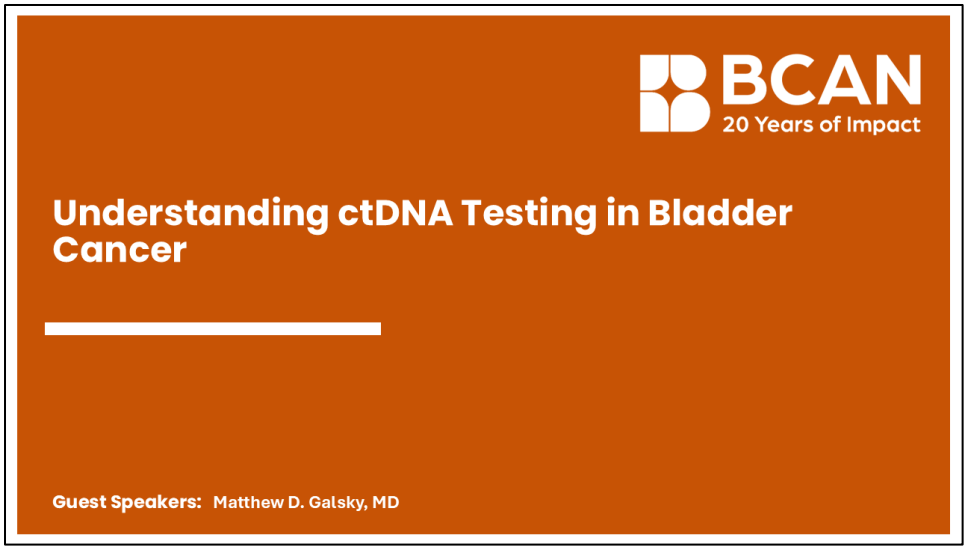
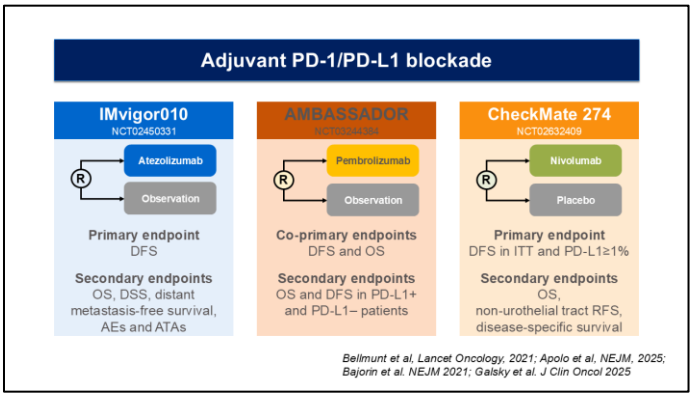


Dr. Matthew Galsky:

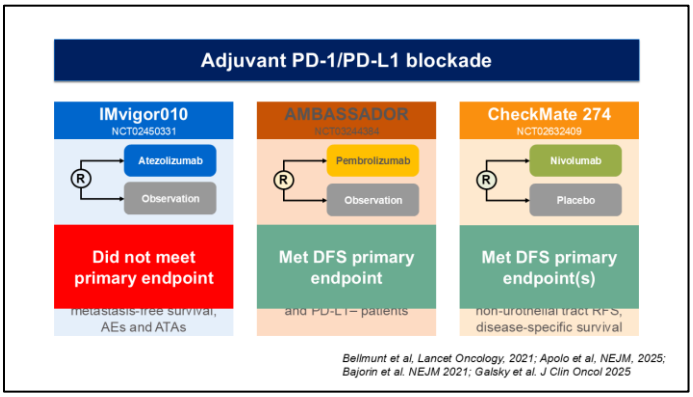


So the story in parts starts the story with ctDNA in parts starts with the recent adjuvant studies that have looked at the role of immunotherapy after cystectomy for bladder cancer. There were multiple data sets, some from specific investigators that had been working on this for, for, many years that had been published prior to some of these clinical trial data sets. And those really showed that ctDNA might be telling us something that some of our other testing wasn't. But some of these data that I'm going to show you now solidified that this testing really might have a role in clinical decision making. And so, we have three studies that have asked whether or not we should give immunotherapy after cystectomy for bladder cancer, an attempt to eradicate microscopic cancer in case that had spread, and to try and decrease the risk of recurrence of bladder cancer after cystectomy. One of those studies is called IMvigor010, and that used a PD-L-I inhibitor in immunotherapy called atezolizumab.

There was another study very similarly designed called AMBASSADOR that used a immunotherapy called pembrolizumab, and then a third, which I used an immunotherapy called nivolumab called CheckMate 274, these studies didn't necessarily show us the same results even though they were designed pretty similarly.

Dr. Matthew Galsky:

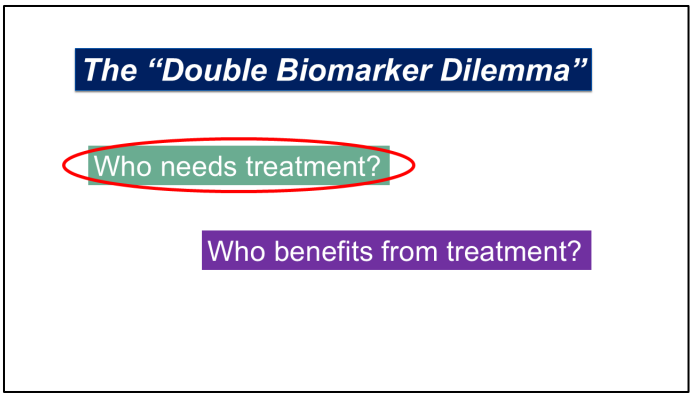
The study with atezolizumab didn't meet its primary endpoint. The study with AMBASSADOR showed that there was an improvement in disease-free survival if you give pembrolizumab after surgery versus just observation. And the study with nivolumab showed that there was an improvement in disease-free survival with nivolumab versus a placebo after cystectomy.



Based on the results of CheckMate 274, nivolumab was approved by the FDA in this adjuvant setting. And so we have three studies. One of them didn't show the same results as the other two. That always raises the question, is this the right approach? Is the concept here, right? Are these just differences between different trials, different drugs? And I think, you know, there are all of those potential explanations for these findings.

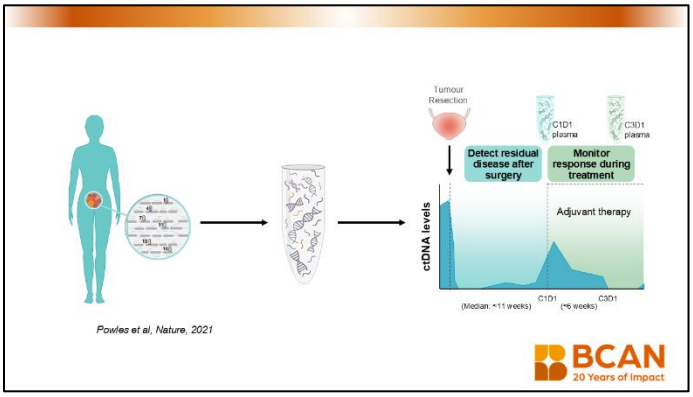
Dr. Matthew Galsky:

But another explanation is just that this is a really challenging space to potentially show a benefit with therapies. And it's a challenging space because as I was getting at before, after cystectomy for bladder cancer, of course we know that a lot of patients are cured already. And so nothing that we give patients after they've had cystectomy for bladder cancer, if there's no microscopic metastatic spread of cancer, nothing that we do can improve that outcome further if they're already cured with surgery alone. And we know that a subset of individuals in these trials based on the controller observation arm do quite well. And so that tells us that a large subset are cured already. And so when we study drugs in this setting, we really are flying a little bit blind in terms of not knowing necessarily who needs treatment.



There are features under the microscope in the surgical specimen or if lymph nodes are involved under the microscope in the surgical specimen, that might impact the risk of microscopic spread. But that's just telling us about risk that's not actually measuring microscopic spread still present in the body. And so this has really been a challenge in the adjuvant space in terms of selecting which individuals actually need additional treatment. And that's why it's also hard to show a benefit in clinical trials. And some clinical trials might not show the same thing. One trial might be enriched in more individuals who were cured with surgery alone, making it harder for a medication to show a benefit in those individuals who actually needed the treatment.

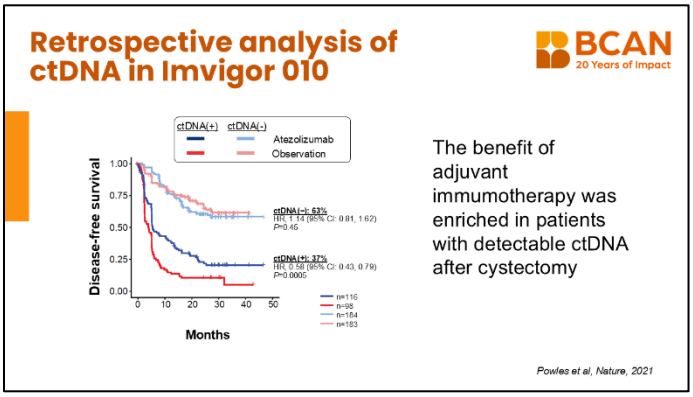
Dr. Matthew Galsky:



And so that's where ctDNA potentially has come in terms of being able to help us understand that. And again, the assay that's been used in a lot of the studies is an assay called Signatera.

And this is a tumor-informed test like I was referring to, the primary tumor undergoes DNA sequencing to check for alterations in the DNA. In the case of this specific study, 16 of those mutations or alterations in the DNA are selected to make this blood test this bespoke blood test to check in the blood for whether or not this abnormal DNA can be found. And so this was done, this test was done in that study that I showed you that didn't show a benefit with immunotherapy after surgery, after cystectomy in a retrospective fashion, meaning the study had already been done. We already had the results of this study. And then there were samples, blood samples that were obtained during the course of that study. So the investigators went back and they said, would the results have been different if we knew after surgery who had detectable DNA in the blood tumor DNA in the blood versus patients who did not?

Dr. Matthew Galsky:

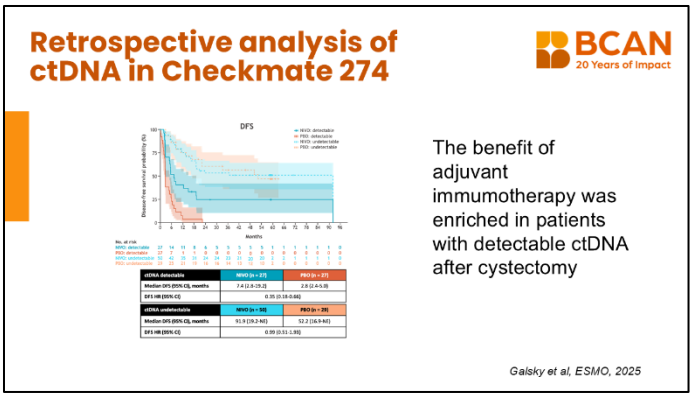


And this is what they found. They found that in... These are called Kaplan-Meier curves. And what we're looking at is time in the X-axis and then the likelihood of cancer coming back in the Y-axis. And when the lines go down, that means that there's a higher probability that cancer has come back. And so when there's a split between two curves, that means that one arm has a lower likelihood of cancer coming back than the other. And so here you see the results of that study now split out by individuals who had detectable ctDNA after surgery. This is a single blood test at a single time point, so, the first blood test done after cystectomy. You can see if the DNA blood test is detectable than patients who were treated with immunotherapy have a better, a lower likelihood of cancer coming back than patients who were treated with or who were followed with observation.

But if the ctDNA was undetectable, it was harder to show that split between these curves. Now this is retrospective data. As I mentioned, a look back and looking back when we think about

medical advances is incredibly important because it gives us the ideas about what we need to test. But there is a difference when we look back versus whether or not when we test something moving forward, a retrospective study versus a prospective study. We try not to inform medical decisions based on retrospective studies because there's always the potential for some bias. So this really gave us the sense that maybe this technology could help us with this difficult problem.

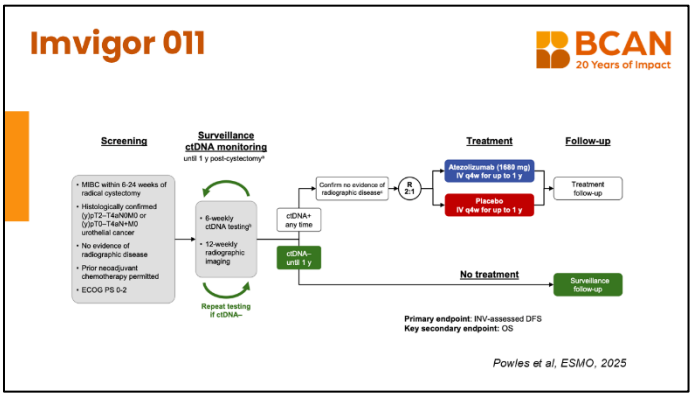
Dr. Matthew Galsky:



And then we replicated this result in one of the other studies that I showed you, this study with nivolumab, another immunotherapy that was given after cystectomy versus placebo in a phase three study that did meet its primary endpoint and led to an FDA approval for nivolumab.

And here you can see the colors are a little bit different, but the results look quite similar. If there's detectable ctDNA, there seems to be a lower risk for cancer coming back versus placebo. Whereas if there's undetectable DNA, it's harder to show a difference between those curves.

Dr. Matthew Galsky:

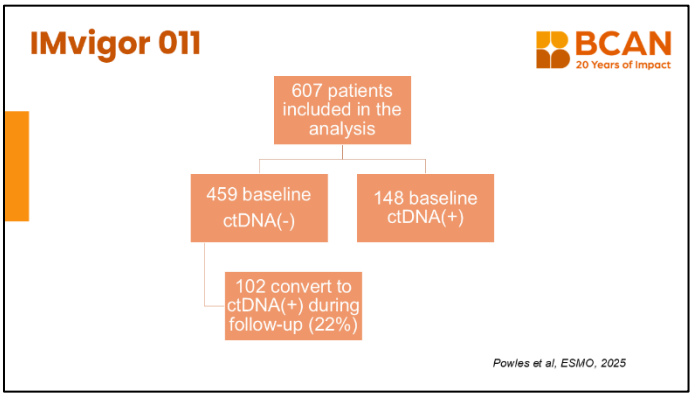


So that's retrospective data. We need prospective data and we really don't have in any cancer, we really have very few prospective studies testing the clinical utility of using ctDNA for clinical decision making that is making a decision based on ctDNA leads to some improvement in patient outcomes. We don't have a ton of those studies across different tumor types, but they're coming. But bladder cancer is actually one place where we have this data, and this is called the IMvigor011 study. And we just saw the results of this study presented at a European oncology meeting and in published in a major journal now, and this is the design of this study.

So, individuals had cystectomy to treat bladder cancer and based on the features under the microscope, what we refer to as the pathological stage, if there was felt to be a high risk for recurrence, then patients could enroll on this study and they underwent ctDNA testing. If their testing showed abnormal tumor DNA in the blood, then they were randomized to receive immunotherapy with atezolizumab versus placebo. So essentially taking that retrospective finding that I showed you and trying to replicate it in a prospective manner, a nuance to this

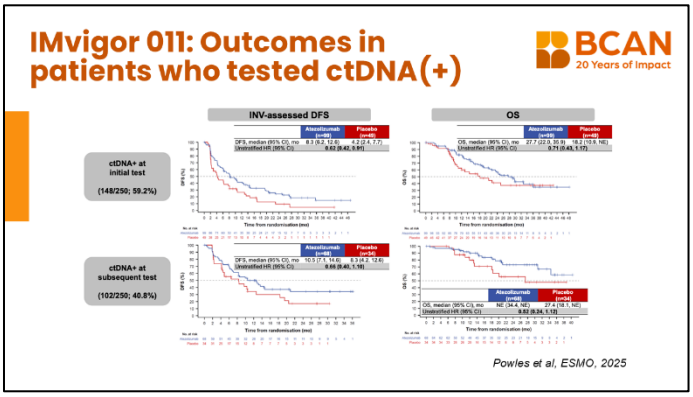
study, an interesting element of the design is that patients underwent ctDNA testing every six weeks for the first year. And if their initial test was undetectable, but during that course of that year it converted to detectable, then they could enter the randomization. So there's really two groups of individuals for whom immunotherapy was tested, those with DNA in the blood right after surgery, and then those who had undetectable DNA in the blood initially. But during the course of monitoring, it became detectable.

Dr. Matthew Galsky:



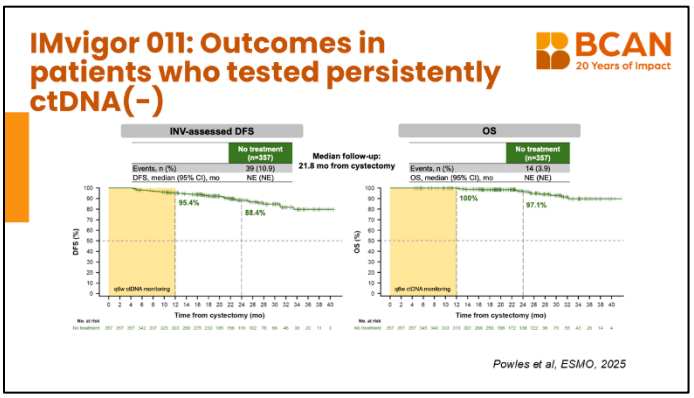
And I'll show you the results of this study here. So a moderate size phase three study, 600 individuals were included. You can see the breakdown of the DNA detectability after surgery. So the majority of individuals actually had undetectable ctDNA after surgery, a subset had detectable on that first test. And then you can see during that year of follow-up among the individuals who had undetectable ctDNA, a subset of those, the DNA becomes detectable. So we know that that baseline test is very important from a prognostic standpoint, but it doesn't completely exclude the chance that that test could become detectable over time. And that's one of the potential roles of serial monitoring of this test.

Dr. Matthew Galsky:



What I'm showing you here is the result of giving immunotherapy versus placebo in individuals who had detectable circulating tumor DNA, either at that initial test or in the lower curves in the lower half of the slide, if the DNA test was initially undetectable and then became detectable. And you can see the blue lines are immunotherapy, the red lines are placebo. And basically if you look at any of these lines, the immunotherapy curve is higher than the placebo curve showing that in individuals with detectable circulating tumor DNA, either right after surgery or during the course of follow-up treatment with immunotherapy versus a placebo decreases the likelihood of a recurrence. So that was a promising result that really reinforced the result from the retrospective studies.

Dr. Matthew Galsky:

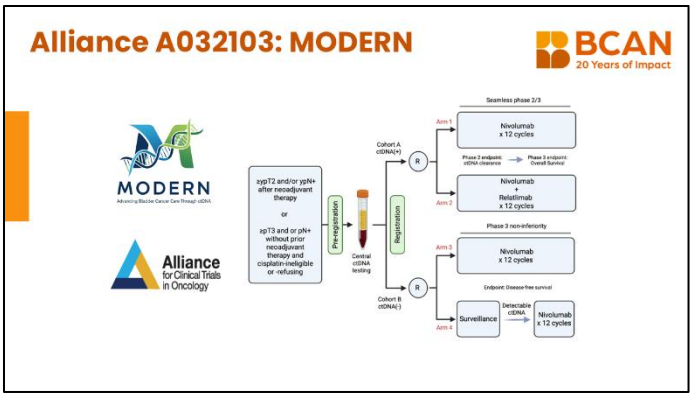


Another interesting finding from the study, or I should say important finding rather, is that I mentioned that during that year there was ctDNA testing serially.

And what I'm showing you here is the likelihood that a cancer is detected on a scan during follow-up in the absence of the DNA test becoming detectable. So in other words, what's the likelihood that if the ctDNA test is continuously undetectable, what's the likelihood of seeing something on a scan before the test becomes detectable? And you can see here it's really quite low. It's not zero, but you could see about 5% individuals who have an undetectable ctDNA test during that first year had a recurrence diagnosed in the absence of the ctDNA test becoming detectable. So when it's negative and stays negative, that's a favorable prognostic indicator. I think what we don't know from this study, we know that if there's detectable ctDNA, that probably indicates that additional treatment should be considered. And that's what I had mentioned before in my overall conclusions before I went through the data.

But what I don't think this data necessarily tells us yet is if there's undetectable ctDNA after surgery, whether or not individuals, all individuals should be monitored or whether or not individuals should get immunotherapy right after surgery. And the reason that I say that is this study shows us that if treatment is initiated at the time that the DNA test converts to detectable in that subset of individuals for whom it does treatment versus a placebo is beneficial in that setting. But that's not necessarily the same question as whether or not immediate immunotherapy after surgery is better than giving immunotherapy later in the subset of individuals who have the test convert from undetectable to detectable. That's a nuance, but it's an important nuance in terms of clinical decision making.

Dr. Matthew Galsky:



So, based on all of that, based on what we know about ctDNA testing from those clinical trial data sets that I've showed you so far within the US Cooperative Group system, these are clinical trials that are supported by the National Cancer Institute, and this is through the Alliance Cooperative Group within the United States, we're doing a study called MODERN, and MODERN is enrolling individuals after they've had cystectomy.

And based on the findings under the microscope, if there's felt to be findings that place individuals at risk for the development of recurrence, then patients undergo ctDNA testing just like I showed you in that last clinical trial. But what happens after we get that result is slightly different in this study. In this study, if there's detectable ctDNA within the blood, then individuals are randomized to receive standard immunotherapy with nivolumab. That's FDA approved treatment versus a combination of two immunotherapies really asking the question, should we be giving two immunotherapies versus one immunotherapy in individuals with detectable ctDNA after surgery? If there's undetectable ctDNA, then we're asking whether or not it, it's just as good to monitor individuals and treat with immunotherapy if the ctDNA test converts from undetectable to detectable versus treating everyone with immunotherapy immediately after surgery. So those are the two questions that are being addressed.

A different question, if there's detectable ctDNA after surgery, what's sometimes referred to as a treatment escalation question. And then in individuals with undetectable ctDNA after surgery, we're asking a de-escalation question, is it okay to monitor individuals and only treat in the setting where the DNA blood test becomes detectable.