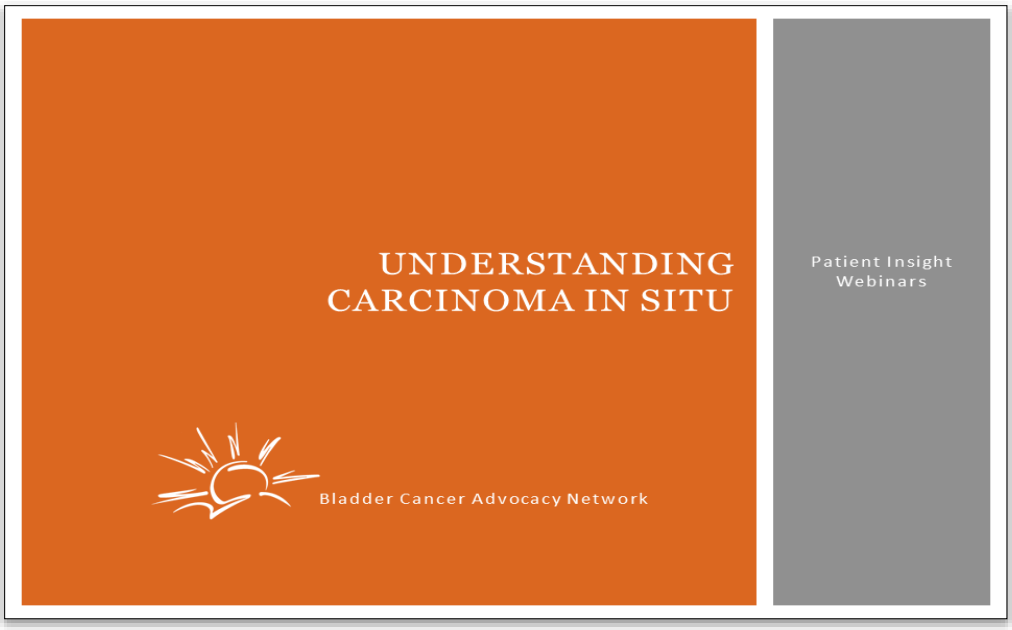
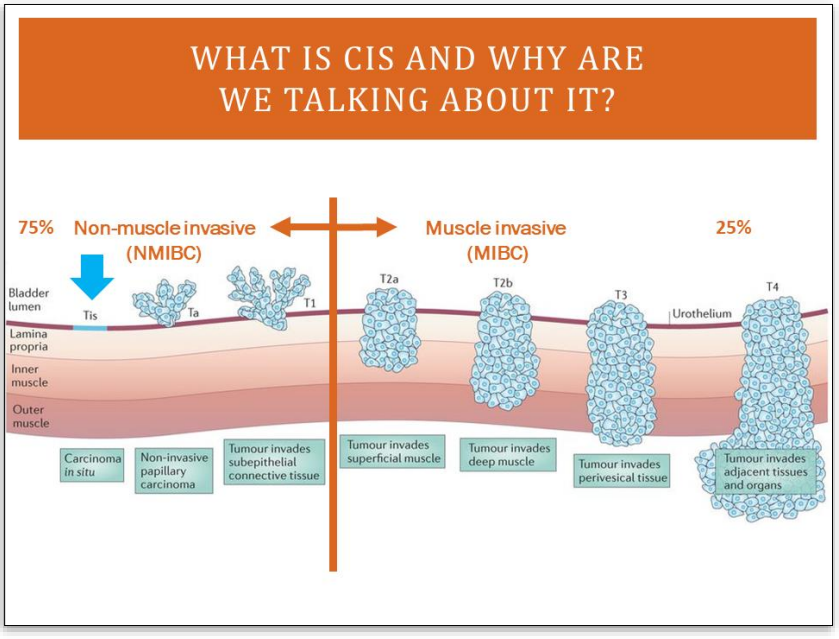


Stephanie Chisolm:

Early stage tumors in non-muscle invasive bladder cancer, or NMIBC, are generally confined to the lining of the bladder, and they may be papillary and look a little bit like tiny finger-like clusters or flat, velvety patches known as carcinoma in situ or CIS. Tumors that are CIS have a very high rate of recurrence and possible disease progression, so it's really helpful to understand your diagnosis. Tonight, BCAN is welcoming urologist Peter Black from the Vancouver General Hospital in Canada and Pathologist Dr. Hikmat Al-Ahmadie from Memorial Sloan Kettering Cancer Center in New York City. We're delighted to have you here to help us talk about CIS and what patients need to know. So welcome to both of you. I'm going to turn my video off, and Dr. Black, if you want to take it away.

Dr. Peter Black:



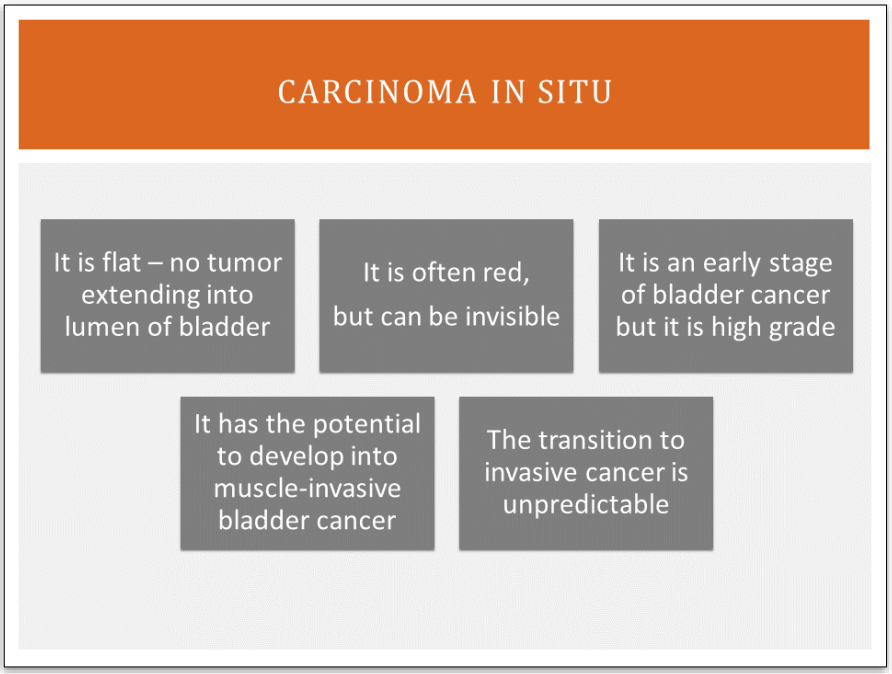
Terrific. Thank you, Stephanie, very much for the invitation and the opportunity. I think I can speak for Hikmat as well, that we're both passionate about bladder cancer and so we're always very happy to explain what we know and educate, so hopefully the attendees tonight will find this interesting. Hikmat and I are going to go back and forth a little bit. I'll start and then we'll switch to Hikmat and we'll carry on like that. What is carcinoma in situ? Stephanie alluded to it a little bit at the beginning. We have this broad spectrum of disease stages for bladder cancer from the very superficial on the left and carcinoma in situ is also called TIS. It's just right on the surface of the bladder, to tumors that

actually extend into the lumen of the bladder but still sit on the surface. That's the Ta just next to that.

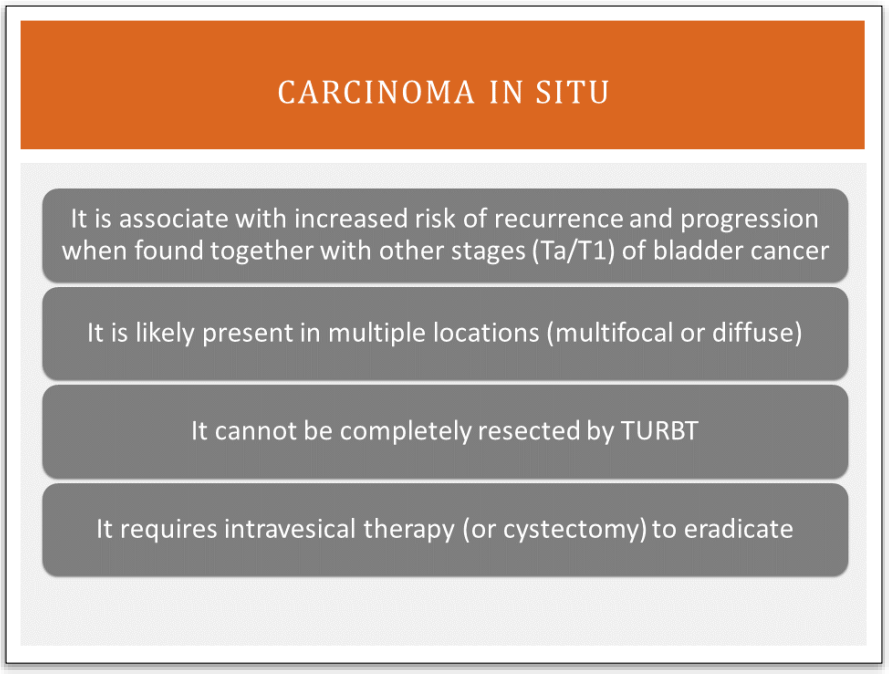
Then, the T1 tumors that are already invading the first layer of the bladder wall. Those three together are all non-muscle invasive, but there's a difference between actually invasive T1 and the other two that are really non-invasive. And then on the right, we have the muscle-invasive, which we're not going to talk about this evening.

Dr. Peter Black:

There's special things about carcinoma in situ that make it really worth having a dedicated session just on this. So it's a flat tumor, there's no tumor extending into the lumen of the bladder. It's often red and velvety, but sometimes it's invisible, so we struggle to see it in a lot of patients. Although it's an early stage of bladder cancer, it is high grade. By definition, it's always high grade and therefore, it has a significant malignant potential. It has the potential to develop into muscle-invasive bladder cancer, so even though it's early stage, we take it very seriously and we treat it quite aggressively because we need to eradicate it. One of the problems that we also struggle with is that this transition from a non-invasive, superficial carcinoma in situ into a potentially invasive muscle-invasive tumor is very unpredictable.



Some other key features is that it's often... if it's found together with the other non-muscle invasive bladder cancer stages, so Ta and T1, it indicates a higher risk of recurrence and progression. The fact that the cancer could come back or it can turn into something more invasive if there's carcinoma in situ there. It's an additional risk factor in patients who have other disease stages.



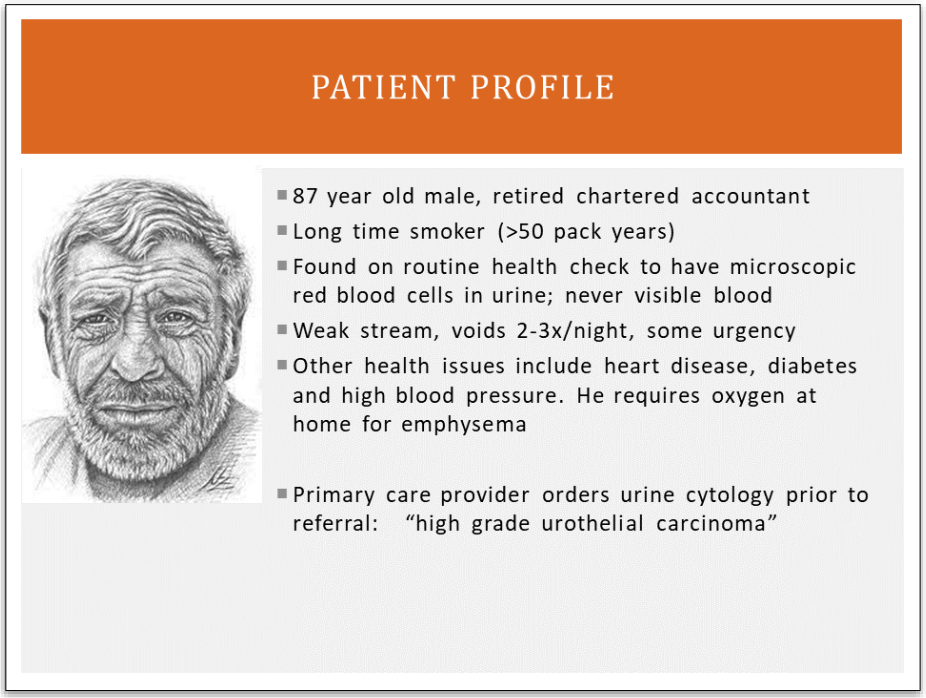
Carcinoma in situ we assume is multifocal, so it's at different locations in the bladder, or even diffuse, meaning anywhere you would sample the bladder wall, you would find carcinoma in situ. That's a very

important and special feature. The other tumors generally we consider to be confined to what we see and what we resect. Because it's multifocal and diffuse, we general believe that we cannot resect it completely with a transurethral resection, which again, is different from the other bladder tumors that typically we can resect at least all visible disease.

Dr. Peter Black:

Since we can't resect it, we either need to treat it with intravesical therapy such as BCG, or we need to remove the bladder if we really want to get rid of it all. That's why it's really tricky and a bit different than the other bladder tumors.

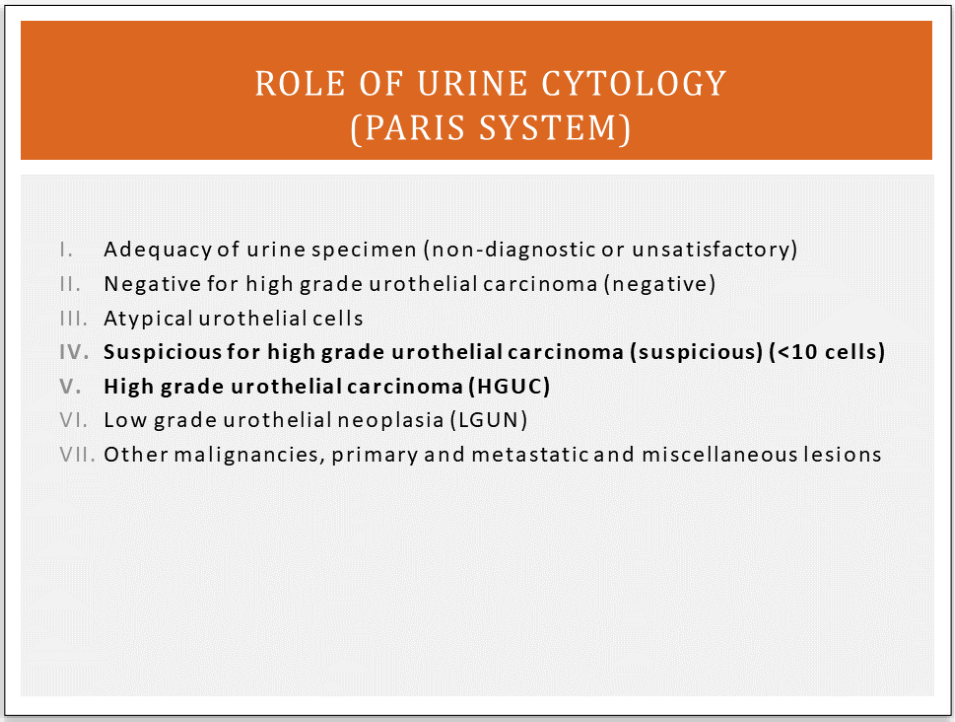
I'm going to present a patient profile, and then Hikmat and I will go back and forth a little bit about some of the issues related to this patient's bladder cancer. So this patient is an 87-year-old gentleman, retired chartered accountant, who has been a longtime smoker, more than 50 pack/year history of smoking. He was found just on a regular check with his primary care provider to have red blood cells in his urine under microscopy. He's never seen blood himself, so there's no gross hematuria, just microscopic hematuria. He has symptoms that are common for his age. Nothing particularly severe or noteworthy, extreme. He gets up a couple times at night. There's some urgency to void. He has very significant other health issues. He has heart disease, diabetes, high blood pressure, and he is even on home oxygen because he has emphysema also related to his smoking history. One thing that is relatively clear when we look at this gentleman is that he's probably not going to be fit from a general health point of view to undergo radical cystectomy to remove the bladder. We're not necessarily at that point yet, but I just wanted to highlight that in his medical history. His family doctor, based on the microhematuria and the risk factors, sent his urine for urine cytology. Can you go back for a second? The diagnosis of the urine cytology is high grade urothelial carcinoma. Hikmat's going to run us through a little bit what that means.



Dr. Hikmat Al-Ahmadie:

All right. Thank you, Peter for the lead-in and hello everyone. Thanks for tuning in and spending the evening with us. It's my pleasure to share this hour with you and talk to you about urothelial carcinoma in situ and different aspects of the disease, and hopefully you'll find it helpful. As the story started, a quick test is urine sample. It's a very easy type of specimen to get. It just requires the urine sample that you may get in the clinic or the office. It is sent to the pathology department. It's spun down, just to concentrate the cells in that container, and then it will give us a better opportunity to detect any atypical cells in there, and when slides are prepared, the tissue is stained, then we'll be able to have the

ability to visualize cells that can tell us what they constitute, they're benign or reactive or abnormal cells, and we follow a system. There is a system.



These type of specimens have been studied for a long time and there are criteria that we apply every time we analyze these type of samples. And then we follow the most recent system is the Paris System, and it has different categories and every case, every urine sample that goes to the lab, the result come back with one of these categories. Of course the red flag would be, or when we highly suspect a urothelial carcinoma is in these two categories that are in bold now, the number four and five. Either we see some atypical cells that are atypical enough to suspect urothelial carcinoma but there are not as many of them as you would want. The cutoff has been made as 10 cells. If you see more than 10 cells that are very atypical, and I'm going to show in some of these features what we mean by atypical in the next slide, these are what you say suspicious. When you see these atypical cells in a quantity that is high enough, and as I said, this is typically more than 10 cells, then you can render the diagnosis of high grade urothelial carcinoma.

Dr. Hikmat Al-Ahmadie:

During cytology, you will be not able to say in situ or papillary because most of the times, that designation requires more architectural assessment rather than individual cells, so I'm going to show you example how the urothelial carcinoma cells in the urine may look like.

Yes, so this is a high magnification image from a urine cytology specimen taken from under the microscope. These are the individuals, as these dark purple or blue structures in the middle of the picture. These are the nucleus, the nuclei of tumor cells. You can see how they're different in size and shape, different in the darkness quality just of the chromatin quality. They have indentations, projections. These are all atypical features that do not resemble normal cells that we are very familiar in how they look like, and with these type of features altogether, with the abundance of these cells in the urine sample, we can comfortably say that this is cyg-diagnostic of a high grade urothelial carcinoma.

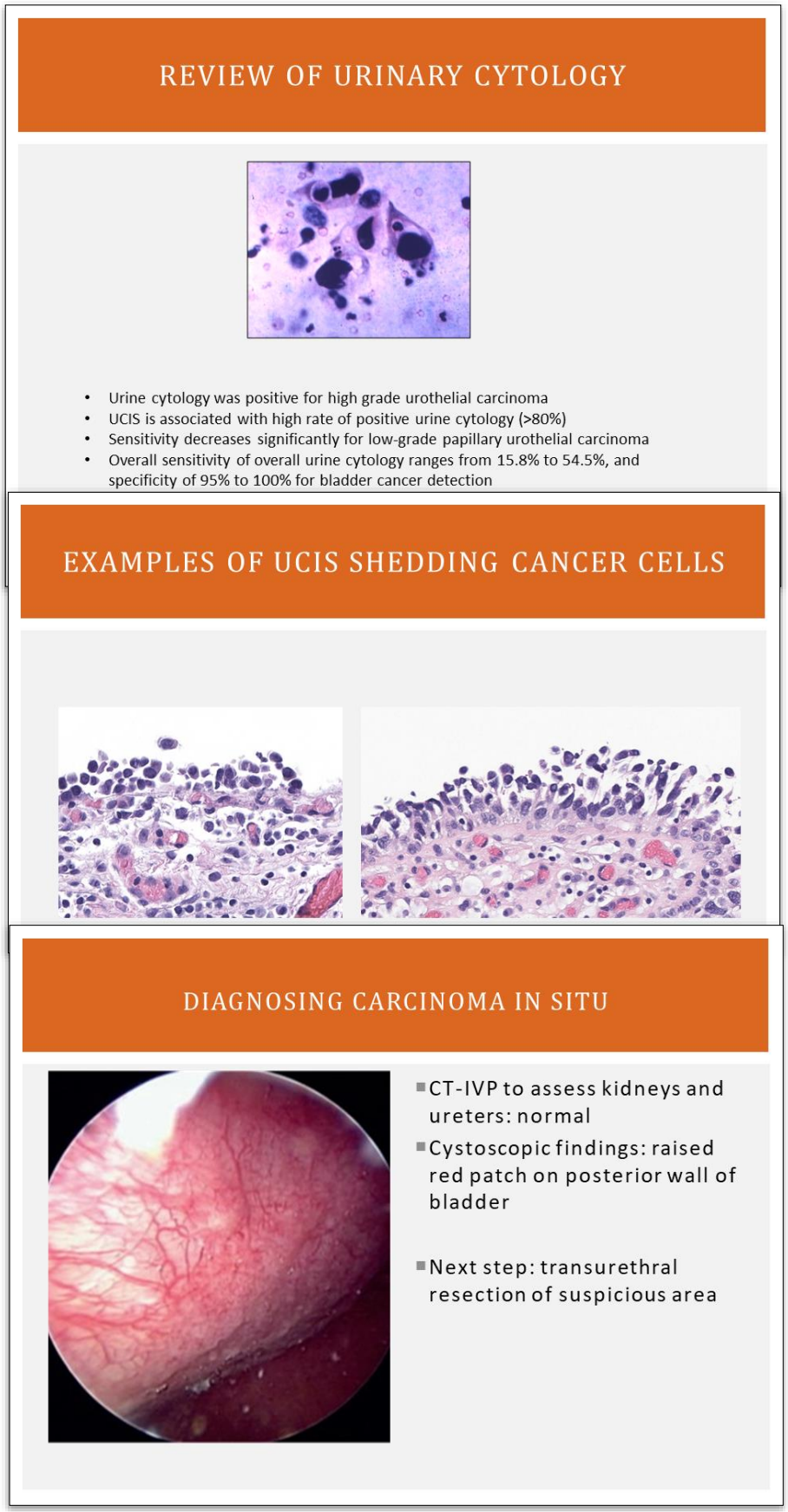
Dr. Hikmat Al-Ahmadie:

Urine cytology, as I said, it's a very simple process. It's a simple test. Doesn't require much. It's not invasive. You don't stick a needle or anything, just a urine sample in the office. It's very sensitive for high grade urothelial carcinoma. It's less sensitive for lower grade lesions, because again, you need to see atypical features that are obvious here. In low grade lesions, you don't see the same features. And it's also specific, which means if you don't see these atypical cells, you have a high confidence that you're

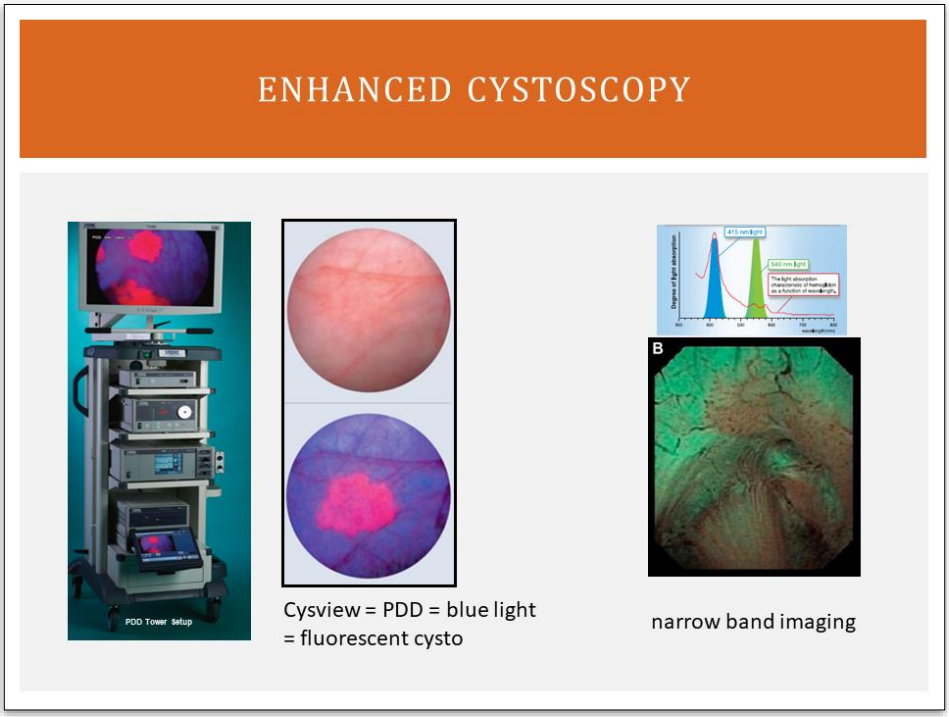
not seeing a high grade urothelial carcinoma, and that's why you can say it's very specific. If we go to the next slide, just to show you where these cells might come, this is a tissue section. This is a biopsy. There's also the biopsy processed in a similar way, a little bit different because you have to prepare, you have to fix the tissue and cut the sections and make them into a slide. As you can see at the top, in both these images, all these dark cells that have different sizes and shapes, these are all malignant cells. With this tissue section, I can easily call it urothelial carcinoma in situ because I have architecture. But as you can see, the individual cells are coming off of the main tissue fragment and they'll be sloughed off or shed into urine, and that's what will make up the positive urine cytology. I'll turn it back to you, Peter, I think, for the next set of slides.

Dr. Peter Black:

Great. Yeah, I think from the urologist's perspective, the urine cytology is so important because we often don't see carcinoma in situ. That's something we'll come back to while we're talking. This gentleman had a CT scan. He had blood in his urine, so that's part of the usual workup, and everything was normal with the kidneys and ureters. Remember that carcinoma in situ and indeed any cancer of the bladder, you can get very similar lesions in the renal pelvis and the ureters, and then upper tracts. On cystoscopy, however, there was a red patch on the back of the bladder. It was a little bit raised. It wasn't the typical sort of cauliflower tumor that we often see, but it was certainly suspicious, especially given



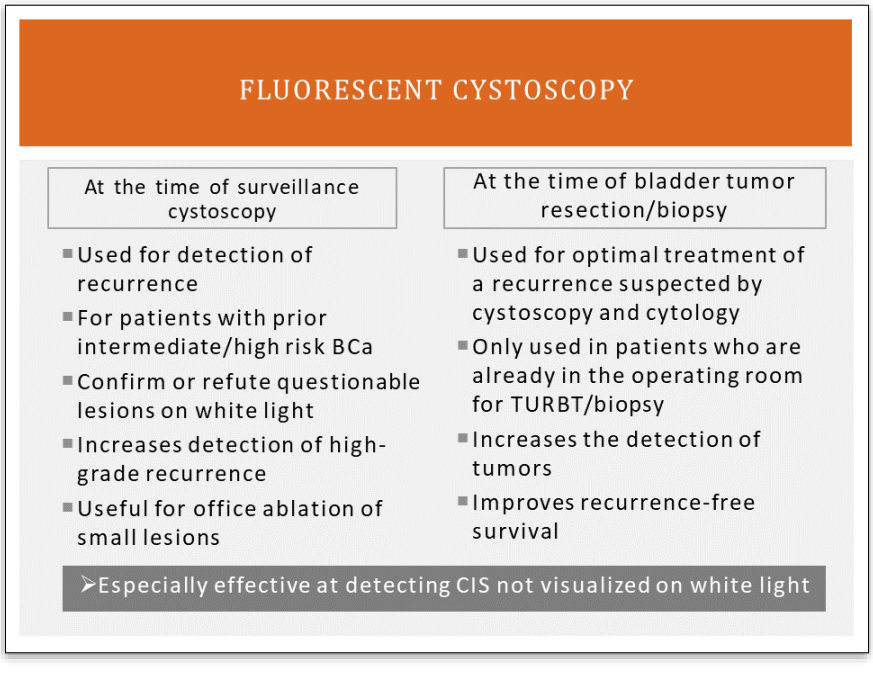
the positive cytology. So this gentleman we took to the operating room for a resection of this area that you see in the micrograph here. Next slide please.



An important consideration, again, this is particularly with carcinoma in situ, is the use of enhanced cystoscopy. There are two different methods with which we can enhance a cystoscopy. On the left-hand side, you see the cysview, which is the trade name. It's also called blue light cystoscopy or fluorescent cystoscopy. PDD is often used in Europe as a terminology. It's photo-dynamic diagnosis. But here, you put a substance into the bladder prior to the surgery, prior to the TRBT, and it's taken up and metabolized by cancer cells so that these cells then fluoresce. When you shine a blue light on it, a fluorescent light, or, well, a blue light, the cells will fluoresce and they appear a bright pink. They really stand out. We know that we can detect more tumors this way, and especially more carcinoma in situ. Up to 40% more carcinoma in situ. It's particularly valuable, again, because we often overlook them on regular white light cystoscopy.

Dr. Peter Black:

On the right-hand side, we have narrow band imaging, which is a little bit different. It uses filters to pull out the blue and the green wavelengths that really accentuates blood vessels and changes in blood vessel patterns. Vascularity will often accentuate tumors so that we can see them better. It's a little bit easier to use because it's just a flip of a switch on the device and it doesn't require putting anything into the bladder, but the evidence for its use is not as widespread. Next slide.



I just want to highlight some of the differences in fluorescent cystoscopy. And this is I think particular to the US market actually, because it's not necessarily available everywhere, but you can actually do fluorescent cystoscopy at the time of a surveillance cystoscopy in the office. If a patient has had a prior bladder cancer and they're undergoing their routine cystoscopy, you can do fluorescent cystoscopy that, for example, is not available for us in Canada. Or, you can do it, which is more common, more universal, you do it at the time of a

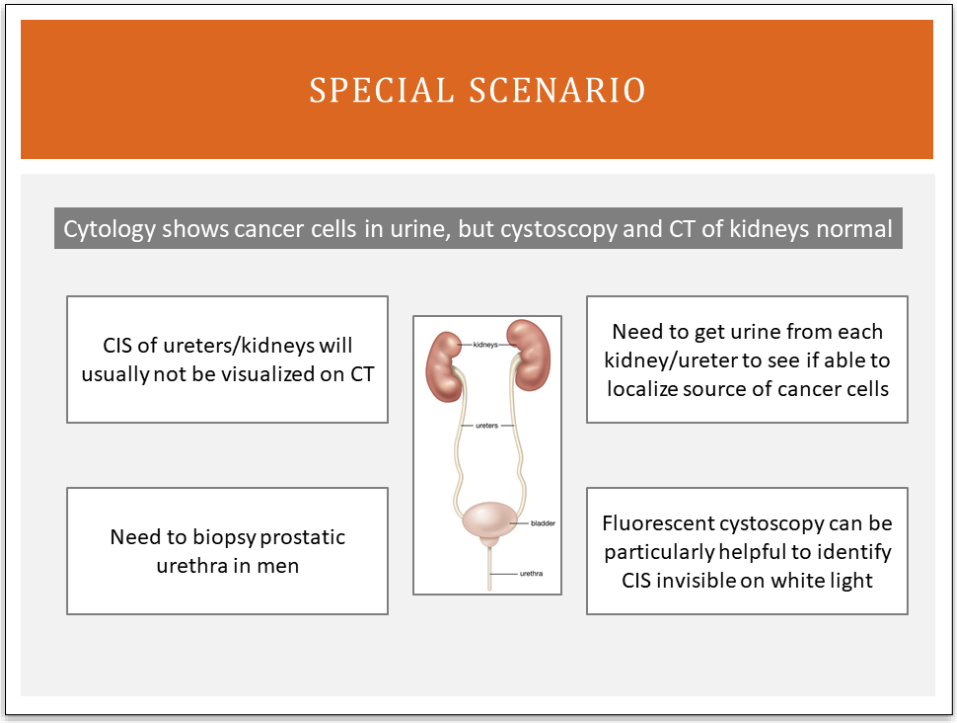
broader tumor resection or biopsy, so if a patient has an identified tumor, you go to the operating room, you give them the reagent beforehand and then you use it at the time of resection. So there are two different uses, and I think as patients and caregivers, you need to be clear on the differences.

On the left-hand side, if you're talking about what we do at the time of surveillance cystoscopy, so it's used to detect a recurrence. It's primarily patients with intermediate and high risk disease. So they they've had tumors before. Based on the prior tumor characteristics, we know that they're high risk for recurrence and we especially do it early on in their disease course. So if they had a tumor three months ago and it's the first look, then that might be a time when we do it. We always have to consider the cost and the treatment burden of all these tests. We can also do it, for example, if we see something on white light that we're not, and white light's that the usual cystoscopy, if we're not quite sure what it is, this might help us decide, yes, we need to biopsy that or no, we don't need to biopsy it. And overall, there's especially one very good American trial that shows that it enhances the detection of high-grade recurrence.

Dr. Peter Black:

On the right-hand side now, when we're using this at the time of resection, there's very good data from multiple trials, North American and European trials that tell us that we'll detect more tumors, we'll resect more thoroughly, patients will have a lower risk of recurrence, and that's a very important endpoint. In particular for what we're talking about, we'll also find more carcinoma in situ. Next slide.

I wanted to highlight one specific scenario that comes up routinely and is particularly relevant for carcinoma in situ. I know there was already a question in the chat or the Q&A about carcinoma in situ of the upper tracts. Upper tracts are the ureters and the renal pelvis. One scenario that we see sometimes are patients who come in to the office with a urine cytology that clearly shows abnormal cells, so it says high-grade urothelial carcinoma, so there are malignant cells there, but we don't see anything on cystoscopy and we don't see anything on a CT scan.



There, we have a specific algorithm that we run through and we know, for example, that carcinoma in situ is something flat in the ureter or renal pelvis is not going to be visualized on CT scan. We might not even see it, actually, when we look at it with a camera. We can't do the fluorescent cystoscopy in the upper tract, so it can be particularly tricky.

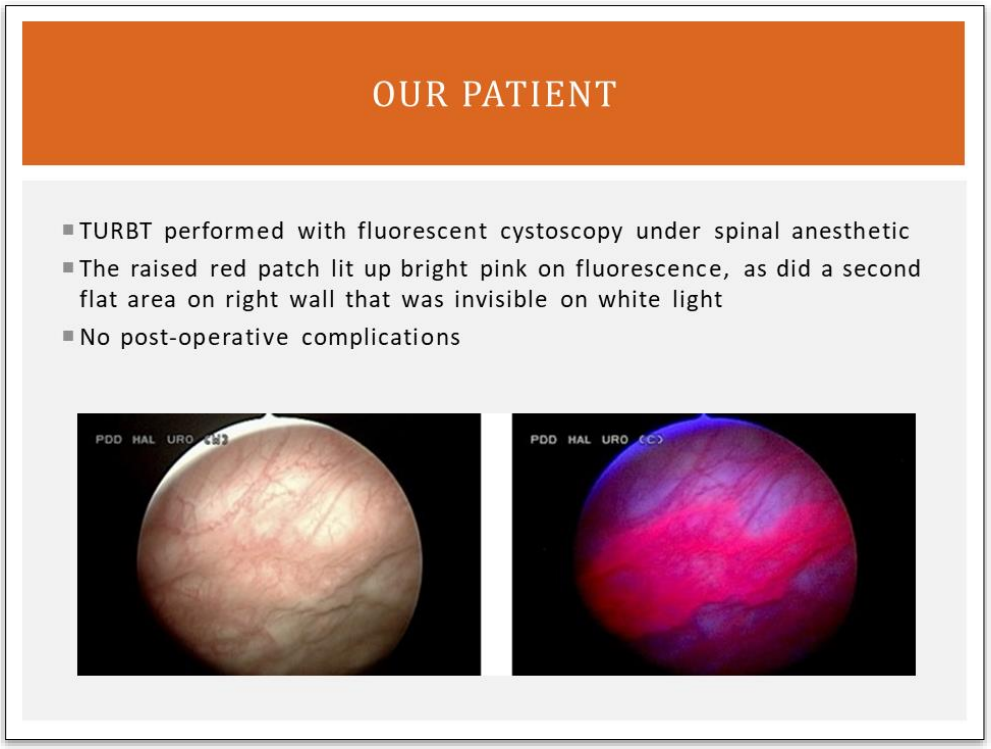
So what we do is we go to the operating room, we get urine from the right ureter and separately from the left ureter so that if it is coming from one side or the other, we can determine that definitively. If we

find urine, let's say from the right renal pelvis, then the cytology is positive, but we don't see anything with the camera and we don't see anything on CT scan, then we call that carcinoma in situ. We assume there's carcinoma in situ there even though we don't see it.

Dr. Peter Black:

The other thing we do, then, so, if in a patient like this it's still more likely that it's actually from the bladder and we're just not seeing it. So in the bladder, we will do the fluorescent cystoscopy and we'll biopsy whatever we see there. And then in men, we always have to consider that it could be coming from the prostatic urethra, where you can also get carcinoma in situ. It can be quite tricky. Upper tracts, bladder, and prostatic urethra. Next slide.

So our patient, you'll recall he had the positive cytology and the red patch from the posterior bladder wall. We took him to the operating room for a resection and we used the fluorescent cystoscopy. It lit up the patch that we saw, but it also lit up a second patch bright pink. And you can get an idea from these pictures here, which of course are not actually from this patient. I stole these, but on the left-hand side, the regular white light, you don't really appreciate much with even an experienced eye, yet on the right-hand side, it's really night and day. It's very clear that there's something there. So this lesion was resected in addition to the other one that we saw, and the patient did well with the surgery, no complications. Next slide.

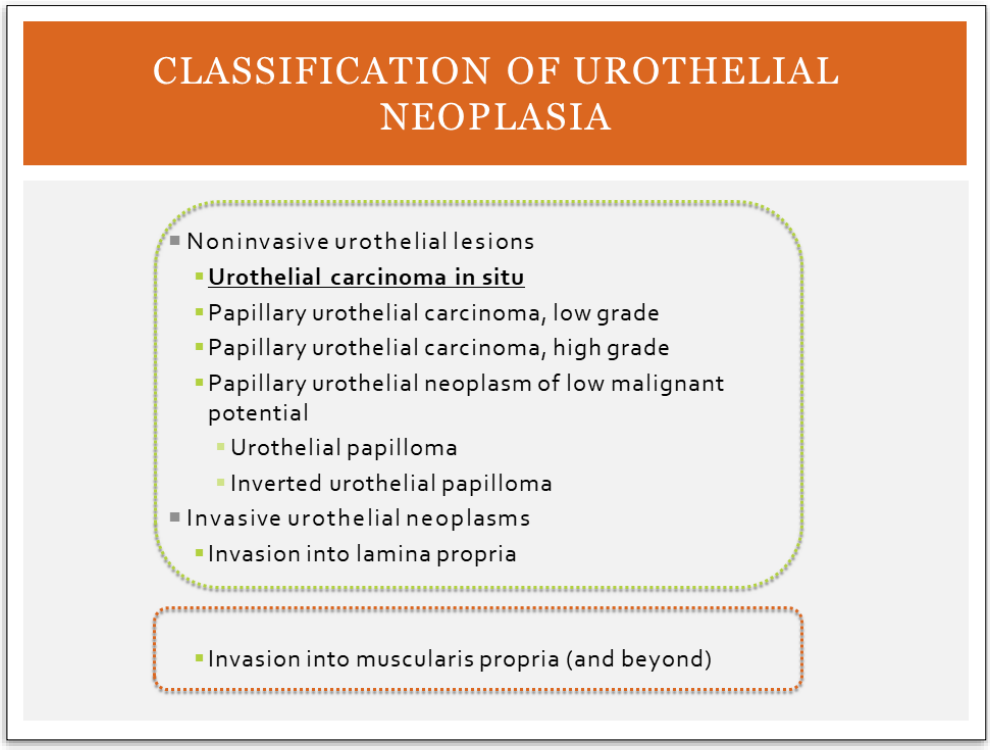


Dr. Peter Black:

So we sent the sample off to Dr. Al-Ahmadie, who's going to go through the pathology for us.

Dr. Hikmat Al-Ahmadie:

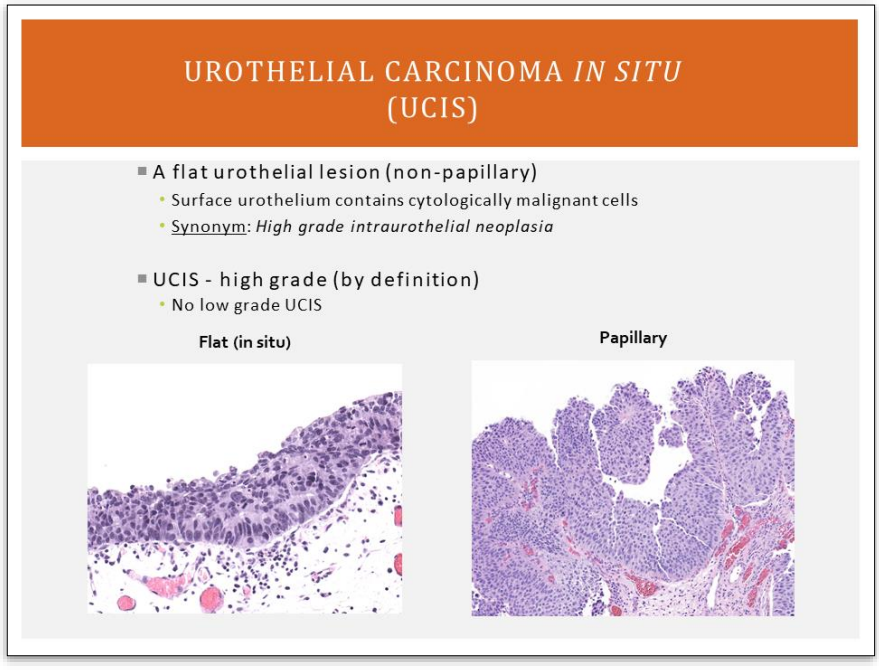
All right, so this is kind of a step-wise process. Every sample that is removed in the clinic or the office goes to our pathology department, and in the next few slides, I'll just walk you through some of the process and some of the classification or how we make the diagnosis, how we look, how we evaluate these specimens. And then at the end, I'm going to show you a slide that would represent that actual biopsy from this gentleman.



Once we start the evaluation of any bladder sample, in our mind is when we find a malignant process, where can we ask the question, where can we fit it from the current specification? And as Dr. Black alluded to in the beginning, you have non-invasive spectrum of tumors that are in the non-invasive category, and those can be the carcinoma in situ, which is the subject of tonight's discussion, and then you can have papillary lesions that can be low-grade and high-grade as opposed to the in situ, which is always a high-grade. There are some rare benign tumors that we call them papillomas. They have distinct morphologic features, and then the tumor starts invading. And then the tumor can invade into the lamina propria, which is the most superficial layer of the bladder wall, or the one underneath the surface lining. And then the deep invasive disease. This is our main challenges. Where can we fit this lesion that we identify on these histologic sections in any of these categories? So if we go next slide.

Dr. Hikmat Al-Ahmadie:

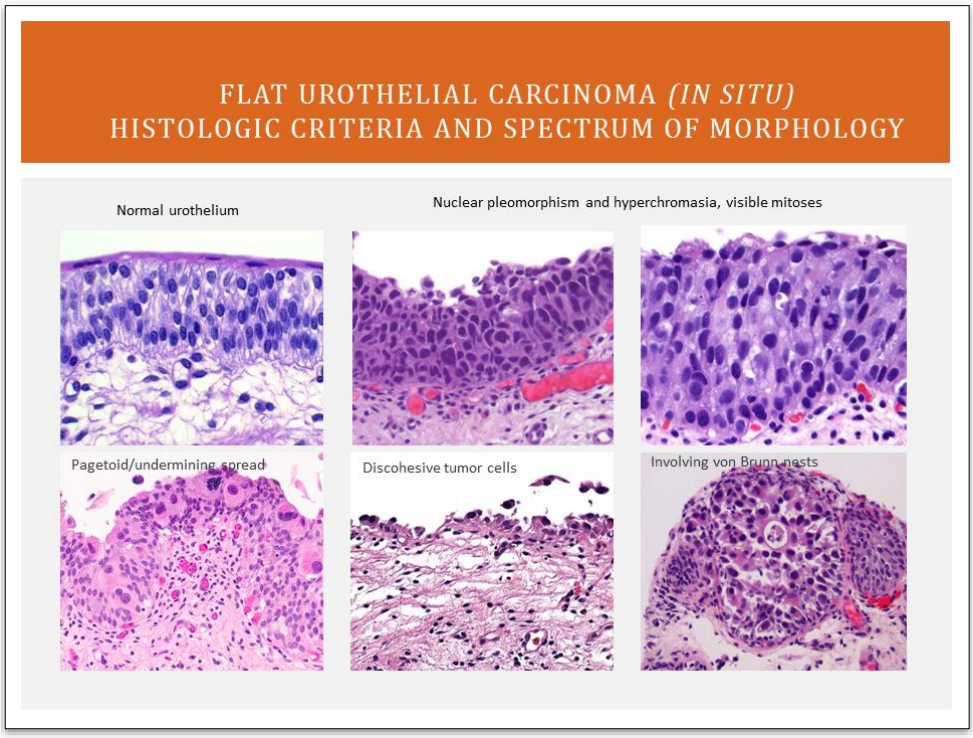
This is one we come into. We identified a malignant process. It's a urothelial carcinoma now. Can we place it into in situ flat disease or papillary? This is what it is. You look at the image on the left, this is the definition of urothelial carcinoma in situ. We've identified these cells that are very atypical. Again, the features that we use are the variation in the size and shape, the variation in the coloring of these tumor cells, how they're



spaced. Are they overcrowded? Are they not respecting each other's borders?

Then, there are some other features that I may point to and I apologize if these may sound too technical, but I'm happy to kind of discuss or answer any questions if you think and if it might need more explanation. When the cells divide, they form a structure called mitosis, which is a sign of dividing in cells and a growing tumor. You can see a lot of mitosis in these tumors as compared to the picture on the right, which is the papillary tumors, which are basically a finger-like projections, and this is how the tumor grows into the lumen of the bladder. It's simple recognition of the pattern that can tell you it's a flat versus papillary disease, and these are all things that are [inaudible 00:23:04]. You can see it's be definition high-grade disease, and it's a tumor that's just growing as if on the flat, the surface of the bladder. Next slide.

Dr. Hikmat Al-Ahmadie:



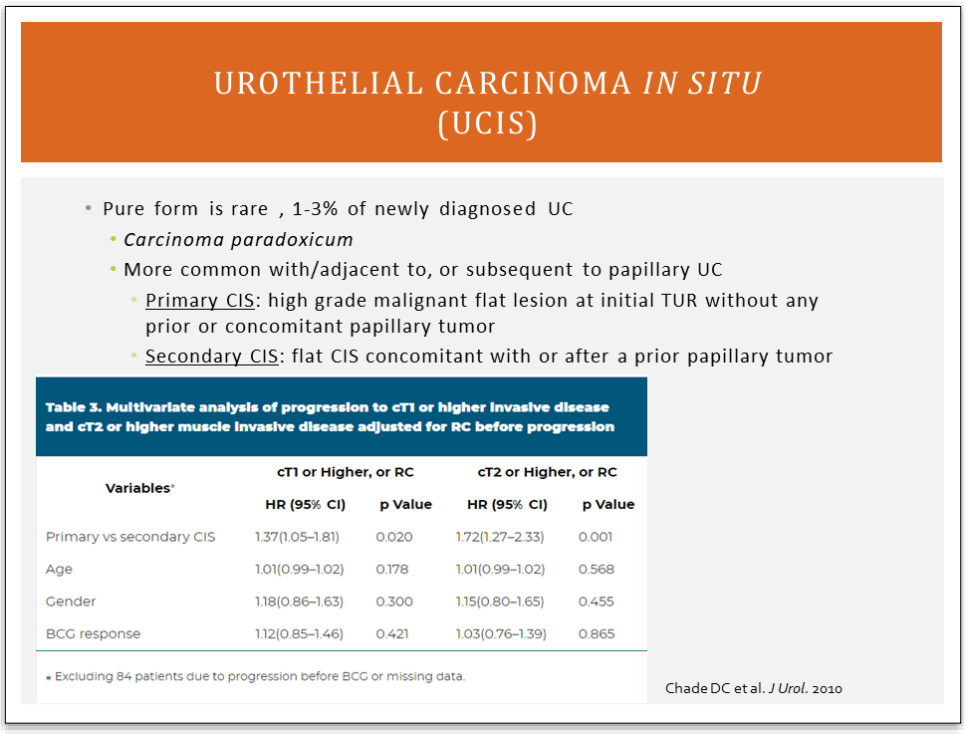
Fortunately and unfortunately, just to make things difficult and challenging, urothelial carcinoma in situ can come in different forms and shapes. These are just different examples. I just wanted to show you, just to kind of put an image to the name, so to speak. We always rely on reference to the normal urothelium, which is the image on the top left. Here you can see it doesn't take much, you can see, comparing this image to all the other five images. You could see how we use the reference normal urothelium to help us identify abnormal lesions. Normal urothelium is very orderly structure, multiple layers of different types of cells start from the base all the way to the top. All of these cells are important. They have normal functions in the bladder. For example, this middle layer can be four, five cells thick, but it can become two cells thick if the bladder is distended and becomes flattened.

All the other images on the right and the bottom, these are different shapes of urothelial carcinoma in situ. Again, highlighting the features that we rely on and we use: the variation in the size and shape, the discoloration, the growth pattern. Some of these patterns can be deceptive, like the one on the top left. These large cells are individual tumor cells just growing along the normal urothelium. We use the term spread, but this could be challenging and this could be one of these examples where not many tumor cells go into the urine, for example, because these cells are just growing under normal urothelium, compared to the picture in the middle and the bottom. This is when you have a lot of dis-cohesive, disjointed tumor cells that can easily shed into the urine.

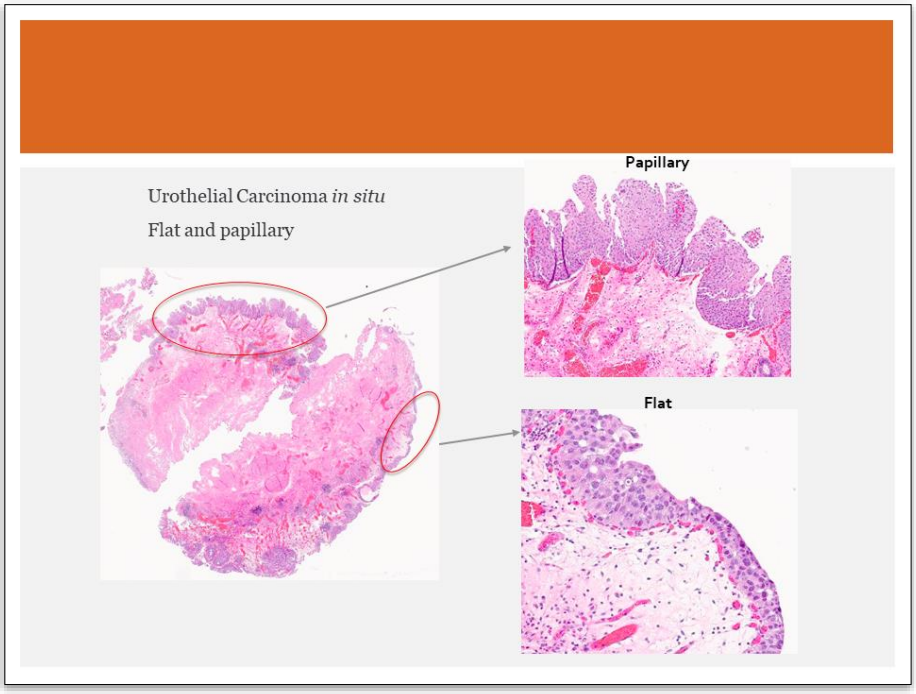
Dr. Hikmat Al-Ahmadie:

The last image on the bottom right, this is a tumor, carcinoma in situ, that is involving some normal invaginations in the bladder. Sometimes all the surface urothelium is normal. You don't see any malignant cells, but you could see these tumor cells inside this invagination. Sometimes that can make it more difficult to treat. If we go next slide.

As much as we want to believe or we want to think of urothelial carcinoma in situ as distinct from papillary tumors, a lot of times they coexist or they overlap, coexist or they develop after one another. These are some terms that we use, primary urothelial carcinoma in situ or primary CIS is one. On the first presentation, it is urothelial carcinoma in situ with no associated papillary tumor, or you can have secondary CIS, where there is urothelial carcinoma in situ developing concomitantly with or after a prior diagnosis of a papillary urothelial carcinoma. Of course there's always attempt to try to link this to differences in outcome or responses to types of therapy even though it's not necessarily conclusive yet, but it seems that some people believe that primarily urothelial carcinoma in situ may be associated with a worse response to rate to these conservative treatments. Next slide.

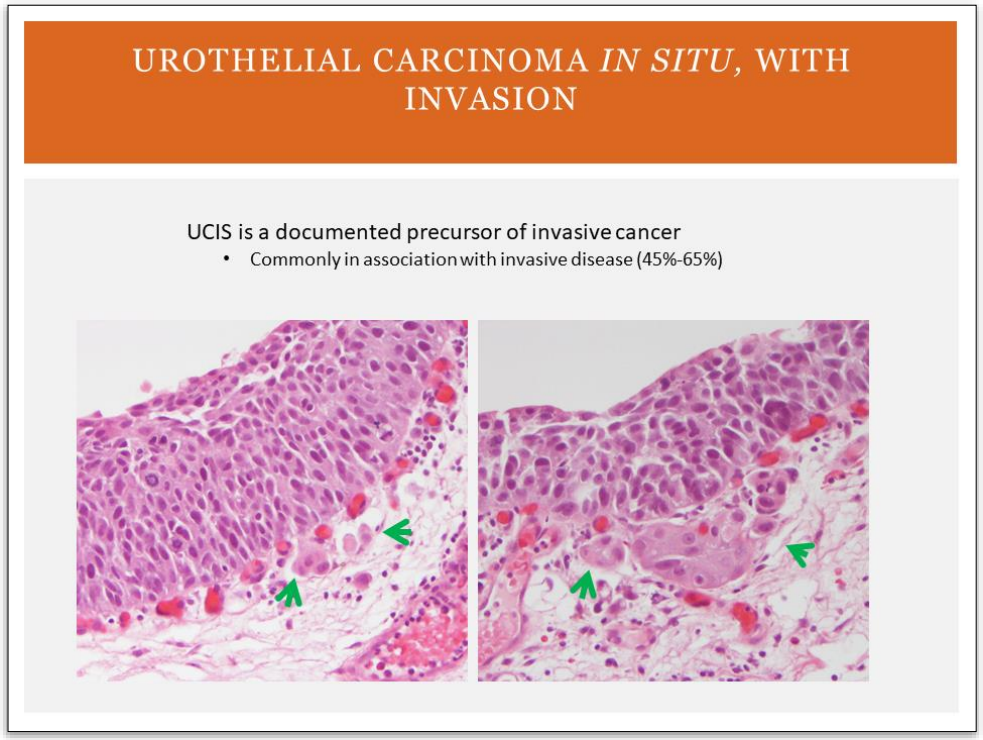


This is one example I wanted to show you, is as I said, as much as we like to keep them separate and unique, sometimes they coexist. This is one example here. This is the same TUR specimen from the same individual. You can see them grouped together, coming together within the same container from the same patient. If you look at the higher magnification picture on the right top, this is a papillary tumor. You can see these finger-like projections sticking into the bladder wall, out the bladder lumen, versus the picture on the bottom, the growth is very flat, just along the surface urothelium. That's the distinct growth pattern for urothelial carcinoma in situ. So a

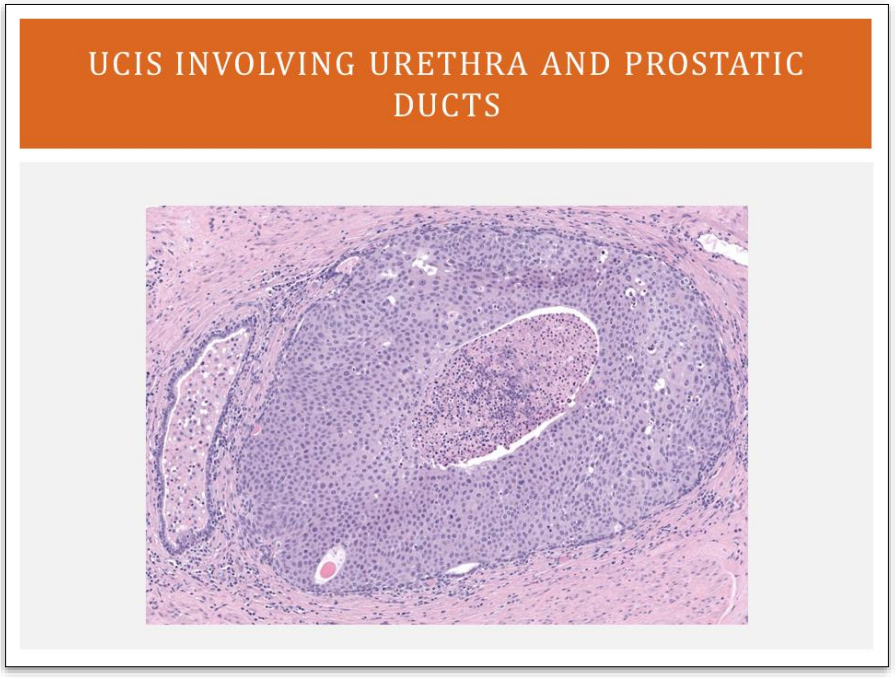


papillary tumor and a flat disease can coexist within the same specimen. Next.

Dr. Hikmat Al-Ahmadie:



And of course another important thing, when we assess, when we evaluate the urothelium here, and every time we see urothelial carcinoma in situ, the next most important thing is to determine whether there is any amount of invasive disease or whether this tumor is purely non-invasive urothelial carcinoma in situ. We look carefully at the base of the surface urothelium and we are carefully evaluating the stroma underneath it, these layer that are loose underneath it. When we start seeing these individual cells that are highlighted by these green arrows or arrow hats, that's when we start calling this invasive disease. Depending on the level of invasion and the amount of the invasive disease, we can call it focal superficial, and if it goes deeper than the first layer... if it remains in the first layer, you call it laminal invasion or T1 disease, as you might hear about it. And if it goes further deep into the bladder wall, it may invade the muscular layer, where you call it muscle-invasive bladder cancer. These features are not depicted here on the slide, though. Okay, next. I think we went back, so... Yes, next slide.



Yeah, and so this is another example that Dr. Black alluded to that could present challenges in evaluating urothelial carcinoma in situ. This is a tumor that is involving... here, what we see in the picture is involvement of prostatic ducts, which are immediately underneath the urethra. This is a tumor that kept kind of crawling along the surface urothelium, involving the urethra and went all the way into the prostatic ducts. It's not invasive, it just keeps colonizing all these ducts and makes it difficult to detect sometimes, difficult to remove and difficult to treat overall. Next.

This is going back now to the actual case here in this presentation. This is the biopsy from the gentleman, and as you can see, after I showed you all the slides, now everyone should be able to recognize that yes, these cells are very atypical. They're different sizes and shapes, different colors. They don't respect each other's borders. There are a lot of variations amongst them. This is diagnostic of urothelial carcinoma in situ. Next. Yeah, that's yours. Back to you.