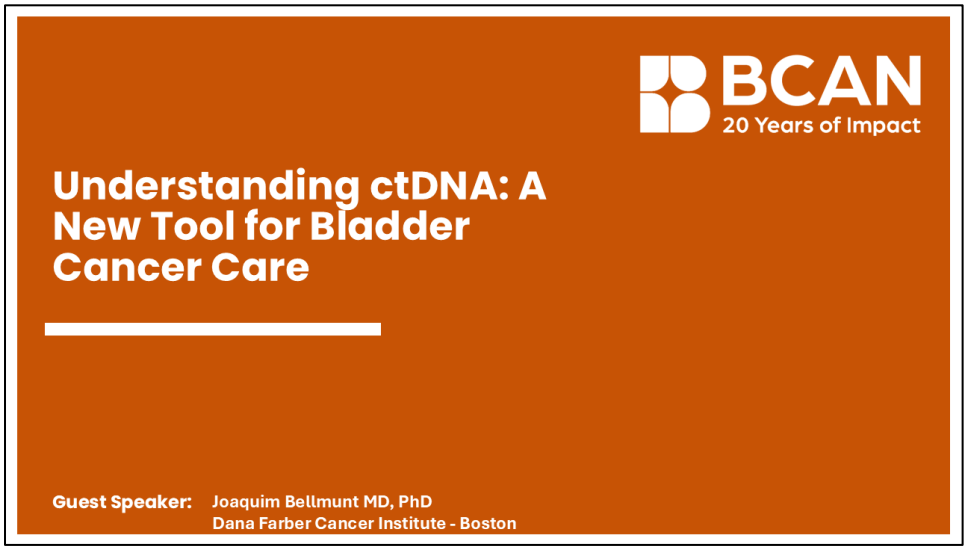

Stephanie Chisolm:

Thank you. That was so comprehensive and detailed. I absolutely appreciate it. We actually had a number of very excellent questions that I'll be asking you. I just think it was just wonderful that you know we now have this tool to use at baseline to check post-neoadjuvant chemotherapy and then also to be used for surveillance. You know, in terms of if a patient were to ask, what would you tell them in you know in terms of using this test, could that make them feel more confident in their treatment by being able to monitor? Is that something that you use to tell patients an idea that you use to get patients to consider this?

Dr. Joaquim Bellmunt:

Yeah, so obviously this is still not yet in the guidelines of and what I'm going to talk is trying to move ahead of the guidelines. So all the times we, we have new data that is you need to implement and explain the patients just before it's approved by FDA, whatever. But yeah, I think, I think I need to congratulate Natera because it's helping patients to get this tests done. I'm not doing marketing, but it's really... Patients need to forget about the issues with the insurance and so on. So obviously there are like limitations on the number of tests that you can use, it need to be done beyond six to eight weeks. But for example, I can tell you a clinical case.

So a real patient, he received it wasn't an upper tract tumor, so he received surgery and then he received adjuvant therapy. He was a high risk patient and imaging was a negative. And he came to me after adjuvant and said, "Well, what can I do to make sure that this tumor is not coming back? How can we detect earlier if this tumor is coming back and add for example, immunotherapy?" "Oh, it's not a standard." Well they say, "Or something else that could help

me." And this patient now has had this Natera platform being performed every, every eight weeks. Now it's one year so and the patient is pretty comfortable saying, "well, imaging is negative." Obviously we don't skip imaging. But also when he receive... He all times call me, "Oh, because" and he received the results before me. So that's amazing, so because the company is providing these results for patients that are anxious obviously to know these results and this patient after a year.

So I present this lecture that I get at SUO and say, "Now after a year you have only the chances of recurrence based on this clinical trial is only 10%, 12%." So and he was quite relieved saying, "At least my ctDNA is not coming positive." It's an additional tool to reassure that things are going well.

Stephanie Chisolm:

Thank you. So you're there at one of the premier cancer institutes in the world, Dana-Farber Cancer Institute, and I know that many of our colleagues that are working at large academic centers are using these tests regularly. What would you say to patients who are being seen at the community level? How do they ask their doctors who may not be as involved in this process and understanding the benefit to find out if they could get this test even at the community level?

Dr. Joaquim Bellmunt:

Well I believe that in a patient that has had surgery that is not a really, really high risk and the patient is doubtful if to receive adjuvant treatment. So that's a way to say, "Okay, you don't want to receive." We'll see the results have been IMvigor011 are going to tell you, ask more about that. But if a patient say, "No," it is reluctant and to convince the patient say, "Well, maybe if you don't want, let's monitor the ctDNA. But if the ctDNA is coming positive, that's mandatory that you need to receive adjuvant therapy before the any imaging is coming positive." So I think it's a way to get to know more about the status of the disease. It's like in fact we are checking for minimal residual disease that is not detectable by any type of imaging right.

Stephanie Chisolm:

Right so you can see it before it even shows up. So another question I'm sure many patients ask you, "Is this covered by my insurance and will it still be, do you have to pre-authorized to get this test done?" How does this happen in the real world?

Dr. Joaquim Bellmunt:

Yeah, I think in the, it's pretty clear in the perioperative setting, this is what I have focused on that space, it's pretty clear, the results are pretty clear that is prognostic predictive and it's helpful to understand a bit more how the biology of the disease evolves. So and I haven't had any big problem. I've mentioned that the company's helping a lot on solving the issues with insurance. And even they say that sometimes they assume if the insurance is not paying because the priority is the patient and I need to congratulate them, just even they have a

mobile way just to get the blood test done at home, the patient doesn't need to go to the hospital. So I think that's an advantage for the patient. Usually as mentioned, if you request this test in a frequency that is every two months, it's likely that no one is going to complain if you explain the rationale.

So obviously we have talked in this perioperative space the surgery after surgery, but now we've done we have been collecting ctDNA in patients receiving bladder preservation, right. And that's very important. Say, "well, you give you chemotherapy or you give chemoradiation therapy, and you know you get imaging, you get cystoscopies, and you are never sure if still there is some cancer tumor cell hidden in the thickness of the bladder right because you have not removed the bladder." And that's a you see these changes in the bladder, there is bladder wall thickening. Sometimes the MRI is saying there is a highlight in this bladder, is the ADC is high, is there inflammation, is tumor? In these cases, I think ctDNA even it's not standard, but we are now using that in patients that are willing to preserve their bladders, saying, how can you make sure that... Because imaging has limitations in patients that have received chemoradiation therapy, how can we optimize and make imaging...

Obviously we're not going to make decisions with the ctDNA only in these cases, but like close follow up or additional measures intensifying biopsies just to go to areas where initially you would say, "Oh, let's wait for three months." Patients don't like that, no one likes just to wait three months to know if things change, right. With that maybe you can get more information and to make decisions. That's, that's a setting that I think is emerging. And even there are now trials designed to preserve the bladder based on the negativization of the ctDNA before deciding, deciding to preserve the bladder. So a patient is coming say, "well, standard of care is neoadjuvant chemotherapy, removing your bladder," but obviously no one wants the bladder to be removed despite neobladders, whatever.

So, and if you are asking for bladder preservation, you can say, "Well, this patient has responded pretty nicely to the neoadjuvant chemotherapy, but is there something else that might help?" And despite the RIT-URVT and so on. And if a patient has negativity on ctDNA with response on the RIT-URVT, maybe you can consider this patient for bladder preservation and not remove the bladder. This is going to be explored in a clinical trial. So that's that's the future of how we can use ctDNA.

Stephanie Chisolm:

So I have a really excellent question from a patient. "I'm four years with no evidence of disease after having muscle invasive disease and having my bladder and prostate removed in 2021 based on traditional CT scan of the chest, abdomen, pelvis contrast. Is it possible to start using ctDNA for ongoing surveillance instead of the traditional CT scans? How do I get the DNA profile from my original small cell carcinoma? Would that be stored in my doctor's file?" So that's really a question about is it possible to do tumor-informed even if you don't have the actual bladder with the tumor, or does he need to just look at tumor-naive tests?

Dr. Joaquim Bellmunt:

Yeah, so I don't know, this might be a patient of mine maybe because I have the same patient, same patient. It was a young patient with small cell that nothing has been exploring in this setting. This patient received chemotherapy and immunotherapy and then had surgery, and then after two and a half years or three years of follow up, the patient came to me and said, "well, can I check my ctDNA?" I said, "You need to know the natural history of the disease, right." So initially we know that the median time to disease recurrence is like 18 months or 20 months, maybe now with the chemotherapy is, is more, but if you have been three years without disease progression so and everything has been negative, so continue with imaging likely it makes sense.

Going back to ctDNA it's not a perfect tool. That's the other thing. So it's sensitive but it's not 100% sensitive, not 100% specific. So and then the rationale is in a small cell lung cancer, if the patient is free of disease four years, I think there is no role. Obviously you want to explore more, but I haven't seen in my life. Everything is possible in medicine obviously, but I haven't seen very few patients like progressing after three years from muscle invasive. Maybe they develop a new tumor, that's a different thing. But from the same tumor it's exceptional to see recurrences. There are cases.

So I think that you could discuss with the oncologists and say, "Was my tumor really, really high risk? I received resistance therapy. There is still residual mass that we don't know." But after five four years in a small cell likely you are... You never, you never use that free of disease or cure right. Meaning I wouldn't be like supporting that, but obviously it's a way to say, "well, let's add on," but not it's not going to exclude doing imaging. Imaging is the standard of care. That's the important thing here. I don't know if I answered the question, I know that... So happy, happy offline just to answer more detail.

Stephanie Chisolm:

No, there's there's so many good questions that are in here and a lot of them, you've covered so much in your webinar, so I'm not going to answer ask all the questions. In terms of looking at the difference between, again, tumor-informed, tumor-naive, you were talking about urine tests before. I mean for people with non-muscle invasive that aren't necessarily having a tumor that they could see present. And if they didn't have the tumor tested when they had that TURBT, what could be what would be the preferred type of CT testing for them at that stage if they still have their bladders?

Dr. Joaquim Bellmunt:

Well so all the times, that the good thing of these Natera is that you can go back and the tumor that was resected, you can use it with germline, that germline is the regular blood, right and create a customized customized platform to monitor. But this is in blood. In the urine, in the urine obviously there are different other ways to capture ctDNA, these and these methylation tests sometimes are much more useful or like tumor-informed tests looking for a panel of mutations. We know that non-muscle invasive is enriched for FGFR3. So there are

customized panels that are exploring specific genes that are frequently mutated and you are looking in this urine, the presence of these DNA with these mutations or alterations. So those are areas that are actively being explored.

Important thing that we need to remember. So the urine need to be well-collected. So there's a lot of contamination. There are like there are specific straight tubes, so you cannot collect the urine with a regular tube because you want to have the DNA well-preserved. And those are nuances that I have not I haven't covered obviously, but that's important. So these need to be done in a place where people is expert and know how to handle all these samples because, yeah, there might be false positives. So, but nowadays in patients that have received treatment for non-muscle invasive, in addition to the cytology and the FISH test. So also the urine DNA has been proven like helpful. But this is research still, we are not we're getting there. So hopefully we'll get there.

Stephanie Chisolm:

Yeah. So again, they're looking for very different things when they're looking for ctDNA than when they do this cytology test. With urine cytology, they're actually looking for the cells, whereas you know from what I understand, they're looking actually now for the actual DNA, not necessarily for the cells to see if there's any evidence of those mutations that are common in, in this. Okay, great.

Dr. Joaquim Bellmunt:

Usually, usually you do the same as in blood. So the urine, you centrifuge the urine, the pellet is used to look for cytology, and the supernatant is the one that might contain the DNA, the tumor DNA, and this is what it gets processed. You extract the DNA and then you analyze if these DNA has a the specific mutations with the test that you're looking your using.

Stephanie Chisolm:

This is really, again, amazing. Let me just ask you to end with just one general thing. What would be one fact you want to impart on all of the patients that are here, we have over we've had close to 100 people on this call today, really looking at what do you see as the future of bladder cancer because of this growth of ctDNA understanding?

Dr. Joaquim Bellmunt:

Yeah, I think, I think it's here to stay, the ctDNA. So as mentioned, this is what I said, I said there are different platforms and the platforms are improving. So I met with a some people at Natera said well now "We are now developing platforms that instead of these 16 like bespoke mutations, we are looking for like 80 or maybe 700," meaning the sensitivity and specificity is going to increase with this test. Obviously we need to study in the proper place and like these trials I have mentioned. So this trial was crazy, the IMvigor011, because it was the time that the Nivolumab and Pembrolizumab were approved here in the US, but this, this trial could only be completed in places where people didn't have access to nothing. And then at least they were

able... And it was randomized 2:1, meaning at least let's let's benefit as maximum as possible patients.

So yeah, it was a painful trial if you see from now, right because it was... Now wouldn't be ethical, but it's what the regulatory authorities require right. And now maybe this is going to change or establish in your standard of care in patients with the ctDNA. And as mentioned, we are going to hear that like this year. Sorry, I cannot say more the result of this trial. But no, no, I think that the the field is evolving. I mentioned just briefly that there are all... So you look for methylation DNA, you can look for mutations, you can look for epigenetic marks.

So now we are able, because whenever the DNA is shedding the blood. So usually the DNA is wrapped to the nucleosomes, and what can be captured is the nucleosome. So when there are the nucleosomes are the ones that regulate the opening or closing of the chromatin. And now we are able to capture that. We have what we call histone marks. So you are able to precipitate the histones and then you can read what's the DNA that is in between these histones and say, "well this specific transcription factor, this gene is presenting this patient." So it's the techniques are evolving. Yeah, it's amazing. It's amazing really. It is difficult to follow. I can tell you, so.

Stephanie Chisolm:

It's just been a very exciting, exciting time for bladder cancer. There are more treatments now available. Again, because we know more about these tumors down to that microscopic DNA level where we know what's going to work. It can certainly help prevent patients from having expensive treatments that might not offer any benefit by understanding more about how those tumors need to be treated. So what is your recommendation? Like how long do people need to keep getting this test? Maybe if you have five years of nothing, is, what's the what's the option? What do you think it should be going forward? Should they be tested every year, every other year? How often do you have these tests done?

Dr. Joaquim Bellmunt:

So nobody knows. Now, as mentioned, as mentioned, nowadays what I do is like if the patient has received, depending on the type of treatment, if you say, well, the median time to, to, for disease recurrence in this trial is like 18, 20 months. I said, well, let's go beyond that point. Maybe let's test for two years, three years, but it doesn't make sense to test beyond five years, for example, right? Because, so all I, this need to be linked to the biology of the disease, the type of treatment receive, because personally we don't have rules on what's the best frequency to test these patients. As mentioned the in these trials, we were testing every six, eight weeks. That was the timeframe. The technology is very important. So if anyone is interested, we present these data at ASCO. There is an educational chapter that I am happy to share with whoever that explains all these nuances on processing different types of tests. So that, yeah, I don't have time just to go through because it's a bit complicated.

Stephanie Chisolm:

Great. Thank you so much. I really think this has been a very informative program. I know that our patients are really going to appreciate having this as a tool.