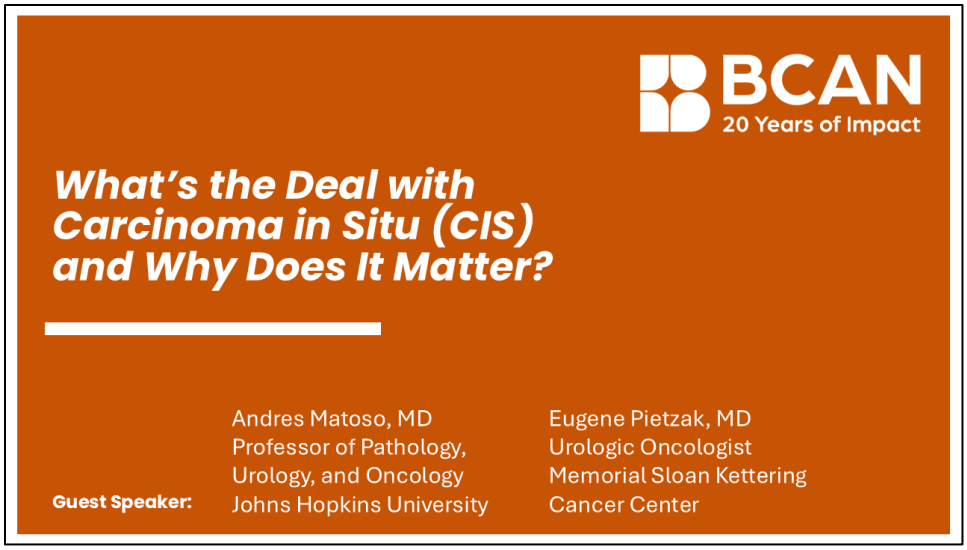
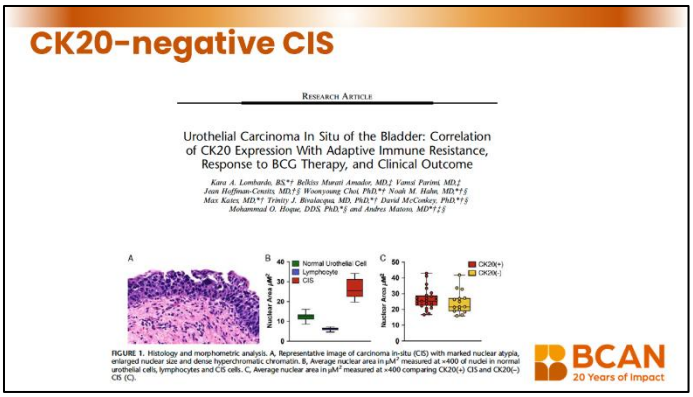


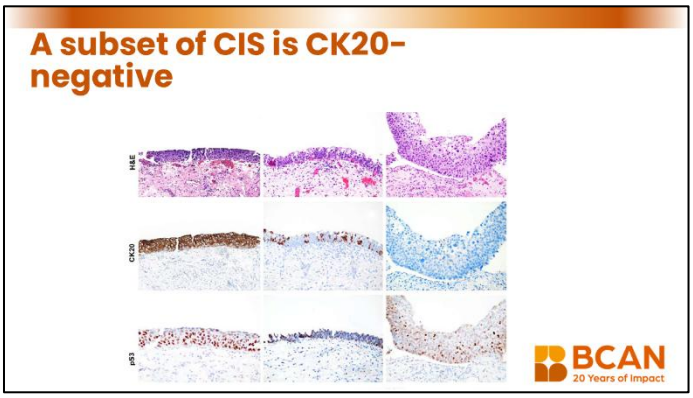
Dr. Andres Matoso:

Now I mentioned that not all cancers or CISs are positive for Cytokeratin 20, and we conducted a study to look at the Cytokeratin 20 negative CIS that is approximately 20% of the patients have that Cytokeratin negative CIS agent.



Dr. Andres Matoso:

And you can see here the thickness is increased, the nuclei are large, there is loss of polarity, so it has all the features I described of CIS, but it does not express CK20.



Dr. Andres Matoso:

So, in about 20% of the patients the CIS cannot be diagnosed either with the use of immunohistochemistry. And when we looked at the survival curve of those patients, they have similar progression and death due to disease secondary to CIS that is CK20 negative. So for those pathologists that rely too much on immunohistochemistry, they could be missing up to 20% of cases of CIS.

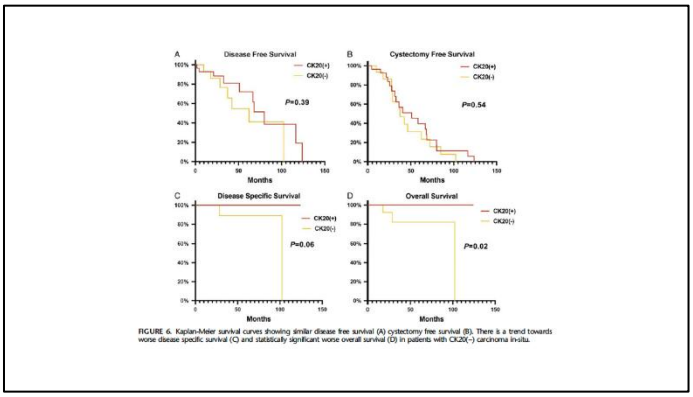


FIGURE 6. Kaplan-Meier survival curves showing similar disease free survival (A) cystectomy free survival (B). There is a trend towards worse disease specific survival (C) and statistically significant worse overall survival (D) in patients with CK20(−) carcinoma in-situ.

Dr. Andres Matoso:

Also, from the pathology perspective, and this is more merging into the sign of therapy, we looked at the immune system and how that could affect response to BCG or therapy, and basically patients with increased PD-L1 expression are enriched in the group of patients who do not respond to BCG. So some additional immune microenvironment studies could help better select which patients could be best candidates for BCG versus BCG plus other type of therapy.

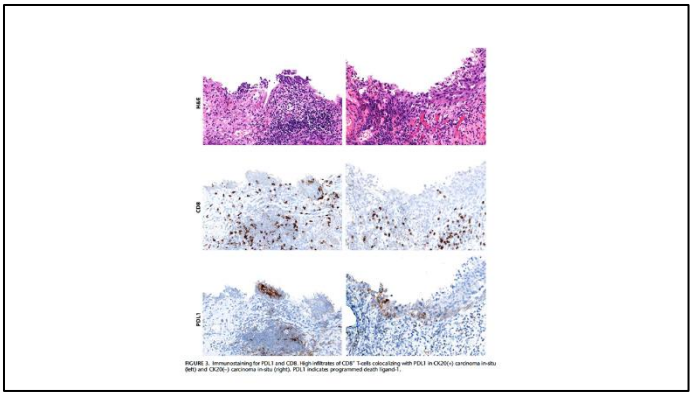
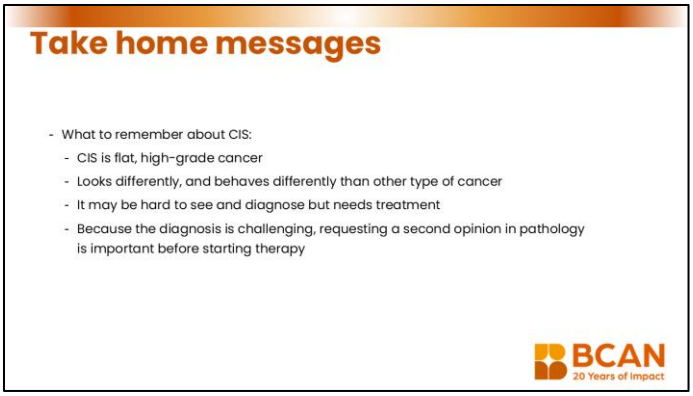


FIGURE 3. Immunostaining for PDL1 and CD8. High infiltrates of CD8+ T-cells colocalizing with PDL1 in CK20(+) carcinoma in-situ (left) and CK20(-) carcinoma in-situ (right). PDL1 indicates programmed death ligand-1.

Dr. Andres Matoso:

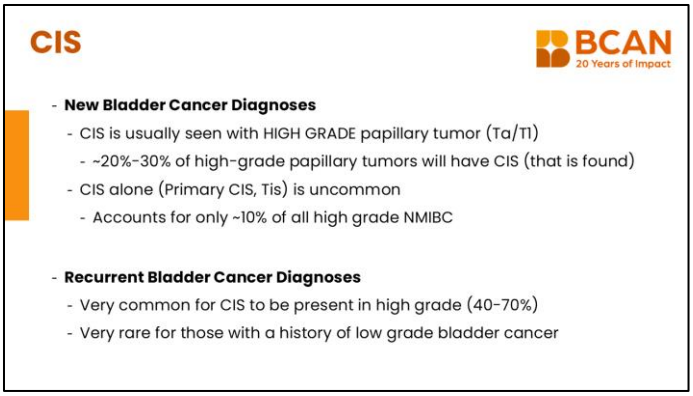
So as a summary of my presentation and what I would like you to remember is that CIS is flat, high-grade cancer always by definition, looks differently, behaves differently than other types of cancer. It may be hard to diagnose because we don't see it, but then also when we see it and we take a biopsy there are very different tricky situations that could prevent a definitive diagnosis in multiple instances, and in those situations we always recommend getting a second opinion because of the high implications that this diagnosis could have and that could impact therapy and the outcome. And with this I'm finishing my portion of the pathology, and I pass it on to Dr. Pietzak. Thank you.



Dr. Eugene Pietzak:

Hi, so as mentioned, I'm Eugene Pietzak, I'm one of the urologic oncologists focused on bladder cancer at Memorial Sloan Kettering. It's nice to see everyone, thanks for joining tonight.

Dr. Eugene Pietzak:

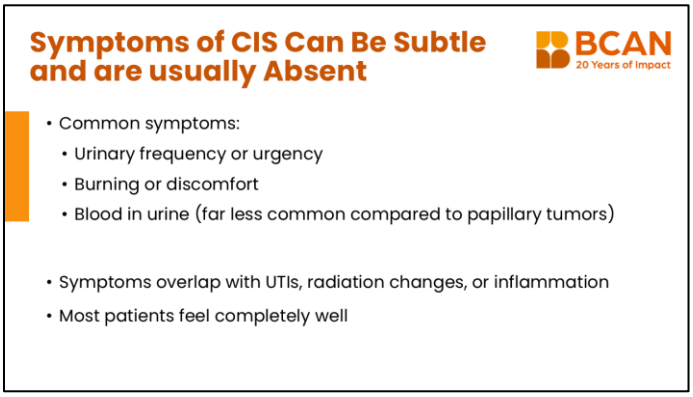


So, I'll talk more about the sort of clinical implications of carcinoma in situ, as Dr. Matoso just gave a very good overview of the pathological implications. Clinically at least, so carcinoma in situ for someone with newly diagnosed bladder cancer, it typically exists more commonly with papillary tumors, the sort of lumps that was just described where it's more visible. It's fairly uncommon alone at the initial diagnosis. So of all the individuals diagnosed with high-grade, non-muscle invasive bladder cancer, somewhere between 20 to 30% of individuals will have a high-grade papillary tumor with carcinoma in situ, where somewhere around 70% of people will not have that CIS component.

And as was well emphasized, a lot of it also depends on the pathologist report and their read, and that varies from different centers and different pathologists reviewing it. But pretty consistently, in the literature at least, carcinoma in situ alone as an initial diagnosis is pretty uncommon, it's typically less than 10% of individuals. And we'll discuss, but usually that may be missed for a while and because some of the symptoms may be more irritative urinary symptoms, urgency, frequency, burning with urination. Because there's not a visible mass seen it is not uncommon for urologists in the community to sort of treat that as a urinary tract infection, and so those individuals tend to have what appears to be a bit of a delay in their diagnosis.

So after someone with initial high-grade, non-muscle invasive bladder cancer that potentially gets treated with BCG, which we'll discuss in some of the subsequent slides, carcinoma in situ then begins for those that have recurrent cancer to become more prevalent, and it almost continues to increase with high-grade recurrences where it reaches somewhere between 40 to 70% of individuals when they sort of develop this more BCG resistant disease. And it is fairly uncommon for someone with a low-grade bladder cancer to develop carcinoma in situ in a subsequent recurrence, but that is something that is potentially possible but typically it's associated with high-grade recurrent disease.

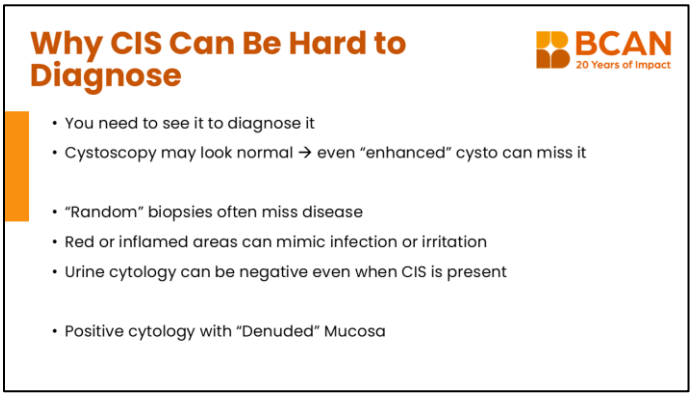
Dr. Eugene Pietzak:



And it is more common in the recurrent situation to be alone just carcinoma in situ in the papillary tumor, the visible lump tumor, are sometimes not present in the recurrent setting and it's just the carcinoma in situ only.

So, it is somewhat more resistant to treatment for various reasons, and that's in part because the endoscopic resection, the scrapings, are less effective for carcinoma in situ. So as I sort of alluded to, the symptoms of carcinoma in situ, they're typically absent, most people with carcinoma in situ besides having say blood in the urine, which is more common with papillary tumors, the symptoms seen are typically urinary urgency, and frequency, and burning. And a lot of these symptoms overlap with urinary tract infections, radiation changes that someone may have received or inflammation, but most patients typically feel well. And as I think most people on this call probably know, blood in the urine is the most common presenting symptom for bladder cancer in general, but that's less common for the flat carcinoma in situ just because they don't have as robust as a blood supply.

Dr. Eugene Pietzak:

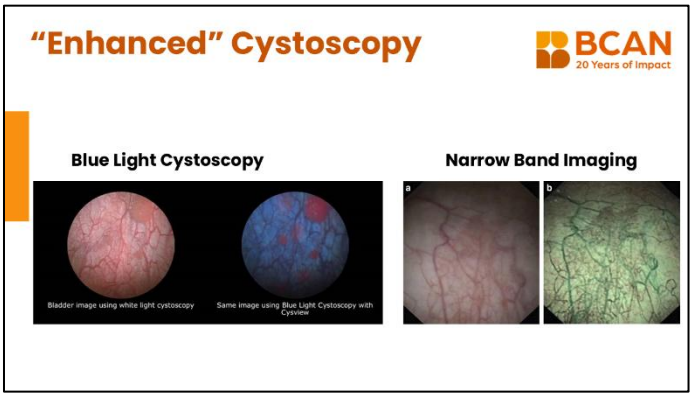


So, you know, carcinoma in situ from a clinical standpoint where we're doing cystoscopies is actually pretty hard to identify sometimes and the cystoscopy may look relatively normal, and we have to often use these technologies known as enhanced cystoscopy, which I'll show you in a subsequent slide what that means exactly, but it's special light filters, either narrow band imaging or blue light cystoscopy that may better highlight features of carcinoma in situ, but sometimes we can see these flat velvety type lesions and identify them. And you know, even though we may suspect there's carcinoma in situ, even if we do what are sometimes referred to as random bladder biopsies, we will not be able to actually identify it. Or sometimes what is carcinoma in situ may be dismissed for either inflammation or just a sort of an aspect from infection or vice versa.

So it can definitely be very challenging and we will check urine cytologies, which are basically the urine sample that gets spun down and look to see if there's cancerous cells in the urine underneath the microscope, that's called a urinary cytology. That may sometimes be positive

or it may sometimes be negative even when there's carcinoma in situ. So there's not a perfect test clinically to determine whether or not there's carcinoma in situ present, and we're really dependent upon the urologist visibly seeing an area to biopsy because the first step in the management, you have to find it before the pathologist could even identify and classify it as carcinoma in situ, which can be very challenging. And sometimes because of the way carcinoma in situ is, it may get shed into the urine. And when we do the biopsies it may come back with what's known as denuded mucosa, meaning that lining Dr. Matoso just showed you, has already shed into the urine and there are no more carcinoma cells present. And so it can be a challenging clinical entity to diagnose.

Dr. Eugene Pietzak:



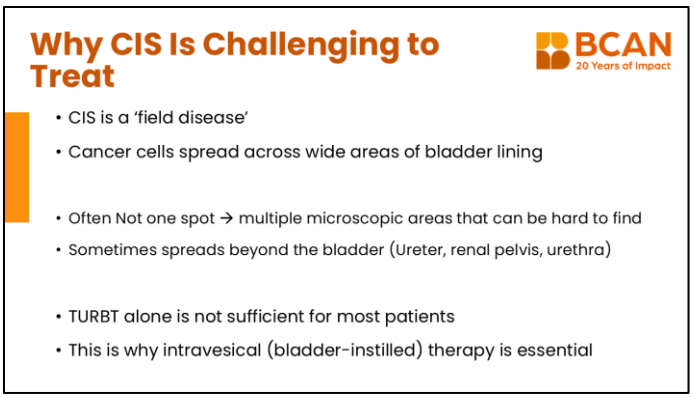
And these are just some examples that I actually pulled from the internet, because oftentimes I'll tell you the images don't ever really look as good as this.

All right, so this is a white light cystoscopy over here. You can hopefully see that this is a papillary tumor, it's this lump you could visually see. These are smaller ones, these are quite obvious and in the dynamic cystoscopy these are very evident. But what's a little bit more subtle is if you go to the blue light where this is, it's basically you can think of it as like a fluorescent dye that gets instilled about an hour before the procedure and cancer preferentially takes it up, and then under a special blue light wavelength they fluoresce, as you could see here, and they show a bright orange. And so what is a little bit more evident is that this area over here, these little orange patches, these fluorescent patches, that's carcinoma in situ. And if you look back on the white light you can kind of see where those core respond to, but they're definitely more subtle on white light, it is far more challenging to identify it. So this is a nice bit of technology to do.

And this is another form enhanced cystoscopy called narrow band imaging, this is just changing the filter on the camera source itself, does not require that extra installation, and it's often a little bit more subtle, and I would say probably users need more experience compared to blue light where it's more obvious on blue light cystoscopy. But if you noticed over here you could kind of see that this is a flattened lesion over here, and then on narrow band imaging the narrow band really highlights the capillaries, the blood supply that's around there, and you get a little bit more of this blue-green contrast, and you could see the borders of where the carcinoma in situ is. So these are very helpful adjuvants in order to identify what needs to be biopsied, but the urologist needs to be thinking about these and they need to be looking for these very diligently. And these are things that if someone's doing a cystoscopy very quickly, or if there's a large tumor that's very obvious, it's papillary that

they're focused on and they're not looking for carcinoma in situ, this is something that could easily be overlooked.

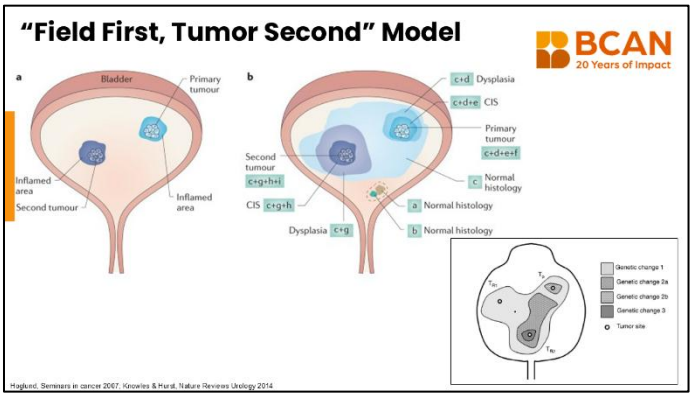
Dr. Eugene Pietzak:



So, carcinoma in situ, as I mentioned, it's difficult to diagnose, and it can be very challenging to treat as well. At least the way that I conceptualize and think about carcinoma in situ, it's a disease of the lining of the bladder, and it's often not just in one spot. The way that I think about it is the carcinogens that led for the bladder cancer to develop, the carcinoma in situ to develop, the entire lining of the bladder, as well as the ureters and the urethra, were all exposed to that and so they're all prone to developing it because of that carcinogenic insult. And so it sometimes can spread beyond the bladder into the ureters, the renal pelvis, the urethra as well, especially with treatments like BCG and other treatments, intravesical treatments into the bladder that do not reflux up into the ureters or into the urethra itself. So those are things that the urologist needs to be mindful of to look out for essentially.

So the TURBT, the transurethral resection, may be sufficient treatment for papillary tumors because they could visually see it, but it's often insufficient for carcinoma in situ, and that's why the intervescal treatments, the treatments into the bladder, are so essential.

Dr. Eugene Pietzak:



So, these are some cartoon versions of at least how I think about the cancer. And so just to sort of demonstrate, if you look over here in this lightish blue area, like the lining of the bladder in this area is probably more of this dysplasia or pre-dysplasia scenario that Dr. Matoso was just talking about, and then they accumulate more mutations. And what may only be visible is this papillary tumor, but sort of the surrounding urothelium, the surrounding lining of the bladder, may have carcinoma in situ or may have dysplasia, sort of the pre-carcinoma in situ type

entity. And it can be very diffuse, it can be spread out, it can be multifocal, and it makes it more challenging to diagnose as well as treat.