Colorectal cancer next on HER2 horizon

Karen Titus

May 2023—Behold the common coin. Note its two sides, its easy flippability.

Here is Joseph Pizzolato, MD, with the first coin toss. Given the expanded use of biomarkers with a variety of tumors, and constantly evolving assays, how hard is it for medical oncologists to navigate testing?

“It’s not difficult at all now,” says a cheerful Dr. Pizzolato, medical director of the comprehensive therapeutic unit of Sylvester Comprehensive Cancer Center, University of Miami Health System, as well as medical director of the Aventura satellite at Sylvester.

With third-party companies integrating test ordering directly into electronic medical records, he adds, “It’s getting even easier to order tests and see the results.”

Agreed, says his colleague Rhonda Yantiss, MD, director of surgical pathology, Department of Pathology and Laboratory Medicine, University of Miami Miller School of Medicine. And therein lies the problem. “It’s kind of a mess,” she says.

In practice, precision medicine is becoming both more and less precise. This is playing out in a number of scenarios, but these days HER2 testing in colorectal cancer offers an especially vivid example of the complexities labs face.

Stage four colorectal cancer itself is complicated. Some patients will respond to the EGFR inhibitors such as cetuximab. But lacking a correlation between EGFR immunoexpression and a therapeutic response, “there isn’t any value to performing immunohistochemistry for EGFR when considering a targeted therapy,” Dr. Yantiss explains. “We do look for downstream alterations in EGFR-mediated signaling, particularly *KRAS* mutations,” since the presence of such mutations renders EGFR-targeted therapies ineffective. Close to half of colon cancers have such mutations. “Regardless of whether the receptor is blocked, if everything downstream of that is turned on, that cellular mechanism is still going to be running.”

HER2, which belongs to the same superfamily of tyrosine kinases as EGFR, is adding to the story. Some patients with wild-type *KRAS* still have cetuximab-resistant tumors and, in this situation, a lack of therapeutic response may be due to *HER2* amplification. In these cases, patients may respond to a combination of agents that target the HER2 receptor.

Recent clinical trials have established the usefulness of HER2 in advanced colorectal cancer therapies, says Antonia Sepulveda, MD, PhD, professor and chair, Department of Pathology, and medical director of the George Washington University Hospital laboratories. One of the first was the HERACLES trial, which looked at trastuzumab and the EGFR/HER2 inhibitor lapatinib. Another trial, MyPathway, evaluated trastuzumab and pertuzumab. The Mountaineer trial used trastuzumab and tucatinib, a combination that received accelerated FDA approval in January. Yet another trial, DESTINY-CRC01, looked at the antibody-drug conjugate trastuzumab deruxtecan. Other trials are ongoing.

Dr. Sepulveda’s summary of this work is sweet as well as short: “These trials offered significantly improved objective response rates on these patients.”

The number of affected patients, at first blush, might seem small. HER2 is present in three to five percent of colorectal cancers, says Dr. Pizzolato. That sounds small but isn’t, he says, noting that five percent is another way of saying “one in 20.” As a GI oncologist, “I certainly see more than 20 different colorectal cancers a week.”

In addition, Dr. Sepulveda says, the number rises, to about 14 percent, among cases that are *RAS/RAF* wild-type. “That’s a level of frequency that becomes more relevant in clinical practice.”

Dr. Rhonda Yantiss and Dr. Joseph Pizzolato at the Sylvester Cancer Center at the University of Miami Miller School of Medicine. Of cancer biomarker testing, Dr. Yantiss says pathologists need to be the gatekeepers. "But that means we have to improve our own efficiency and actively engage our oncology colleagues," she says. [Photo by: Joshua Prezant]

Stage four CRC is dire. Targeted therapies can work for many patients who didn't or no longer respond to cetuximab. Physicians and patients are eager to test for HER2 overexpression or amplification.

"This is pretty exciting as we begin to understand more and more we've got good targets for HER2 in colon cancer," says Dr. Pizzolato. "We're getting much better at finding niches for targeted therapies. But we can't get better unless we test"—not only to plan first-line and subsequent-line therapies, he says, but also to look at clinical trials options. "Testing allows us to pick our regimens wisely."

But test how?

And given that recent data indicate the majority of labs are not testing for HER2 in these cases, an equally big question looms: Why not?

Testing for HER2 seems to be a self-evident step, but like a recipe instruction to "Season to taste," there's nothing formulaic about it.

A 2021 CAP survey (Hagemann IS, et al. *Arch Pathol Lab Med.* Published online Dec. 20, 2022. doi:10.5858/arpa.2022-0229-CP) assessed current testing practices for ERBB2/HER2 in colorectal carcinoma as well as endometrial serous carcinoma. For CRC, 20.2 percent of responding labs (239 of 1,185) performed in-house HER2 testing.

Of the remaining 946 labs not doing HER2 testing, 8.4 percent said they planned to start in 2021, 7.2 percent planned to start after 2021, and 64.2 percent said they had no plans to start.

The results weren't necessarily surprising to coauthor Anna Yemelyanova, MD, who instigated the study as a member of the CAP Molecular Oncology Committee. "Our expectations, unfortunately, were fulfilled," says Dr.

Yemelyanova, chief of gynecologic pathology and professor of pathology and laboratory medicine, Department of Pathology and Laboratory Medicine, Weill Cornell Medicine.

Such low numbers are concerning to medical oncologists as well. Says Dr. Pizzolato: “I can’t stress this enough: We have to keep testing. Less than 100 percent doesn’t cut the mustard. We need every single person tested in the metastatic setting.”

For those that were doing testing, 82 percent of them did so only at the clinician’s request. Less than 10 percent (8.4 percent) said they performed it for all metastatic cases. The most commonly used primary test was IHC (82.3 percent), with reflex to ISH for equivocal results.

Dr. Yemelyanova

The survey also pointed to variable practices among labs that were doing testing—again, this was not a surprise, says Dr. Yemelyanova, given the lack of formal guidance for HER2 testing in CRC. That forces labs to extrapolate and adopt practices from other tumor types that may have no relevance to CRC, at least based on some of the clinical trial data.

Breast cancer testing algorithms, for example, have benefited from abundant data, multiple rounds of guidelines, and refinements in how results are reported. Attempts to expand testing to other tumor types, including CRC, are playing catch-up as targeted therapies take off. “We have to be careful about extrapolating practices to other tumor types as they might be biologically different tumors,” says Dr. Yemelyanova, “and the well-established trial-proven algorithms that work for some tumor sites may not work for others.”

Moreover, she notes, as the breast guidelines evolved, the thresholds for scoring have changed significantly. Unless pathologists are tracking updates closely, it can be hard to stay current, creating another challenge for anyone trying to adapt breast thresholds to colorectal cancers.

And though the guidelines for gastroesophageal and GE junction testing may seem to be applicable to CRC, some clinical trials, such as HERACLES, used a different scoring scheme, Dr. Yemelyanova says.

In the CAP survey, 49.4 percent of labs reported using 2016 CAP/ASCP/ASCO HER2 guidelines for gastroesophageal adenocarcinoma for scoring CRC HER2 cases, 30 percent followed the scoring used in the HERACLES trial, and 16.3 percent used the ASCO/CAP 2018 guidelines for breast carcinoma.

The lack of formal guidance may contribute to labs’ hesitancy to test, says Dr. Yemelyanova. “For validation in the lab, you absolutely cannot run a clinical trial,” although labs can try to duplicate the testing algorithm and scoring thresholds used in the trials.

One of the common approaches to validation is to send out specimens to a reference laboratory, then validate tests performed in-house against the external results. But those reference labs are also practicing in the setting of the absence of the formal guidelines, she says. The same drought will affect the entire kingdom—the royals, landed gentry, and peasants alike. “It’s the same sort of void,” says Dr. Yemelyanova. “It’s a major obstacle.”

Fundamentally, Dr. Sepulveda says, testing in CRC—when it’s done—can follow the well-traveled path of immunohistochemistry, FISH, or SISH. Also being added to the mix are next-generation sequencing panels that provide copy number amplifications of *HER2*. “It’s already being widely used in the clinic,” Dr. Sepulveda says.

Many laboratories have applied knowledge gained from experience with GE junctional and gastric adenocarcinomas to HER2 testing in CRC, says Dr. Yantiss. The ToGA trial mapped out the criteria used to assess immunohistochemical expression of HER2 among GE junctional and gastric adenocarcinomas, she says, as well as when to perform *HER2* in situ hybridization when immunostain results were equivocal. But the scoring system was not revalidated for CRC.

The HERACLES trial was the first big study to look at HER2 immunoexpression and *HER2* amplification in colon cancer. "The authors created an algorithm for assessing the immunostain results and when to reflex to in situ hybridization, and also defined situations when you didn't need to do that," says Dr. Yantiss. These criteria are somewhat different from those endorsed by the CAP, ASCO, and others for assessing upper GI tract cancers. Although there is a fair amount of overlap between the testing criteria used in the HERACLES trial and those promoted by the CAP, Dr. Yantiss says, they differ with respect to recommendations for in situ hybridization in equivocal cases.

Most of the trials follow the 2016 CAP guidelines for gastroesophageal adenocarcinoma. By this criteria, Dr. Sepulveda notes, a tumor is considered positive if it is HER2 IHC 3+ positive, or when it is HER2 IHC 2+ positive and positive by FISH.

The HERACLES trial, however, stands apart from the others, Dr. Sepulveda continues. A tumor is considered positive if it is HER2 IHC 3+ in greater than 50 percent of the cells, or (as in the other criteria) is HER2 IHC 2+ and amplified in greater than 50 percent of cells by FISH. "Importantly, the NCCN guidelines decided to recommend the use of HERACLES criteria," she says, adding, "We can argue that." These are stringent criteria. "There are some data from trials that seem to indicate that these very strict criteria do not seem to lead to better outcomes" compared with using the criteria in the 2016 CAP guidelines, Dr. Sepulveda says.

Guidelines for CRC molecular testing, including HER2 testing, warrant an update. The ASCP/CAP/AMP/ASCO guidelines were published in 2017, i.e. pre-HER2 (Sepulveda AR, et al. *Arch Pathol Lab Med.* 2017;141[5]:625–657). Dr. Sepulveda, the lead author on those guidelines, says, "We are in discussions to update them."

Dr. Sepulveda

Dr. Sepulveda says she suspects most pathologists are reporting 10 percent positive cells (as recommended in the 2016 criteria), not 50 percent. Regardless, she says, when reporting HER2 CRC results, "I would suggest that it's important to indicate what percentage of tumor cells are positive to help the oncologist decide what drug combination they would be using for that patient."

There might be another stair to trip on as labs consider expanded HER2 testing: HER2-low. A recent study (Lang-Schwarz C, et al. *Pathol Res Pract.* 2023;244:154417) looked at more than 300 CRC cases (stages one through four). Nearly half were defined (using the HERACLES criteria) as HER2-low (IHC 1+ or 2+/FISH negative). Compared with HER2-negative cases, says Dr. Yantiss, "they tended to have more tumor-infiltrating lymphocytes, which we know is a good prognostic indicator, as well as less tumor budding, which is an adverse prognostic indicator.

"It's too early to tell if this is going to mean anything in the colon, but people are talking about it," she says.

Dr. Sepulveda spies another fork in the road. Until now, the standard HER2 testing practice of doing IHC, followed by FISH, has worked well and stood the test of time, she says. But the old order could be upended, given the eagerness with which medical oncologists are turning to NGS panels.

Dr. Pizzolato says he and his colleagues would like to see pathologists order NGS automatically at the time of surgery. “It’s enormously helpful when the patient comes to us and we know who has a *BRAF* mutation, *KRAS* mutation, *NRAS* mutation, DNA mismatch repair status, and who has *HER2* amplification. In modern-day oncology, this is part of the diagnosis, just as much as knowing the difference between adenocarcinoma and squamous cell carcinoma.”

On the other side of that truth lies an equally important one. “There’s still very limited data in terms of studies looking at correlations of *HER2* copy number gains by NGS compared to results of immunohistochemistry,” Dr. Sepulveda says. One study showed that IHC 3+ expression correlated with copy numbers of six or greater of the *HER2* gene. “But there are many different NGS panels,” she says. “So we don’t know yet how universal or how standardized the copy number threshold can really be, or whether it has to be based on each specific panel,” given that each panel could entail different technical and/or informatics pipelines for determining gene copy number. In short, copy number gain reported by NGS will need to be standardized and guidelines established for clinical practice as well as for other biomarkers in CRC.

Even as the shift toward NGS appears imminent, “It is not a miracle test,” Dr. Sepulveda says. “Test results in general are stronger if we make use of the armamentarium of assays that provide complementary information,” including IHC, FISH, PCR, and single assays, such as microsatellite instability.

“Pathologists tend to stay on the conservative side,” Dr. Sepulveda says, “and if they’re not asked to do the test, they won’t do the test.” Medical oncologists might be eager to pick up the pace, however. “They may send NGS testing to commercial laboratories,” she says, “because they just want to do the large NGS panel that local labs do not do in-house.”

As Dr. Pizzolato notes, easy ordering is a boon for him and his colleagues. But where oncologists see a fluffy omelet, pathologists may be looking at a dozen broken eggs.

“Our oncologist colleagues feel comfortable sending out NGS tests elsewhere, bypassing the local pathology laboratories,” Dr. Sepulveda says. This invites potential testing duplication, confusion, and delays. “We don’t know, did the oncologist order this test already, in some other lab?” Dr. Sepulveda asks. “I see that trend,” she says. “I see the commercial laboratories going to the oncologists’ offices and offering direct services.”

Among her concerns is the effect on turnaround times and impact on patient care. Send-outs almost invariably take longer and may delay treatment decisions.

Another issue is the pathologist’s education in academic laboratories. “If all these tests are going to be sent out, how are we going to train our pathologists of the future?” she asks.

In the early days, molecular testing had an all-roads-lead-to-Rome air about it. What *wouldn’t* end up in an institution’s molecular lab? More recently, however, the testing seems to be heading out of town on every road possible.

Even the fundamental-to-pathology issue of how to handle equivocal IHC results appears to be evolving. In the CRC HER2 setting, if a patient didn’t meet the eligibility requirements because the lab was using the more stringent HERACLES criteria, they might miss out on a successful treatment.

That’s the theory, anyway. In practice, however, “I’m not really sure it matters that much,” says Dr. Yantiss. “In the modern era, so many of these patients with advanced-stage colon cancer are getting these large-panel NGS assays that include copy variant numbers as well.” This will likely pick up *HER2* amplification that was missed because FISH wasn’t performed.

Dr. Yantiss is more concerned about another issue. When testing is sent out, “Labs don’t want to have to go back and face the block,” she says, speaking as if it were a firing squad. “Each time you face the block you lose a lot of tissue, or they need a lot of tissue for the send-out test.”

Dr. Yantiss would like to see pathologists and oncologists on the same page, she says, in terms of the situations for which clinicians will want an additional workup. So, for example, every time there's a stage four CRC, oncologists will want workups for *KRAS* and other mutations, as well as druggable targets and HER2. "Then when we get a biopsy of a tumor deposit, we can, at the time we create the block, cut unstained slides as well, then do those stains up front so the information is ready—and we don't have to face the block again."

She outlines a not-uncommon scenario, particularly given the recent rise in colorectal cancer among younger patients—that of a woman in her early 30s. "That's a devastating diagnosis. We routinely perform additional studies, such as DNA mismatch repair protein immunohistochemistry, to evaluate for Lynch syndrome and inform clinical decision-making." As the case unfolds and more questions arise, oncologists and patients may request next-generation sequencing with a large panel of genes that requires an additional 15 to 25 blank slides. There may be other requests that include some combination of immunohistochemistry and/or assessment for circulating tumor DNA, "often coming sequentially," says Dr. Yantiss. "So you'll get a request for 15 unstained slides, and then three weeks later there will be another request for another 15 to go to another lab.

"And with these small biopsies," she continues, "you can end up with nothing left in the block," which can be a huge problem when, a few months down the road, someone finds something else that might be a potential druggable target.

All of which puts labs in a terrible situation. "Nobody wants to be the one who can't perform the linchpin assay, the one that would have changed the course for the patient," Dr. Yantiss says.

As many pathologists are now seeing, at least one reference laboratory offers its testing directly to oncologists through the electronic health record. "They can order tests through pathology, or directly from the [reference lab] through the chart," Dr. Yantiss says.

She reports that these menus are very user-friendly, designed to make it easy for even nonspecialists to figure out what tests to order. "So, basically, the reference laboratories create a situation in which it is easiest for the oncologists to simply order everything, right?" says Dr. Yantiss. "The [company] does everything for them, which makes it even more desirable." That often leads to redundancy, she says. When the oncologist orders NGS, they might fail to notice that they are also ordering an IHC panel that includes mismatch repair proteins, PD-L1, and HER2. "Which are things we have already done, in many cases, internally. So there's a huge waste of resources."

As she considers this all, Dr. Yantiss is firm: Pathologists need to be the gatekeepers of testing. "But that means we have to improve our own efficiency and actively engage our oncology colleagues."

It's almost impossible for labs to keep up. She recalls the 50-gene NGS panel offered at her previous institution, Weill Cornell, where she was chief of gastrointestinal pathology. "It was not a suboptimal panel—it had almost everything on it that is druggable today, including extensive *RAS* testing. But if you're an oncologist or a patient and see you can choose a 50-gene panel, or a 150-gene panel, or a 500-gene panel, which one are you going to choose?" she asks.

That tendency is not going to change, she says. "Cancer is a life-altering diagnosis. As oncologists help their patients process that information, they want to pull out all the stops and get as much information as they can possibly get to devise the best possible treatment regimen. Oncologists are never going to say, *I'll just take the 50-gene panel.*"

Pathologists need to play the role of regulator, Dr. Yantiss says. "The waste associated with duplicity of testing for various molecular alterations and repetitive immunohistochemical stains can be cut down if pathologists take a more active role in overseeing the testing that is performed and limiting the amount of material that is sent for molecular studies."

Can that work? Yes, Dr. Yantiss says. "We're in the process of doing that here." Though outside companies may have little incentive to let local pathologists take back the reins, her clinical colleagues can see the benefit. "We're

trying to get our oncology group to all agree on one company to send their testing to. Once done, we will control the menu options and get better control on the amounts of material we send out."

Dr. Pizzolato is sympathetic to the cause. "Pathologists are the smartest people in the room, but they face a lot of impediments," he says. "The biggest one is when there's a lack of consensus among practicing oncologists. That can be daunting for pathologists."

How do you get oncologists to agree? Replies Dr. Pizzolato: "Ahahahaha." Pressed further, he concedes, "It's like herding cats."

"Our department doesn't currently offer in-house molecular testing," Dr. Yantiss says, "but even if it did, we'd likely be having the same conversation. The problem doesn't go away just because you offer in-house testing."

If oncologists can come to an agreement on where to send testing, the next step will be to negotiate to have the lab be in charge of test ordering, she says. "Then we can control how much material goes out and what the tests are."

If if if. Dr. Yantiss has seen firsthand how difficult these negotiations can be. "Everyone has their favorites," she says. Nevertheless, "If you can show the oncologists that we can decrease the turnaround time on these send-outs by three weeks on average, that's substantial. If we are chasing around, pulling all these slides, trying to find cases all the time, we are delaying the ultimate testing."

Like others, Dr. Yemelyanova sees the day-to-day challenges and can no longer separate them from the larger forces shaping them.

Pathologists who are involved in biomarker development and clinical trial support, she says, "definitely need to do a better job reporting exactly the algorithms and thresholds used." Until formal guidance is developed, "that can serve as evidence and something for labs to adopt or lean on."

Not one to mince words, she adds: "That's definitely on us as pathologists as a community.

"But that said," she continues, "our clinical colleagues often do not involve us early enough in the trial design." CRC and HER2 may be making the latest headlines, but it happens across the spectrum of biomarker testing. "The nightmare of PD-L1 is one example," Dr. Yemelyanova says.

The goal is for the pathology community to work with the clinical oncology community at the stage of trial design, she says. "Everybody thinks about discovery and, yes, it's exciting to live in an era of rapid discovery." But mountains of data aren't enough. "We rarely think about the test adoption in the field until we are ready to implement. And that needs to be thought of early on." In other words, don't ask where you're heading after you've been on the highway for days.

Everyone bears responsibility, she adds. "We need to recognize the complexity, the speed of development, and the need for thoughts about testing adoption early on—*not* after the fact." It simply doesn't work to await FDA approval before asking, *Where are we going next?*

That's the ideal world, "which we're obviously not in," says Dr. Yemelyanova. Instead, the perpetual chase continues, like watching Lucy and Ethel trying to keep pace in the chocolate factory.

Without these broader efforts, though, "Pathologists are put in the situation where they're forced to report on something that may be questionable," she says.

She's a realist, as is everyone. But the reality is getting much bigger for those in practice. While not dismissing the importance of pathologist-oncologist conversation, "At that stage it's also too late," says Dr. Yemelyanova. "There is FDA approval. There is a drug. They need a result. How can you argue with that?"

If a pathologist were to raise issues, the pushback could be strong, she says, and understandably so: "The reply

from my clinician might be, *I don't have anything else to give. Let's give this a try.* And it's a reasonable thought, absolutely. I understand this is often the last resort in treating advanced-stage tumors," Dr. Yemelyanova says. "We all want to give the patient a chance while maintaining high biomarker testing standards."

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