# Head and Neck Cancer Awareness Month

April 12, 2024

**Becca Battisfore:**

Welcome to the latest edition of the College of American Pathologists' CAPcast. I'm Becca Battisfore, content specialist with the CAP. In honor of April being Head and Neck Cancer Awareness month, our guest host, Dr. Gladell Paner, will be talking with two surgical pathologists who specialize in head and neck pathology.

To start things off, I thought I'd give a little background information on this topic. There are many different types of head and neck cancers. The most common affect the squamous cells in surfaces that line the head and neck such as the mouth, throat, and voice box. It can also affect salivary glands as well as sinuses, muscles and nerves. Now that I've defined what is considered a head and neck cancer, it's also valuable to know what is not and that would be cancers of the brain, eyes, esophagus, thyroid, and skin. Head and neck cancers account for about 4% of cancer cases in the United States, or an estimated 67,000 cases annually. Screening and prompt attention to concerning symptoms are important to treating head and neck cancers. This month's cancer awareness episode will focus on how pathologists and biomarker reporting templates contribute to providing care teams with the information they need to make treatment recommendations.

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

**Dr. Gladell Paner:**

My name is Gladell Paner. I'm speaking from the great windy city of Chicago. I am working at the University of Chicago as a GU pathologist. In the past six years, I had a pleasure of working with the CAP Cancer Committee and the Pathology Electronic Reporting, or the PERT, committee. I am also involved in the creation of the CAP Gleason grading courses. Used to be part of the CAP Advanced Practical Pathology Program or the AP3 for prostate cancer and I'm happy to announce that we have exciting new CAP products and courses that will be out this year, so stay tuned for that.

**Dr. Raja Seethala:**

Hi greetings. My name's Raja Seethala. I'm a head and neck and endocrine pathologist at University of Pittsburgh. I've been on the CAP Cancer Committee for almost a decade, both on head and neck and to variable extents on endocrine for the Cancer Protocols, but I'm also part of the CAP biomarker working group and the CAP HPV testing in upper digestive tract cancer expert panel.

**Dr. Nicole Cipriani:**

Hi, I'm right down the hall from Dr. Paner here in the University of Chicago. I also do head and neck as well as associated endocrine pathology and bone and soft tissue. Very excited to have recently joined the CAP Cancer Committee and I will be working on those predominantly endocrine but also hope to participate in the head and neck and VST checklists as well, so looking forward to it.

**Becca Battisfore:**

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

**Dr. Gladell Paner:**

Thank you, Becca. Dr. Seethala, our first question is for you and this is to give us a background overview of biomarker testing for head and neck cancer. So the question is, can you describe how biomarker testing for head and neck cancers has evolved in recent years and where you see it going in the near term?

**Dr. Raja Seethala:**

Absolutely. With the exception of HPV testing, back initially during the first iteration of the biomarker protocol, biomarker testing for head and neck cancer was initially largely restricted to diagnostic purposes and classification. In the past few years, this role did take off. There are numbers of defining supportive molecular alterations for several sites, including salivary gland, sinonasal tract, all across the head and neck, and this has expanded almost exponentially. Tied to that to some extent though is testing has then also taken on more of a prognostic and also even theranostic role as a increasing number of these molecular targets. The molecular operations offer targets, so to speak for intervention. I think this trend towards a therapeutic application in general will persist in the next few years. I think also in the era of immunotherapy, other things will become more important. Biomarker testing, querying the tumor microenvironment, it will become more nuanced rather than a single test. It may be multiplex and we can talk about that later as well. And this is increasingly incorporated into a clinical workflow not only from a trial perspective but also actually as a baseline.

**Dr. Gladell Paner:**

Thank you. Raja. And I believe about five years ago there was a CAP guideline for HPV testing in the head and neck.

**Dr. Raja Seethala:**

That is correct.

**Dr. Gladell Paner:**

Yeah. And of course that was also kind of pivotal integrated into the CAP biomarkers, the recommendation that came out from that.

**Dr. Raja Seethala:**

Yeah, exactly. That was a key component. Arguably it's the most important biomarker testing that we do currently for head and neck and there are updates to the guidelines underway as if you check the website and we have been working on that. That should be out soon, hopefully.

**Dr. Gladell Paner:**

That's great, thank you. Raja, I'd like to switch to Nicole, and this is at the practice side of things, the application. Nicole, can you talk about the most used biomarker test and this includes predictive and prognostic biomarkers for head and neck cancers that is used in your practice or our practice?

**Dr. Nicole Cipriani:**

Our practice, exactly. Perfect segue. Right, so I think the first thing that came to my mind was certainly HPV testing as you said. And so the options for HPV testing in head and neck as advocated specifically, either mostly in the oral pharynx or in cancers of unknown primary in the neck would be things like p16 mRNA in situ hybridization or in certain cases where the subtype might need to be known might be something like DNA PCR or even sequencing in fact, not only related to portending good prognosis in these patients, but for the potential to enter into clinical trials, which we also have here ongoing at the UofC for deintensified therapeutic regimens. Probably the second one that popped up in my head that I know is not on this list but is becoming equally as common is PD-L1. I think this answer goes for probably every single organ system, but it is very, very commonly requested by clinical services for eligibility and immunotherapy regimens. I think the other therapeutic markers that we are asked to perform came to my mind for things like salivary neoplasms in terms of androgen receptor, HER2 or even targetable fusions like NTRK, potentially even RET. But depending on the tissue type and tumor morphology, our testing may be directed in any of those veins.

**Dr. Gladell Paner:**

Thank you. Nicole, how about examples of diagnostic biomarkers that you're using?

**Dr. Nicole Cipriani:**

Diagnostic biomarkers might include things for either salivary or sinonasal neoplasms in which distinction from benign to malignant might be useful such as MAML, MAML2 in mucoep in the differential diagnosis of a benign cystic lesion in sinonasal cancers, as I'm sure we will get to eventually in terms of pinning down epithelial versus mesenchymal neoplasms like for example at adamantinoma-like Ewing sarcoma, which may masquerade as a carcinoma. So in those cases, testing may be implicated.

**Dr. Gladell Paner:**

Thank you. Nicole. I'd like to switch to Raja. Raja, I believe you work with the team to develop the CAP biomarker protocol for head and neck cancers. Can you tell us how it was created and what work goes into keeping it to current advances in the science?

**Dr. Raja Seethala:**

In about 2016-2017, the first head and neck biomarker protocol was created. We created it as a paper version with a limited set of tests mainly for diagnosis and classification when it was modernized around 2019, 2020 roughly. When we initiated the effort to convert it into an electronic format, we expanded the number of tests that are included biomarkers, and we covered both prognostic and theranostic applications.

As Nicole previously indicated, AR, NTRK fusions and alterations were included. The creation and maintenance of this protocol is done as with many CAP protocols and initiatives through the efforts of content experts as well as the biomarker work group who supervises this. Basically the overarching goals were to ensure practicality for all users harmonization of syntax across different protocols and also compliance with the existing guidelines like HPV testing that we talked about already, and as well as standardized reporting of markers such as PD-L1 or even HER2 across different organ sites whenever possible.

Obviously it's not possible for every site. There may be individual site specific criteria, but we try to, as part of the biomarker work group, we try to keep all those aspects in mind when creating and maintaining a protocol. The content expert along with the biomarker work group and staff will meet at least monthly, sometimes even more frequently by electronic mail. Then we'll create the updates in both the content, the format, and now at this point since it's electronic, we package it into a usable ECC type format. The updates generally have been determined by priority for the biomarker work group and as well as need. And despite all the efforts we're actually behind in terms of the markers that we cover. Technically speaking, just because the field has advanced so much, there are a lot of markers that at least in a busy head and neck practice setting that we utilize very frequently that are not exactly covered. The good news is that structurally reporting of many of the markers is very similar basically. So you have a fusion, you have different mechanisms to report it depending on what assay you've utilized. So a novel fusion can be modeled in the same fashion that inevitably next updates we'll cover these. I think we got all the important markers that are frequently used.

**Dr. Gladell Paner:**

Thank you, Raja. And I'd like to emphasize to our audience that the head and neck biomarker reporting template is available for free at the CAP website and I'll take this opportunity to thank and acknowledge your co-authors. You have an amazing group of excellent pathologists here. So in addition to Raja, the other authors of the head and neck biomarker reporting template are Frank Schneider, Alexander Baras, Brett Baskovich, Patrick Fitzgibbons, George Birdsong, and Joseph Khoury. And they work with all these individuals and they are amazing group of pathologists. And at this point I believe for the biomarker protocols, not one of those are required. So they are optional at this point. And having said that, I'd like to switch to you Nicole, and this is from the user's perspective of the biomarker protocol. What benefit do you see in having a biomarker protocol for head and neck cancer in routine practice?

**Dr. Nicole Cipriani:**

Yeah, I think the benefit in these protocols as similar to the actual cancer checklist is certainly standardized reporting as well as standardized interpretation of whoever's reading those reports. And so we as pathologists have interobserver variability in terms of how we interpret things and clinicians may have a variability in terms of how they read our reports. And so the more we can standardize from either of those ends, I think the better, especially when patients, as we all know in today's day and age, transfer institutions or get second opinions or third opinions or fifth opinions. And so both for clinical teams to be able to have access to all data and for pathologists to be able to read other pathologist reports and make appropriate recommendations for treatment. And I think not only in terms of active patient care for pathologists and treating physicians to be on the same page for different institutions to be on the same page, but also in the future if we retrospectively look at these cases on investigative or research protocols that we can make meaningful comparisons between patients and between institutions based on the reported data and not have to necessarily repeat some of these biomarkers if curious for research purposes.

**Dr. Gladell Paner:**

That's a very good point, especially that we don't have that much data on some of these biomarkers, especially the newer ones. So let's talk about this specific biomarkers and let's start with the HPV testing. And this is a question for you Raja. Can you describe the HPV testing for head and neck squamous cell carcinoma and why do you use a surrogate marker in p16?

**Dr. Raja Seethala:**

HPV testing as we've come to know over the past couple of decades, at this point, it's very critical to defining marker for oropharyngeal squamous cell carcinoma critical for prognosis and staging. As Nicole previously mentioned, it's also a key component of the workup of carcinoma of unknown primary, and that's actually confirmed in the AJCC Eighth Edition Staging where it is carcinoma HPV positive if a carcinoma of unknown primaries identified with the status and no primary is it's codified as T0 or pharyngeal. Right? So there is now an emerging role for sinonasal squamous cell carcinoma. There's probably enough evidence at this point that it is reasonable to do HPV testing on sinonasal squamous cell carcinomas. Nasopharyngeal carcinoma, at least in the Western population remains a point of debate in terms of HPV testing, but other sites at this point are not key sites where HPV has a significant prognostic role.

So HPV testing is not at this point recommended for other head and neck sites. In the context of oropharyngeal squamous cell carcinoma though in the western population, most of the US practice settings, you asked why p16 is utilized as a surrogate. Well, not going into the biology and detail for this question, but let's just say as a surrogate marker, it performs exceptionally well given the high attributable fraction in these populations. So the prevalence of HPV is very high, so p16 performs very well in this context. It's also faster and cheaper than HPV specific testing modalities, making it very efficient. It covers a lot of things with one simple immuno stain and with most practice settings in the United States at least and Canada as well, the quality of the antibody is fairly standard, reliable and consistent. So it's robust from a technical standpoint as well.

The problem is that when you get outside the oropharynx in other scenarios or in situations where you have no room for error, like clinical trial settings, you need to know HPV status. The understanding now is that the attributal fraction and also the standards at which you want to be certain regarding the reliability of this marker as a surrogate are such that you need to do specific testing, particularly if you're doing it outside of the oropharynx. And as I mentioned before, you really shouldn't in most scenarios, but sometimes it happens in niche cases as far as treatment, despite its significant prognostic and staging role, actually HPV NCCN guidelines at least for the standard treatment, are mostly identical for HPV positive and HPV negative tumors. But again, as Nicole had alluded to earlier, the issue of de intensification or deescalation of adjuvant therapy is important and determined largely by HPV status.

And that's kind of the main thing. And since there are so many trials out there and a larger proportion of patients now than before are on some sort of clinical trial, whether we're not even talking about the key trials like ECOG3311 or the ORATOR trial, but basically this almost becomes, it is almost like a standard given in many practice settings where you have patients go on to clinical trials so frequently, aside from deintensification, the other thing is the advent of transoral robotic surgery for small tumors. So low risk features than an HPV positive oropharyngeal squam can be deescalated while the treatment can be at least.

**Dr. Gladell Paner:**

Thank you, Raja. So I'd like to switch to you, Nicole, and this is in the practice setting. Can you share your experience with interpreting IHC or immunostains for p16? Do you have issues with the cutoffs? As you know traditionally when we say positive, there's some staining, we say negative, there's no staining, and in this case there's some staining, yet we call it as negative. So can you share your experience with interpretation p16?

**Dr. Nicole Cipriani:**

Definitely I think in the appropriate anatomic site and tumor morphology of non keratinizing, if it's an HPV cancer, the p16 is generally diffuse and strong and some people have called it sort of block like or wall to wall. And in those contexts it's certainly not challenging to interpret if it's completely negative and you have a good internal control such as associated dendritic cells. I think that's equally as easy to interpret. However, the challenges are if you are lacking in any appropriate internal controls, I would be mindful we've had some cases of false negatives where the stain just didn't work, but also the internal control was not present. So be mindful of that. Be mindful if there is, as you commented, this sort of patchy expression or sometimes I like to call it checkerboard expression, which is not diffuse and not strong, and the current guidelines are diffuse strong nuclear and cytoplasmic in at least 70% of tumor cells. And arguably if it doesn't meet that, then some additional confirmatory testing like in situ hybridization for messenger RNA E6/E7 might be quite useful or confirmatory testing might be useful if the morphology and result of p16 don't correlate, right, so if it appears keratinizing and it's strongly p16 positive or if it appears non keratinizing and it's p16, arguably negative in those cases, additional testing might be warranted.

I think that the options we have, right, as already mentioned are the insight to hybridization for the messenger RNA, which many labs have and is also equally quick to do in a couple of days, but things like PCR or even in certain labs tumor sequencing, DNA sequencing next gen might pick up DNA from HPV as well. So those are some higher level options to be employed in certain circumstances. I did want to address the issue of p16 in cell blocks and cytology specimens. And so it is known that p16 in cell blocks does not work as well due to perhaps alcohol fixation or other variables which may be not the same as formal and fixed paraffin embedded tissue. And so p16 obviously strong is a good sign in a cell block, but p16 might have false negatives or false weeks in cell blocks. So we might advocate for in situ hybridization specifically in these circumstances because of the limitations on cell blocks. And that's the one caveat that I did want to make, especially because this is how the carcinomas of unknown primary present. And so just a note for the cytopathologist out there.

**Dr. Gladell Paner:**

So for the in situ hybridization, when do you order this for whenever you have equivocal, for any equivocal immunostain reading or do you perform this as a reflex test when you have that result?

**Dr. Nicole Cipriani:**

My practice here is probably somewhat different than what might be done in a general scope, but because we have clinical trials here, we pretty much do all of the testing. We do the p16, we do the ISH and we do the confirmatory sequencing because, one, our clinical teams want to know pretty much right away. So I personally don't do it as a reflex in equivocal cases because then it delays by even a couple days. So I just do both upfront so we have the information and then do the sequencing as a longer term solution because the clinical trials for these patients are important. However, if this is not in a standard setting, I would say yes, a reflex in the equivocal cases or the non sort of matching cases would be the way to go rather than as I do now, which is just order in both upfront.

**Dr. Gladell Paner:**

Thank you, Nicole. How about you, Raja? When you order the in situ hybdrization?

**Dr. Raja Seethala:**

Our practices are very similar. So again, the margin of error that clinical trials would like is much lower than standard practice. So for clinical trials, we'll just do p16 and HPV are usually RNA and in situ hybridization. Otherwise for other scenarios, discordant morphology and p16 results carcinoma of unknown primary non-conventional utilization of p16 and HPV testing, let's say for neuroendocrine tumors or non-squamous cell carcinomas for instance. Those will be all scenarios where you need to follow it up with a more specific testing of some sort. Now actually the new preference, at least at this point because of the commercial availability is RNA and in situ hybridization.

**Dr. Gladell Paner:**

So just to clarify this for our users of the head and neck biomarker protocol, so we will have the p16 IHC, and then for confirmation confirmatory testing, you can basically select any of these other molecular testing. And the most common or I would say the best testing will be that mRNA in situ hybridization. Is that accurate?

**Dr. Raja Seethala:**

Okay. Yeah, that is correct.

**Dr. Gladell Paner:**

Alright.

**Dr. Nicole Cipriani:**

And at least the current vendors now I believe cover 18 subtypes, so you will pretty much catch all of the relevant high risk types.

**Dr. Raja Seethala:**

Right. Yeah, for GYN, apparently internally in our institution detection rate went from 95 to 98%.

**Dr. Gladell Paner:**

Now I like to switch to another biomarker testing, which is EBV testing and this is for Raja. Tell us about the EBV testing for head and neck cancers and can you do IHC or in situ hybridization or both for this biomarker?

**Dr. Raja Seethala:**

Yeah, that's a good question. I had to go way back to think about it. Why don't we do any sort of IHC? So as we know, EBV testing is not only important for lymphomas, but in the head and neck it's mainly done for nasopharyngeal Carcinoma also has a role in carcinoma of unknown primary workup as well and can be treated in a similar fashion to HPV. In fact, the paradigm for EBV testing was in existence before HPV testing was in vogue. So it's interesting parallel from that perspective. Testing can also be useful and a small subset of sinonasal carcinomas, true sinonasal, not just nasal pharynx with extension to sinonasal tract as well as rare lymphoepithelial carcinomas of the salivary gland. A subset of those are EBV associated. So talking about proteins, I had to go way back to when I was a resident to think about testing for EBV associated or related proteins like LMP2, et cetera.

So why don't we do that in nasopharyngeal carcinoma for instance? Well, from a diagnostic perspective, none of these markers are sensitive enough, not by a long shot, they're just don't perform as well. They have been evaluated in the context of prognostic value within nasopharyngeal carcinoma, but they fall flat if you're going to apply them for a diagnostic purposes, at least for a nasopharyngeal head neck type malignancies. The other thing about EBER, which is different from most other in situ hybridization is that it's been around for a while. It's been around since the nineties actually mid to late nineties. And it's been used in lymphomas as we know, as well as carcinomas outside of head and neck, so subset of gastric carcinomas. So this test is not like the new in situations, it's more readily available to, and it's applied beyond head and neck sites. So there's not as much of a kind of penalty to do an EBER versus other newer in situ hybridization assays that are provided by limited number of vendors.

**Dr. Gladell Paner:**

Thank you, Raja. And I believe Nicole, we have this available at the UofC. We're using this test as well, right?

**Dr. Nicole Cipriani:**

Yeah, we have EBER ISH.

**Dr. Gladell Paner:**

How critical is assessing for not expression in poorly differentiated head and neck carcinomas? When do you do molecular tests to search for not rear rearrangement and besides its prognostic value, thus finding not expression or rearrangement matter therapeutically?

**Dr. Nicole Cipriani:**

Thanks. Yeah, I think this is one of those very rare but very clinically relevant diagnoses that if you don't think about it or you don't search for it, you will never make the diagnosis. And so to think about it and to search for it, especially if it can be diagnosed on an immunostain, I think is important. And so in a high grade neoplasm of primitive morphology, sometimes showing squamous differentiation and sometimes not sometimes in young people and sometimes in older, sometimes in the head and neck, sometimes not. So we kind of have to keep this on our mind a lot of times and a lot of places because we understand that it is no longer midline and it is no longer just head and neck. And so in cases of a high grade neoplasm, a nut immunostain can be applied with pretty good diagnostic accuracy with the kind of speckled nuclear expression.

If smaller institutions or even mid-range institutions do not have it at their own lab, it can be a send out test, which usually can come back in a few days as we perform the stain, send it back to you and you interpret it. And I have a low threshold for doing not if I am finding the diagnosis challenging as you said, because it potentially portends a poor prognosis for these patients. And if diagnosed these patients can actually, there are these bet or sort of brood domain, extra terminal inhibitors that patients can enter into treatment for that may prolong their life, may not cure them, but may prolong their life. And as far as we know, at least at this point, the actual nut partner may be less important for prognosis. I know there was some debate on whether the variant partners were or were not more or less aggressive. I dunno, Raja if you have any thoughts on that, but as far as I know the word out now is that it may not.

**Dr. Raja Seethala:**

Yeah, from a prognostic perspective, I agree with you. I'm not sure that it's that discriminatory at this point for prognosis.

**Dr. Nicole Cipriani:**

Right. And interestingly, as an aside, some mesenchymal neoplasms or sarcomas are now being demonstrated to have not rearrangements. So kind of another expanding use for this marker.

**Dr. Gladell Paner:**

Let's talk about the biomarkers for salivary gland and we have a laundry list of biomarkers here, and this is for you, Raja. Can you tell us about the diagnostic biomarkers used for some of the salivary gland carcinoma and share your experience on interpreting it slides using these different diagnostic markers? And when do you perform molecular studies for these biomarkers?

**Dr. Raja Seethala:**

Great question. I'm not biased or anything. That's why there are more salivary biomarkers on the biomarker template than anything else, right? Truth is salver gland tumors are so diverse, there are a number of distinct entities in a limited volume in a patient anatomically. So over the past decade or even 15 years at this point, we kind of witnessed a paradigm shift. So the understanding that most monomorphic salivary glands have a reproducible driver alteration, often a balanced translocation allowed us to leverage that to create testing not only by FISH, by RT-PCR for fusion transcripts and now by NGS essentially, interestingly enough, those were the initial testing modalities for these fusions and alterations that are defining the variety of different salivary tumor types. But now we have immunohistochemical surrogates for these as well, as well as chromogenic mRNA and in situ hybridization. As a class, I would say that IHC, you can consider it a sensitive screening marker for most of these alterations.

It's not that specific. So whenever you have the luxury and availability of material for most markers, it's a good idea to follow it up with more specific testing. Just like with HPV, the pretest probability, if it's high you can use it as a surrogate, but if it's not, which is the case for most of these, given the performance of a lot of these immunostains, it's better to follow up either with FISH, NGS, or something else. One exception I'd say for IHC is NR4A3, it's a marker of a acinic cell carcinoma. It's not frequently utilized because it's not necessary. Acinic cell carcinoma is usually readily diagnosed morphologically, but very useful with limited sampling core biopsy FNA, and it actually outperforms FISH. Most of the literature, including stuff from our institution supports that. As Nicole mentioned earlier, MAML is a high yield FISH testing on the other hand.

So FISH testing is, in contrast to IHC, more specific but not very sensitive. So we understand as with many other molecular alterations, whether they be fusions or other types of alterations involving the same gene, the story is not just one set of translocation partners, right? So we describe lots of different partners including NUT sometimes including MAML2 sometimes, and you'll see that break apart FISH and even fusion FISH, these are all very specific but not sensitive for the diagnosis. And then if view NGS as kind of like the final word, depending on how the panel is constructed, it can be very robust. It can cover a lot of the fusions and can give you additional information, but at a premium cost. So in many situations it still is outperformed by cheaper testing. Sometimes FISH actually is better sometimes, and our NR4A3 immunohistochemistry is better than doing next gen sequencing because a fusion is not a fusion transcript, it's a juxtaposition of enhancer elements to hijack NR4A3. So from a methodologic standpoint, the algorithm to find this type of fusion is not necessarily that straightforward right now we also have in situ hybridization, particularly MYB for adenoid cystic carcinoma is promising, kind of takes the best of both worlds. The sensitivity of IHC with enhanced specificity more similar to FISH and it's quick pathologists can look at it and read it and the cost is probably going to end up being intermediary between IHC and FISH so to speak.

**Dr. Gladell Paner:**

Thank you, Raja. I like to switch now to the last group of biomarkers and these are for nasal malignancy and this is for you Nicole. Can you tell us about the biomarkers used for nasal malignancies and is immunostaining sufficient to detect these biomarkers?

**Dr. Nicole Cipriani:**

Yeah, so similar to our burgeoning understanding of salivary diagnostic mutations infusions, similar thing has happened in sin nasal cavity both with epithelial or epithelial malignancies as well as spindled. And so we've already discussed NUT, but I think others to bring up in terms of when immunostains or fusion testing can be used for prognostic or therapeutic include things like the SWI/SNF deficient carcinomas for INI and BRG1 immunostains, which are essentially poor prognosis, the HPV33 related multi phenotypic cancers, which might inversely be a good prognosis, the new, the DEK-AFF related sinonasal carcinomas that mimic sinonasal papillomas. So this might be a diagnostic difference here. As mentioned earlier, the sort of adamantinoma-like Ewing sarcoma, which has keratin expression, but in fact has EWSR1-FLI1 gene rearrangement and so would be classified as a sarcoma and treated as such.

And then our waist basket, what we used to think of right as SNUC, sinonasal undifferentiated carcinoma, in fact now has recurring IDH2 mutations. So probably an entity in and of itself. And so as we tease out all of these specific things for diagnosis, some of them come out as prognostically relevant for better or worse prognosis. And I do just lastly want to mention the spindle cell neoplasms including things like the biphenotypic sinonasal sarcoma with PAX-MAML gene rearrangements in which IHC for PAX3 can be used, which is apparently somewhat better than PAX8. These are also quite rare, but reflective of the PAX rearrangement, remember that rhabdo is also on the potential differential. So something to consider with that and where fusion testing or molecular confirmation of the partner might be useful. Solitary fibrous tumor, which we see outside and inside of the sinonasal tract. But STAT6 is a great immuno marker for that often of the differential diagnosis with something like a langio in which beta-catenin and nuclear expression would be useful for that. Most of these are surgical malignancies and surgery upfront will be important for all of them with varying use of subsequent therapy following, but mostly diagnostic for the sinonasal cavity.

**Dr. Gladell Paner:**

Thank you, Nicole. I find it interesting that practically express in biphenotypic sinonasal sarcoma we used commonly in penial cell carcinomas in the gene. Now I like to switch to a question for both of you. This is about, so based from our discussion, it looks like the future looks bright for biomarkers for head and neck cancer and both of you cited some examples of biomarkers that not even here in this the current biomarker protocol. And for that we are looking forward for the new version of the head and neck cancer biomarker protocol. So my question for both of you is what excites you on the horizon for biomarker use in head and neck cancer?

**Dr. Nicole Cipriani:**

What's exciting is our ability to more finely discriminate these overlapping neoplasms and actually utilize ancillary tests to refine our diagnoses and be able to therefore understand what the prognosis is for these patients. And I think a lot of what's to be gained is actually going to be in both retrospective and prospective studies of what we now know and therefore hopefully better guide our ability to diagnose and treat based on what we now know and what we are yet to know.

**Dr. Gladell Paner:**

And for you, Raja, are you excited too?

**Dr. Raja Seethala:**

I'm excited today. You guys got me excited, but for me the excitement is not necessarily about what new marker. I'm not saying that it's not exciting, but there will be new markers diagnostically prognostically and theran agnostically for a variety of different tumor types and we're going to get better at teasing out these subtypes versus different entities altogether. I see this as inevitable, but what really excites me a lot is how we utilize testing to arrive at this, how we implement this. So from that perspective, I think increasing usage of multiplexing, maybe it doesn't have to be chromogenic, it could be a IF platform and also pairing it with the digital image analysis. Okay, so improving the precision. So it's not just about the markers, it's how they're utilized. Obviously right now for the most part, this is currently in a research setting. We have only a very few clinically available quantitative image assisted assays in our institution. But this will increase and it'll not just be single marker, it'll be multiplex. We'll be looking at not just the tumors themselves but the tumor microenvironment. That's kind of the excitement in the scientific community, particularly in head and neck.

**Dr. Gladell Paner:**

Thank you both. I have another question here. So this is in a situation where someone wants to incorporate or set up these biomarkers in their own laboratory, in your opinion, and this is a question for both of you, what do you think are the main challenges when setting up in-house biomarker testing in the laboratory? I'll start with you, Nicole.

**Dr. Nicole Cipriani:**

I mean, I think because of the sometimes rarity of some of these diseases, a lab may not want to go through the effort to validate and implement some of these markers if the incidences of these tumors in their hospitals, in their regions is small. And so that's probably one of the barriers to implementation that I've seen is just extent, an ability to actually test on a meaningful basis and keep these markers up to date. Aside from that, we haven't so far been talking about techniques that are very challenging. We've been talking about immunostains and in situ hybridization and FISH and most sort of sequencing. So it's techniques that are available, but tumors that are potentially rare.

**Dr. Gladell Paner:**

And for you, Roger?

**Dr. Raja Seethala:**

Yeah, I agree entirely with what Nicole had said, especially in this current climate, financial climate or given the shortage of laboratory personnel across multiple practice settings and institutions. It's the usual, it's staffing budget and priority. And keep in mind most of the biomarker panel here, these are all LDTs, aside from maybe HER2 and PD-L1, if you include it, that's part of the generic biomarker panel. But so this imposes an additional burden on labs because of the validation requirements. And as Nicole said, for certain tumor types or for certain tests, the volume would not necessarily justify bringing it in.

**Dr. Gladell Paner:**

Thank you, Raja. So considering the factors that you both mentioned, so a follow up question to that, if we look at all this biomarkers in the head and neck protocol, so which of these biomarkers would you recommend to our colleagues in the community practice?

**Dr. Raja Seethala:**

I'd prioritize the basics, p16, EBER are a must. I think it's reasonable to consider HPV mRNA in situ hybridization for E6/E7. Those are the big, I would mention HER2, but it's already likely available to most laboratories because of breast, right? So other mid-tier or even luxury markers, if you had a larger head and neck volume, even in a private practice setting would include for salivary AR and MAML FISH in some form of MYB and TRK testing that will cover all the more common salivary type malignancies for the nasal tract. I think NUT plus the SWI/SNF pathway alteration. So SMARCB1, SMARCA4, you probably don't need SMARCA2 or ARID1A, but at least those big two are going to be important. Maybe even IDH testing, if there's an immuno, I haven't had much experience with it. I don't know if you have it at your institution, we do it by NGS, but that might be worthwhile as well.

**Dr. Nicole Cipriani:**

Yeah, I agree with all of the above and we've mentioned the big hitters, but there are some ancillary immunos that we haven't mentioned that can help, that can aid in a lot of these diagnoses, including if you may have something like an SMA for a myopericytoma, even if you don't have a beta catenin, but most likely you also have a beta catienin and things like that, right? So I think the big ones have already been named, but there are also smaller, more common ones that can potentially aid in diagnosis. And that's a whole nother two-hour episode.

**Dr. Gladell Paner:**

Think you, Nicole. So that's all the time we have for today. Thank you, Nicole. Thank you, Raja for sharing your insights and experiences on biomarkers for head and neck cancers. We learned a lot today. So final words, Nicole?

**Dr. Nicole Cipriani:**

Final words are the pathologists are very important in clinical care. And I would encourage you to actively take a role in utilizing and interpreting biomarkers for the clinical teams, whether or not your lab performs them or not, you are integral in assisting with that in patient care.

**Dr. Gladell Paner:**

And Raja?

**Dr. Raja Seethala:**

I agree entirely, this is our domain, so don't let others take it over or else decisions will be made for you. And that will be to the patient's detriment, honestly, because we are positioned to interpret these results better than anyone else perhaps. But don't forget about morphology. The ultimate supervision of these testing modalities is ensuring that you're ordering them appropriately and that requires a good morphologic acumen.

**Dr. Nicole Cipriani:**

Right on.

**Dr. Gladell Paner:**

Right on. I agree 100%. And I'll pass this to you, Becca.

**Becca Battisfore:**

Thank you, Dr. Paner, for leading the conversation, and to Dr. Seethala and Dr. Cipriani for sharing your insights on the biomarkers for head and neck cancers. And I want to thank you all for listening to this CAPcast. You can find links to the CAP's biomarker reporting protocols mentioned during the episode in the episode description along with other Cancer Protocols. If you have questions or comments about any of the protocols, please email Cancer Protocols@cap.org. And for more information about the CAP visit cap.org.