# Colorectal Cancer Awareness Month

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**Becca Battisfore:**

Welcome to the latest edition of the College of American Pathologist CAPcast. I'm Becca Battisfore, Content Specialist with the CAP. In this episode, Dr. Gladell Paner will be talking with experts in the field of gastrointestinal pathology.

Colorectal cancer is the third most common cancer, yet it is one of the most preventable when detected early. It is highly treatable. March is National Colorectal Cancer Awareness Month, which raises awareness about this disease emphasizing its prevention early detection, improvements in diagnoses, and advances in personalized treatment options. This is particularly important as recent studies have shown an increase in colorectal cancers in younger patients. This month's Cancer Awareness podcast will focus on how the CAP supports pathologists in developing their reports using cancer protocols and more recently biomarker templates.

Before we get into the questions, let's learn more about our guests. Dr. Paner, we'll start with you.

**Dr. Gladell Paner:**

Good afternoon and welcome to the Colorectal Cancer Awareness Month CAPcast. I am Gladell Paner speaking from the windy city of Chicago. I'm a GU pathologist at the University of Chicago, and in the past six years I had a pleasure of working with the Cancer Committee and the Pathology Electronic Reporting or the PERT committee.

**Dr. Dhanpat Jain:**

I'm Dhanpat Jain. I'm a pathology professor at Yale University School of Medicine. I have been practicing GI pathology for over 25 years and have been at Yale University, which is the tertiary care medical center with an attached cancer center. And I have been involved with the Cancer Committee as well for the last few years and I have been involved with the PERT committee as well as work on developing various Cancer Protocols for the CAP. So thank you for having us here.

**Dr. Adam Booth:**

Hi, I'm Adam Booth. I'm a academic GI pathologist at Washington University School of Medicine in St. Louis. I've been involved with the CAP for several years and I currently serve on the House of Delegates Steering Committee, the Federal and State Affairs Committee, and the Councils on Government and Professional Affairs and Membership and Professional Development. And happy to be here. Thanks.

**Becca Battisfore:**

Welcome and thank you all for joining the podcast. Dr. Paner. I'll let you take it from here.

**Dr. Gladell Paner:**

Thank you, Becca. So our topic for discussion today is on biomarkers for colorectal cancer, and I'd like to start with you, Dr. Jain, and this is just to give us a background on biomarkers for colorectal cancer. Can you describe to us how biomarker testing for colorectal cancer has evolved in the recent years and where do you see going in the term?

**Dr. Dhanpat Jain:**

I think that's a very good starting point as most of us who have been involved in this field that about 15 or 20 years ago, there were no biomarkers available for testing in colorectal cancer, at least routinely. Then the testing for microsatellite instability came in and initially it was unclear as to which test is the best test for the microsatellite instability. And centers were testing for both microsatellite instability by PCR as well as by immunochemistry. And the various criteria evolved during that time as to who should be tested for these biomarkers. Now we have come a long way. Biomarker testing for microsatellite instability most often by immunohistochemistry testing for the DNA mismatch repair enzymes has become a routine process and actually it has become sort of a universal testing for all newly diagnosed colon cancer. So this is a big progress in this field. Now in addition to MSI or DNA mismatch repair enzyme testing, other biomarkers have also entered the field and gradually are incorporated into routine workflow and guidelines are continually evolving to incorporate them in the routine practice.

**Dr. Gladell Paner:**

If we look at this time evolution, Dr. Jain, we're looking here like the past two decades. For us, we are in GU pathologists and at this point we're actually behind you guys. We don't even have a GU biomarker protocol. So historically this went through for how many years, this whole process that you mentioned?

**Dr. Dhanpat Jain:**

Yeah, I think I can date myself because when I started as a fellow, there were no biomarkers in colon cancer. And when I became a faculty, we were talking about some of the biomarkers around 2000 and gradually these evolved, as I mentioned in last few years, the guidelines from various societies and templates for reporting these biomarkers and standardization of the techniques have all evolved such that I think testing of some of the biomarkers have become fairly routine while some others continue to be modified and evolve as time goes by.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. And this is for you, Dr. Booth. So this is on the practice side of things. Can you talk about the most commonly used biomarker tests and we can include predictive and prognostic biomarkers for colorectal cancer that is used in your practice?

**Dr. Adam Booth:**

Sure. Thank you, Dr. Paner. The most frequently used biomarker test is that for colorectal cancer specifically is that as Dr. Jain mentioned about mismatch repair testing for microsatellite instability. And that's most commonly performed using immunohistochemistry for those mismatch repair proteins. And that's where we evaluate for loss of nuclear expression.

**Dr. Gladell Paner:**

Thank you, Dr. Booth. The panel that you have right now is basically just a MMR test?

**Dr. Adam Booth:**

Yeah, currently that's the standard of care is on new diagnosis of colon cancer to perform either MSI testing or MMR IHC. Recently, HER2 testing by immunohistochemistry has been recommended for metastatic colorectal cancers. And that's something that we see on requests sometimes.

**Dr. Gladell Paner:**

We can talk about the details of this test later, but at this point I'm going back to you Dr. Jain. So I believe you work with a team to develop the CAP biomarker protocol for colorectal cancer. Can you tell us how it was created and what work goes into keeping its current to advances in the science? And I'd be curious because you have a laundry list or you have several biomarkers in the list. In your opinion, which of the biomarkers in the protocol you consider as the most clinically essential?

**Dr. Dhanpat Jain:**

Yes. I think the development of this standardized reporting or biomarkers has been a great service provided by the CAP and it has taken several years to get to this current format. And actually I cannot take any credit for developing this biomarker template because it was developed more than 10 years ago by a lot of luminaries in the field who realized that testing for biomarkers had a lot of issues in practice where people were using different technologies, different reporting system, different criteria, interpreting these results was a problem and comparing results across laboratories was a problem and clinicians were confused and it created a lot of practical issues for treating physicians. So to address many of the issues, CAP had put together this team to develop this biomarker template, which I was involved in updating more recently with Dr. Burgart, Dr. Chopp, Dr. Bellizzi and others. And this template I'm sure will be continuously updated as new data becomes available, new guidelines become available, new information becomes available. And all I can say is that this is a moving field, one of the fastest moving field. In fact, awareness of all the changes that are taking place in this area are critical to people who are out there in practice, both from the pathology side and both other from the treating side as well.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. And thank you for your amazing work with your team, with the team for updating the biomarker protocol for colorectal cancer. And I just like to mention to our audience that the biomarker protocol is available for free. You can access it any time in the CAP website and also in that same website there is a email there in case you have questions regarding the content of the protocol. And at this point, Dr. Jain, I believe that the biomarker protocol is not a required protocol unlike the colorectal resection protocol.

**Dr. Dhanpat Jain:**

That's true.

**Dr. Gladell Paner:**

Yeah. So having said that, I'd like to go back to you, Dr. Booth. This protocol is important but not currently required from the user's perspective for pathologist. Can you tell us what benefit do you see in having a biomarker protocol for colorectal cancer in your routine practice?

**Dr. Adam Booth:**

Sure. Thank you, Dr. Paner. Yeah, I think bluntly it's like a standardization, right? And the uniformity in reporting of biomarker results, which can ultimately hopefully translate to facilitating better communication between us and those treating physicians so that the patients can get that next step wherever that may go. So if they have one biomarker result and we're able to demonstrate that clearly and concisely and accurately based on the information that's provided in the reporting protocol that can better facilitate patient care.

**Dr. Gladell Paner:**

Exactly. And that's the reason why we have these protocols and only for biomarkers, but also for resection specimens and biopsy specimens.

**Dr. Adam Booth:**

I always like to tell the residents the protocols are great because they tell you exactly what you need to say. And then also the explanatory notes are great there because it shows you why you need to say it. So it can be great for your everyday practice.

**Dr. Gladell Paner:**

Yes, absolutely. I agree to that. Let's go to the specifics of the test. So this is a question for you Dr. Jain. Can you describe to us the mismatch repair testing and when is this test indicated As a GU pathologist, the first thing that always comes to my mind of course, is Lynch syndrome to everybody, but I think this test is also now used to determine therapy. So I would like for you to expand on that as well.

**Dr. Dhanpat Jain:**

That's true, and I think earlier you had also asked about which are the most important tests in the colon cancer field as a biomarkers and testing for microsatellite instability is probably the most important and most currently used biomarker in the field of colon cancer. Initially this sort of property of the tumors was evaluated using a more cumbersome, technically challenging PCR-based assay and it tested the actual microsatellite instability. However, with time people figured it out that actually testing for the DNA mismatch repair enzymes by immunohistochemistry, which is much more readily available and much more easy to perform, gave almost the same results. And over time, the mismatch repair enzyme immunohistochemistry has actually become the first test to use the biomarker and also test for microsatellite instability indirectly in clinical practice. And as I mentioned earlier, testing for microsatellite instability earlier was based on certain guidelines or criteria which were initially called Amsterdam criteria, which was modified and went to several changes.

But now because of so many issues, particularly the lack of sensitivity of those criteria and the implication for therapies, the testing has become a universal test for all newly diagnosed colon cancer. And as I mentioned, this is now done with the testing for DNA mismatch enzymes or DNA mismatched proteins as we call them. And this test is available in most of the laboratories who can perform immunohistochemistry. It's an relatively easier test to perform and interpret. And now with a lot of experience, people have figured out even the commonly encountered problems with this test and how to overcome those issues. So I would say that this is something that is one of the most commonly used biomarker tests in the community as well as all practices, basically all kinds of centers. And initially this test was designed to identify patients with Lynch syndrome, which is the habitable cancer syndrome with very high risk of colon cancer. But it soon became apparent that this test also is important in response to certain therapies. For example, patients who have microsatellite unstable tumors are less likely to respond to 5FU-based chemotherapies. On the other hand, the tumors that are considered microsatellite unstable respond very well to certain immune checkpoint inhibitors. And these are two major factors in the therapy of colon cancer that are based on the testing for microsatellite instability or the DNA mismatch repair proteins.

**Dr. Gladell Paner:**

That's very interesting from in the course of time since it's used for Lynch syndrome now it becomes a predictive marker as well. And I'd like to go back to you Dr. Booth, so I'm sure you are interpreting, you are reading this IHC slides for MMR. So can you share us your experience in interpreting these slides? Do you have issues with any of the MMR protein IHC? To me personally when I'm reading IHC and the positive result is a negative staining, I'm a little bit cautious about that, especially we have to make sure that the first, the stain is working before we try to say that, oh, it's, it's really negative. So can you share us your experience? And the other thing is also some scenarios like if the staining is patchy or heterogeneous, how do you deal with those situations?

**Dr. Adam Booth:**

Thank you, Dr. Paner. Fortunately, so we're looking at four different IHC slides and that's for expression of those four different nuclear proteins, those mismatch repair proteins. And oftentimes it's usually pretty easy to interpret fortunately, and that's because based on what the protocol says, any positive reaction in the nucleus of a tumor cell is considered intact expression. So that alleviates a lot of the concerns that we might have regarding tumor heterogeneity and expression patterns regarding false positives, typically the slides will have a positive control tissue on there, so some other colon tissue that's known to be positive or to have expression. And then typically it's nice that typically background lymphocytes and background normal colonic mucosa can also serve as a positive internal control so that if you do see that loss of expression or a microsatellite or you see that loss of expression or loss of staining in the nucleus, you know that you can trust that answer. And if you're on the edge or you come up with something equivocal, maybe one thing you may repeat, the slide may reevaluate the stain.

**Dr. Gladell Paner:**

That's very helpful. Dr. Booth, those are good advices. I'm just curious, when you have a control tissue, do you put it together on the same slide with a test tissue?

**Dr. Adam Booth:**

So typically it would be on the same slide, but it would read further away from where the test tissue is from the patient's sample so that they can clearly be distinguished.

**Dr. Gladell Paner:**

Okay, thank you Dr. Booth. And I have a follow-up question for you as well. So we talked about the IHC staining for MMR proteins and when you are ordering the MSI testing, do you perform the IHC staining concurrent with the MSI testing or you ordered MSI testing after the IHC staining. And in terms of the MSI testing, do you have it now or do you send it out to a reference lab?

**Dr. Adam Booth:**

So in my practice, so I ordered the IHC first and use that. They do have the MSI testing in-house if needed, but I don't do it concurrently. I may too, if there's a discrepancy that I'm concerned about or I'm getting standing results that are questionable. So I may do the MSI testing then.

**Dr. Gladell Paner:**

Okay. Yeah. And I would like to loop that question to you, Dr. Jain, how about for you? How do you order these two tests?

**Dr. Dhanpat Jain:**

Yes. I think our practice is same as Dr. Booth mentioned, and I think that is more or less the most commonly used algorithm for testing for microsatellite these days. As I alluded to earlier, when the tests were evolving, people were doing both concurrently, but with time we realized that both tests are concurrent with their results. Over 95% of cases where you don't have to do both. And because of many advantages that DNA mismatch repair enzyme expression by IHC provides, this has become now the first go-to test for most laboratories. And we use the MSI testing by PCR by exactly the same kind of situations with Dr. Booth mentioned. Whenever there's a problem with IHC, the results are difficult to interpret or there's some other clinical concerns, we will try out the MSI testing.

**Dr. Gladell Paner:**

Thank you Dr. Jain. Let's go to a situation here. Say we got a result of MSI testing and it tells us that this is MSI high. My question is, does it occur only in Lynch syndrome or it can occur in a sporadic setting? And can you tell us the test how to distinguish that this test is because of a germline mutation or it's basically in a sporadic setting?

**Dr. Dhanpat Jain:**

So surprisingly, or contrary to one might think from our prior discussion, MSI testing identifies this subset of colon cancer where while the main objective earlier was to identify Lynch syndrome, actually vast majority of patients do not have Lynch syndrome. If one looks at the percentage of colon cancers that are microsatellite unstable, about 15% of the colon cancer are microsatellite unstable. Of that only about two to 3% of the patients have actually Lynch syndrome. So the vast majority of microsatellite unstable cancers are not Lynch syndrome. And this is largely because of the promoter methylation of MLH1, which is one of the DNA mismatch repair genes that causes silencing of this enzyme leading to the MSI high phenotype. And this can be easily identified or tested in clinical practice by other looking for DNA hypermethylation of MLH1 or another surrogate marker, which is looking for BRAF V600E mutation, which again identified about 70% of these non Lynch syndrome MSI high colon cancers.

Very, this is our sort of routine practice that whenever we have a patient with microsatellite unstable cancer where the DNA mismatch repair MLH1 is lost by immunochemistry, we will automatically test those samples for DNA hypermethylation of MLH1. Earlier we were testing the BRAF V600E mutation for the same purpose, but as it turns out the hypermethylation is a slightly better assay and we moved on from the BRAF testing to the hypermethylation assay very recently.

**Dr. Gladell Paner:**

So how often do you see this MSI high phenotype in the situation of sporadic colorectal cancer? Just to give us a numbers percent.

**Dr. Dhanpat Jain:**

Cases? Yeah, so about 15% of all colorectal cancers turn out to be microunstable and as I mentioned, only two to 3% of that population is only Lynch syndrome.

**Dr. Gladell Paner:**

That's actually surprising because we always talk about Lynch syndrome for this test. So I'd like to go back to you Dr. Jain, and this is regarding the interpretation of the results. And in a situation where there is a discrepancy between the MMR IHC test and the MSI testing, how do you deal with that discrepant results?

**Dr. Dhanpat Jain:**

I think that's a very good question. And as we all know in pathology, none of the tests are ever a hundred percent sensitive or specific. And it's true for both of these tests. DNA mismatch repair immunohistochemistry as well as micro stable by PCR and with experienced people have figured out that these tests are concurrent in about 95% of cases and somewhere around 5% or less. There are discrepancies between these two test results. And these are due to various phenomena. I mean any test can fail because of technical reasons, but there are other biologic reasons for these results to be discrepant. There are situations where the immunoreactivity of this four commonly tested DNA mismatch repair proteins is preserved due to the nature of the mutation while the function of those proteins is lost. So in a sense, when one tests such tumors for DNA mismatch repair immunohistochemistry, one might find preserved staining implying normal function, while the MSI testing may give a result which says that the tumor is microsatellite high.

So that's one of the situation where the results can be described. And the other situation is people recognize that certain defects in one of the enzymes, particularly micro MSH6, may result in a result in a defective or abnormal result by immunohistochemistry while the tumor may be micros stable. And this is also recognized that that may identify a separate population of Lynch syndrome, which could be missed by the MSI testing. So there are situations like that they're not very common but do occur in practice. And one of the solutions is that whenever these results in practice are not in sync with the clinical scenario or to what we are seeing in front of us under the microscope, the next best thing is to send out the test for the germline mutations which will pick up all those patients of Lynch syndrome. And there are all the array of situations where people have figured out there may be other rare or uncommon DNA mismatch repair produce that we don't test for and they may be abnormal are not picked up by our standard four panel of DNA mismatch repair proteins and we would miss those by our chemistry examination. As long as people are aware of these discrepancies and know about the, I think these should not cause any problem in clinical practice.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. So we're at less than 5%. There are several scenarios or causes that can result with this false positive testing and I would like to loop this question to you, Dr. Booth, if you have any comments for the same question?

**Dr. Adam Booth:**

No, I would just echo what Dr. Jain mentioned. So yeah, it can be, like you said, any lab test can, it's not a hundred percent oftentimes. So having different modalities that we can compare and hopefully come up with the same answer or either, and then follow that up with germline testing if necessary, gives us that opportunity.

**Dr. Gladell Paner:**

Yes, thank you Dr. Booth, and I think I may add to that, just the first probably instinct is to check the labels, right? They're matching if they're testing the same specimen from the same patient. So let's now switch to another biomarker. Let's talk about the RAS. Dr. Jain, can you tell us about the RAS mutational analysis and their current use?

**Dr. Dhanpat Jain:**

Yes, I think the KRAS gene testing was one of the next markers biomarkers that came into clinical practice after the microsatellite instability. And initially people were testing only for the KRAS mutations, especially some of the hotspot mutations that sort of predicted response to some of the anti EGFR directed therapies. Soon it became clear that it was not only the KRAS but in the entire RAS pathway map kinase pathway and some of the downstream molecules alterations could result in the similar kind of phenomenon. So with time, this mutational analysis was sort of expanded to include RAS pathway and a couple of other molecules which are more downstream trying to evaluate the patient for potential of anti EGFR therapies.

**Dr. Gladell Paner:**

Thank you Dr. Jain. And I would like to go back to you Dr. Booth. This is about receiving this request for this biomarker, particularly for the RAS. So in your practice, how do you receive this request to test RAS for colorectal cancer? Does it comes from the clinics or you just have a say reflex bordering to have this test for certain stage of tumors?

**Dr. Adam Booth:**

So for this, we'll do usually by clinician request, so the oncologist will contact me and request that test and then that's a send out for us.

**Dr. Gladell Paner:**

And in my experience, I did a bit of GI before, I mean this is okay if they ask one test a week or something like that, but if it becomes multiple or several, the request becomes several. It can be sometimes it can be, I don't want to use the word disruptive, but it can be in our practice. So is there a mechanism in your practice like they order it say in epic and then there's a notice for you guys or they just notify you through emails the way we did it before?

**Dr. Adam Booth:**

Yeah, so different places that do it, different ways I guess handle these kind of send out tests, but so we'll get the request or they'll at the same time as they, maybe they'll contact me about it. They'll also contact our send out test staff, support staff and so that we can coordinate, make sure I select the right block so that we can do the testing on the appropriate tissue slide as well. But as you mentioned though, it can be kind of burdensome at times with the different sendout tests, so trying to streamline that process does help. And at the same time though, as we get these requests, it also is good to sometimes maybe that test is not indicated, so have that conversation with the oncologist as well to get a little more of the clinical history or the background behind it. So maybe you can guide them as well as maybe this test instead or so on.

**Dr. Gladell Paner:**

That's a very good point. Yeah,

**Dr. Dhanpat Jain:**

So I would sort of add that I think this has been our practice as well for quite some time where we always did DNA mismatch repair or MSI as the starting biomarker test for all colon cancers and only do the other biomarkers like the RAS testing or HER2 know based on the request from the clinicians. But as time has gone by and things have evolved and standard of care is also changing and for the other reasons that you mentioned about the delay and the confusion created by who is ordering where their orders going through, we have put this in our reflex testing scenario now where any patient with advanced colon cancer, especially metastatic colon cancer, automatically gets tested for the RAF panel as well as HER2 new. And this has certainly not only streamlined the process for us but also cut down the turnaround time because these now have become the standard of care for most of the cancer centers. Were seeing advanced colon cancers very frequently.

**Dr. Gladell Paner:**

That's a great advice to our listener, Dr. Jain. So you said in the metastatic setting and only or high stage T3?

**Dr. Dhanpat Jain:**

Yes. So I think this indications has evolving and I think some of them are applicable to high stage colon cancer stage three or more, but certainly for metastatic colon cancer. So at least we routinely test these tests by reflex ordering on metastatic colon cancer and other situations we wait for the clinicians to tell us if they need the test.

**Dr. Gladell Paner:**

Thank you. Doctor. I'm not familiar with the actual the test itself, but can this be done also on cytology specimen, like a lymph node of a sample, the metastatic colon cancer, can it be done or only it needs tissue?

**Dr. Dhanpat Jain:**

I think you're talking about all the biomarkers or only the KRAS. So I would say that actually probably all the biomarkers including the DNA mismatch repair and microsatellite instability can be done on cytology specimen. The problem is that in cytology specimen you get sometimes a much scanned material and interpretation of some of the tests which are immunohistochemistry based can be challenging. So we do test even those tests on cytology material sometimes when they're biopsying a lymph node or liver metastasis. But certainly the mutational analysis for RAS pathway can be done on cytology material much more easily and not a problem.

**Dr. Gladell Paner:**

That's good to hear. Dr. Jain, I like to move to another biomarker, HER2, and this is for you Dr. Jain. Can you tell us about the scenarios where pathologists or clinician might order HER2 testing in colorectal cancer and are there anti HER2 for colorectal cancer that is approved by the FDA?

**Dr. Dhanpat Jain:**

So I'll start with your last question first that yes, there are FDA approved therapies for HER2 positive colon cancer and that demands that we test colon cancer or HER2 expression. And this is generally for again, advanced colon cancer or metastatic colon cancer. And as I mentioned earlier now, this has become a more or less reflex testing for us for patients with advanced colon cancer or metastatic colon cancer. And we follow the guidelines that have been set by the CAP in terms of how to test and how to report HER2 new in colon cancer, which again remains an evolving field. This is an area which I think people still struggle with in some ways, but we have come a long way from where we started.

**Dr. Gladell Paner:**

Thank you Dr. Jain. I believe this is one of the latest addition right in the biomarker protocol, if I'm not mistaken, the HER2?

**Dr. Dhanpat Jain:**

Yes, HER2 has been a recent edition in the biomarker and the CAP along with the ASCP also worked on providing some guidelines as to how to report HER2 new in the gastric and GE junction cancers. Interestingly, some of the same guidelines are now also applicable to colon cancer in addition to some other guidelines that have been published. So I think people who are looking towards how to report or how to evaluate HER2 new in colon cancer, they can look at the cancer reporting template which mentions these guidelines as well as look at the other publications that also clarify some of the issues that are problematic in this area.

**Dr. Gladell Paner:**

Thank you Dr. Jain. And since this is a relatively new test, I would like to go back to you, Dr. Booth and ask this question, do you perform HER2 testing for colorectal cancer in your practice? And if you do, tell us, share your experience in interpreting IHC stain for HER2. When do you call it as positive?

**Dr. Adam Booth:**

Sure. Yeah. So typically on request, and usually per the guidelines it's for metastatic colorectal cancer. So usually on request from the oncologist most frequently and in my practice I've followed the HERACLES diagnostic criteria. So a positive would be considered a three plus, which would be essentially three plus intense staining in cells and greater than 10% to 50%, less than 50% of the cells. So in a circumferential or basal lateral pattern, it does vary. It is a little different than the CAP guidelines for interpreting gastroesophageal HER2 and from interpreting the breast cancer. So that's something to be aware of and so I would urge anyone that's interpreting these and is not familiar with it to check out those guidelines or the protocols so that they make sure they do it appropriately so the patient can or cannot get the treatment.

**Dr. Gladell Paner:**

I'm glad you pointed that out, Dr. Booth. Dr. Jain, if you want to add something.

**Dr. Dhanpat Jain:**

Sure. I think as Adam mentioned, there is some variability in how the HER2 in colon cancer is interpreted. And this is largely a result of different criteria used in different trials and it certainly creates some confusion in the minds of the oncologist as to which criteria are being used. And that's where the cancer synoptic protocol from CAP is very helpful because it has an option of using either of the criteria that are out there in the clinical practice and it helps you to specify which criteria you're using so that people are aware of your positive and negative cutoffs. And I think as time goes by, probably we'll have more updates and more clarity on these criteria. But as of now, I think people are using both the criteria that Dr. Booth met and the HERACLES criteria and the GA criteria. And so far it looks like both have fair good, but they're not exactly identical.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. And this is a follow-up question since we talked about the IHC HER2 test. How about the in situ hybridization? This is a question for both of you. Can you share us your experience about the in situ hybridization for HER2

**Dr. Adam Booth:**

That's going to be reflexively done just like previously done in the gastric or breast, whenever you have an equivocal result and which you can in the HERACLES schema, and so then we would reflexively go to in situ hybridization.

**Dr. Dhanpat Jain:**

Our practice is also the same that anytime we report a two plus or so-called equivocal HER2 result by either of the criteria, it goes for FISH testing by in situ hybridization, and if it is amplified by FISH, then it becomes eligible for the therapy. If not, it's considered then negative.

**Dr. Gladell Paner:**

So equivocal is a two plus.

**Dr. Dhanpat Jain:**

Yes.

**Dr. Gladell Paner:**

Thank you Dr. Jain and thank you Dr. Booth. So there are other markers there in the or biomarkers in the CAP protocol, and this is a question for you Dr. Jain. Can you briefly tell us about these other predictive biomarkers that in colorectal cancer, and I'm not really sure if they are already being used or they're still in the horizon. Can you describe or can you tell us something about this new biomarkers?

**Dr. Dhanpat Jain:**

So I think the current CAP protocol also has a couple of biomarkers that include the PIK3CA and the PTEN. These are molecule or targets that are downstream from RAS and RAF and any mutations in these molecules also make the anti EGFR therapies to be less effective. And hence testing for these has role in the cancer biomarker testing in the field of per colon cancer. However, having said that, I think role of some of these biomarkers is not yet entirely clear and some centers don't clearly test it. At our center, we have a panel that includes all of these biomarkers and we have been very extended panel that goes beyond these biomarkers. So these are tested and the results are available to clinicians and eventually they are in the best position to decide how to use those results and how to modify the therapy based on their interpretation.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. So based on our conversation, the future looks bright for the biomarkers for colorectal cancer. So let me ask this question. What excites you on the horizon for biomarker use in colorectal cancer diagnosis?

**Dr. Dhanpat Jain:**

I know this is a good time for exploring various biomarkers for cancers in general, and I think one can either blame or appreciate the application of Next-Gen sequencing that has become now more widely available, more widely used for testing cancers including colon cancer. So overall, I would say that use of these technologies certainly makes the future very, very optimistic in terms of finding new biomarkers for not only colon cancer but other cancers as well. With regards to colon cancer, I would say that at least as of now, the possible biomarkers that are very promising are limited. I would say to best of my knowledge, I think one of the biomarkers, which is called Claudin 18.2, which is right now being tested in gastric and GE junction cancers and has an FDA-approved therapy is something that is evolving in other GI cancers as well. It may find application in other GI cancers including colon cancer. We have to wait and see, similarly I think application of liquid biopsy and testing for circulating DNA in tumor DNA in blood also is promising and will find application routine clinical practice hopefully in near future. But in terms of very specific single agent or single target based biomarkers in colon cancer right now, to me looks like we are still far away from that. Probably there are many potential things on the horizon, but nothing that I can specifically mention as being highly promising. And for you, Dr. Booth.

**Dr. Adam Booth:**

Yeah, thank you. I think it's an exciting time. I think as Dr. Jain mentioned earlier in his career, they were just developing some of these biomarkers for MSI and MMR and then now here we are. It's kind of standard of care. So hopefully we can continue on that trajectory and towards more personalized treatment approaches for patients as they see who responds best to what. And some of these testing for these different biomarkers and mutations allows identification of better therapies. So as time goes, I hope that these things can be expanded. I hope they can also be integrated into practice in ways that are efficient for everyone to use so that they can actually improve patient outcomes and care.

**Dr. Dhanpat Jain:**

The CAP has played a very important part in this whole biomarking testing field. As the field keeps on evolving and new markers keep on coming on the horizon, the CAP has keeping an eye on what's coming up and developing protocols to address those issues and standardizing the reporting of various biomarkers and bringing this information out to the practicing pathologist. So I would certainly comment the role of CAP in keeping the practicing pathologists informed as well as providing them tools to report these various biomarkers in a fashion that is standardized, easy to interpret and clinically useful to the oncologist.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. I agree with that with your statement 100%. And my next question is, this is at the practice setting. From your experience for the two of you, what do you think are the main challenges when setting up in-house biomarker test in the laboratory? I'll start with you, Dr. Jain.

**Dr. Dhanpat Jain:**

Well, these days in the current environment where there are so many constraints of regulatory agencies, financial constraints, staffing issues, and technological challenges, I would say the setting off biomarkers can be very challenging or sometimes fairly easy for any laboratory. One of the most challenging things is when a biomarker is put into the practice, which has not been very well vetted in the clinical trials or where there are competing technologies to test the same biomarker or there are constraints put by the FDA as to the use of the biomarker in a certain way. This makes a application of those biomarkers in practice very challenging because setting up any tests requires rigorous validation of the test. And whenever there are competing technologies available or competing antibodies available or certain restrictions put by other regulatory agencies, standardizing these tests or making them available to the clinicians becomes difficult.

On the other hand, there are certain tests which are very simple, IHC based tests where you have easy availability of the antibodies or commonly used antibodies with little problem and the ease of interpretation or the cutoff criteria so that are easy to interpret. Those things become much more readily established in any clinical practice and are easy to implement as well as report. So as pathologists, of course, we are always looking at things that are easy to implement and easy report or pass on to somebody who is in a molecular lab where they can put in a machine and get the results out and we don't have to deal with it. So either of those two situations, but that is very ideal setting in real life. I think as I mentioned, there are several challenges that we have to deal with.

**Dr. Gladell Paner:**

Thank you, Dr. Jain. And for you, Dr. Booth?

**Dr. Adam Booth:**

Yeah, I would echo what Dr. Jain said. I think it depends on as far as bringing in a new one, depends on what kind it is. Is it immunohistochemistry stain? Is it like MMR or is it involve other instrumentation or equipment? Is it a molecular test? Is it a PCR based test? So it kind of depends on where's it going to go in the laboratory, who's going to run it. But I think as long as you identify that and then you have the right technicians and pathologists helping to validate that you can get it off the ground. But of course there's the different red tapes and hoops and things you need to jump through to get it correctly done.

**Dr. Gladell Paner:**

I agree. Dr. Booth. Thank you. And this is my last question. So this is kind of sort to wrap up our discussions on the different biomarkers. So from your experiences, which among these biomarkers for colorectal cancer would you recommend to our colleagues in the community practice? So our colleagues that are setting up their labs wants to have the addition in their menu, a biomarker for colorectal cancer. So among these that we discussed, which of those do you recommend?

**Dr. Adam Booth:**

And you got to have the MMR, you're going to have testing for mismatch repair proteins or the microsatellite instability status. So it's really just standard of care on all newly diagnosed colon cancers.

**Dr. Gladell Paner:**

Thank you, Dr. Booth. And for you, Dr. Jain?

**Dr. Dhanpat Jain:**

Yeah, I would echo what Adam said. I think at this point of time, testing for microsatellite instability either by IHC or PCR assay is the minimum required by any practice, whether in community or academic setting. It could be a test that you might be doing on your own lab or is a sendout test, but I think it is now required testing in auto colorectal cancers. The other biomarkers that we mentioned are also sort of now becoming more and more in routine practice as sort of required tests. And it depends on your setting. In advanced cancer centers where you do see a lot of advanced colon cancers, these are becoming gradually routine tests that the oncology expect us to do reflexly in all advanced colon cancers. While in community practice, I think it might still be a situation where you wait for the clinician or oncologist to request because for a variety of reasons. I mean, the patient may not be coming to their center for further treatment or the patient may not be eligible for any other further treatment. So it depends on the clinical setting. And I think these indications and the criteria will continue to evolve that will guide how we do these other biomarkers and some of the other biomarkers that might be evolving on.

**Dr. Gladell Paner:**

I know we can go on and on here, but unfortunately we're running out of time. So I would like to thank both of you, Dr. Jain, Dr. Booth, for giving us your excellent perspective and for sharing your experiences on biomarkers for colorectal cancer.

**Dr. Dhanpat Jain:**

Thank you. Thank you so much for having us on this podcast. It was a pleasure having interacted with both of you. Thank you.

**Becca Battisfore:**

Yeah, thank you so much for a lively conversation and for sharing your insights on the cancer protocols and biomarker templates. And I want to thank you all for listening to this CAPcast. You can find links to the CAP's Cancer Protocols in the episode description along with other resources mentioned during the episode. If you have questions or comments about any of the protocols, please email us at CancerProtocols@CAP.org. And for more information about the CAP visit cap.org.