Hereditary Aspects of Colorectal Cancer

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MS. MAY Welcome back from lunch, everyone. My name is Megan May, and I'm a pharmacist at Baptist Health Lexington. As a gentle reminder after the break, please remember to silence your cell phone. Our first talk is a lecture on "Hereditary Aspects of Colorectal Cancer." It is my pleasure to introduce to you Ms. Heather Hampel of The Ohio State University and Dr. Michael Hall of the Fox Chase Cancer Center.

DR. HALL Thank you.

MS. HAMPEL Thanks, everybody. It's really a pleasure to be here. I want to especially thank *JADPRO* and the organizers of the meeting for inviting me. Dr. Hall and I get to work together a lot on inherited colon cancer, but it's not often we get to speak together to such a great audience, so we're really looking forward to it. Our plan is to start with me talking for 25 minutes more about the genetics of hereditary cancer syndromes, and then Dr. Hall will talk for about 25 minutes on management and treatment of patients with hereditary cancer syndromes. Hopefully, if that works, we'll leave 10 minutes for a good Q&A session at the end.

So without further ado we'll get started. Our learning objectives are I'm sure in the book and hopefully we'll succeed in accomplishing all of these. Here are the disclosures: I do have some disclosures from two of the commercial labs that I do research studies with and serve on their advisory board for.

When you see a patient with colon cancer, this is the flow chart that I go through in my head to decide whether or not they might need further evaluation for hereditary cancer syndrome. The first question I ask is, if you've got that path report in front of you, is there also a bunch of polyps? And what do I mean by a bunch of polyps? If they're adenomatous polyps, then I want to see more than 10. If it's some unusual polyp that you've never seen before or it seems rare like a Peutz-Jeghers polyp or a juvenile polyp, it might not take as many. But roughly the more common polyps you're going to see are going to be adenoma-type polyps, and if you see more than 10, you're going to want to go down more of the polyp pathway and if you don't, then you're going to really be thinking Lynch syndrome. Lynch syndrome is the most common inherited form of colon cancer, so that is the primary syndrome we are trying to rule out with most of these colon cancer patients; the polyposis syndromes are much less common. So we'll start with Lynch syndrome and then do the polyp ones quickly. There's new data suggesting about 1 out of 279 individuals has Lynch syndrome, and if you just take that times the population in the U.S., that's 1.2 million people with Lynch syndrome. That's actually more common than hereditary breast or ovarian cancer, but the public is much more aware thanks to Angelina Jolie and the press of *BRCA1* and *BRCA2* and much less aware of Lynch syndrome. I think it's pretty well known by our patients now that breast and ovarian cancer go together in families and that that's a red flag and much less well known that colon and endometrial or uterine cancer can run together in families. So we have our jobs cut out for us in terms of recognizing this, as the patients aren't necessarily going

to. We know this is an inherited syndrome that causes increased risk for colorectal, endometrial, and other cancers. The next two most common being gastric cancer and ovarian cancer. So this does have some overlap with hereditary breast or ovarian cancer in that they both increase the risk for ovarian cancer. So if that's your patient population, you want to be thinking about Lynch syndrome as well. I think the really important thing about Lynch syndrome is that most of the cancers are really preventable, so as long as we start colonoscopies early enough and do them frequently enough we believe we can keep these patients from getting colon cancer, most of our females with Lynch syndrome will elect to have hysterectomies. And Dr. Hall will talk to you much more about this during his talk, but that means three of the top four cancers could be prevented as long as we get these patients diagnosed.

So what's the problem? The problem is that there are studies now showing that 95% of people with Lynch syndrome in the population are not aware of their diagnosis, so we have a long way to go to try and bridge that gap, and you guys are going to be on the front lines in terms of identifying these patients. The Lynch syndrome is caused by mutations in one of four genes, but you'll hear about a fifth one called *EPCAM*, which really causes Lynch syndrome by causing problems with the *MSH2* gene, so in fact it's really four genes: *MSH2, MSH6, MLH1*, and *PMS2*. And these genes are known as our DNA mismatch repair genes. We all have them and we want them to be working. I describe them to my patients like the spellcheckers in our body, right? As our DNA is replicating to make a new cell, sometimes mistakes happen, that's a normal part of the aging

process. We have these lovely built-in spellcheckers whose whole job it is to recognize when a mistake happens and fix it, so the good genes, we all have them and we want them to be working. In our families with Lynch syndrome, they are unfortunately born with one of these mismatch repair genes not working in every cell of their body because they inherit that mutation from mom or dad. So from the start, they are one step closer to getting a cancer. Luckily that working copy from the other parent is going to compensate for many years, and so we don't really see a lot of childhood cancers with Lynch syndrome; this is much more adult onset.

The odds are high though that with one mutation in every cell, some day over the years when one of those at-risk cells in the colon or the endometrium are dividing, they are just going to acquire a mistake in the working copy, so-called "second hit," and now we have a cell with no more working mismatch repair gene. So as that cell accumulates mistakes naturally, they cannot be fixed, and if any one cell gets enough mistakes or mutations, that cell will become a cancer. So this is basically the pathway for Lynch syndrome patients to develop cancer. And because of that, that's why we see certain characteristics when we look at the patients and their family members, notably earlier ages of diagnosis. If you're born one step closer, it's not going to take as long on average for a cancer to get diagnosed. Multiple primaries. If you have this one mutation in every cell, you could get the second mistake one year and have a colon cancer, and 6 years later this happens in the endometrium and you have an endometrial cancer. So

multiple primary cancers, again, just a red flag that there's probably an underlying cancer susceptibility.

Then of course it's a dominant condition, which you guys know means getting passed down through the generations, so usually you see what we call a vertical transmission pattern, so a grandparent affected, the parents, some aunts and uncles affected, and then it starts hitting that patient's generation as well. That's more concerning, a vertical transmission pattern, than having a couple of siblings affected where if you feel like there is a gene mutation getting passed down through the generations. Now obviously most cancers are not hereditary, but I think the more we are doing testing now that we have these nice panel tests we are realizing much more of cancer is hereditary than we used to think. I would say firmly 10% of colon cancers fall into this hereditary category and again, the vast majority being Lynch syndrome, and 90% are not and those patients are born with all their genes working just fine, but they are aging and their cells are dividing. If one cell gets enough mutations, it will become a cancer; that's going to take a much longer period of time, so you are going to see later ages of diagnosis, one tumor, not multiple primaries, and no family history.

Those are when you're taking that family history in clinic the reason for a lot of those characteristics that you're looking for. You guys know that if it's dominant, if your mom or dad has, it there's a 50% chance you will inherit it. All of the known hereditary cancer syndromes except a couple of the polyposis syndromes are dominant. So you can generally guess it's a 50:50 autosomal dominant condition. There are a few polyposis conditions now that are recessive

though. All right, so what are the cancer risks? Well, it actually turns out it depends on which of the Lynch syndrome genes is responsible in the family. Two are considered much higher-risk genes; those are *MLH1* and *MSH2,* and they lead to significantly increased risk for colon and endometrial cancer. Here you see 40 to 80% risk for colon cancer. That says men, but essentially it's about the same for women as well. Endometrial 25 to 60%, and this compares to the general public, which has a 5.5% risk for colon and a 2.5% risk for endometrial cancer. Here you see tailing in with stomach and ovarian cancer the third and fourth most common cancers. *MSH6* and *PMS2* have much lower cancer risks, and because of that, the family histories often aren't as striking.

These families are a little harder to detect looking for those classic strong family histories with multiple affected at early ages. You see the colon cancer risk could be as low as 10 to 20%, so elevated, but nowhere near what we see with the high-risk genes and the uterine or endometrial cancer risk around 15 to 25%. So that's making things a little bit more challenging, and we're finding a lot of those families now through universal tumor screening for Lynch syndrome. Historically family history was the key to diagnosing Lynch syndrome, but it doesn't work as well, as I said, on those new genes with lower risk. I think if we all saw a patient whose family history looked like this, we would know something was wrong and we'd refer them to Genetics or get some genetic testing, but a lot of the families don't look like that. So the classic old criteria, the Amsterdam criteria were known, you could remember them by 3-2-1 rule. Three cases of Lynch syndrome cancer, so colon, endometrial, or ovarian or stomach, in at least

two generations, one of the patients who was affected had to be a close relative, a first-degree relative, so a parent, sibling, or child of the other two, and one of the people had to be diagnosed under age 50, so the 3-2-1 rule. It turns out this is pretty sensitive, but not at all specific, so a lot of families who don't meet this criteria still have Lynch syndrome. They needed it back when they were hunting for the genes because they were trying to get a group of patients that was sort of really enriched for having a hereditary susceptibility. So they realized that was a little too strong and years ago made Bethesda guidelines, which are a little more relaxed, and this was how to decide if it might be worth doing some tumor screening on the patient's tumor to see if they might have Lynch syndrome. But they are kind of used interchangeably now for when to refer to Genetics to consider Lynch syndrome.

Here you start to see just the early age of diagnosis being enough on its own. Colon under 50 or a person who is over 50, but has a first-degree relative who is diagnosed under 50 with a Lynch syndrome–associated cancer, so then you've got the two case, one being young. Multiple primaries here and that can even be synchronous tumors. So if you have a patient present with two primary colon cancers, that would count. And then the MSI histology. So there's some features that you might see on a path report short of actually screening for Lynch syndrome that are kind of characteristic of MSI pushing borders, Crohn's-like reaction, tumor-infiltrating lymphocytes, and that was in the past before we were actually screening with MSI and IHC tests, one of the ways that these families

could be identified, and then three cases total without regard to an age of diagnosis criteria on the same side of the family.

There is a computer model that's just been updated literally a couple months ago that used to be called PREMM 1,2,6 because it only predicted the likelihood for having mutations in *MLH1, MSH2*, and *MSH6*. It has been updated and it now includes *PMS2* and the *APCAM* gene, so it's called PREMM 5 and it will tell you, it's online, it's free. Google "PREMM 5". It's the first thing that comes up, and it's pretty fast. It asks a series of questions about your patient, how many colon cancers have they had, what was their youngest age of diagnosis, adenomas, endometrial cancer, and then they ask about first- and second-degree relatives and then it spits out a likelihood that the patient will test positive. On the old models and on the NCCN guidelines, it still says that if your patient comes up 5% or higher on this model, you should refer to Cancer Genetics, but the new paper is actually proposing that we can cut that down as low as 2.5%. So we'll see what happens with NCCN guidelines this fall when they get together and we look at that again. That's very easy to use, free, and a nice way of quickly deciding if a patient is likely to have Lynch syndrome, but as we have been talking about, family histories can really be deceiving, so the family size is just getting smaller and smaller, wider use of colonoscopy is pulling things out as adenomas or tubular villous adenomas before they can become a cancer. So that's getting rid of that family history of cancer and it's more a family history of polyps, which people are not good at reporting. Then with *MSH6* and *PMS2*

having lower cancer risk, it can be quite difficult to find them just based on family history alone.

That's why we've started to rely on tumor tests that can be done to screen for Lynch syndrome in the Path. department at the time of diagnosis. The two main ones are either MSI testing, so that's directly testing for microsatellite instability and that needs DNA extracted from tumor and normal tissue and a molecular lab, so less hospitals do this test than the immunohistochemistry test. It turns out it's only positive or high in about 15% of colon cancer cases, but it is positive or high in most tumors from patients with Lynch syndrome, about 77 to 89%, so it's not diagnostic, but it does select a group of patients that are more likely to have Lynch syndrome and could benefit from genetic testing. Far more commonly adopted at most hospitals is immunohistochemistry, where you actually stain the four slides from the tumor with antibodies to the four mismatch repair proteins, and I'm going to show you some slides of that in a minute. The normal situation would be for all four of the proteins to be present, then it is unlikely that that tumor has microsatellite instability and the patient is unlikely to need to see Genetics unless they are young or have polyposis and again. About 20% of the time, you are going to have an abnormal result on IHC.

It is not diagnostic, it does select a group of patients that are more likely to have Lynch syndrome, but not everybody with abnormal screening has Lynch syndrome. Then there's two follow-up tests that are sometimes done if either of these tests come back abnormal, particularly if the IHC happens to be absent for the MLH1 and PMS2 proteins. It turns out the most common cause for having

MLH1 and *PMS2* absent in a tumor is acquired methylation of the *MLH1* promoter. So you can directly test for the *MLH2* methylation in the promoter, but again, that requires a molecular lab and so that's done less widely. As a surrogate the *BRAF V600A* mutation, which most of our hospitals are doing anyway for melanomas and stage IV colon cancers, it turns out 69% of methylated cases have the *BRAF* mutation. so it can be used as a surrogate for doing methylation testing. You just have to keep in mind that if a tumor is missing *MLH1* and *PMS2* and does not have a *BRAF* mutation, they could still be methylated so it doesn't rule them all out, only 69%.

So MSI: they literally just measure five different microsatellites and those are repeat areas in your DNA. One of the most famous ones is called BAT-26, and at BAT-26, there are 26 As or adenines in a row in your DNA, so when your DNA is replicating, it's pretty hard to replicate 26 As exactly, it's prone to slip and maybe it only copies 20 of them. Well, if your mismatch repair genes are working, you would recognize that and correct it. If your mismatch repair genes aren't working, you won't recognize that, you won't be able to correct that, and now you've got a tumor with 20 As instead of the 26 you were born with. So they literally just measure these five different repeats and see if they are the same in your blood and what you were born with as they are in your tumor. If two or more have shifted and gotten a little smaller, that's a sign you have microsatellite instability, and you guys know all of the good things that that now means in terms of treatment, which Dr. Hall will talk to you about. The IHC test is much easier to do because every hospital can do immunohistochemistry stains, and it tells you

which of the proteins is not being produced, which gives us a hint as to where the mutation might be. So most of the time, again, all of the proteins are present. This is a patient that is not microsatellite unstable and is pretty unlikely to have Lynch syndrome.

There is a slight chance they could still have microsatellite instability, but they have about a 96% correlation. But you still might need to refer them to Genetics if they're super young or have polyposis or have multiple primaries or a strong family history because there are other genes that could be playing a role. The most common abnormal result would be the one I talked about where *MLH1* and *PMS2* are absent in the tumor. Here you can see *MSH2* and *MSH6* are present, and so these are pretty easy to read. They are nice brown nuclear staining, and you have normal internal controls, so the normal cells are still producing protein because they have not acquired that second hit, and it's only the tumor cells that have acquired the second hit on their working copy of their mismatch repair genes that have no protein present at all. You're going to see this result about 15% of the time, and most of the cases are due to methylation. About 80% of the time when you see this result, it's due to methylation, so many hospitals automatically reflex when they see this result to either methylation testing or the *BRAF* testing. Here's an example from a recent pathology report at our hospital. Here you scroll down to the bottom in the addendum and you'll see all four of the proteins listed. People use a little bit of different nomenclature here; they are often now instead of "present" using "intact." Intact or present is the good situation, you would like all four of those proteins to be present or intact. If

any of the proteins are missing, they will often say “absent.” Here we have a tumor with *MLH1* and *PMS2* absent, and our Pathology department then automatically ran the *BRAF* test, and here you see it’s negative for the *BRAF* mutation. So this is a case that does need referred to Genetics because there is no *BRAF* mutation, and 69% of patients with methylation will have a *BRAF* mutation, so this is making methylation look less likely and they need to come see Genetics. When we first put this in our hospital report, my one oncologist who I adore would refer them if it was positive for the *BRAF* mutation because normally in Genetics you think positive is the bad thing, right?

But in this case, if there’s a *BRAF* mutation, the case is methylated, and that’s acquired and generally more common in elderly females, and the patient does not need referred. The second most common result would be to have *MSH2* and 6 absent. Very uncommon; about 3% of the time you can assume that tumor is microsatellite unstable and they need to see Genetics because there’s a very high chance there’s a mutation in *MSH2* or possibly 6, and then you can see either *MSH6* or *PMS2* absent alone. These are not common results, but they are again, strongly indicative of a possibility of Lynch syndrome, not diagnostic, but would warrant referral to Genetics.

Now we are going to really quickly go through the polyp syndromes. We are going to look at the adenomatous polyposis syndromes. These are really hard to miss. If your patient has a hundred or more adenomas, then something is wrong. And I think my biggest problem with this syndrome is that because it’s so obvious, they don’t tend to get referred to Genetics. Everybody knows they’ve got

FAP; well, what about their kids? If we can do genetic testing and find the mutation, we can test the kids so they know whether they need to start their colonoscopies early or no. And when I say early, we are talking potentially 10 and 11 year olds, so still important to refer to Genetics even though everyone in the world working with this patient knows they have FAP, send them over so we can find the mutation and help their family members know whether they have inherited it or not. With a weaker form of FAP, attenuated FAP, you can see polyp counts between 20 and 100. In the low counts, it gets a little confusing about whether to refer or not. We generally refer anyone with more than 10 adenomas in our clinic, and lots of them test negative when you test them for these polyp genes, but every once in a while, one of them tests positive. We recently had a 70 year old who had about 16 adenomas; they didn't even start until her 60s, and she had an *APC* mutation. She has a very late onset weak polyposis, but good to find so we can let her family members know who needs increased screening and who doesn't.

One of the newer ones, and this one is recessive, is MAP or *MUTYH*-associated polyposis. It's a great mimicker. You can see full-blown polyposis more than 100, you can see the middle kind of 20 to 100, and you can also see this in early onset colon cancer patients with no polyps. So this one you really detect often on panels because you are ordering a whole panel of colon cancer genes and it comes up. I will just mention that 1 out of 50 Caucasians from Western Europe is a carrier of a *MUTYH* mutation, so that's very common to be a carrier. There is some data that they may have slight increased risk for colon

cancer, but it's unclear whether they need increased screening unless they also have a family history of colon cancer. But it is not that uncommon to get a carrier on a gene test, and it's only when they have two mutations that you see the polyposis. The newest kid on the block, polymerase proofreading-associated polyposis, is caused by mutations in the *POLE* and *POLD1* genes, and these make ultrahypermutated tumors if tumor sequencing is don. We are still learning about these, but they seem to make lots of adenomas, have an increased risk for colon cancer, uterine cancer, brain cancer has been seen; it's really being still defined, but certainly if you're ruling out polyposis, you've got a patient with 10, 20 or more adenomas, you are going to want to check all of these genes on your gene test. The hamartomatous polyposis syndrome: these are going to be those weird polyps that you're not going to see very commonly; less than 1% of all colon cancer patients is going to have one of these syndromes, so these are rare and they are so unusual that the pathology report will probably say something about referring to Genetics.

It doesn't quite take as many polyps to get a diagnosis. If there's even two Peutz-Jeghers polyps, they should be evaluated for Peutz-Jeghers syndrome. Five juvenile polyps should be evaluated for juvenile polyposis syndrome. You see the genes here; there are genes now for all of these syndromes except the last one and that's serrated polyposis syndrome. That syndrome is interesting, and people aren't even quite sure it's hereditary yet or not, but there's WHO criteria and there's certainly increased screening guidelines based on NCCN for people who do meet the diagnostic criteria, but not a lot we can do for genetic

testing. They have a lot of hyperplastic polyps, sessile serrated adenomas, serrated polyps, kind of a mix. And the more mixed polyposis syndromes we see, there's one called hereditary mixed polyposis syndrome just found to have a mutation involving the gene in front of *GREM1*. This has only been seen so far in Ashkenazi Jewish individuals, so Jewish patients with mixed polyposis, they can even look a little like Lynch syndrome. We followed a patient with this syndrome in our clinic who we tested for Lynch syndrome, and we tested for all of the polyposis syndromes and never found anything and finally put them in the research study out of England where they found this gene. So you're not going to pick this up in your non-Jewish patients, but it is common, not common, but it is possibly the diagnosis in an Ashkenazi Jewish family.

Then Cowden syndrome, which most people think of as a breast cancer syndrome, really does have quite a colon phenotype. They make lots of weird colon polyps. A lot of times they are ganglioneuromas actually, and so multiple different types of colon polyps plus big head, plus this family history of breast, thyroid is going to make you think of Cowden and *PTEN*, but the nice part is with these panels, you just order a colon panel, you are going to get every single one of these genes we just talked about covered.

So who should you test? Clinical criteria would be patients who meet the revised Bethesda or Amsterdam criteria we talked about, patients with colon or endometrial cancer diagnosed under age 50. I didn't have colon there because they would meet the Bethesda criteria, but I just want to stress that one. People with PREMM 5 score over 2.5 or 5% and individuals who have a known family

history of Lynch syndrome. We now are doing routine tumor testing in the Path. department for all colon cancer cases, or some groups do all the colon cancer patients that are diagnosed under age 70 and only the patients diagnosed 70 or older who meet the Bethesda guidelines. I find that's tricky for pathologists to know, so most of our pathologists agree it's easier just to do them all. Again, SGO recommendations: all endometrial cancer patients or endometrial cancer patients diagnosed under age 60 or endometrial cancer patients who meet a modified Bethesda guidelines, which includes endometrial cancer diagnosed under age 50.

You're going to just see that on the path report, so if you get a path report with IHC or MSI done and its MSI high or the IHC is abnormal and methylation has been ruled out, refer to Genetics. For polyposis, you're going to want to test for the adenomatous polyposis syndromes for patients who have more than 10 adenomas. For the hamartomatous polyposis syndromes, it's only going to take two Peutz-Jeghers polyps, five juvenile polyps, or a Jewish patient with mixed polyps and we like to start the testing, of course, with an affected person if we can. What tests should be ordered? On the tumor screening test, MSI and IHC should be done, but if you get a referral from a small hospital, it might not be done, so your doc and you might want it ordered especially with the treatment implications. They are generally pretty inexpensive to order MSI or IHC at this point. Then if you get to gene testing, most of us are using next-generation sequencing panels that include many genes, and the colon-specific panels can have between 14 or even 30 genes. Common hereditary cancer gene panels can

have between 25 to 45, and then there's panels as large as 80 genes, which get probably into a lot of rare things you don't need. The cost has dropped dramatically, as I'm sure you're all aware, and we can get panels for as little as $250.00 to $500.00 now, and most of the patients don't see much out-of-pocket costs. There are financial hardship programs at most of the labs now, so if you had a patient you referred in the past who couldn't get testing due to cost or coverage, send them back in because it's changed dramatically.

Due to the overlap between a lot of the polyposis syndromes and Lynch syndrome, panels really are the preferred approach for colon cancer genetic testing. I'm out of time, but a word about early-onset colon cancer. We just tested a series of 450 cases of colon cancer who were diagnosed under age 50 in the state of Ohio and found that 16% of them had at least one mutation in a cancer susceptibility gene, half had Lynch syndrome as you might expect, 8%, but 5% had other moderate- to high-risk colon cancer genes, and 3% had mutations in genes we weren't expecting that aren't traditionally associated with colon cancer like *BRCA1* and *BRCA2* and *PALB2*. And while I cannot say those genes caused the early-onset colon cancer, certainly discovering that is very important for that patient and their family members. So we actually are suggesting on early-onset colon cancer patients that you don't only order colon genes, that you order a broader panel that does include the breast cancer genes as well.

We are now going to switch and hear about cancer prevention and treatment in Lynch syndrome from Dr. Hall. Thank you, guys.

DR. HALL Thanks. Good job. Thanks, that was awesome, Heather. As Megan told you before we started, I am a GI oncologist at Fox Chase Cancer Center and I also run our GI high-risk program. I call it the "GI High-Risk and Other Program" because I basically see endocrine, renal, GI. GI is certainly my focus, but I get a catchall of everything else, anything non-breast/ovary. But my charge here today is I'm going to tell you a little bit about management particularly of Lynch syndrome and how we medically manage these patients, and then I'm going to switch over a little bit and talk to you about current molecular markers in colon cancer and how the Lynch syndrome story is fitting into molecular management of colon cancer.

I also just wanted to say I really want to thank the organizers for inviting me. I think this is an amazing conference with the content, the organization level; it's really been delightful, so I'm very happy to be here. Here's my outline. Just a couple useful terms that I'm going to speak about, and I just want to make sure we are all on the same page. Penetrants: as Heather mentioned, this is the likelihood of developing cancer if you have a particular genetic mutation predicted by a marker is a biomarker that looks at whether someone is going to actually respond to a particular therapy, and we'll talk about a few of those in a little bit. A prognostic marker is one that tells you if someone is going to live longer or not, but may or may not be predictive, and it's important to keep those apart, and we'll talk about that. First I'm going to talk a little bit about how we manage colorectal cancer risks, and what I want to start off with, full disclosure, is that the greatest amount of data we have in how to manage Lynch syndrome

medically really is in the realm of colorectal cancer. Many of the other guidelines and recommendations that are on the national guidelines that Heather and I sit on, the National Comprehensive Cancer Network, many of these are expert recommendations that we have put together from smaller studies or just basically group consensus. So I think that you have to really keep that in mind when you are managing patients.

The most important thing here is that we start colonoscopy in someone who has Lynch syndrome at age 20 to 25 and repeat every 1 to 2 years. There are these rare families where you have very early cancers and you may start even earlier, 2 to 5 years before the earliest case. Colorectal cancer risk can be reduced by intensive screening, but I think one thing you have to be honest with your patients about is this is a disease where we think the classic polyp pathway may be aberrant, and so even though intensive screening is great, it may not pick up every cancer, so there's a reduction in risk, but it's never going to take it to 0. For those patients who may not accept that risk or may have other reasons, we do still think of colectomy as a possible option for these patients. Some patients have that preference, they don't want to get a colonoscopy every year. Sometimes people have had one cancer and then they develop another and there's simply not enough colon left, so they go to colectomy because that's their only option. Also in this syndrome you see polyps that are frankly just not amenable to good surveillance with colonoscopy. I personally have a patient who develops multiple teeny tiny 2- to 3-mm polyps that become dysplastic even at the 2-mm level. That's a polyp that you just can't rely on an endoscope to find

before it develops into cancer, and so many of those patients when they have that intense development of early polyps will decide to have colectomy. Just to contrast that, you probably all know this, but the average-risk person starts colon at 50 with one exception currently being that African Americans are generally recommended to start earlier. Heather already went through this table here, which is the variable penetrants, and again, what I want to point out just as she did is that *MLH1* and *MSH2* are clearly more penetrant genes for colon cancer with higher risks, whereas I would say that we are still learning a lot about these other two genes. Clearly the penetrant seems to be lower, so people have lower risk of developing colon cancer, they seem to get cancer at a later age. We are moving toward gene-specific guidelines, but we just are not quite there yet, so we will use more general guidelines for everyone. People always ask, you know these poor people are getting recommended to have colonoscopy every 1 to 2 years; isn't there some other way we could screen the colon? And you know, there are these other modalities, fecal immunochemical testing, or the classic guaiac test, virtual endoscopy, capsule endoscopy, are these sort of good enough? And I think in conjunction with regular colonoscopy, I think there may be a role for these, but I think there are still a lot of negatives. This is a high-risk group. The pros of this approach would be that it's convenient for patients, it's less invasive, they don't have to undergo anesthesia to do a virtual endoscopy or a capsule endoscopy, but the cons are, again, this is high-risk syndrome and these polyps can be tiny, they can become dysplastic at a very small size and the reality is some of these, many of these modalities are just not sensitive enough to

pick up Lynch syndrome, and then ultimately if you do see something that you're worried about, you still have to have a colonoscopy to go after it.

Many people don't know—I think this is one of the most underplayed parts of Lynch syndrome, is that we do have a chemo-preventive agent in Lynch syndrome and that's the same aspirin you've been taking since you were a child. We have actually known for 20 years that aspirin has preventive properties in colon cancer, and actually not too many years ago the USPSTF came out with recommendations that for adults at 50 to 59, there is a benefit for 81-mg aspirin for primary colorectal cancer prevention. What we learned in Lynch syndrome in the CaPP2 study was that patients with Lynch syndrome who stuck to taking a pretty sizable dose of aspirin, 600 mg a day for 2 years, at the early adenoma outcomes, we were all depressed because actually we didn't see any difference in adenomas and we were like, uh, we just wasted 4 years and lots of aspirin and didn't find anything. But at the 4-year follow-up, what we found was that there was actually a substantial reduction in the risk of developing colon cancer and remarkably other Lynch syndrome cancers. Now I will say that when we sit on the guidelines, we hem-and-haw about this every year; we don't have a follow-up study which has shown similar data; there is a follow-up study being conducted. Everyone always asks us, "Well, what about lower doses than 600 mg in Lynch syndrome?" All I can say is we don't know yet, we are all working on it, we're hoping that data will come out.

Right now what I recommend to my patients is I make that recommendation based on the risks in the family and the amount of risk they are

willing to tolerate with taking aspirin. Obviously it's generally a safe drug, but there are risks that you need to go over with your patient. There are also other risks in Lynch syndrome, and in here, these are risks that are probably familiar to you, especially after Heather's talk. I think the important thing to remember when we are developing how we are going to manage our patients with Lynch syndrome is, again, that the guidelines are simply guidelines. We are making our recommendations based on what we think are the best recommendations for this group of people, but very little of what I present in the next couple of slides is based on randomized controlled data. So when you're making that judgment in the clinic, you have to take into account family history, you have to take into account what the patient in front of you wants, and then use the guidelines to help you make the best decision for your patient. Again, the ones I'm going to really cover are the ones here in the red arrows, which are the more common ones. So endometrial and ovarian cancer prevention: I will say more than any other tumor group in Lynch syndrome, this has been a moving target for us in the last few years, so as we probably all know in the room, for average-risk women, we do not recommend ovarian or endometrial cancer screening. We do know that one group of women in particular, if there is ovarian cancer in the family or if there is a high-risk gene, you may recommend prophylactic oophorectomy. For Lynch syndrome, what we had classically recommended was that at a certain age, all women should have total abdominal hysterectomy and oophorectomy, and I think still for ovarian cancer prevention, that is still very much—we know

that oophorectomy can reduce the risk of or the incidence of ovarian cancer and is beneficial.

The reduction in risk for the endometrial cancer component of risk for women is a little bit more, I would say, questionable. We know that it's an option to have a hysterectomy to lower the incidence of endometrial cancer, but studies that have looked at this have actually not shown a mortality benefit. And we think that's because, or we know that's because, many of these cancers are detected very early, and women have great outcomes even if they are detected with cancer. I think from the side of the patient, that is probably not an easy thing to think about and so what I would say is again, this is a moving target of what the recommendation is going to be. The most recent estimates that have come out of the European group have described still very high risks of endometrial and ovarian cancer, particularly with *MSH2* gene, whereas the other genes in the updated NCCN guidelines, we actually have a table that shows that the risks in some of the other genes are not quite as high at this point based on newer data. So again, all of these endometrial cancer recommendations are really more suggestions; there's no proven benefit to endometrial biopsy, but it's an option. Transvaginal ultrasound can be considered in postmenopausal women; premenopausal women it's not recommended. For ovarian cancer, we know there's no effective screening, but may be considered by the doctor. Certainly women have the option of oophorectomy when they are done childbearing; certainly we know CA 125 is neither sensitive nor specific and we generally do not recommend that.

For gastric cancer, one important thing to remember about gastric cancer is in general, in the U.S. population, rates of gastric cancer have plummeted, and this is probably because of better storage and preparation of food, also lower incidence of H. pylori. So we do believe there is a strong gene environment interaction with gastric cancer, and generally we think in Asian populations there may still be higher risks, but this is a cancer we tend to not see as much in the U.S. population, although the *MSH2* gene and the *MLH1* gene are the higher-risk genes. I think what is the recommendation is that we do endoscopy every 3 to 5 years in this group, and we begin at the age of 30. We certainly test all these people for H. pylori to remove that risk of a gene environment interaction.

Just a couple anecdotes: a lot of my colleagues in GI are sometimes like, we're doing these scopes on people every year and we never find anything and is it really worth the time? But the reality is this syndrome can present in a lot of different ways, and we have had patients who have had *MLH1* carriers with bizarre polypoid masses in their stomach, which ended up being early tumors. Also patients who have high-grade dysplasia and other things and small polyps in their duodenum and in their stomach. I think that what really though has made screening of the stomach complicated is that proton pump inhibitor use is so common in the population, which creates lots of fundic gland polyps and really can confuse the endoscopist when they are trying to go in and determine whether there are premalignant lesions there or not, because you can biopsy fundic gland polyps all day and not find anything that was premalignant.

Skin screening is something that we generally have only recommended for a small subset of patients with Lynch syndrome, although we just heard some recent data at one of our national meetings that perhaps we should at least have everyone get some skin screening in Lynch syndrome. About a third of people with Lynch syndrome have a variant of Lynch syndrome called Muir-Torre, which maybe you've heard of. These individuals will often develop a classic lesion called a sebaceous adenoma or carcinoma, but patients with Lynch syndrome can develop a whole bunch of different kinds of skin findings including something called keratoacanthomas, adenomas, and other things. What we generally recommend at Fox Chase and what is, again, within the guidelines is that you should definitely be questioning your Lynch syndrome patients about skin cancer findings in the family. If there's any question that they could have this Muir-Torre, they should be referred to a dermatologist so that they can have a really good exam and make sure that they don't sit in one of these families that have Muir-Torre variant because if they do, they would really want to have regular follow-up. Although these Muir-Torre skin lesions, like sebaceous adenomas and carcinomas, don't tend to be aggressive and metastatic in the way that a colon cancer can be; these lesions can be locally aggressive, and to remove them can really leave people with a very large skin defect.

We had one patient who we actually had not realized had Muir-Torre variant and actually had to undergo skin grafting and other things for some large adenomas that he had on his body, sebaceous adenomas that we just had not known about. What I'm going to transition over to now though is how Lynch

syndrome has really started to inform how we treat colon cancers. I always say to my colleagues, up until about 2 years ago, most of my colleagues thought it was fascinating that I was interested in Lynch syndrome and I was the Lynch syndrome guy. But the things that I was interested in and studied were not necessarily directly impacting how they practiced oncology, so I was a novelty at best. Then suddenly when immunotherapy hit the market, everyone wanted to hear about MSI and IHC again, so I suddenly became a superstar overnight again. So what have we learned? Colon cancer actually is one of the poster child tumors where we early on had molecular markers that helped us understand how we were going to treat them, and the mismatch repair pathway is a poster child within that poster child.

This is a pathway that we know is associated with Lynch syndrome that as Heather told you, there actually are other people who have mismatch repair who don't have Lynch syndrome. So I'm going to talk to you a little bit about these markers and a little bit about how tumor-sidedness has been incorporated into our thinking about this. The *RAS* family was our first family of predictive biomarkers. Perhaps everyone remembers in 2006, ASCO first came out with a guideline that said, we actually need to test all colon cancers for *RAS* mutations because actually what we figured out was people who were *RAS* wild type had great responses and the people who had *RAS* mutations actually we were harming them by using EGFR inhibitors. So this suddenly, ASCO you know, boom, after the national meeting within a month, there was a guideline out we should not give these drugs anymore to people who have *KRAS* mutations. This

was expanded over the last 2 years to include *NRAS*, which is a smaller group of mutations, actually more common in African Americans, and then also *BRAF* mutations as well. *BRAF*—back to this idea of prognostic predictive, *KRAS* and *NRAS* don't tend to have much prognostic, so they don't have a huge impact on how long people survive, but particularly the *KRAS* exon 2, the first ones we discovered, are very predictive as are these others. *BRAF* though we know especially in metastatic colon cancer, this is a strongly poor prognostic factor. If you have *BRAF V600D* in your tumor, metastatic cancer, that is a bad marker. Those people tend to live half as long as everyone else, but it also is helpful for predicting who's going to respond to EGFR therapy and just so you remember, the two EGFR therapies that are out there are cetuximab, which is a mouse-derived antibody, it's generally given weekly although you can give it other ways, and then panitumumab, which is a humanized antibody.

These mutations—one thing we have learned is although they are pretty strongly predictive, they are not 100% predictive. There is speculation that some *KRAS* mutants may have some response to EGFR therapies, and what we have also learned in a nice paper that came out just probably a couple of months ago that non-*BRAF V600E* mutations and any mutations *BRAF* outside of that actually seem to have a better prognosis than that *V600E*, which is kind of an interesting finding. What has come out at ASCO in the last couple of years has been this new obsession. It's amazing; we've known the colon has a left side and a right side for a long time, but no one kind of really bothered to look at how molecularly these things are different, and so what we've learned is that the

KRAS mutations on the left side seem to respond much more robustly to anti-EGFR therapy than those on the right side. And this was shown both through some retrospective data from CALGB 80403 where survival was getting close to double and also in this large database called ACCENT. So this is an emerging story. I'm certain at ASCO this year, there's going to be more stuff presented about the molecular-ness of the different sides of the colon. I'm going to go back to that a little bit in a few minutes.

So mismatch repair: this is the other thing. This is, as Heather introduced, in Lynch syndrome, mismatch repair is defined by mutations in the mismatch repair genes. A smaller number of those are caused by germ-line Lynch syndrome, but many are caused by somatic mutations or inactivation, particularly of the *MLH1* gene, although others can be involve. And what we've learned in the last couple of years is these are particularly sensitive to the anti-PD-1 and anti-PD-L1 therapies, and there's a number of them on the market now. They are—the big one that we all know is nivolumab or pembrolizumab. These are very powerful therapies and have shown great benefit in these tumors. I'll show you a little bit about that in a minute. Again, the germ-line mutations represent a smaller degree, it's really the *MLH1* methylation which is a bigger group of these, and as Heather said, these tend to be right-sided tumors, they tend to be *BRAF* mutation positive, they tend to be more common in women. And then there's a few other causes also of mismatch repair.

So why are these responsive to immunotherapy? So there's a lot of theories. We think we know the answer to this, but I think this is still an evolving

question. Certainly these tumors produce proteins that are recognized by the body as being foreign, and so when these foreign proteins are sitting around because of all the mutations that occur in the tumor, you get infiltration of these TILs or tumor-infiltrating lymphocytes that come in and they are trying to fight this like you had an infection and it's really a tumor. Also, just the high mutational burden alone even without these cells we know causes the tumor to be recognized by the immune system. These are some of the different things that we know are related to why these tumors seem to be more recognized. But again, there was just a very nice paper that came out that showed that not all tumors seem to be as responsive and it may be that some develop early on mutations in their major histocompatibility complex and so use those mutations to hide antigen presentation from these tumor-infiltrating lymphocytes and so maybe are not as immunoreactive as some of the other Lynch cancers. So again, this is a very evolving story.

But what sort of really blew people away in this field were these data presented by Young Lee and the group from Hopkins just in the last few years. These are some of the first data they presented in the *New England Journal of Medicine*. But actually from the time I had to submit this talk and when this conference happened, they just had some updated data in July and I believe it was in *JAMA*, but showing, essentially mirroring this in mismatch repair deficient colon cancers. You saw a disease control rate, either the ability to shrink tumors or the ability to hold tumors back with stable disease of 90%, whereas in the mismatch repair–proficient tumors, only 11. And they showed some follow-up

data at GI ASCO last year where they also looked at mismatch repair noncolorectal cancers, but GI cancers, and, again, they showed high disease control rates. You know this was such as huge thing that the FDA had an accelerated approval, and actually for the first time in history, I'm sure everyone has heard this, they approved using immunotherapies just based on the molecular type of a tumor. If it's a mismatch repair–high or MSI-high tumor, immunotherapy is an acceptable option.

I think one thing that's really important though to look at these data to realize is this top row. We don't see that many complete responses with immunotherapy and I don't think we really ever will. These are therapies that are more static; they can hold disease back. I don't have many survival curves in here or duration of response curves because we just didn't have time to go through that, but what we see is prolonged disease stability in individuals. It actually occurs very early on, and then sometimes will hold for many, many months, but not that many complete responses, and this begs the question of how long do you need to keep this stuff going? And that question is still unanswered and it's very expensive, but we do need to answer that question.

My last important point here is that colorectal cancer has become like real estate; so it's all about location, location, location, and within that location it's really about the molecular dynamics that are going on in the location. It's not just the neighborhood you live in, it's your neighbors, it's how cool your house is, and whether you mow your lawn or let it go crazy and everyone hates you, but it really is where the mutation is, what part of the colon it's in. We know that the

ascending colon tumors tend to present differently with anemia, they tend to be more mismatch repair deficient, they tend to more often be *BRAF*, which, again, is a poor prognostic factor in the metastatic setting, not a poor prognostic factor in any other setting, but metastatic definitely. These tend to be less chemo responsive; you can sometimes present with these mega tumors that are not responsive, and generally we've seen poorer survival when we've done these analyses as opposed to now the descending colon tumors, which you know present tend to be obstruction and bleeding, so you often present earlier because you're symptomatic earlier.

They have more chromosomal, large chromosomal changes that you have more *HER2* upregulation, which I don't really touch on that much, but is an emerging important biomarker. More often *NRAS* mutant, and that tends to be more clustered in African Americans, which is a story that still hasn't been well explored yet. And then in terms of chemo responsiveness, again, the *KRAS* mutations on the left side have shown really much more remarkably responses to anti-EGFR therapy than the right, to the point that many people, if they are seeing these left-sided tumors and they are *RAS* wild type are starting with anti-EGFR therapy even though you have to coach the patient through the idea that they are going to get a rash and other things, the difference in the degree of response is so striking. I think the last thing about the descending colon tumors I'll mention because it has been so much in the news is one thing we are seeing though is a lot more descending colon tumors in young people, and we don't really have a good explanation of why the incidence of descending colon tumors

is rising in young people, and there's a lot of work going on trying to understand what is the factor? Is it environmental? Certainly we are seeing many of these young people outside of the hereditary setting, it doesn't necessarily look genetic. I have tested a kajillion of these people and I'm not finding any genes, so there's got to be some factor out there that's explaining this. Whether we need to shift screening ages up earlier, there's still a lot of things to define, but that is a current group that we are really trying to understand. I drew a few circles here to say right versus left.

I really want to thank you again for inviting me. It's been great. Thank you.

HEATHER I think we made it with six minutes for questions.

DR. HALL Young lady in pink. Is there a microphone?

HEATHER I think Annie's got a mike.

DR. HALL There's a mike. Okay.

FEMALE What are your thoughts about the gut flora and the new findings we're seeing with response to therapy?

DR. HALL Again I think that is, again, at our national Lynch hereditary meeting, we had a great talk. I think that is still a very underdeveloped area, but certainly there are some clear interactions between what bacteria inhabit your colon, particularly the right colon and the Fuseobacterium and some of the others related to survival. I'll say that it's an area that I think still a lot of oncologists don't know that much about and we don't really know how to account for that right now in terms of how we talk to people about risk or how we treat people. But I think we are going to hear a lot more about that in the coming years.

FEMALE First I want to thank you. This is one of the best presentations I've been to all week, so that's excellent.

DR. HALL Thanks.

FEMALE You had mentioned that aspirin 81 mg 50 to 59 years old. What do you do for the other side of 59?

DR. HALL I think the recommendation focused on that group due to the higher risks of colon cancer and the lower comorbidities in that group. I think then at that point the way the guideline reads is the decision to continue is based on the individual's health status, whether they were found to have polyps and other things about the family history. I certainly think for someone who's healthy, who has good end organ function, there's no reason why you couldn't continue preventive aspirin therapy as long as the physician felt it was safe and warranted.

MS. HAMPEL And I would just say, in our Lynch syndrome patients, we obviously start it much younger than that and though it seems—the CaPP2 data—the longer you use it, the more your risk decreases.

FEMALE Hi. For pancreatic cancer patients, is there any screening guidelines yet for the high-risk patients or patients.

HEATHER There is. There's ironically another study called CAP championed out of the Johns Hopkins group, but they have been looking at endoscopic ultrasound for people who have a strong family history, people with *BRCA* mutations, *CDKN2A*, and the Peutz-Jeghers syndrome I touched on briefly also has a very high pancreatic cancer risk. We haven't been doing that so much for our Lynch syndrome patients, although there certainly are some Lynch

families with more pancreatic cancer than we like to see. There is one published study that looked promising. They found some pancreatic cancers while they were still in the duct, but it's a pretty invasive screening and we don't know when to start, how often to do it. So they're really now targeting the highest risk individuals and trying to do it in a research setting so that we can get more data. And if you want to add…

DR. HALL We've learned a lot about the modalities to screen, and I think MRI and EUS have come a long way, and I think we will get there at one point. As an oncologist, what I see as the two biggest caveats are what we know about the pancreas is really it's a field effect, and if you see a premalignant lesion or something you're worried about in the head and you only go in and take the head of the pancreas out, you're actually kind of doing a disservice. If you want to prevent something in someone who's got a high risk, you should take that whole pancreas out, but the big caveat to that is life without a pancreas is not easy. It's brittle diabetes, it's trying to manage having to take all your enzymes for digestion, so we have to make more progress on that side of things before we are ever going to be able to routinely help people beat pancreas cancer who are at high risk unfortunately.

FEMALE Yes. With your younger patients have you noticed anything with HPV and colon cancer like they have within head and neck?

DR. HALL There have been some studies looking at perhaps whether HPV may be related to colon cancer incidence because that was one that a lot of people thought about, "Ah-hah, maybe this is why we are seeing this rise in

cancer!" And I do think it's still possible, but the studies I have seen to date have not shown that connection. Certainly there's the HPV anal cancer connection, but HPV in early left-sided colon cancer connection, that has not been established.

FEMALE I have a patient who's kind of interesting. She was diagnosed with breast cancer 5 years ago. She had the *BRCA* testing, I think it was 5 or 6 years ago so they weren't doing panel testing at that point. Well, fast forward, she was diagnosed with metastatic disease in the past year and we did next-gen testing on her that ended up showing an *MUTYH* as well as the *ATM* mutation in the genetic tests. So I sent her for—it was in the next-gen, so I sent her for genetic testing and she did have a germ-line mutation in both of those genes also. So our genetic counselor did recommend since the cat's out of the bag now, but just getting her started for colonoscopy. She wasn't 50 yet, so what's her risk with those two mutations I wonder?

HEATHER So *MUTYH*: it looks like it depends if you have a first-degree relative with colon cancer or not. If you have a first-degree relative with colon cancer and you carry one *MUTYH* mutation, your risk might be 10%, which is a twofold increased risk. And NCCN does say if you've got a *MUTYH* mutation plus a first-degree relative to start colonoscopies at 40 and go every 5 years instead of starting at 50 and go every 10. One year we briefly toyed with the idea of having anyone with a *MUTYH* mutation start at 40 and go every 5 years even if they didn't have a first-degree relative with colon cancer. The problem was we got inundated by complaints from people because one out of 50 people carries *MUTYH* and they were like, "Do we need to recontact every *MUTYH* carrier in

our practice?" And now tell them they need earlier and more frequent colonoscopy on fairly weak data. So we dialed it back last year and now only suggest it with the family history. *ATM* we are definitely seeing in our colon cancer cases for sure, and we even see second hits in the tumor that lead us to believe it's causative, but again, it's more of a moderate-risk colon cancer gene. But I think with both of those, starting her earlier and going more frequently seems to make a lot of sense.

DR. HALL Your question though brings up a even deeper important question, which is that the more genes we test and particularly the more moderate penetrants genes we identify, we're going to start finding lots of people in the population, and I'm sure Heather does and in my practice as well, who carry multiple things. And it then really makes you wonder whether the testing, the approach that was taken with your patient 5 years ago or however long ago it was, should be the approach moving forward. For instance, if someone is Ashkenazi Jewish and has breast cancer, does it really make sense to just test for *BRCA1* and 2 at this point? Because we know that there's a variant in the *APC* gene, I1307K, common in that population, which is going to raise their risk of colon cancer. In my eyes, you shouldn't just test people for those two *BRCA* genes because you may be missing other risks. So I think as we get more sophisticated, as testing gets cheaper, I think what we are going to see, as Heather mentioned in her talk, is more panel-based testing because there's a lot of risks out there and you've got to know which ones you have.

FEMALE Thank you.

DR. HALL Thank you so much.

MEGAN Thank you, Ms. Hampel and Dr. Hall. Once again, this presentation was part of our Smarty Program. Participants please be on the lookout for an e-mail tonight with questions about this session. We'd appreciate your prompt response.

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