Episode 19 - "Beyond the Basics: The Pathology of NET" with Dr. Andrew M. Bellizzi

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Lisa Yen:

Welcome to the LACNETS podcast. I'm your host, Lisa Yen. I'm the LACNETS, Director of Programs and Outreach, as well as a caregiver and advocate for my husband who is living with NET. In each podcast episode, we talk to a NET expert who answers your top 10 questions. This podcast is for educational purposes only and does not constitute medical advice. Please discuss your questions and concerns with your physician.

Lisa Yen:

Welcome to the LACNETS Podcast. I'm really excited to introduce our special guest for today, Dr. Andrew Bellizzi. Dr. Bellizzi is the Director of Gastrointestinal Surgical Pathology at the University of Iowa, the only designated NET Center of Excellence in the United States. He's also the Director of Diagnostic Immunohistochemistry Laboratory.

To say that Dr. Bellizzi is an internationally recognized expert in pathology is an understatement. He is an uber expert, particularly in NET pathology, and particularly in the area of immunohistochemical biomarkers, which is a mouthful. And he introduced 100 new immunohistochemical tests during his time as the director of the immunohistochemical laboratory at the University of Iowa. He conducts clinical and translational research, mostly in oncopathology with a major effort in GI and neuroendocrine neoplasms. He's the chair of the College of American Pathologists Immunohistochemistry Committee, and he trains residents, fellows and medical students. He's an internationally recognized speaker, and he's absolutely passionate about pathology. We're really excited to have you here with us today. And Dr. Bellizzi, if you don't mind telling us a little bit about yourself.

Dr. Bellizzi:

Thank you, Lisa. Thank you so much for having me. Immunohistochemistry, I love immunohistochemistry. If you're having trouble with it, you can say "IHC." Let's see, what should I say about myself? How about how about this? I'm a diagnostic surgical pathologist. I say that I'm the Oracle and my main function is to

translate the truth from the patient through their tissue. Then the truth I sort of can translate into a report that I share with the team of patient-facing clinicians so that they can have the best information to take the best care of the patient. And I also would emphasize that as a pathologist, I'm an integral member of the care team in the context, especially of that multidisciplinary tumor board. So I participate in those multidisciplinary tumor boards, explain the pathology. I always say that pathologists have unique insights into the biology of disease because we spend all day looking through that microscope but that we have this important role in diagnostic medicine as part of the care team.

What else should I should I say? I'm from Connecticut originally. And I just got back from a short trip to Connecticut to give grand rounds at another academic medical center, which is something that I really, really enjoy doing, meeting folks, exchanging ideas with folks. And then conveniently since I'm from there, I spent a couple days with my mom and visited with my grandmother. We're recording here today on November 21, 2022, which happens to be the anniversary of my dad's birth. He was born on November 21, 1951. And he recently passed away of lung cancer. He died on the date of Joe Biden's inauguration. And when Lisa had suggested some possible dates to record, I thought it would be nice to record talking about cancer on the anniversary of my dad's birth.

You know, I spent a lot of time teaching, lots and lots of different learners of all different types and I recently gave a lecture to our dental students about general principles of cancer. And I had reflected to them that I think that my career has taken the direction that it has where I really focus on the diagnostic aspects of cancer from a clinical perspective in my teaching, and in my scholarship, probably deeply influenced by my family history, and my dad's dad had also died of lung cancer and I imagined that sort of set the set this ship sail pointed off in that direction.

Lisa Yen:

Wow, you said a lot there. And there's so many different directions we could go. But well, first of all, just remembering your father and your grandfather, and so sorry for your loss. What an amazing way to honor your family, your father today and through your work. All that you do. I think that many people probably aren't even aware as there being a field of oncopathology. Could you explain a little bit what that is and how you even got into this field and particularly in NET?

Dr. Bellizzi:

Sure, we could talk about oncopathology. Let's define oncopathology and talk about how I became a neuroendocrine pathologist. Oncopathology is the pathology of tumors. One can study many things, but I suggested, probably because of my personal history, that I was drawn to the study in all its aspects—the clinical aspects, the educational aspects and the research aspects, I was drawn to the study of tumors as opposed to the study of inflammation.

Another point of me calling myself an oncopathologist is, I'm emphasizing that I'm broadly interested in the study of cancer. And maybe that leads us to neuroendocrine—how did I become a neuroendocrine pathologist? And why did I become a neuroendocrine pathologist?

So the most special thing about the University of Iowa Hospitals and Clinics when I got here 11 years ago was the multidisciplinary group that studied and took care of patients. And I think that's important—studied *and* took care of patients. Lots of places take care of patients but also was engaged in the scientific inquiry of neuroendocrine tumors. Everything is always about relationships and people. And

then I came here and saw this group and knew that I was going to be involved in a lot of things, but I wanted to cast my net with the NET doctors, pun intended. And I started doing research in neuroendocrine tumor pathology where I had done, I can't even say that I had done none, I had done some when I was at when I was at the Brigham, but now it's a major focus of my research effort. Again, the reason that the group is so strong for the vast majority of the members of the group, it's 100%, of what they're focused on all day, every day. They eat, sleep and breathe neuroendocrine tumors.

Lisa Yen:

I love this idea of what you said, it's important to not just study, but also take care of people. That you as a pathologist, even if patients don't think about it, you are taking care of them too.

Dr. Bellizzi:

Absolutely. That's literally why I'm here. That's the single most important thing that I do, take care of patients. I take care of patients. I look at these pathology specimens, I translate what's going on in their body that's been removed by a medical doctor or a surgeon, and we make slides out of it and I look at these slides. I translate that information into a complicated report that I help the patient-facing clinicians understand, so that they can convey the information to the patients and also have it be intelligible to the patients.

Lisa Yen:

Yeah, you're the Oracle, as you said, what a perfect metaphor for it. So that the patients can better understand what you do, let's go back to the other big word that we're talking about: immunohistochemistry. What is it and what do patients need to know about it?

Dr. Bellizzi:

So immunohistochemistry is a technique. It's "histochemistry" with the word "immuno" tagged to the front of it. So what's histochemistry? How do we do diagnosis at the light microscope? We can start there.

Immunohistochemistry is a special technique. It's an ancillary diagnostic, it's something that we add on to the main histologic examination of tissues. So what's the main histologic examination of tissues? Cells or tissues removed from the human body and it goes to the histology lab and if it's small enough, we process the tissue directly. If it's a larger sample like a resection—pancreatectomy, ileal resection, we examine that tissue with our eyes, and we have tools like scissors and knives where we can cut into and dissect the tissue. The pathologist or pathologist designee, they examine the tissue and they decide if it's a larger sample, what to sample. And from a resection, we'll take sections that are about the size of a nickel. And everything has to get processed. Everything has to get fixed in formalin and dehydrated and embedded in paraffin so that we can make glass slides. So after the tissues processed and embedded in paraffin, we cut sections using a microtome, which is a meat slicer. But we take the paraffin block, and we screw it into the microtome, and we advanced the blade toward that tissue and we cut incredibly thin sections. Three, four, five microns thick, which is so thin that five microns is the diameter of a red blood cell. So these incredibly thin pieces of tissue that we place on the glass slides. Then we stain them with hematoxylin and eosin. That's the standard histologic process and hematoxylin is a blue dye and eosin is a red dye. So this hematoxylin is blue, and it stains nucleic acid. And that's advantageous because the nuclei of cells stain

with hematoxylin. And then eosin is a red dye. And that stains proteins and the cytoplasm of cells generally stains pink or red. So we have a blue-purple dye hematoxylin and a pink-red dye eosin. Then that slide, I put it down on the stage of my microscope here. And I put my eyeballs in there. And through tens of thousands of hours of repetition, I'm able to translate again that image into words. I can say, “This is a well differentiated neuroendocrine tumor,” for example. Or “This is a normal colon biopsy.” Or “This is ulcerative colitis,” or “This is celiac disease,” et cetera, et cetera, et cetera.

Then there's histochemistry. Sometimes H&E is not enough. And histochemistry is this fascinating field where again, empirically, folks have figured out that different types of mainly dyes, and we probably do maybe 20 or 30 different histochemical tests in the histology lab, that mainly that different substances, inorganic materials react with certain substances in the tissues in unique ways to produce colored products. So we do histochemistry for stuff like iron or mucins. And these are really useful tools in the diagnostic laboratory.

And then there's immunohistochemistry, which I think is a play on histochemistry. Immunohistochemistry is a technique. We are able to specifically label proteins and do the immunohistochemical reaction and produce a colored product. I can give an example and I can briefly describe the immunohistochemical technique. One of the advantages of the IHC technique is it's incredibly flexible as long as you have a primary antibody. The primary antibody is an antibody like you make antibodies, I make antibodies. The sources of the antibodies we either get them from rabbits or mice. These primary antibodies are directed against something. So we inject something like ki67, or part of ki67 into a rabbit or a mouse and it generates an immune response. And we use those antibodies produced by that immune response, we extract them and purify them. And we end up with a primary antibody that's specific for something like ki67. Or in the neuroendocrine space, keratin, chromogranin A, synaptophysin, INSM1, SSTR2A. So we apply that primary antibody. Then we apply a secondary antibody that is studded with enzyme called HRP, stands for horseradish peroxidase. Then we add a substrate called DAB, diamino benzene. And wherever the enzyme is, wherever the HRP is, the DAB reacts with it, and it precipitates a brown color. And the specificity is conferred by the primary antibody. So the antibody to ki67 recognizes wherever ki67 is on that tissue but the primary antibody reacting doesn't do anything. It's the secondary antibody studded with the enzyme, adding the substrate. Wherever that primary is bound to secondary, wherever that DAB encounters that HRP in two dimensions, that's where brown color will be deposited. Then the pathologist will visualize the slide and say, “Oh, there's brown signal in these nuclei or this cytoplasm or these cell membranes of this population of cells or that population of cells.

So immunohistochemistry is a technique that pathologists use to visualize protein expression in the context of tissue morphology. It's such a wonderful technique that I love so much that it's really the main focus of my entire academic career and my career as an educator. I'm a neuroendocrine pathologist for sure, but I am like, *the* immunohistochemistry pathologist. And our test menu has about 300 different analytes so there are about 300 different proteins that we can visualize with the immunohistochemistry technique. And a main focus of my role as the local immunohistochemistry lab director is to increase our test menu with the best tests that are out there. And *the* main focus, like what my grand rounds was about were a bunch of novel immunohistochemical tests that I invented and introduced into the diagnostic space. My main research interest is in creating novel new immunohistochemical tests to help pathologists make better diagnoses. I'm either trying to bring up tests for a diagnostic application where no test existed in that space before. Or if there's a test in that space, to make a better test, one that is more sensitive or more or more specific. So immunohistochemistry, not quite in a nutshell.

Lisa Yen:

If I'm understanding this correctly, the immunohistochemistry is the technique of applying the staining to find the protein. And it matters to neuroendocrine tumor patients because you're helping make that diagnosis by finding certain proteins on the cells of the tumors.

Dr. Bellizzi:

Yep. On the cells or within the cells.

Lisa Yen:

Or within the cells. So the things that have come up in our conversation are things that might be familiar, the chromogranin. So I wanted to ask you some more clinical things.

One question is, what do you think when you see that there are two different ki67 or ki67 in the same tumor specimen. For example, one institution might report it a ki67 of 3 and the other one might report it as 10. So what do you think? What goes through your mind?

Dr. Bellizzi:

So, we have to break the problem down. How do we end up with a ki67 proliferation index? And then maybe we can talk about what that is. First, the ki67 proliferation index is the proportion of tumors that are ki67 positive, which is the proportion of tumor cells that are in the cell cycle that are non-G0. And it's a ratio of the total number of brown staining tumor nuclei over the total number of nuclei times 100 to give you a percentage. And it ranges from zero to 100. And G1 is less than 3%, and G2 is three to 20%, and G3 is greater than 20%. Another thing that's worth mentioning is that actual number, the actual ground truth of the proliferation index, is more important than the grade. For example, a tumor with a ki67 proliferation index of 2.9 is going to behave a lot more like a tumor with the ki67 proliferation of 3.1, even though it's right at the G1/G2 arbitrary junction. 3.1 is going to be a lot more like 2.9 and dissimilar from a tumor with a ki67 proliferation index of say 17%, even though 3.1 and 17 are both are both G2.

Breaking down an explanation as to why you might get multiple ki67 results from the same tumor, we have maybe three components. We have heterogeneity of ki67 expression in space. And then we have variability variation, lack of interchangeability of immunohistochemistry biochemistry. And then finally, we have IHC readout. And let's break down each of those three pieces.

If case is examined at one hospital, to make a second diagnosis, that second pathologist can reexamine the same slides. Or they could make their own set of slides out of the initial paraffin block. And if they examine the same slides, then the difference in the ki67 proliferation index might be due to readout. And that's how the pathologist goes about assessing the ki67 proliferation index. And there are different ways to do the readout. You could do it by eyeball estimate, which is really common and inaccurate. So, you and I could look at the same ki67 image and I'd say, “What do you think this is?” And you might say, “five,” and I might say, “two.” So that's an explanation as to why there would be heterogeneity.

Another way to do it is to actually formally count. For years and years and years, I would take a picture. I can take a picture of a field in the tumor and ask you to count the number of brown nuclei and the total number of tumor nuclei. And we could disagree, because we could disagree on whether the nucleus is brown or not. We could disagree on whether we consider the degree of brownness of that nucleus to be

sufficient to warrant counting it. We could disagree on whether a cell should be counted in the denominator, whether it's a tumor nucleus or not. And then a third way to get to do the readout is digitally. A manual count of a camera-captured image might differ from a digital count. These are all on the readout side multiple reasons to have variability around the actual value that's in the report.

The biochemistry is super important. If I run a ki67 assay in my lab, and pathologist X runs it in her lab, the biochemistry of our assays could be similar, but really no two immunohistochemistry assays are interchangeable. I talked about all these steps, like the primary antibody and the secondary antibody, or also known as detection and the chromogen. And there are other steps in the assay as well. And all of us in our labs have our immunohistochemical assays tuned slightly differently, and in some cases radically differently. So that's the IOC biochemistry reason for there to be variability, the readout.

And then the last thing to mention is the heterogeneity. We could be examining the same slides. We could agree that we're going to do a manual count of a camera-captured image. We could look at the same slide and I could take a picture of one area of the slide because I think that this is the highest staining area and the other pathologist might take a picture from a totally different field. So we might differ in selecting the hotspot to even count.

And then another thing around the heterogeneity is, if I'm making a new set of slides from the paraffin block directly. And these are all five microns, super thin slices. But even from one five-micron slice to the next deepest five-micron slice, the tumor that we're examining is not identical. And there can be subtle differences from five-micron to five-micron increment and really radical differences from millimeter to millimeter through the tumor. So multiple reasons for there to be more than one ki67 proliferation index in an individual case.

And then I'd say from the patient's perspective, if you're curious as to why there might be a difference in your case, I'd say it behooves you to ask the pathologist how they generated the ki67. What assay did they run? What clone did they use? The most commonly used ki67 clone is called “MIB1.” So what assay? And then how did you do the readout? Did you do it by eyeball estimate or manual counting of camera at captured image or digital image analysis? And then if it's read in a second lab and they're different, same questions. What assay did you use? Or did you use the original slides? What kind of readout did you do? And if they can't answer those questions, maybe send to a third lab.

I'd say even though maybe it's distressing for it to be 2.9 and 3.1, I'll go back to that example because it straddles the grade. When they're so similar, it's easy to wrap one's head around why they might slightly differ, because really a ki67 proliferation index is no different than like serum glucose or sodium, which if you get it done in different labs might be 140 and 142. It's more disquieting when it's 2 and 3 and 7. It's of interest to try to identify why any value would be an outlier from the other values, especially if the management is going to be driven by the number. So maybe if your oncologist is going to treat your tumor differently if the ki67 is really 7%, but you have other reads that are 2 and 3%, it behooves one, the patient, and the clinician, to get to the bottom of why there might be a discrepancy.

Lisa Yen:

Yeah, that's helpful. So bottom line is, we can ask. Patients may not even know that they can ask this, “How did you get to that number?” and ask the clinician or ask the pathologist that.

So, the next question is maybe a twist on that. The first the previous one was, “What if with the same tumor, two different pathologist or two different institutions had different interpretations?”

What if one person had two to three different biopsies with different ki67s? So say it was 3, 5, 8. How do you get different numbers?

Dr. Bellizzi:

So this is back to the issue of heterogeneity. The ki67 proliferation index is not evenly distributed across the whole tumor. And all we can do is take a sample, and then we have to decide how to take a sample. If there's only tissue block of tumor, it's easy. We do ki67 on that one tissue block. But if there are five or ten blocks with tumor, it's impractical to do a ki67 on every block, so we have to decide. And I can say our practice at the University of Iowa is in patients who get resected, we do ki67 immunohistochemistry on one block of primary because patients, especially with ileal, often have multiple primaries. And we choose the largest. So if a patient has four primaries, and they’re 3 millimeters and 5 millimeters and 1.2 centimeters and 2 centimeters, we'll do it on the 2 centimeter tumor. One block of primary, one block of lymph node metastasis or tumor deposit. Patients will often have more than one metastasis, and we'll choose the largest. Then if there's a distant metastasis, often a liver metastasis, there might be multiple liver metastases, we stain one block of metastasis. We direct it toward the largest. The reason for that is there is data that the ki67 proliferation index is correlated with size. So, it's more likely to be higher in tumors that are larger. It's not one to one. You know, in that patient with 3, 5, 1.3, and 2 centimeter primaries, the two centimeter primary doesn't always have the highest ki67 but it's apt to.

We stain primary, regional, and distant disease. The reason we do is we've seen that the grade is often discordant. It's discordant about a third of the time between matched primary, regional, and distant disease. And 25% of the time, it's higher in the metastasis and actually 10% of the time, it's highest in the primary. So that's why we don't abandon the primary. I do the biggest one, because it's usually going to be highest there. I do the metastasis in addition to the primary because if it's going to be higher in one than the other, it's usually higher in the metastasis. But we still do the primary as well.

It's all patient centered, and it's all outcome centered. And the reason to stain all these samples is, wherever the highest ki67 is and whatever the highest ki67 is, that's the most important number that'll be associated with prognosis. So if the primary is G1 but the lymph node met is G2, the tumor is going to behave more like G2. The clinical course might be more aggressive. It's all heterogeneity over and over and over again—within a single slide, between different tumors from the same patient. Spatial heterogeneity. But another idea is temporal heterogeneity, because patients might recur. So we do ki67 in diagnostic biopsy. We do ki67 on the resection in primary, regional, and distant disease. If the patient recurs and if the recurrence is biopsied, we’ll always do ki67 in the recurrence. In a recurrence, say the primary was G2 and the ki67 was 5% and the patient's progressing and a biopsy is repeated. And it's still well differentiated neuroendocrine tumor. Our clinicians are going to want to know, is the ki67 still 5%? Or is the ki67 now 10, 15, 20, 30, 40%, which correlated not one for one, but as a marker for the pace of the disease.

Another reason for the ki67 to be different between samples is these cancers, they're dynamic. They're not static. They're always changing. They're evolving. One of the concepts around oncopathology that I try to transmit to beginning students is oncopathology is human evolution. You know, every tumor cell is like an animal in a population. And the tumors are constantly subjected to selective pressure, especially if

you treat a patient with chemotherapy. It's survival of the fittest. So, a reason for the ki67 to change and generally to increase over time is therapies are sort of a double-edged sword. They knock back the tumor, but simultaneously they apply selective pressure to the tumor that often ultimately selects for subclones of tumor that are fittest to survive, and that's the biologic basis of clinical recurrence. And it's common for those tumors that have been selected for their survival advantage to have higher ki67 proliferation indices.

Lisa Yen:

Wow, there was so much that you said there and so many good points. And I think especially the whole thing about heterogeneity. Just like no two people are the same, no two tumors are the same. And interesting that you say that because we want to treat to the highest ki67 for the good of the person, you're saying a quarter of the metastases, the tumors that have spread, are usually higher ki67 than the primary tumor site.

Dr. Bellizzi:

And higher by grade. It's even more common if you're trying to distinguish 2.9 versus 3.1. But in a G1 primary, in our experience in hundreds of cases, the regional and distant disease is going to be G2 and occasionally G3 25% of the time. So that's a thing for a patient to advocate for if they get resected and they have lymph node mets and liver mets, you can maybe press your clinician or your pathologist if a ki67 is only been done in the primary, “Do you mind adding a ki67 in the lymph node met and/or the liver met?” It’s often of importance to the treating oncologist.

Lisa Yen:

That's a helpful little tip there. And you already addressed my next question about re-biopsying it, but you did bring up G3 tumors and I know that this is something that you're passionate about, and you do a lot of work in. So, this there's been a lot of discussion about well differentiated versus poorly differentiated G3 tumors. How are they different and how do you figure it out?

Dr. Bellizzi:

There's well differentiated neuroendocrine tumor, and there's poorly differentiated neuroendocrine carcinoma. And then we're going to talk specifically about well differentiated neuroendocrine tumor grade 3. Those would be well differentiated neuroendocrine tumors with ki67 proliferation indices greater than 20%, and/or with mitotic counts greater than 20 per 2 millimeters squared. But nearly always, a tumor makes its grade based on the ki67 proliferation index. So we could focus on NETs with the ki67 proliferation index greater than 20%.

And then there's poorly differentiated neuroendocrine carcinoma. When I teach about this concept, I use the example of a bird and a bat. Birds have wings and bats have wings. These two organisms have evolved the ability to fly. But genetically, birds and bats are entirely dissimilar, right? Birds are like evolved from dinosaurs and bats are closer to like mice and squirrels, but they both have wings. And the wings in this analogy is neuroendocrine differentiation. So, neuroendocrine tumor and neuroendocrine carcinoma are fundamentally different tumor types. They’re as different as the non-neuroendocrine tumor types, adenocarcinoma and squamous cell carcinoma. But they do have this common phenotypic feature, this

neuroendocrine differentiation, which manifests morphologically, and we use these biomarkers, the synaptophysin and the chromogranin, to highlight the neuroendocrine differentiation.

That being said, even though birds and bats are different, there are individual organisms where it's really, really easy to tell that's a bird and that's a bat. But there are some examples where on the H&E, the linchpin to our diagnosis, when we look at the slides, it's hard to tell whether it's a neuroendocrine tumor or neuroendocrine carcinoma. That difficulty in making that distinction almost never occurs in NET G1 or NET G2, it's almost always in the context of NET G3. They're fundamentally, biologically different.

And that's where immunohistochemistry can come in and then what I call “my shtick” is next generation immunohistochemistry. So not only do I like to build immunohistochemical tests, I like to build immunohistochemical tests that give us information about the underlying molecular genetic state of the tissue or tumor. NETs and NECs are fundamentally different in their genetics. And so, some of the immunohistochemical tools that I use to parse this very important differential diagnosis take advantage of protein correlates or immunohistochemical correlates of molecular genetic events.

So poorly differentiated neuroendocrine carcinoma, the type example. When you close your eyes and you hear poorly differentiated neuroendocrine carcinoma, the tumor type you should think of is small cell lung cancer. And the molecular genetic hallmark of small cell lung cancer is biallelic, the alleles on both chromosomes, inactivation of TP53 and RB1, which are two super famous genes, tumor suppressors, and in small cell lung cancer. And poorly differentiated neuroendocrine carcinomas, they're famous for rising in the lung, but they can arise in any organ like the pancreas, for example. Very uncommon in the midgut actually. But you can make a NEC, a neuroendocrine carcinoma, in any organ, and they usually have biallelic inactivation of these tumor suppressors. And I have immunohistochemical tests for these.

We do p53 immunohistochemistry. And we do RB immunohistochemistry. And there are different patterns that I might see that correlate with mutation status. So instead of doing molecular genetics like NGS, we do a few immunohistochemical stains that inform us as to the molecular status of the tumor that helps with NET versus NEC. There are a bunch of other tests that I do too. One of the things that I like to convey to the pathology learners is that tumors tend to recapitulate cells or tissues native to the organ in which they arise. A NET, even a NET G3, if it's arising in the ilium, it's going to have phenotypic characteristics in common with the neuroendocrine cells in the ilium. If a neuroendocrine tumor, even grade three, arises in the pancreas, it's going to have phenotypic characteristics in common with islet of Islet of Langerhans cells, the native neuroendocrine cells in the pancreas.

And then how do I get toward phenotype? What's the fundamental irreducible characteristic of phenotype? It's driven by the developmental biology of the organ. They're called transcription factors. They're proteins that get switched on that tell a cell to make a bunch of other proteins. But they're sort of master regulators of the cell’s phenotype. So I talked about p53 and RB for the genetic piece. And then I do a bunch of immunohistochemistry for transcription factors that are specifically expressed by neuroendocrine cells in the ileum versus the pancreas versus the rectum and tumors that are derived therein. A pancreatic neuroendocrine tumor, for example, will express PAX6 and Islet-1, which are two transcription factors that are expressed in the islets of Langerhans. The retention of that differentiated, developmental neuroendocrine program gives me information that this is likely a tumor versus the carcinoma which tend to have lost all of those markers that link the tumor to some cognate normal cell.

Lisa Yen:

So the NEC looks nothing like the normal cells next to it.

Dr. Bellizzi:

Right. And neuroendocrine tumors in the pancreas, for example, significantly resemble on the H&E, the islets of Langerhans. And looks like is reflected in the immunophenotype. So the results of the immunohistochemistry. The way that the tumor looks that's its morphologic phenotype and the results of the immunohistochemistry are its immunophenotype. The neuroendocrine tumors have an immunophenotype in common with the islet cells or the serotonin expressing cells in the midgut, etc etc.

Lisa Yen:

Well, I'm amazed by your curiosity and your dedication. And what really stood out to me too is that you said you could do a few IHC stains. And I know that a lot of people do genomic testing and next generation sequencing, but you could get some of this information by doing some stains.

Dr. Bellizzi:

And I'm asked to speak on that a lot. The role of immunohistochemistry versus molecular pathology, or the future of immunohistochemistry in an increasingly molecular pathology-centric, NGS (next generation sequencing)-centric diagnostic and therapeutic space. I always emphasize that immunohistochemistry and molecular diagnostics are highly complementary. And there's advantages and disadvantages of both techniques. But a lot of my immunohistochemistry tests, they are just another way to examine the same thing. So instead of doing NGS and looking at TP-53 and RB1, I can do immunohistochemistry. And the results are so highly correlated with the NGS results that that unless there's another reason to do NGS, it's not necessary to do molecular. Right now, I would say the main role of molecular pathology is in informing therapeutic targets. But, especially in the NET space, there really aren't any therapeutic targets that we can glean from NGS.

In neuroendocrine carcinoma, there are a few and they're rare, but they're such good therapeutic targets and the prognosis of neuroendocrine carcinoma is so bad that I say it behooves one to know the results. And in neuroendocrine carcinoma, what you want to try to find is MSI high, high level microsatellite instability. Or by immunohistochemistry, we say deficient DNA mismatch repair function. And then another one would be NTREK rearrangement, which you could get by NGS or FISH, or Pan-TRK is the complimentary immunohistochemical assay that one could do, Pan-TRK positivity. Those are, I call them “homeruns.” So once in a while in a neuroendocrine carcinoma, you find one of these really superior oncogenic targets.

NGS also helps diagnostically. It could theoretically help in NET of occult origin. 25% of neuroendocrine tumors present as usually liver metastasis, often extensive liver metastasis. And the primary site where the tumor arose from is not clear based on clinical presentation, physical examination, imaging studies, CT chest/abdomen/ pelvis, MRI, even the next step in a staging workup for a NET would be somatostatin functional imaging, so DOTA-scan. Octreo-scan in lesser resourced settings or even 10 or 15 years ago before DOTA-scans or gallium scans were pretty well dispersed out in the community. There are patients who even on a gallium scan the primary doesn't like light up.

But there are certain instances where NGS results would potentially inform the site of origin in a NET of occult origin because certain mutations arise in certain sites of origin. Mutations in ileal primaries are really rare, but pancreas is famous for having several genes that are that are frequently mutated like ATRX and DAX, MEN1, and genes related to the mTOR pathway like TSE1 and 2. So, if somebody handed me an NGS report from a NET, I might be able to look at that report and say, “Oh, I think this is probably a pancreas tumor.” But instead I do that with immunohistochemistry because most neuroendocrine tumors, like for ileum, all neuroendocrine tumors, you're not going to find any mutations. So I use immunohistochemistry. And for the NETS, I mainly use immunohistochemistry that's an algorithm that I developed based on markers that I selected based on this developmental biology approach where I'm trying to find very accurate targets that are informed by the developmental biology of the cells and tissues. So I use things like CDX2, which is a transcription factor that's expressed by the EC cells, the enterochromaffin cells, the cells that make serotonin. The cells that ileal and jejunal and many appendiceal neuroendocrine tumors recapitulate. Tumors are trying to be some normal cell type. So we use CD2, PAX6 and islet-1, I've highlighted for pancreas. We use SACP2 for rectum. We use OTP for lung. And usually we hit with that panel. But I've got I've got backups and backups to backups, and sometimes I strike out.

We also use immunohistochemistry for NEC site of origin assignment. And there's not as many useful markers. Though I will highlight that in the poorly differentiated carcinomas, the most important distinction is “Did the tumor arise from an organ?” I say visceral. So, it's there either of lung origin or extrapulmonary visceral origin. So visceral or cutaneous. And the distinction of cutaneous from visceral origin is super important because it entirely informs in a metastatic tumor the therapy. Because cutaneous neuroendocrine carcinomas, they're called Merkel cell carcinoma are among the most responsive to checkpoint inhibitor therapy, which is this new class of immunotherapy that's been deployed for the last five years and is used in tons and tons of different tumor types, but it really makes Merkel cell carcinomas melt away. And visceral neuroendocrine carcinoma is not so much. So implied by this discussion is site of origin is important in metastasis of unknown primary because the primary site, even in metastatic disease. Metastatic disease is bad, right? But metastatic ileal neuroendocrine tumor is going to behave differently than metastatic pancreatic neuroendocrine tumor. For example, they have different prognosis. They have different survival curves.

And then of course, it informs therapy. So there are therapies that a patient would be eligible for if they had a pancreas primary that they would never get if they had an ileal primary. And at some centers, like definitely at the University of Iowa, we're always going to try to resect the primary in metastatic neuroendocrine tumor. Jim Howe is our surgeon. He wants to know in this metastatic neuroendocrine tumor of occult origin, should I do an exploratory surgery intending to remove, usually it's ileum or pancreas. And in the vast majority of cases, at least 95% of the time, I can tell him with an incredibly high degree of certainty using this immunohistochemistry what surgery to do. Because it's often clear. If the somatostatin functional imaging is positive and it's clear, that's fine. But it's often, “Oh, the somatostatin receptor imaging, there's something in the mesentery but the SUV, the signal intensity, it's intermediate, it's not quite bright enough. There's also this little spot in the pancreas. I'm getting mixed signals. Andrew, can you give me more clarity?” I can often say, “DOTA-scan be damned, this is definitely an ileal primary. Or this is definitely a pancreatic primary.” Or sometimes, every few months, I say, “You know what, I don't know.” And then we talk about what to do in the tumor board.

Lisa Yen:

Wow. This is like a masterclass of how and why pathology is so important, how it guides treatment and even surgery so that the surgeon knows where to go.

Dr. Bellizzi:

Pathologists often say that the role of the pathologist is to guide the surgeon's hand.

Lisa Yen:

Yep, you're the Oracle. The other point that you made about these stains, I think a takeaway is the patient can ask about what IHC stains were done and…

Dr. Bellizzi:

Sure

Lisa Yen:

…and if can be added on to their pathology specimen.

Dr. Bellizzi:

Yeah, and there's gold standard. You mentioned that we're practicing at this ENETS Center of Excellence So just because all the stains haven't been done, it doesn't imply that the diagnosis is incorrect. But I would say there is sort of a floor gold standard, which is a ki67, a keratin to make sure that it's epithelial, mainly to distinguish well differentiated neuroendocrine tumor from paraganglioma/pheochromocytoma, which are other neuroendocrine neoplasms. This is another example actually of birds and bats. They're both chromogranin and synaptophysin positive, but one is keratin positive and one is keratin negative and getting that diagnosis right is really important. So ki67, keratin. And then making sure that it's actually neuroendocrine, and that's your chromogranin and synaptophysin. Mainly, INSM1 is especially useful in poorly differentiated neuroendocrine carcinoma. Then if you do get a second opinion, that lab might want to do their own immunohistochemistry. I like to do my own ki67 immunohistochemistry because of all the issues with heterogeneity and biochemistry and read out that we talked about. But they might have a unique University Medical Center-centric approach to diagnosis and there might be additional pieces of information that they want to glean from one pathology specimen.

Lisa Yen:

So you are already kind of addressing what I was thinking, which is a lot of patients at this point would be thinking, "Oh, well, what if I want to get a second opinion? What if I'm one of the 25% that don't know where my metastatic tumors from the liver originate? Or maybe I'm seen at a hospital and they didn't go into this much detail? Or have a G3 NET or NEC? How would I get a second opinion?"

Dr. Bellizzi:

When people reach out to me, including family members, but the public at large reach out to me and they have a new diagnosis of cancer, I always recommend getting a second opinion. When do I recommend a second opinion? If the diagnosis is rare. If there's any diagnostic ambiguity. If the clinician is uncomfortable treating the condition. If the hospital-based setting is uncomfortable treating the condition. One of the disadvantages of medicine in the United States is there's incredible heterogeneity in access and quality to care. For me, access to healthcare is a human right, but not every doctor even feels

that way. I'd say the vast majority do. Especially if you as a patient feel uncomfortable with what's going on, if you have that sense, that's when you should consider getting a second opinion.

And the second opinion has lots of pieces to it. You can get second opinion just on your pathology. But usually when people reach out to me and they're potentially interested in a second opinion, it's also potentially worthwhile to get a second opinion about your whole case. And to be seen at a different place to have your history revisited, your imaging revisited, your pathology revisited. So when folks reach out to me, they might be interested in a second review of their pathology, or they might be interested in just being seen at the University of Iowa Hospitals and Clinics. The pathology diagnosis drives at everything. So, if it's a rare tumor—we quip that neuroendocrine tumors are the most common tumors at the University of Iowa, and it's almost the case. But that's the reason it's awesome if you have a neuroendocrine tumor if you can have me review your pathology or if you can be seen by our multidisciplinary team because we see so many cases. The idea is that outcomes are better at places that see a higher volume of cases. [It] doesn't mean you're going to have a bad outcome at a lower volume place, but it's really all about your comfort.

And then, how do you get your case seen? You reach out to the medical center and you make an appointment. So you could make an appointment with our neuroendocrine clinic. You can reach out to me if you want just your pathology seen, and I'll connect you with our consult practice. I see a large volume of cases in second opinion. Most of the requests for pathology review from the outside come from other pathologists. They say, “Andrew, we're seeking your expert opinion in this GI tumor or this neuroendocrine tumor.” Or they're generated by clinicians. I said if the patient's uncomfortable, maybe they should seek a second opinion. Sometimes the pathology report that the clinician gets doesn't quite jive with what they think is clinically going on. Or the pathology diagnosis is rare, and the clinician, they say, “I know our pathologists are awesome, but this is a rare diagnosis. I would feel more comfortable taking care of my patient, knowing that this was seen by somebody more expert.” If you want your pathology seen, you reach out to me by email, and I connect you to our consult practice. Or if you want to be seen at the University, I connect you to Kimberly Miller, the nurse that that runs the clinic.

And then, it’s not fair. Some people have better coverage than other people. And that's why it's useful to interface with our practice managers. They'll help with the billing. Sometimes they'll bill the referring institution. Sometimes they'll bill insurance. And then, it's up to the patient. Sometimes it's going to be a direct bill. And it's not the most expensive thing, but it's non-trivial, especially because we charge per immunohistochemistry test. We might be talking about a bill in the potentially in the one to $5,000 range, probably closer to the $1,000 range. Eyes wide open when you seek any second opinion what the coverage environment is like.

Lisa Yen:

Like you said, it's not nothing. Here's a practical question: How long are pathology specimens typically stored?

Dr. Bellizzi:

I get questions about this all the time and there's all different kinds of pathology specimens. There's what we call the wet tissue. In a biopsy, everything gets submitted and processed. And I talked about this process of making the slides. But in a resection specimen, I suggested that we only take small samples

from the whole. And there are guidelines for tissue retention that are set by the College of American Pathologists, which is one of the main pathology professional organizations with a really, really multifaceted mission. But one of the most important things that the College does is accreditation of laboratories. So, you want to work at a CAP-accredited lab. You want your pathology specimen to be read out by a CAP-accredited lab. So, the CAP dictates retention periods for different things. For that wet tissue for your pancreatectomy, for example, we hold on to the wet tissue for at least two weeks after the pathology report is signed out. And then what happens? Usually it's a question from friends or family. They say, “Oh, I had my gallbladder out, what happens to that?” And for most tissues after two weeks, we discard them. It's medical waste, and we discard the tissue and we actually we incinerate it. Functionally, we cremate the tissue. But we can't give you your cremated gallbladder, right? Because it's gonna get aggregated with other similar tissues.

And then there's the glass slides and the paraffin blocks. And the tissue retention guideline for slides and blocks is 10 years. And 10 years is the floor. And the reason this is important is the CAP sets these guidelines and to be accredited, there are hundreds of checklist items that the labs have to subscribe to and be able to demonstrate when an inspector comes. After 10 years, your slides and blocks might get discarded, especially in the community. In the community, your slides and blocks will likely be discarded after 10 years. The reason they'll be discarded is 10 years is the floor and storing stuff takes space and space is money. Every month they're discarding the stuff from 10 years and one month ago. An academic medical center almost always retain stuff for longer. We have glass slides in the file back to the 1910s or 20s. And I don't know who had the bright idea but in 1973, at the University of Iowa, some leader decided, “Why are we throwing this tissue away? This seems like a really invaluable biospecimen resource.” And so, now we keep slides in blocks forever.

I'll mention one or two other things. One other thing is, throwing away tissue blocks is a tragedy. Because these biospecimens are invaluable, and they're useful in so many different ways. One group that’s savvy to this is the NIH. They fund a program called the “SEER RTR.” RTR stands for residual tissue repository. And SEER stands for “Surveillance Epidemiology and End Results program.” So, this is the main cancer epidemiology program of the government. The SEER has a program called RTR where, basically they'll pull up to the community pathology labs with a truck and say, “Any slides and blocks that you're going to discard, we’ll take. Instead of incinerating them, we'll take them. And we'll file them and sort them and annotate them and make them available as a biospecimen resource to investigators who have to apply for use of this material and have to pay for use of this material, but instead of it going in the garbage.”

You'd never know whether your blocks are going to the SEER RTR or not. Up to you, but if I was a neuroendocrine tumor patient, even if I was at an academic medical center, I would reach out to the pathology lab and say, “Hey, I had a resection five years ago or seven years ago or 10 years ago. And I'm curious about the paraffin block. What are your laboratories’ tissue retention policies?” And if they say, “We retain blocks for 10 years, and then we discard them.” I would say, if the choice was for that block to go into the garbage, I would rather have that block in my hands. Because it might be useful to you, as a patient, someday.

These tissue retention policies, they haven't changed for a long time. It's probably been decades. And I think that they're sort of due for a change because the idea was 30 or 40 years ago, if a patient had cancer in 10 years, they were going to be dead. And this paraffin block, it's good for an H&E diagnosis. But now 40 years later, we have immunohistochemistry and molecular diagnosis and predictive markers and

personalized healthcare. And I think that probably the 10 year is not long enough. I would see in the next 5 or 10 or 15 years, it being 15 years or 20 years. So if it's gonna go in the garbage, and it's 10 years out and you're a happy NET patient, I would get that block in your hands, because it would potentially be useful someday. There might be a new test that we might want to do on that block in a year or two years or five years that we would never have thought to do 10 years ago.

Lisa Yen:

Wow, what a helpful practical tip. I guess I have some phone calls to make after this. That's actually a really good transition to the last question, which you're touching on how much things have changed and how many advances that there have been. I'm really curious what future advances you look forward to that would really bring hope to the patient population?

Dr. Bellizzi:

I mean, this is why we're here, right? To advance the standard of practice in neuroendocrine tumors. It's why I'm so excited to be at the University of Iowa and a member of this multidisciplinary care team. I think what I'm excited about are advances in therapy, and the ability to increasingly match a therapy to the specific patient and to the specific patient's tumor. And right now, the tools are pretty crude around prognostication and prediction. What I foresee, it's this translational molecular pathology. Discoveries that are made in basic and translational science. For example, in ileal neuroendocrine tumors, there are studies that haven't really been applied to the diagnostic space where ileal neuroendocrine tumor under the microscope, it looks one way. But there are three or four main molecular subtypes of ileal neuroendocrine tumors, and we can distinguish them based on genetic abnormalities and promoter methylation. What I can envision, in even five years is an entirely different standard ancillary testing that's mainly molecular based that will give us a lot more information than the ki67.

We know that not all ki67 2% tumors behave the same. Some are incredibly indolent and patients live with them for decades. And others, they grow and they rapidly progress. And so, right on the horizon are much more precise predictive tools. Then the ultimate goal is to have a specific therapy for every patient. And my job as IHC guy, it's my Twitter handle, is if there are any opportunities to develop, build, implement immunohistochemical tests that are of use in the diagnosis, prognostication, or prediction of neuroendocrine tumor patients, to be there to develop that test, to implement that test. That's what I'm hopeful for. And that's what's changed in the last in the last 10 years. Ten years ago, it was just ki67, chromogranin A and synaptophysin. And Lisa, you mentioned in the intro, as the lab director, I have developed and implemented 100 new immunohistochemical tests. And probably 20 of them are in the NET space. And a lot of them right now are for diagnosis. But the goal is for more of them to be for prognostication or prediction. And a couple examples are, we do somatostatin receptor subtype-2 immunohistochemistry. And that's mainly in the neuroendocrine tumor space. But one of the most recent impactful neuroendocrine carcinoma markers that we developed and implemented is immunohistochemistry for CXCR-4, which is very similar to SSTR-2. It's a bio-theranostic marker. That is, it's useful for diagnosis and therapy. So we built this marker that's correlated with the results of a nuclear medicine test and is potentially correlated with the results of a specific peptide receptor radionuclide therapy. And so hopefully, more of those.

Actually, it came up on Twitter yesterday, and then it came up at signout today. Someone was asking about SSTR-5. So SSTR-2 is one of the somatostatin receptor subtypes. It's highly expressed by

neuroendocrine tissues and tumors. And its expression is the basis of the octreoscan and the DOTA scan, is why patients get somatostatin shots. That's why they get octreotide and lanreotide. It's why they would get Lutathera. But there's not just one somatostatin receptor, there are several. And we're working on immunohistochemistry for SSTR-5. Potentially to highlight tumors that might benefit from different cold peptide therapy that would be the scientific basis for groups developing PRRT to patients whose tumors preferentially expressed five instead of two. Insulinoma patients, a subtype of pancreatic neuroendocrine tumor, they're famous for making a lot of SSTR-5 and little to no [SSTR]-2. And it's all incremental. We'll do it one test at a time, and we take care of one patient at a time. And hopefully when I come back on the pod in five years, I can say that we've implemented 200 immunohistochemical tests and 40 are in the neuroendocrine space. People always say that there's no better time than today to be practicing medicine. But it's always the case because the fields always advancing. And I'm super privileged to be at such a lovely academic medical center that I can be at the leading edge of, of discovery and mainly implementation of novel diagnostics and therapies. The pillow that I lay my head on is oftentimes the neuroendocrine pillow.

Lisa Yen:

Thank you so much. I mean, what you're talking about that is so exciting. Better tools for clearer answers so that we can have more personalized and tailored treatments. And we thank you for all your hard work in that space. It's something that we can rest assured someone else is worrying about it, so we can worry less about this.

Dr. Bellizzi:

Thank you for the opportunity. Pathologists are mainly behind the scenes. Pathologists, in general, are introverted types. And one of the reasons that I'm a famous-y pathologist is I'm not so quiet. I appreciate the opportunity to put pathology's best foot forward. So I appreciate this opportunity to know you, to interface with you to help you advance your goals and to honor your values through this really important podcast.

Lisa Yen:

Thank you so much. This is very educational, informative, and hopeful. And it's really amazing to learn about all the different ways that pathology and what you do really helps us. So, thank you again, and we look forward to seeing you in the future in person.

Dr. Bellizzi:

Thank you.

Lisa Yen:

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