



## Understanding ctDNA: A New Tool for Bladder Cancer Care

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Guest Speaker: Joaquim Bellmunt MD, PhD  
Dana Farber Cancer Institute - Boston

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### Stephanie Chisolm:

So today's topic, as I mentioned, is understanding ctDNA. It's a new tool for bladder cancer care and why is it so important? You know it's a simple blood test that looks for traces of DNA in your bloodstream and now we're going to hear about why it's becoming really important for patients with bladder cancer. Dr. Bellmunt has been a friend of BCAN for many, many years. And I don't know that this was even on your radar 20 years ago when BCAN got started, was it, Dr. Bellmunt?

### Dr. Joaquim Bellmunt:

Yeah, no, no, absolutely. We're discussing, I joined the first meetings of BCAN long ago when, when we didn't have any white hair yet. So here we are.

### Stephanie Chisolm:

Right. Yeah, which is just amazing. So much has happened in the last 20 years. So, this just can help detect cancer earlier, it can show whether your cancer treatment is working, maybe even spot signs of recurrence. For patients with muscle invasive or advanced bladder cancer knowing your ctDNA can really help to help them decide on more and specific treatments options for them and more personalized treatment, and maybe a greater peace of mind because you know you're doing everything you need to do for your particular tumor type. So we are really delighted to have Dr. Joaquim Bellmunt here. He's an associate professor at Harvard Medical School and director of the Bladder Cancer Center at the Dana-Farber Cancer Institute in Boston. Dr. Bellmunt will explain how ctDNA works and why staying informed about this new tool can really make a difference in your care. Professor Bellmunt is

also a senior researcher at the Instituto Hospital del Mar de Investigaciones. I'm not going to say this, can you say it, Dr. Bellmunt?

**Dr. Joaquim Bellmunt:**

So this is from Yeah, I still have my lab, all lab in Barcelona at IMIM Hospital del Mar, yeah.

**Stephanie Chisolm:**

Great. So you really do pop back and forth and are very engaged in global bladder cancer care. As a genitourinary medical oncologist, you practice as a principal investigator in so many clinical trials and we really appreciate your time and sharing your expertise. I'm going to turn the screen over to you. If you want to share your slides now, you're welcome to do that.

**Dr. Joaquim Bellmunt:**

Yeah.

**Stephanie Chisolm:**

And I'm going to turn off my camera and I'll be in the background and we'll get to questions and answers at the end of today's program.


**Dr. Joaquim Bellmunt:**

All right, excellent. Thanks Stephanie for the kind introduction and as mentioned, it's a pleasure to be here on these 20 years of impact of BCAN. As mentioned, I, I joined the first the first think tank meetings like almost yeah 15, 17 years ago and I have been involved in collaborating, so that's a great, great effort and I want to thank Stephanie, Allison, Patricia for making this lecture to happen despite I cancel because of some unexpected commitments.

So yeah so I'm going to explain you a bit what's the present status of the ctDNA as a new tool for bladder cancer care.

**Dr. Joaquim Bellmunt:**

So, some of some of the things that I'm going to explain that are listed here, likely some are not fully accomplished, so but we are trying just to learn a bit what ctDNA is and how it can help find cancer. That's one aim. Also, how it can help doctors choose or change treatment. Still we are not there, but we are getting there. How it may show if cancer comes back before even the scans can show that, and how it helps patient feel more in control of their care. So those are the main aims. As mentioned, we are heading there. So we still are not fully accomplished all these aims.



**You'll Learn:**

- What ctDNA is and how it helps find cancer**
- How it can help doctors choose or change treatment**
- How it may show if cancer comes back—before scans do**
- How it helps patients feel more in control of their care**

**Dr. Joaquim Bellmunt:**

So first I'm going to introduce several terms just to make clear what we're talking about. So the first term is the liquid biopsy. Liquid biopsy is a biopsy, but instead of getting tissue, we are getting blood and we are trying to analyze the blood if there are cells there. So on the first documented evidence of a solid tumor material in peripheral blood is coming from this

**New technology is unlocking the century-old promise of the 'liquid' biopsy**

First documented evidence of solid tumor material in peripheral blood

Thomas Ashworth  
Pathologist  
1869  
Melbourne, Australia

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paper that is 1869 in Australia where this was a patient that died and then in the blood they were able to capture cancer cells. So this has the first documented like paper or, or, or experiments just showing that the tumor is like spreading and circulating in the blood.

**Dr. Joaquim Bellmunt:**

And then going back again to the term liquid biopsy. Liquid biopsy is a wide term. And in the liquid biopsy, meaning doing a biopsy in the blood, we can find different components. And you can see here in this cartoon in the right side you can look for ctDNA or cell-free DNA, and we will talk about what's the difference between cell-free, it's cfDNA, or ctDNA.

**Cell-free DNA is one component of liquid biopsy**

- Material in body fluids can serve as practical, minimally-invasive biomarkers
- Cell-free DNA in blood plasma is an established analyte that does not require specialized purification protocols
- Cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) is distinct from circulating tumor cells (CTC)

Catherine Aïx-Panabières and Klaus Pantel, Cancer Discovery 2021

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That's very important is that I have underlined the T and the F. But you, you can look for circulating tumor cells, you can look for extra vesicles, proteins. So so many things under the concept of liquid biopsy. So what we're going to talk about is the cell-free DNA in the blood plasma and this is a very easy to obtain like a research and there we can capture the cell-free DNA and the cell tumor DNA, the ctDNA.

So three concepts here, cell-free DNA, circulating tumor DNA, and this is completely distinct from the what we have heard on circulating tumor DNAs. And I, I could say that cell-free DNA is like whatever DNA is floating around is named cfDNA and the one that is tumor specific, and we have tools to identify that these DNA, because of mutations and copy numbers is a tumor DNA, and then we have these CTCs.

### Dr. Joaquim Bellmunt:

So we are going to focus on the concept of cell-free DNA. Cell-free DNA, that's the first thing meaning is DNA that is found in the blood and this mainly coming when the cells die, like they are going through like what we call apoptosis. The DNA inside the cells are shed into the blood. And we know that the the normal cells die too. So when the majority of the cell-free DNA that is found in the blood is coming from normal hematologic precursors, and usually we see fragments of a 167 base pair.

**Properties of cell-free DNA (cfDNA)**

- Apoptosing cells shed cfDNA into blood
  - Most normal cfDNA is from the blood lineage
- Median fragment length is 167bp (the unit of the nucleosome)

Plasma and cells free DNA  
White blood cells  
Red blood cells  
Cell-free DNA

Van der Pol and Mousiani. Cancer Cell, 2019; Murtagh and Calkins, Nat Genet, 2016; 2022 Turmalkian Saldiver (NFT)

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### Dr. Joaquim Bellmunt:

So very, very, very tiny fragments. And the amount of DNA that is found, of cell-free DNA that is found in the blood is between 5, 10 nanograms per mL of plasma, meaning that is very, very tiny amounts that we are now having technologies that are able to capture this DNA that is floating, isolated this specific CT, circulating tumor, DNA and obtain results and provide guidance.

**Properties of cell-free DNA (cfDNA)**

- Apoptosing cells shed cfDNA into blood
  - Most normal cfDNA is from the blood lineage
- Median fragment length is 167bp (the unit of the nucleosome)
- Average person will have 5 – 10 ng DNA in 1mL of plasma
  - ~1500 diploid genome copies

*Collection protocols are important: control pre-analytical variables, prevent further lysis of white blood cells*

Phallen et al., Science Translational Medicine 2017

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### Dr. Joaquim Bellmunt:

So another thing is that there are the ctDNA, circulating tumor DNA, there are different tests and now are commercially available. And obviously we all the time that we're talking about ctDNA, the first thing that we need to ask is which tests are you going to use to monitor my ctDNA? And there are tests that are mainly focusing on detecting tumor, detecting cancer, but there are other ones that are in fact going not further, in a different way. They are characterizing which type of cancer cells are there.

**One size does not fit all when selecting a ctDNA test**

- ctDNA tests have emerging impact across oncology
- Test design varies by clinical goal: **DETECT** or **CHARACTERIZE** cancer
- Sensitivity and specificity remain sub-optimal in some contexts
- New **epigenomic technology** is boosting sensitivity of detection tests and permitting cfDNA lineage phenotyping

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So this is completely two different concepts. And obviously the techniques are improving and we know all the companies that are investigating on that, they are increasing on sensitivity and specificity, but as still of now still we are a bit suboptimal. So those are not 100% sensitive

and not 100% specific. There are new evolving techniques that I'm not going like to present at the epigenomic techniques that are emerging trying to like better characterize the cell ctDNA floating in our, our, our blood.

**Dr. Joaquim Bellmunt:**

So three, three, main groups of areas on how the CT, the circulating tumor, DNA test might help managing cancer. The first is that we can improve cancer screening. And we know this paper in the right in patients that these predisposing cancer syndrome Li-Fraumeni. Cell-free DNA was used to identify early appearance of cancer.

And also we are presently using for a concept that is named minimal residual disease. And minimal residual disease is a concept coming from hematological malignancies, leukemias that there were tests that we were able to detect like very tiny amounts of translocations for example in chronic myeloid leukemia. So this is also now being done in a, in a, solid tumors like bladder cancer. That's the first group.

**How can ctDNA tests help manage cancer?**

- Improve cancer screening
- Detect minimal residual disease
- Monitor for response to therapy
- Estimate cancer aggression
- Predict treatment efficacy
- Identify resistance mechanisms
- Characterize biology and evolution

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**Early Cancer Detection in Li-Fraumeni Syndrome with Cell-Free DNA**

**Multi-gene Methylation Assays for Tissue-specified Detection of Circulating Tumor DNA in Patients with Uterine Cervix Cancer**

**Dr. Joaquim Bellmunt:**

The second group, we can use ctDNA as a way to monitor the response to therapy. As you can see in the paper in the right, plasma ctDNA as a treatment response biomarker in metastatic cancers, a correlation with the resist criteria, the classical radiological criteria, and also we can estimate how aggressive is a cancer, a tumor. So in the right you can see here this paper for Nature Communications 2021 in unselected first-line metastatic urothelial cancers, patient having low ctDNA, all these patients were doing much better than patients having high ctDNA.

**How can ctDNA tests help manage cancer?**

- Improve cancer screening
- Detect minimal residual disease
- Monitor for response to therapy
- Estimate cancer aggression
- Predict treatment efficacy
- Identify resistance mechanisms
- Characterize biology and evolution

**Plasma ctDNA as a treatment response biomarker in metastatic cancers: evaluation by the RECIST working group?**

**ctDNA % is prognostic**

**Unselected first line mUC (n = 71)**

Vandekerckhove G et al., Nat Commun 2021

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**Dr. Joaquim Bellmunt:**

The third group of concepts where we can use the ctDNA is predicting treatment efficacy, also identifying resistant mechanisms. And now this is being used for example in tumors like lung cancer that we have drugs that are under treatment, the tumors change their phase and they create new mutations. And through monitoring the blood we are able to say, "This treatment is no longer responding." Then we capture the changes that the tumor has developed and then we implement a new therapy for this resistance. So we can characterize the biology and the evolution.

**How can ctDNA tests help manage cancer?**

**LOCAL**

- Improve cancer screening
- Detect minimal residual disease
- Monitor for response to therapy
- Estimate cancer aggression

**METASTATIC**

- Predict treatment efficacy
- Identify resistance mechanisms
- Characterize biology and evolution

ERBB2 (HER2) amplification

FGFR3 gatekeeper mutations post-FGFRi

Temporal sub-clonal shifts

Vandekerkhove et al., Nat Commun 2021; Clinton et al., Cell Rep 2023; Herberits et al., Nature 2022

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So a lot of areas where CTNA is helping on making decisions, assessing the, the prognosis, and establishing the best treatment for patients.

**Dr. Joaquim Bellmunt:**

As mentioned, the CTNA test is not that one size fits all and we mentioned that. So there are tests that are going to help us to detect cancer and it's not going to provide in general any additional information, say, well, "You have malignant cells in your blood." Or there are others that are like characterizing more specifically the type of mutations or the type of genomic alterations that this tumor is having.

**ctDNA test choice: one size does NOT fit all**

**DETECT**

- Smoke detector
- Must be highly sensitive
- Does not inform on fire properties
- Human-designed machines

**CHARACTERIZE**

- Fire Investigation
- Informs cause & properties
- Understand how to extinguish
- Requires human expertise

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**Dr. Joaquim Bellmunt:**

So focusing on detecting and detecting is a way to say well, I want to make sure that this, this, tumor is in complete remission and you want to make sure that there is no minimal residual disease. For that, there are two different type of, of platforms. One is what is called a tumor-informed. This is a customized, customized, is a bespoke platform where the we create a personal... We create, the company who's doing that is creating a personal test to follow this

**DETECT ctDNA: minimal residual disease**

**TUMOR-INFORMED**

- Uses information about the individual cancer from tissue
- Pros: specific and established
- Cons: time and cost

**TUMOR-NAÏVE**

- Relies on *de novo* detection of ctDNA features (e.g. mutations)
- Pros: quick; can also screen
- Cons: lower specificity

Personalized test developed

Same test for all patients

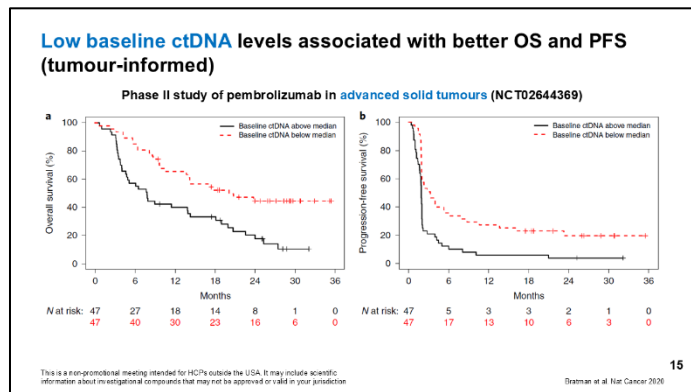
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patient. That's an example of the Natera Signatera platform. So this is specific test. Obviously you need to, and we're going to discuss more about this platform that is widely used in bladder cancer.

And then there are different platforms that are tumor-naïve, so meaning those are not platforms that are personalized. So this is like we know that in bladder cancer there are specific genomic alterations that are well-known based on the TCGA work and so on. And we create platforms to look for specific mutations or genomic alterations that we know are highly prevalent in bladder cancer. And this is using the same test for all the patients. It's not, that's different that tumor-naïve to the tumor-informed where we are using a very highly personalized approach to monitor the minimal residual disease. Now, as mentioned, minimal residual disease, very important we're going to discuss its role for example in the, in the, follow-up after surgery in patients with muscle-invasive bladder cancer.

**Dr. Joaquim Bellmunt:**

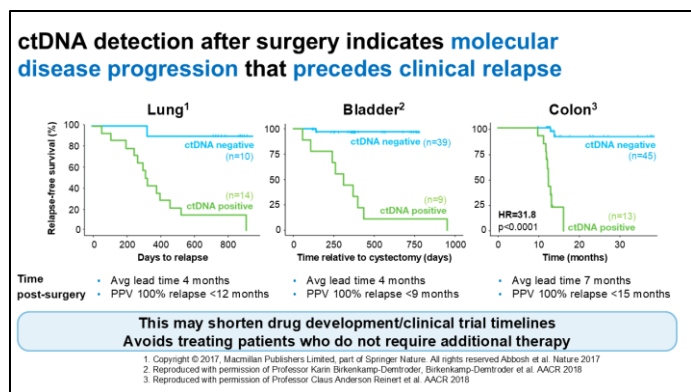
So, let's let's go back a bit. When several emerging studies, these show the benefit of using the ctDNA. So you can see here ctDNA detection after surgery indicating the what we call the molecular, this is progression. And this is seen before you see a clinical relapse meaning until the radiographic images are coming positive. And these are publications from 2017, 2018. We can



see here that in lung patients that the ctDNA was negative, no no cells found after surgery, the relapse-free survival was higher than patients having ctDNA positives meaning DNA material from the tumor floating in the blood. The same for bladder, the same for colorectal cancer. So this is not only applicable to bladder, but as you can see to so many different tumor types.

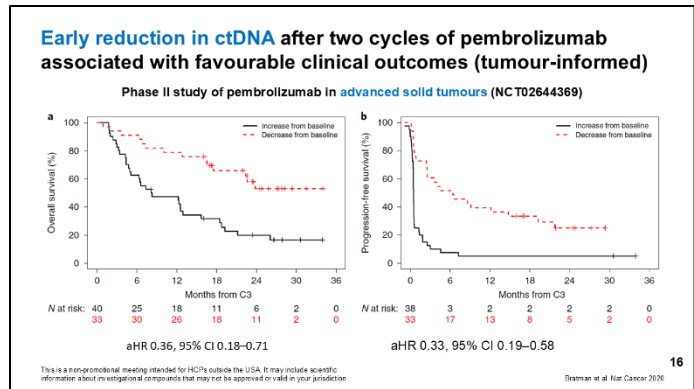
**Dr. Joaquim Bellmunt:**

Also we mentioned that before, and this coming from a phase two study of Pembrolizumab in advanced solid tumors, it was shown that low baseline ctDNA levels are associated with better survival and progression free survival is a way to monitor the tumor burden. So if you have less cells in your blood, your outcome is going to be much better.



**Dr. Joaquim Bellmunt:**

And obviously if this is like  $\alpha$ , like a decrease by the treatment, and this is something that we see in here, this is the same study in these initial studies. As mentioned this was published in 2020. We can see that patients receiving Pembrolizumab. If there was a reduction of the amount of circulating tumor DNA in the blood, those patients were doing much better. So it's a way to predict monitor if the patient is responding well or not responding well under treatment. In this case it's for immunotherapy.



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