally, they’d like to see six to eight pieces, though they concede this can be difficult to obtain. Many are willing to do so when asked. Says Dr. Hsieh: “Generally, the endoscopists we have interacted with are quite receptive. However, it may be more difficult to reach endoscopists who primarily scope at the private clinics.”

Dr. Hezel agrees, adding that he and his medical oncologist colleagues are well aware that pathologists need sufficient, high-quality samples to work with. “A clear test result is of such significance that I think most oncologists would consider getting more tissue—redoing a biopsy—if asked,” he says.

Dr. Hsieh says he needed to correct pathologists’ misperception that it’s preferable to work with a larger, resection specimen, which meant some pathologists didn’t bother sending biopsy material. “But the patient might not even get a resection. They might wait until they die of the disease. And we know from the original studies that biopsies are good, and biopsies do not decrease the rate of detection of HER2 positivity,” Dr. Hsieh says. Even if a resection is done, it may be months later—again, time that most gastric cancer patients can’t afford to squander.

Dr. Streutker notes that the advent of GI HER2 testing is also calling long-held lab logistics into question. “People

obsess about breast," she says, noting concern about ischemic time and the need to cut breast specimens quickly. "And then the stomach sits there marinating in its own acid." If biopsies were always available, the issue might be moot. But that's not the case, and both she and Dr. Hsieh suggest that as personalized testing expands, pathologists will need to more carefully consider how all specimens, not just breast, are fixed.

Not everything boils down to a difference. In breast cancer, Herceptin was first shown to benefit those with metastatic disease; when clinical trials moved to an earlier stage, it became evident that Herceptin was beneficial in the adjuvant setting. "I think we're going to see a similar evolution in gastric cancer," Dr. Hicks says.

Most obviously, oncologists with GI patients want the same thing breast experts do when it comes to results: a clear, yes-no answer that will help them decide the next step in the patient's care, says Dr. Hezel. "Most medical oncologists who are not molecular characterization gurus simply want to know if there's enough strong evidence to act."

Despite their long history of interpreting HER2 testing, breast pathologists are unlikely to take over GI cases completely. But given GI cancer's relatively low numbers (some 21,000 new cases are diagnosed each year in the United States), it won't be easy for every lab to develop GI HER2 expertise.

Cancer Care Ontario's recommendations include a statement that those who interpret gastric HER2 need a "fair amount" of GI knowledge, says Dr. Hsieh. "Not necessarily be a specific GI pathologist, but have spent sufficient time training specifically in GI HER2 interpretation." It's a commonsense approach, he adds. "But that's not something that always happens at all institutions."

GI HER2 is not as easy as it looks, says Dr. Streutker. "It's a fussy enough test that people should only do it if they do it quite a bit. People should be looking at at least 100 cases a year—even if you're not signing out 100 cases—to stay good at it."

Alternatively, Dr. Hsieh suggests that an institution have a "good three-digit number," with frequent internal consultations within the department. "We've been telling our colleagues at other institutions to think of it as a new test. Don't think of it as HER2; think of it as gastric HER2, which is a separate test," he says.

But not every institution has its own GI specialists. CCO made a conscious decision to limit the number of sites where GI HER2 testing would be done; given the lower number of cases, the intent was to make sure those reading it would be able to maintain their expertise.

GI and breast pathologists are working more closely together at Rochester, a fact Dr. Hezel appreciates. "It's helpful to have both groups provide reference and be open to help with interpretation," he says.

Dr. Hicks says that while GI pathology is not part of his practice, it's become part of his career. "I will still be brought a slide [by a GI colleague] and asked, 'What do you think of this?'" And while the GI pathologists do the IHC and then decide if FISH is needed, the FISH itself is done by the breast experts. So even the technical staff, who are more familiar with breast cancer, have had to adapt to doing gastric samples. "The techs all say gastric's a lot tougher to read and score than breast is," he says. Challenges include finding the right area to examine and distinguishing inflammatory infiltrate from tumor cells. One approach advocated by both Drs. Hsieh and Hicks is to use the light microscope to look at the morphology before ISH is done.

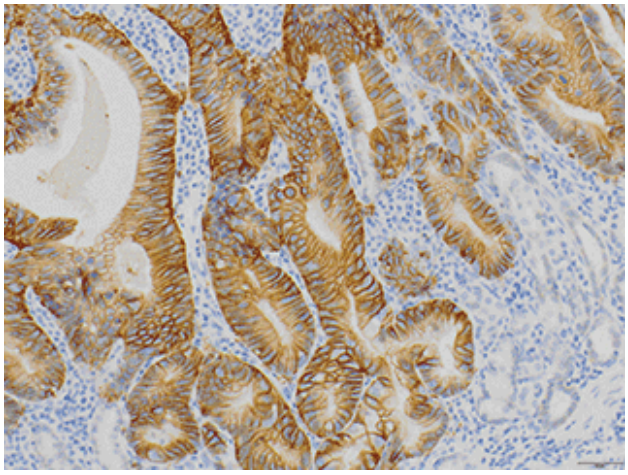


Fig.3 Gastric cancer with HER2 amplification/overexpression frequently exhibits basolateral or lateral reactivity with IHC (shown above); this is in contrast to HER2 amplified breast cancer which exhibits complete membranous reactivity.

At Sunnybrook, notes Dr. Hsieh, the ISH for GI cases is read by a GI pathologist. His lab also does SISH (silver ISH), which is used for the majority of cases. FISH (also read by the pathologist) is used as a backup. At St. Michael's, DISH (dual color ISH) is used.

Each method has its own peculiarities, he says. With FISH, "It's hard to know where you are on the slide, in my opinion. So if you use FISH, it's often quite helpful to have a good idea in your mind what the regular histology slide looks like in advance to help orient you." With SISH and DISH, which are both brightfield methods, it's easier to recognize the structures, he says.

Like a mini-lesson in relativity, GI HER2 testing also challenges notions of time.

On the one hand, though it may be a hoary saying, time is of the essence.
For patients whose life expectancy is a mere six months, "Spending three or four weeks mucking about to get the [HER2] test is unfair," says Dr. Streutker. She sees oncologists who are frustrated by having to wait for the test. "If we're going to do this test, we need to have it ready for the oncologists. It needs to be a reflex."

Indeed, says Dr. Hsieh, medical oncologists, who've been at the vanguard of this testing, express virtually no questions or confusion about the testing, other than, *How can we get labs to do the testing as early as possible?*

On the other hand, despite that enthusiasm, labs should be cautious about rushing into GI HER2 testing, says Dr. Hsieh. Move too fast, he says, and labs could easily run into some of the same problems they ran into during the early days of breast HER2 testing, including so-called scoring drift. Labs need to think hard about whether it makes sense for them to offer testing.

For those who decide to offer it, Drs. Streutker and Hsieh have one final piece of advice, which will no doubt resonate with fans of the old TV police drama "Hill Street Blues."

Says Dr. Streutker: "Be careful out there."

Adds Dr. Hsieh with a laugh: "Yes. That's the best way to put it—be careful."

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