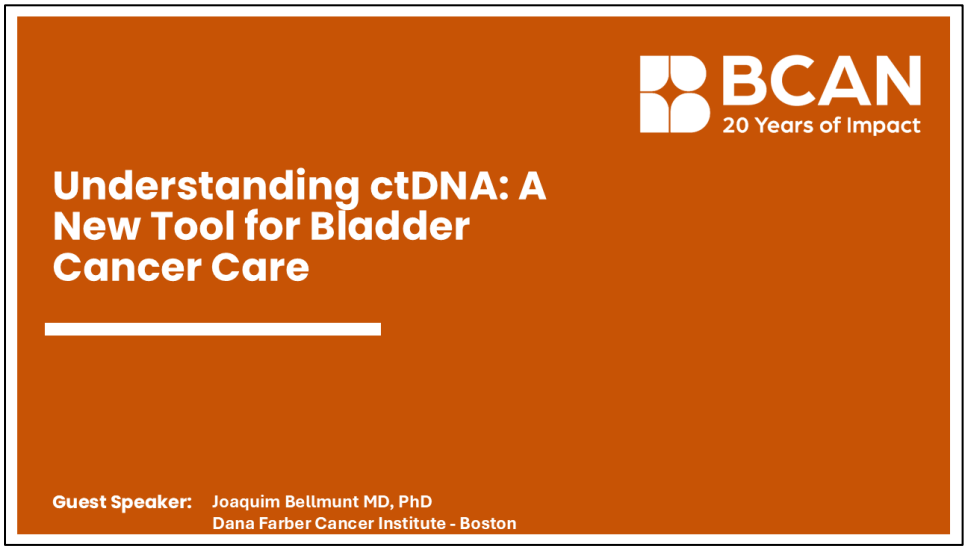
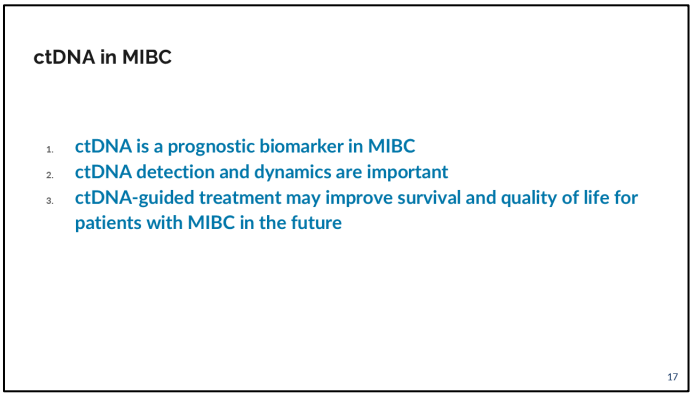


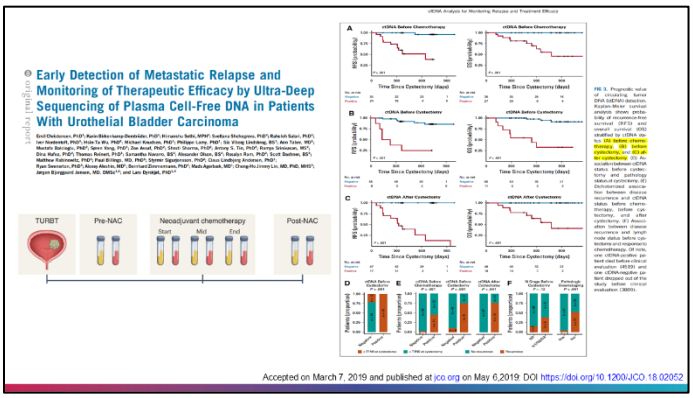
Dr. Joaquim Bellmunt:



So let's focus on the area where the ctDNA has been expanded and we have done a lot of work on the that. That is the muscle-invasive bladder cancer. Just to refresh your memory, muscle invasive is when the tumor is invading the muscle of the bladder, it requires neoadjuvant chemotherapy or neoadjuvant chemotherapy nowadays, followed by surgery. You need to remove the bladder or sometimes we can use radiation therapy. And then after that, patients need to receive complementary adjuvant treatment after the surgery. So that's the setting of muscle invasive.

And the use of ctDNA has shown to be a prognostic biomarker and it helps us to understand the dynamics of how the treatment evolves and then obviously can be used, as, as Stephanie was mentioning, to guide treatment to improve, survival quality of life, or maybe to select patients that don't need to receive additional therapy. So this is a still not definitive, but we are working on trials just to make sure that all these endpoints and are fully established in the management of muscle-invasive bladder cancer.

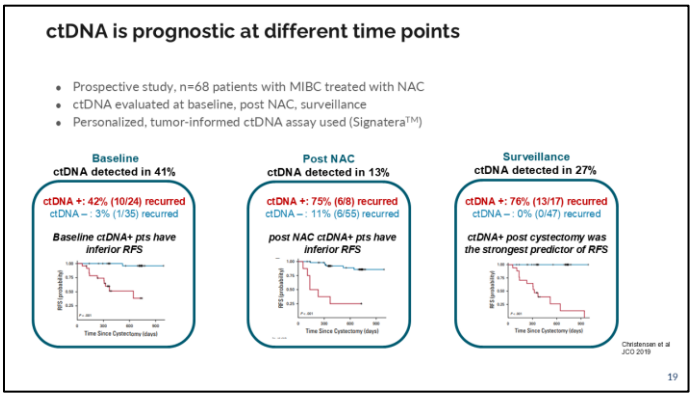
Dr. Joaquim Bellmunt:



So here is the first paper and now I'm going to focus my attention on bladder cancer specifically. So this is the first paper that did show that using ctDNA, and this was using this Natera Signatera platform, is helpful to predict response to neoadjuvant chemotherapy, outcomes after surgery, and then monitoring the patients after the resection of the, of the, of the bladder tumor. So you can see here is that in this perspective follow up of patients, so authors and this is from Denmark, they did collect plasma before the patients are starting receiving neoadjuvant chemotherapy. Some plasma samples were collected in during the treatment when they were receiving neoadjuvant chemotherapy. Patient had surgery here and then these patients were followed with a plasma collection.

And just to clarify, the plasma, so if you centrifuge the blood, you are going to find the red blood cells in the bottom, you are going to find the white blood cells in the middle. And then the plasma is what is the the supernatant, and this is where we can extract the, the, ctDNA. So this is what you see here in these tubes like there, there are two different, two different, sorry, two different, two different layers. So yeah, so this report, this is a JCO, 2019, so the this was with chemotherapy.

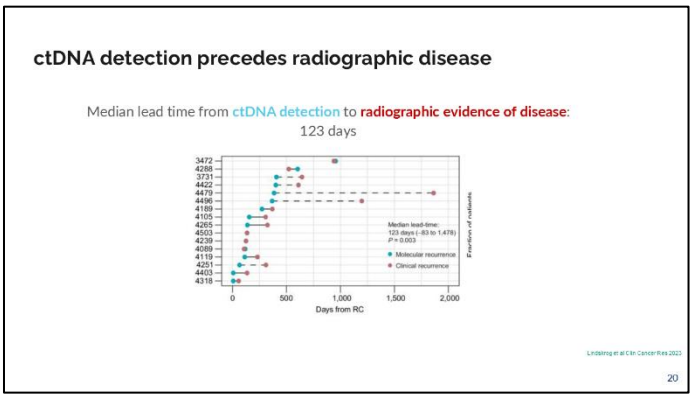
Dr. Joaquim Bellmunt:



And what this trial did show, they demonstrated that if you have ctDNA positive at baseline, the likelihood of have recurrent disease was higher, 43% versus 3% in patients being negative. While the patients were receiving neoadjuvant chemotherapy, if the ctDNA was positive, 75% of patients did recur despite receiving neoadjuvant chemotherapy.

And the same for surveillance. So if the, if the, ctDNA was positive, and this was seen in 27% of patients, the, the, likelihood of recurrence having positive ctDNA was 76% versus 0% in patients having ctDNA negative. So no, no, no minimal residual disease found in the blood, no circulating tumor DNA. So as mentioned, this is the first like report published in Journal of Clinical Oncology in 2019 telling us that the ctDNA is a useful tool just to predict what's going to happen in terms of outcome.

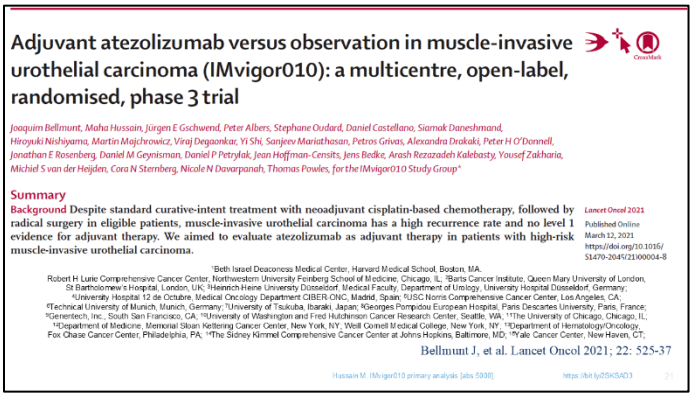
Dr. Joaquim Bellmunt:



And just to say, well, what's happening, what's the timeframe where you see a ctDNA positive before you see clinically evident disease in the, in the scans? So in this study, in this cohort, it was so four months before the CT scans or the MRIs became positive, ctDNA were positive before and having normal imaging. So the ctDNA positivity predates or predicts what's trying to be seen in radiographs or in imaging at least at the median time of four months or 123 days.

So with that still is not yet there. Maybe early implementation of therapy or likely this might might mean a lot in terms of improving subsequent survival or, ortherapeutic benefits. We are now exploring all these areas. Still having a ctDNA positive doesn't mean, oh yeah, you need to change therapy. Maybe in the adjuvant setting we're going to discuss that maybe it's applicable there and we have trials ongoing. But in general in other settings, you all the time want to get like proven evidence of the disease is there. And now we are using PET/CTs that are much more sensitive, meaning that this timeframe in between positivity for ctDNA and positivity for imaging is going to be shortened.

Dr. Joaquim Bellmunt:

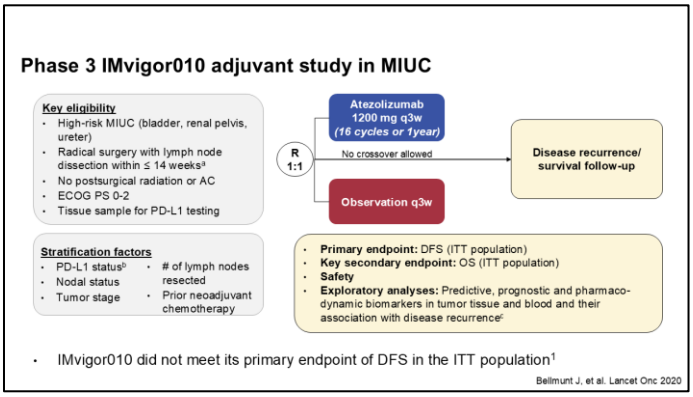


So moving specifically for how the ctDNA in immunotherapy through the patients in this muscle invasive came up. So here you have this, this manuscript. This was published in Lancet Oncology. And now you know that there are two drugs that are approved for treatment of muscle-invasive bladder cancer, high risk muscle-invasive bladder cancer after surgery, meaning in the adjuvant setting. We have Nivolumab FDA-approved based on disease-free survival benefit. Also, Pembrolizumab.

There were, and those was based on these two trials that came back positives, the AMBASSADOR and the Checkmate 274. And there was a third trial that people has forgotten. It was lead by myself. But this trial using Atezolizumab in the same setting, meaning in the adjuvant setting versus placebo. This trial was negative. So yeah, there might be reasons, maybe it's not the drug itself that is not working.

Dr. Joaquim Bellmunt:

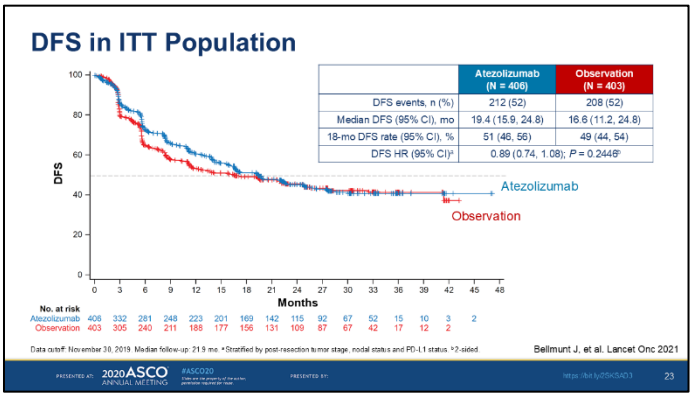
And here you can see the design of this trial. So patients with muscle invasive after having received neoadjuvant chemotherapy having had surgery, if there, there was a still disease, they were randomized at that time to receive immunotherapy versus observation because there was no standard at this time point.



So still these three trials were running together, and the main point was disease-free survival as you can see here.

Dr. Joaquim Bellmunt:

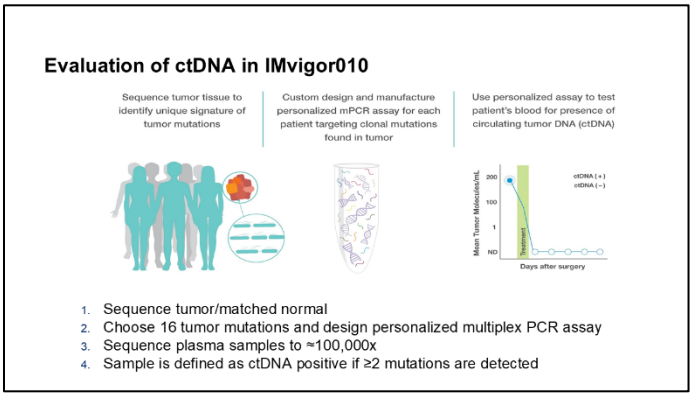
And as mentioned unfortunately there was no benefit although there was some initial benefit in here patients receiving Atezolizumab, but in the end this was not statistically significant. So this trial was negative. But all the times when we have a trial that is negative, we, you can do a deep dive and try to find if there is something that we can obtain from this trial?



So from this trial we prospectively collect plasma samples...

Dr. Joaquim Bellmunt:

Before starting treatment with adjuvant Atezolizumab and during treatment. And we were in retrospect with... Tom Powles was the lead on IMvigor. We start to analyze the ctDNA in those patients receiving Atezolizumab and also in the patients that didn't receive Atezolizumab in the, in the observation arm. So here's a summary of how this, this, platform is working. So this is a personalized platform, it's a bespoke assay. So these need to be done specifically for each one of the tumors for each one of the patients.

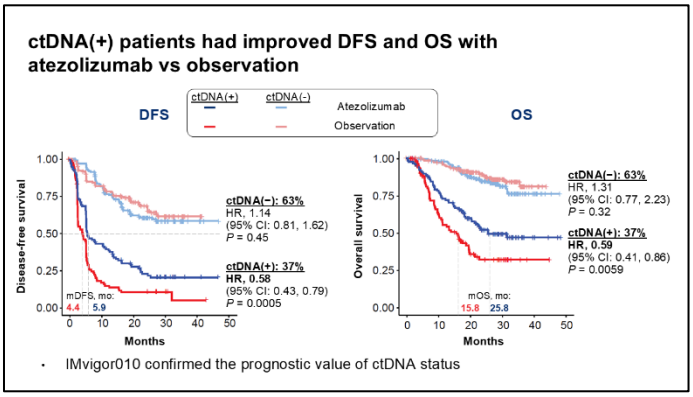


And the way that it's working is that in the very beginning we get the tumor from the cystectomy and we sequence the tumor and also we sequence the matched normal. And what we do is we merge this data in order to identify which mutations are completely different and not found in the normal tissue, in the germline tissue. And with that, a 16 tumor-specific mutation platform is made. So you need to do next-generation sequencing in the very beginning, sequencing the tumor, sequencing the normal. Likely this is much, much, much expensive, right.

But then once you have identified these 16 tumor-specific mutations, you design a personalized multiplex PCR assay that is being used sequentially to check the system mutations in the peripheral blood of the patient. So this is much more cheaper, it's simple, and with that you can follow the, the, ctDNA. And this ctDNA we are not going to know which specific mutations that the patient have, the aim here is to detect if there is minimal residual disease. And in the very beginning when this trial was designed, we considered ctDNA positive is if more than two of these 16 mutations that are tumor-specific, patient-specific were detected. Nowadays, there are different ways to measure. There is a more quantitative way. This platform now is being optimized just to look for more than 16, and also we can characterize the type of mutation. But this is the way that the initial Natera Signatera platform was used for this trial and also for the other one, the neoadjuvant chemotherapy trial.

Dr. Joaquim Bellmunt:

So, so what we found when we analyzed the samples of these patients that were randomized to receive immunotherapy versus observation, we saw that in this IMvigor010 trial we confirmed the prognostic value of ctDNA. So you can see here the two slopes here, those are the patients with ctDNA negative, they do much better whatever the treatment they receive.

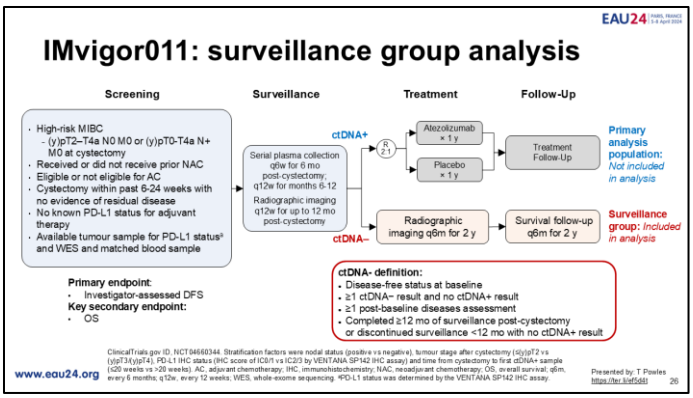


You can see here that the blue is atezolizumab, the red is observation. So here these patients having ctDNA negative, they do much better than the patients that had overall ctDNA positivity as shown here.

So confirming the value prognostic, the prognostic value of ctDNA in this setting. But the most interesting thing, and as mentioned the IMvigor010 was a negative trial, but when focusing our attention here in these patients being ctDNA positive, we saw that patients that receive Atezolizumab, that is the blue line, did much better. And you can see here with the p-value statistically significant compared to the patients that were on observation suggesting that maybe, maybe immunotherapy was really helpful in patients being ctDNA positive. And this was seen for disease-free survival and the same for overall survival.

So this data was finally published in Nature, one of the highly prestigious journals and because it was a change on the paradigm, but obviously this was retrospective, this was in this IMvigor010 trial and we were willing to confirm that this was real in a prospective trial.

Dr. Joaquim Bellmunt:



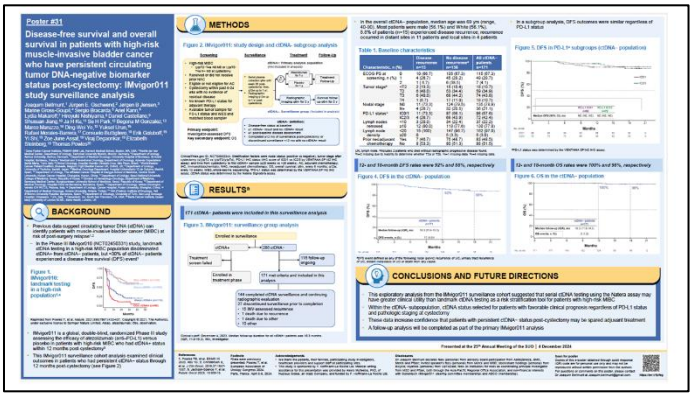
And this is why we designed the subsequent trial, the IMvigor011. So the IMvigor011 was trying to test if in patients being ctDNA positive, randomized... And as mentioned this patient is the same, patient receive chemotherapy, surgical removal of the tumor, there is nothing on imaging so they are considered to be like free of disease, and then you check the blood and say, "Well, this patient that is free of disease after surgery, the ctDNA is positive," and these patients when the ctDNA was positive were randomized 2:1 to receive Atezolizumab or placebo.

This trial obviously now would not be ethically ethical because we have standard of care here, but at that time that when the trial was designed, so there was, this was the only way to demonstrate that ctDNA positive patients were benefiting from Atezolizumab. This trial completely accrual. We're going to see the results of this that is the main part of the trial in the next coming meeting this year. So this is going to be very nice to see if the ctDNA is identifying patients that benefit from receiving immunotherapy versus placebo.

But obviously in this trial we need to screen patients for ctDNA. And then obviously there were. There were a cohort of patients that the ctDNA was negative and we decided just to follow these patients followed for with radiographic imaging as a standard of care and surveillance without no treatment. So this group is what we define as ctDNA negative patients, meaning patients that after surgery there is no evidence of ctDNA positivity in the blood. So those patients are patients that are disease-free, negative ctDNA. And we monitor these patients. We have been monitoring, as mentioned. This is in a clinical trial. The trial is focusing on here, but those are the patients not included in the trial. And we have We did monitor these patients for more than a year.

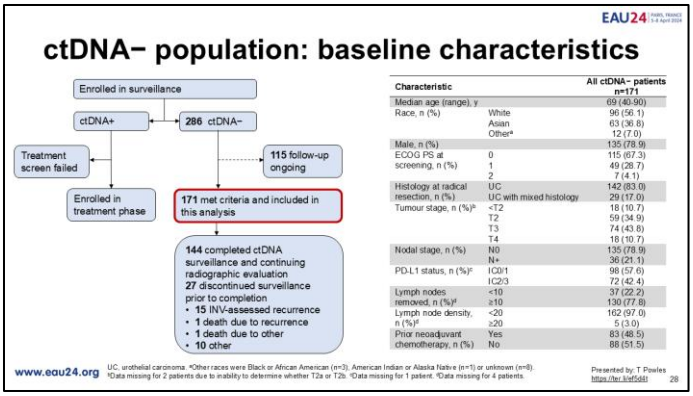
Dr. Joaquim Bellmunt:

And the results, those were presented at AUA. I present this data at the SUO last year.



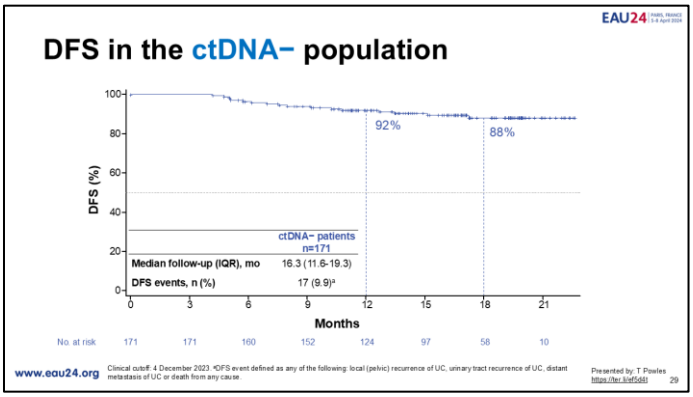
Dr. Joaquim Bellmunt:

So let's summarize the findings. We are talking on these patients that are high-risk patients that the surgery has gone well, everything is being removed, imaging is negative, but and they have the ctDNA is negative. So we identify 171 patients meeting this criteria having follow-up for at least one year monitoring every six, eight weeks ctDNA. So continuously being negative, negative, negative, negative. It's not a single time point that is the one that we obtain in the IMvigor010.



Dr. Joaquim Bellmunt:

So what we can see here in this population of patients with negative ctDNA, the disease free survival is at a year and a half 88%, meaning still there are patients that recur. This is what I'm saying that this test is not 100% sensitive or, or, specific. Still 12% of these patients recur. But in patients that have received surgery, so in addition to getting scans, this is quite reassuring. If the ctDNA is negative and as far as the ctDNA is negative for a year after sequential analyzing the ctDNA, the likelihood of these patients to recur is like say 12%. So it's quite reassuring.

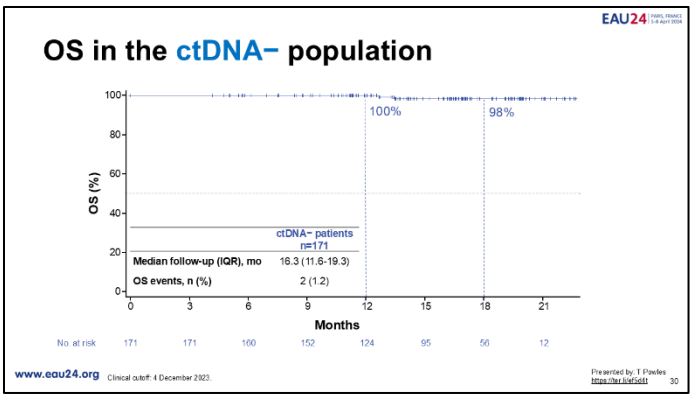


Presently I'm using that for patients that even that they have received adjuvant chemotherapy or immunotherapy and it's an additional way to the imaging to say, "The

ctDNA is negative after a year," so meaning the good news is that the likelihood of recurrence is really, really low. And I'm this patient has mentioned in the trial, those were high-risk patients.

Dr. Joaquim Bellmunt:

So the same for survival. Survival still is short so we cannot say that, but for disease-free survival, I think it's very important to say,



Dr. Joaquim Bellmunt:

"well, these patients that, that have had serial ctDNA testing, they have, this really having greatest utility than having only a landmark ctDNA testing." So serial monitoring is to predict with patients after a year I my, my have chances to recur and these chances based on this trial is 12%.