“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Brian McCloskey and Brad Power
August 24, 2022

“You need more than just one signal - a lot more.” Peter Kuhn

“15 years ago, the model systems were mice and cell lines. With liquid biopsies, we have been able to start using humans as our model system.” Peter Kuhn

Meeting Summary

Peter Kuhn, PhD, Dean’s Professor of Biological Sciences and Professor of Medicine, Biomedical Engineering, Aerospace & Mechanical Engineering, and Urology, University of Southern California; Founder and Chief Scientific Advisor, Epic Sciences; and Stephanie Shishido, PhD, Director of Clinical Research, CSI-Cancer, University of Southern California, led a discussion on the cutting edge of liquid biopsies and their ability to inform patients about their disease (“Do I have cancer?”), predict progress (“How bad is the diagnosis?”), guide treatment decisions, (“Active surveillance vs. prostatectomy?”, “Will my treatment work?”), and monitor progress (“Is my treatment working?). Liquid biopsies hold out the promise of less invasive, lower cost, and more frequent capture of data about cancer and its environment, which could revolutionize treatment.

- ***How does liquid biopsy technology work?***

  Liquid biopsies refer to collection of bodily fluids for analysis, usually blood draws, but can be of any fluid, including bone marrow aspirates. The analysis can look for “circulating tumor cells” (CTCs), which are cancer cells that split away from the primary tumor and enter the circulatory system (metastasis); or “cell-free DNA”, fragments of DNA released into the bloodstream when cells die. The DNA signature of healthy cells is very different from cancer cells, called “circulating tumor DNA” (ctDNA).

- ***What can patients learn from a liquid biopsy, especially prostate cancer patients who are already getting blood draws to monitor their PSA biomarker?***

  We tend to focus on the tumor cells, but there’s a lot of information in the environment around the tumor cells we can get from liquid biopsies that can help us understand the tumor and its dynamics. For example, a blood test can tell if a patient has an androgen receptor variant (AR-V7), indicating that he will not respond to androgen-targeted therapies (such as abiraterone, enzalutamide, or apalutamide).

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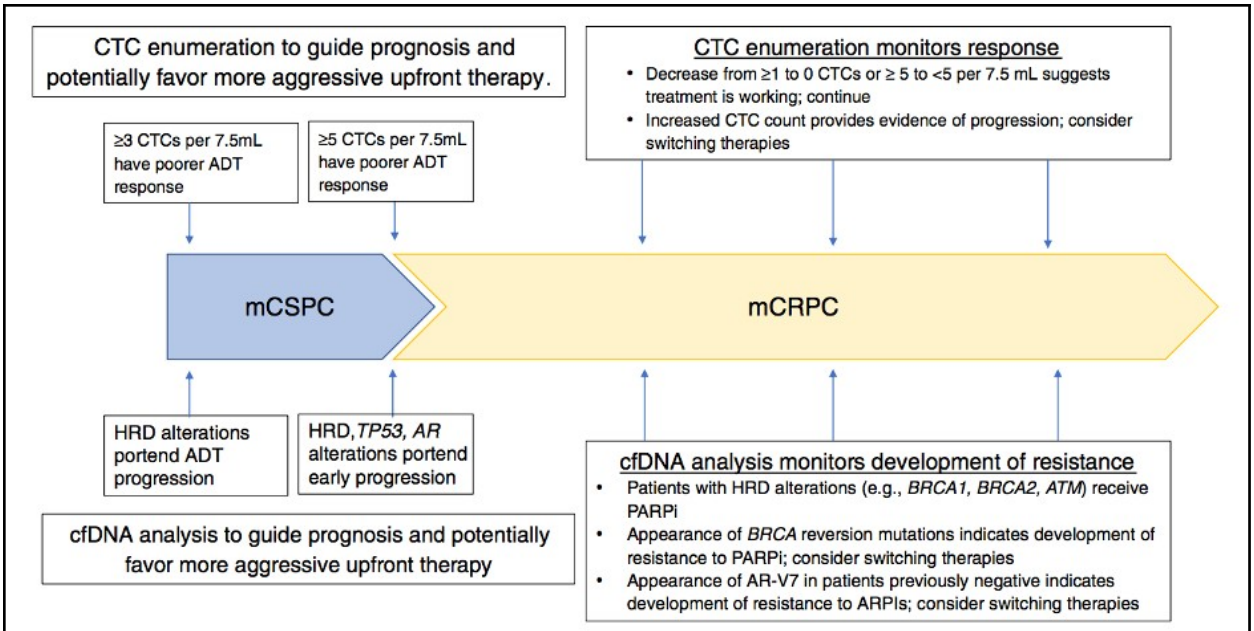

Fig. 1 Proposed model for incorporation of liquid biopsies into clinical management of metastatic prostate cancer

From UroToday, August 18, 2022, “Integration of Liquid Biopsies in Clinical Management of Metastatic Prostate Cancer - Beyond the Abstract”, Written by: Varsha Tulpule, Gareth J. Morrison, Mary Falcone, David I. Quinn, Amir Goldkorn; Division of Medical Oncology, Department of Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.

- ***How close is the promise of this new technology to widespread access?***

  This technology is in an early research phase that seems like “The Wild West”. We don't have clarity on which types of these liquid biopsies are relevant for which types of tumors at which stage in their progression. It’s hard to find practitioners who are nimble and are willing to do serial liquid biopsies so we can be addressing genetic changes along the way. It's often hard to get these and other tests done, and they can be expensive.

- ***What's next?***

  In prostate cancer and other cancers, like breast cancer, patients have lots of treatment choices. We need to maximize the amount of information we can extract from liquid biopsies to make decisions that will yield better patient outcomes. Over the past three years there has been progress in analyzing liquid biopsies, from looking only at circulating tumor cells to understanding other cancer dynamics. There is research in combining multiple tests, e.g., cell-free DNA analysis, circulating tumor cells analysis, and single cell genomics analysis of the circulating tumor cells, enabling better targeting of cancer treatments. And new biomarkers are being researched in liquids which can predict disease progression and target cancer cells, such as a replacement for the PSMA PET test.

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doctor about your specific situation before pursuing any health care program, treatment, product or other course of action that might affect your health.

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Meeting Notes

SUMMARY KEYWORDS

liquid biopsy, cancer, cell free dna, patient, cells, ctc, test, disease, super, blood, question, slides, imaging, wild west, androgen, waldo, rick, research, bit

SPEAKERS

Mike Yancey, Robert Ellis, Saed Sayad, John Laird, Jeff Waldron, Peter Kuhn, Rick Stanton.

Robert Ellis 00:04

I'm patient number five in the Prostate Cancer Lab. Our guests are Peter Kuhn and Stephanie Shishido. We're going to be talking about liquid biopsies, which I am very much interested in. Liquid biopsies enable less invasive, more frequent, and lower cost data about disease, which could revolutionize treatment. We're going to be talking about the cutting edge of liquid biopsies in their ability to inform patients about their disease, predict progress and guide treatment decisions. Peter Kuhn, PhD is founder and Chief Scientific Adviser at Epic Sciences, which develops liquid biopsies blood tests to inform cancer patients on their best course of therapy. Dr. Kuhn is the Dean's Professor of Biological Sciences, Professor of Medicine, Aerospace and Mechanical Engineering and Biomedical Engineering at the University of Southern California. He also serves as the Director of the USC Michelson Convergent Science Institute in cancer and the Deputy Director of the Convergent Science Virtual Cancer Center. Dr. Shishido is currently a postdoctoral researcher at the USC Michelson Center for Convergent Bioscience, focusing on the identification and characterization of circulating tumor cells from the liquid biopsy received from clinical trial cancer patients. Her research interests focus on the interactions between the neoplastic cell and the microenvironment

Epic's Comprehensive Cancer profiling approach uses multiple technologies to provide up to three types of analyses all based on a single blood draw. Their proven and proprietary circulating tumor cell capabilities along with circulating tumor DNA and immune cell analysis allow for a clearer and more efficient picture for both prostate and breast cancer. Their approach reveals both genotypic and phenotypic data and can offer the most actionable personalized liquid biopsy available, which is specifically of interest to us. The AR-V7 test identifies patients who are likely to be resistant to androgen-directed therapies such as abiraterone, enzalutamide, or apalutamide. The liquid biopsy test for AR-V7 is the first and only Medicare-reimbursed clinically validated predictive CTC liquid biopsy test for metastatic castrate-resistant prostate cancer.

Peter Kuhn 03:01

Stephanie has retired from her role as a postdoctoral fellow, and given the success of what she has done during that time is now Director of Clinical Research within CSI (Convergent Science Institute)-Cancer. She is responsible for a substantial portfolio of clinical research applications that range all the way into CAR-T therapies for prostate cancer. We won't talk about this today. That is for a for a future conversation and why it is that we are involved with what we think are the best groups in the world working on this and how we think that we can hopefully help and support them in their mission to enable CAR-T therapy in prostate cancer, which has been one of the challenges up until now. Today is really meant as a discussion and to provide information.

I want to be most helpful to you guys. I have slides because that's what an academic does. I have 30 slides that might be useful for this conversation, but I am just as happy, if not even happier, if this is simply a conversation driven by questions that you have when you hear the key words "liquid biopsy." Let me start by asking, "What is it that comes to mind?"

Rick Stanton 06:04

It was 2017. I was at Human Longevity. I heard about this company called Grail, and I thought, “This will never work”. It's like finding an attack submarine in deep water in the ocean, which is a job that I did. Then I was just amazed. I've told my wife who's just had breast cancer. She's under care at UCLA. She had a lumpectomy, no genomic sequencing. I have been listening to “The Drive” podcast by Peter Attia, and his interview with Max Diehn from Stanford regarding circulating tumor cells. It came to me that my wife should have her primary tumor sequenced so that someday, circulating tumor cells will be able to identify reoccurrence before imaging. I said, "You need to do this”, but it hasn't happened yet. I just had this conversation with her yesterday. I've been enthralled with Max Diehn's great podcast, and it's helping me understand the power of liquid biopsies. I'm under care at UCLA, and these things aren't even discussed. I'm also under the care of Dr. T at Providence Saint Johns, and I'm hopeful that he will leverage new technologies like liquid biopsies and spatial phenotyping.

Jeff Waldron 08:46

I've been working a little bit with a woman in France, Catherine Alix-Panabières. You may know her. One of the many interesting things is the distinction between ctDNA and CTCs, or cell-free DNA and ctRNA. I worked a little bit with Mass General. I'm in the Boston area. We worked a little bit with Torpedo Diagnostics, which had a CTC launch that then got rolled back into Mass General, but not for original detection of cancer, but looking for remission or recurrence of cancer, particularly liver cancer, so they're using CTCs to do that. It's interesting to see the way the applications are rolling out in some of these areas and to try to better understand the distinction between some of them. I'm not sure if that's in your scope for today.

John Wadude Laird 10:05

I'm a medical doctor and medical advocate. I'm not a treating physician. My role is really to identify testing and treatment resources. My questions are coming from that perspective. One, it seems like this is the Wild West. I don't have clarity on which types of these liquid biopsies are relevant for which types of tumors at which stage in their progression. The second concern I have is finding practitioners who are nimble and are willing to do serial liquid biopsy so we can be addressing genetic changes along the way. And thirdly, some of these tests are more robust. If you're getting live tumor tissue plus blood liquid biopsies. It's often hard to find a patient who can get all that done at the same time. And fourthly, the cost of these tests is out of bounds for most people, especially if they're done on a serial basis.

Peter Kuhn 11:30

This is great because each of you hinted at important aspects. Max Diehn is a super good friend and colleague. We are working together on an early lung cancer detection program. That's another super fun, super important conversation.

Jeff, I'm sorry you're stuck in Boston. I eventually escaped from the northeast. I did my tour at MGH with Mehmet Toner and Dan Haber, one of my absolute favorite competitors. Publicly, we

chew each other apart, but privately, we are good friends. I have a huge amount of respect for them.

Catherine Alix-Panabières is one of our counterparts in Europe. She has done lots of super interesting things, but I'm German and she's French, and those countries have always had a challenging relationship. :) It's a little bit more of the Wild West over there in France.

Part of the commentary around Wild West is also important when it comes to the context of what it is that they do at comprehensive cancer centers versus at some of the private clinics. I am a scientist, obviously, and I am all for research, but please be thoughtful around what is evidence-based, and have a true understanding of the risks that we are taking when we are going with early-stage technologies. It's important because that's a big part of this sea change in liquid biopsy that has really changed the face of cancer research. 15 years ago, it was all about model systems and mice and cell lines and all this stuff. With liquid biopsies, we have been able to start using the human as our model system. It doesn't get any better than doing discovery together with the patient with the tumor from the patient. That's exactly what cancer research needs to do. We have seen this huge change of really making real progress by doing basic discovery with the patient, then validating it, and then getting back out into clinical care with a validated product. This is complicated. It's going to feel like, "Oh my god, this is too expensive, or this will never work. Hey, wait a minute, somebody failed so they're pulling it back in.” Or companies say, “What's going on?"

Peter Kuhn 15:07

This is a tribute to Rick because there's something important that has happened as we worked our way through the last 20 years of research into liquid biopsy. If you saw a little postage stamp excerpt of this picture, you might think, "What is that, Peter? Is it a broken slide? There is no information there. This is noise. Is that something in the ocean?” Then if you zoom out, you

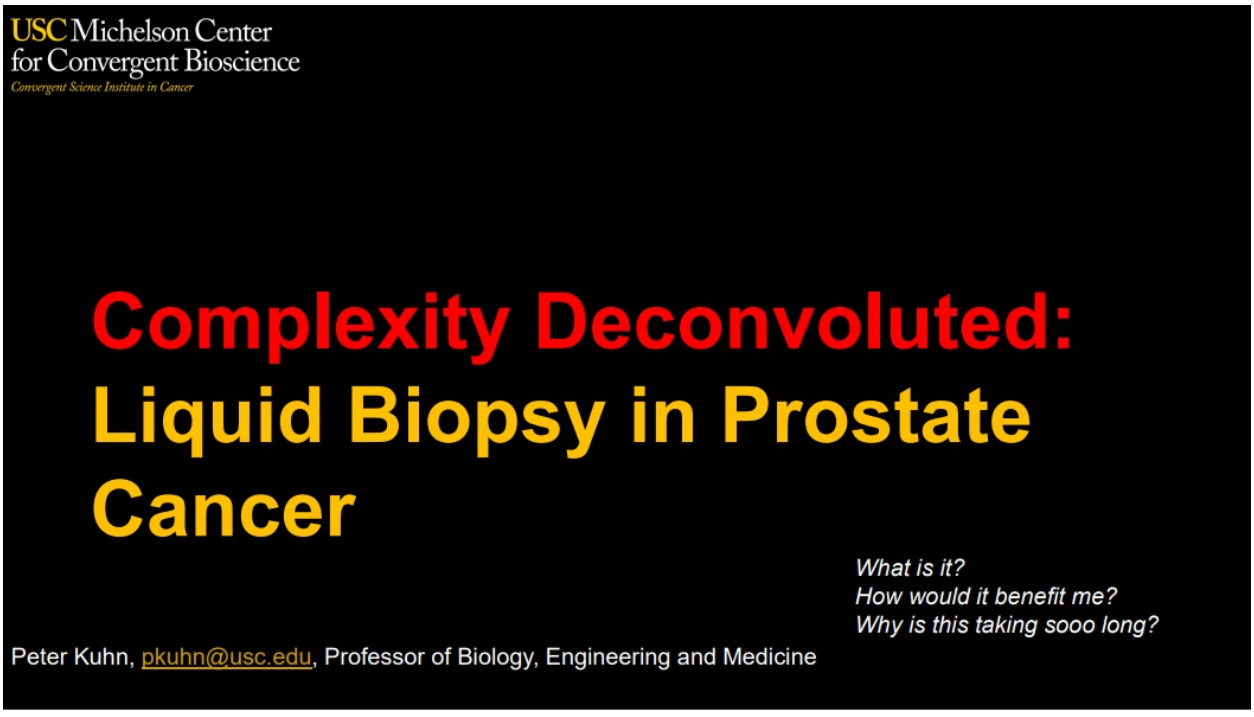
might see a second boat, and that second boat is a little bigger. “Huh?” Then if you zoom out further, you see a bunch of boats. “What is it?” Eventually, you should see there's a chopper overhead. This is a whole fleet. “Wait a minute. If these guys are all here, the actual carrier can't be far away.”

Rick Stanton 16:16

I worked on the sonar system that was dropped from that helicopter and would go 40 miles in front of the carrier group looking for deep threats. We used Sikorsky helicopters. I designed all of it, and it was a hard problem. This is hilarious.

Peter Kuhn 16:45

This is a really hard problem. We started off by doing nothing else but looking for the carrier only. Because the carrier is the big problem. That's our big hit. That's the one we need to put out of commission. Everybody else is there to protect it and work with it. But at some point, somewhere there is enough signal around to say, “No, the carrier is there. I know it's there. I might not be able to see it right now. But I know it's there. I'm going to come back to this bit over and over.” When I only see this small ship, there's very little certainty on whether the full carrier group is there, or the carrier is there. As I'm adding information, I get to higher and higher and higher certainty. Somewhere in there, I know I need to strike. But it's always this risk balance because typically our information is incomplete. I want to always keep that in mind.



I'm a professor at USC. The reason for all the other titles is simply because I was recruited into USC to build something that is really focused on patient benefit. It's super unique. There are not very many places that would support this. (None of the Boston schools. Sorry, Jeff.) None of the Boston schools could ever get there because their ego gets in the way. That's true for Northern California as well because it's too much about empire building. The creativity within Los Angeles is really allowing us to be driven by patient benefit. It's a fantastic place to do it. It's a great way

to make progress. I love teasing our colleagues at the outset, but of course, we work very closely with all of them around the country.

Disclosure

- **Founder and Chief Scientific Advisor to Epic Sciences**
- **Founder and President at Cansera**
- **Advisor to Earli, Sampling Human, Quest Diagnostics**

- **MOST IMPORTANTLY:**
  - **I am just a physicist.**
  - **I am not a physician. I am not healthcare provider of any kind.**

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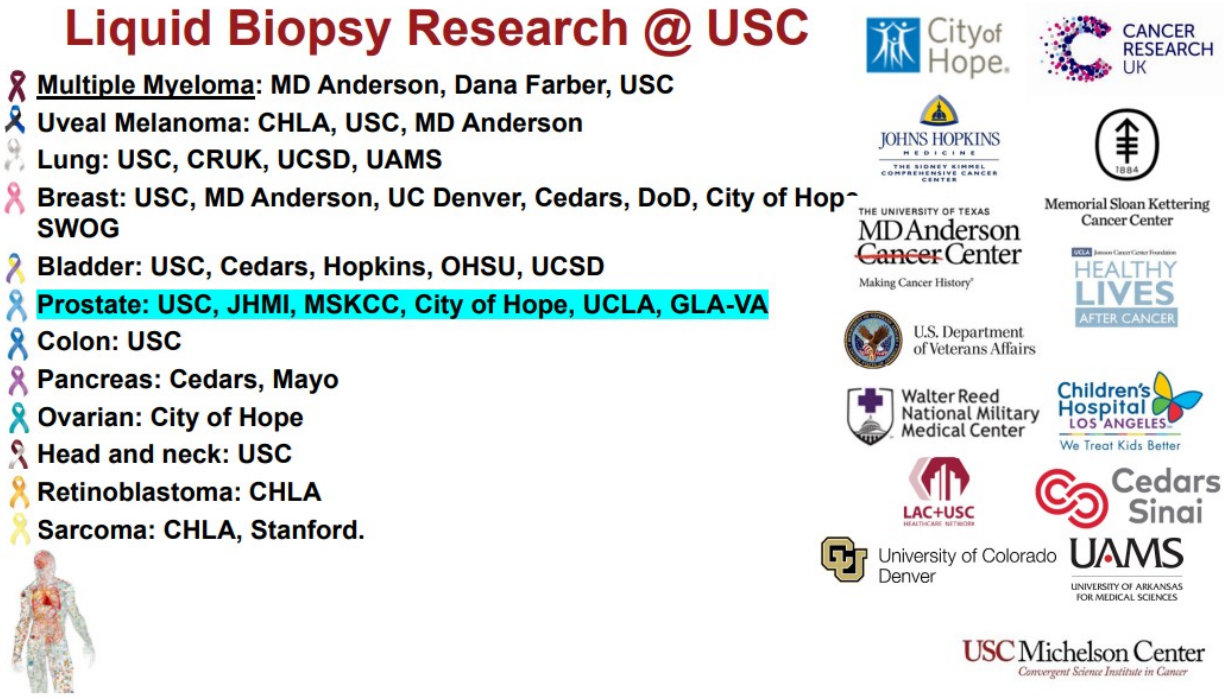
I have some quick disclosures. We started Epic Sciences with technology to be developed in the lab. Cansera is a digital health company, and I'm advising a bunch of other companies. But most importantly, I'm just a physicist. I am not a physician. I am not a health care provider whatsoever. What I didn't put on this slide is I never took a biology class either. All this information, everything that I'm going to say, comes from a straight up science perspective.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Our goal here is to maximize the amount of information we can extract from a tube of blood. Let's not get hung up over this analyte or that analyte. It's just information. What we want to do is maximize the amount of information that we can access. I'm going to come to what we have done in the past in terms of research, and what w



e have put into the clinic.

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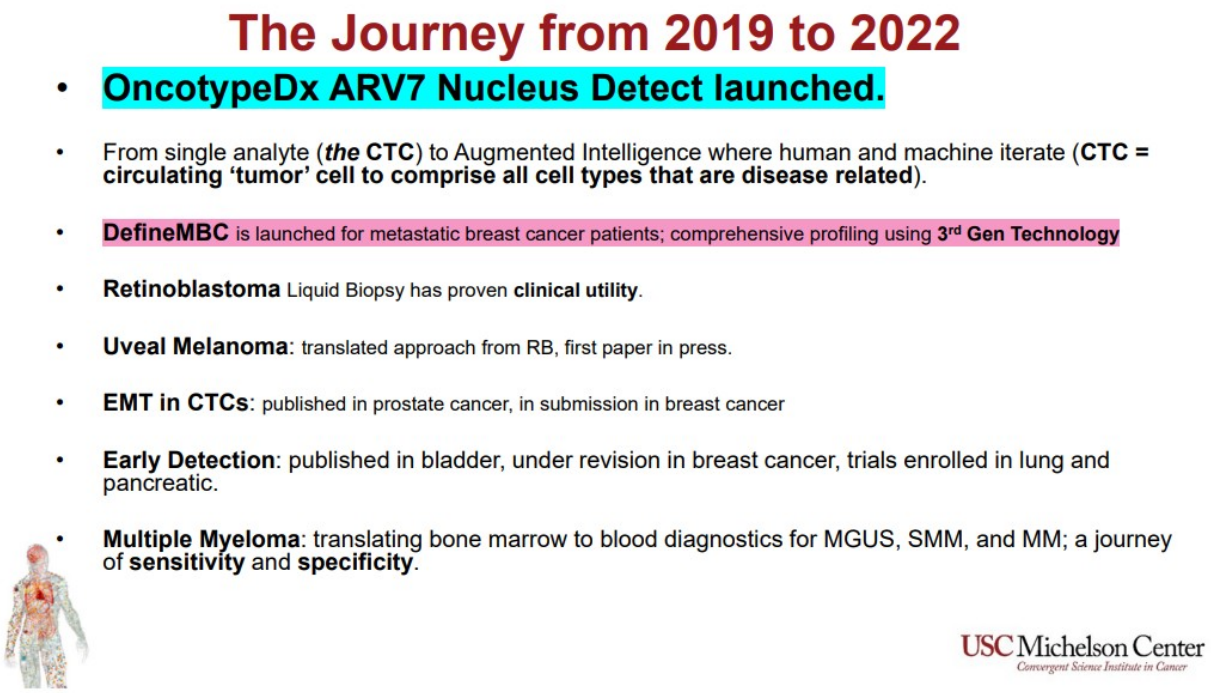
Within our program at USC, and this is true for other research programs in the liquid biopsy space, is that we typically work across several different diseases. It is a large portfolio of research programs. It's important because it allows us to learn across cancers. We can leverage a whole other super cool conversation to be had of, “What does that mean? How does that work? And how do we make progress?” On the right-hand side, you see we work with groups from around the country to do data exploration. Our multiple myeloma program, for example, is with our colleagues at the Farber in Boston because they're just absolutely fantastic colleagues to work with.



Over the past three years we made this progress from looking at the carrier only to being able to start understanding the carrier group. We started off only looking for the CTC and a lot of the first-generation technologies are still sort of in that world. But because of the science that we have now done as a community over the past two decades, we started realizing there's a lot more information to that. The first clinical test is out there, and we're going to talk about this in a few slides. That is the Oncotype DX ARV-7 nucleus detect. We are typically quite good at abbreviations and fun keywords but this one I'm not particularly proud of because it's just way too complicated. Bottom line is if your prostate cancer has the variant 7 of the androgen receptor, it will not respond to a or targeted therapies anymore. It's as simple as that. And I'll show you the data for that in a moment.

Rick Stanton 22:23

Can you elaborate on that a bit. I don't know what that really means.

Peter Kuhn 22:26

I will do that in a couple of slides because it's important. It's an important part of what it means and what the consequences are of that. Just like you mentioned with your wife who has breast cancer, we have a second product that was launched by Epic called DefineMBC. We love DefineMBC from a research perspective because it's the first test that brings together cell free DNA analysis with standard CTC analysis and single cell genomics of the CTCs. It’s super cool, brand new, super-hot. For a while, it will be expensive but that's just a transition period that we must get through, and then we'll figure out how to go from there.

We have done work in retinoblastoma. That was our first foray into children's disease. Uveal melanoma was a big step for us working on cancers other than the prostate and other tissue cancers and the carcinomas. With respect to early detection, the key players are Grail and Thrive which come to mind here. They looked only at all the associated signals that they can find within the ctDNA (circulating tumor DNA). Rick, if you would have nothing else but sonar, it would probably not be good enough. You need more than just one signal - a lot more. Our argument is exactly that. We need visuals, we need sonar, we need intelligence. 10 years ago, I would've said this is completely nuts. This will never work. Today, I would have to say that I think we are on a super exciting path.

LBx in Prostate Cancer

- **LBx is a liquid sample (not a test).**
- **The liquid sample is most of often blood but could be bone marrow aspirate and other liquids.**
- **LBx contains analytes that reflect the immune system and the body’s connected highway of information and transport.**

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In prostate cancer, let's cover a couple of basics. Liquid biopsy is just the liquid sample. It is not the test. There's a lot of confusion around that, but that's really all it is. Most often it's blood. But in prostate cancer in particular, bone marrow aspirates are pretty darn powerful. If I can prove the utility of it, then I think one could argue it's very worthwhile. Liquid biopsy contains different analytes. It's a tube of blood. It has red blood cells and white blood cells. Your entire immune system has the plasma, which contains all the proteins like PSA, but it also contains your cell free DNA. Then it has the wafer cells, which are those that are connected to the tumor itself, the circulating tumor cells and all the associated ones.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

LBx in Prostate Cancer

- **Do I have cancer?**
  - **Diagnosis.**
- **How bad is it?**
  - **Prognosis**
- **Will my treatment work?**
  - **Prediction of response.**
- **Is my treatment working?**
  - **Response monitoring.**

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What is it that a patient wants to know? It's straightforward. “Do I have cancer? Yes, or no?” “How bad is the diagnosis?” That's my prognosis. “Will my treatment work?” I'd like to know that not just because it's expensive, but also because I have only so many shots on goal. However, I want to think about this. I really want to know once I'm on this treatment, “Will my treatment work?” “100% of the time” is not the right answer. I need to have some sort of response monitoring.

LBx in Prostate Cancer

- Do I have cancer?
  - Diagnosis.
- **How bad is it?**
  - **Prognosis**
    - **In prostate: active surveillance vs. radical prostatectomy.**
- **Will my treatment work?**
  - **Prediction of response.**
    - **If my prostate cancer is AR driven, the AR inhibitors will work.**
    - **If my prostate cancer is not AR driven, AR inhibitors are likely a waste of opportunity.**
      - But it is a bit grey there because ‘how good is the measurement of AR?’
- **Is my treatment working?**
  - **Response monitoring.**
    - **Once you see a 1 cm^3 spot on a scan, that is about 1 billion cells. It would sure by nice to see earlier.**

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“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

When it comes to how bad it is in prostate cancer, one of the basic choice points is active surveillance versus radical prostatectomy. That sure would be nice to know with much higher confidence because we know that somebody who is truly a Gleason 6 doesn't really have to worry about it. We worry about it because truly Gleason 6 is really complicated. Will my treatment work? If my prostate cancer is AR-driven, the AR inhibitors will work. Great. If my prostate cancers are not AR-driven AR inhibitors are likely a wasted opportunity. But it gets a bit gray there, coming back to Rick's point about ARV-7. We'll get to that. Is my treatment working? Traditionally, that's all been done with imaging, but imaging isn't straightforward. Rad onc's say that imaging sensitivity is getting better. They say they're using a cubic centimeter roughly, but that's a billion cells. That's a lot of cancer cells. It would sure be nice to know a little earlier than that, if detecting a recurrence earlier does not directly mean that you will have a better outcome. This is a really important point again about this Wild West. Just because I can detect something does not mean that I can improve the outcome. We on the science side must prove this. Two decades ago, experiments were done with imaging in breast cancer that essentially showed that whether I identified the recurrence a bit earlier or a bit later makes no difference in terms of the overall survival of these patients. But that was because we didn't have that many drugs available. Every drug gives you an episode of life. If I only have two drugs, I get those two episodes. If I took them a little early or a little later did not make much of a difference because they were all very similar drugs at the time. This is different today. We have lots of treatment choices. We need to figure out how to be smart about these treatment choices. How do we generate the evidence that proves that we will reach better outcomes?

Which drugs relate to which marker? Again, androgen receptor relates, of course, to androgen deprivation therapy and the androgen receptor signaling inhibitors, such as Abiraterone, Enzalutamide, and the whole cohort around that. PSMA is a new marker for lutetium 177. It's really important to think our way through that. What does that mean? There are lots of consequences even when it comes to imaging. We used to call rising PSA in the absence of evidence of imaging a biochemical relapse. Now with PSMA imaging, you see that recurrence much earlier. Let's make sure that we get benefits for the patient out of this.

The next one is measuring the cell free DNA. I will come to that in a couple of slides. ATM/ BRCA1/BRCA2 mutations are a predictor of response to the PARP inhibitors. Again, important, if the AR inhibitors are not an option. What are my alternatives? Let's turn to ARV-7, or the androgen receptor. Think of this like a fuel pump. Testosterone is your fuel. Fuel comes in and then drives the engine. Now I want to stop the engine after cancer. How do I do that? I can shut down my androgen production through androgen deprivation therapy, which is step one. If I just throttle that down, does that work? Because if I provide less and less fuel, the engine should die. But the engine starts becoming much more efficient. It needs less and less of that fuel due to improved fuel efficiency. What should I do next? Let me screw with the fuel pump itself. I can block the fuel pump, and that's what abiraterone and enzalutamide are all about. Two slightly different mechanisms, but they all screw around with the fuel pump. The next thing that happens is this variant seven, where it starts becoming completely independent of testosterone, androgen. Now we have a whole different problem. Up until now, the only alternative is to switch over to the taxanes.

Peter Kuhn 31:32

That means we go in and carpet bomb this whole thing. It's ugly. It's a set of shitty drugs, but that's the best we've currently got. Now, the reason why all of this is super important, is that when that test came out and we started realizing just how big that gap was in terms of survival, pharma and biotech companies jumped all over this and started building drugs specifically for that patient population. Nothing is approved yet, but this is going to come hopefully sooner than later. That cycle of science and research and development really must be appreciated. It's so super important. That is how we make progress because suddenly there are going to be new drugs that are essentially these androgen receptor degraders. They just eat up the entire receptor. The science is just super cool, and this is driven by the fact that we now know how to measure that. There is aggressive prostate cancer and that's complicated. I'll come to that in a moment. Rick, does that, in part, help regarding your question about ARV-7?

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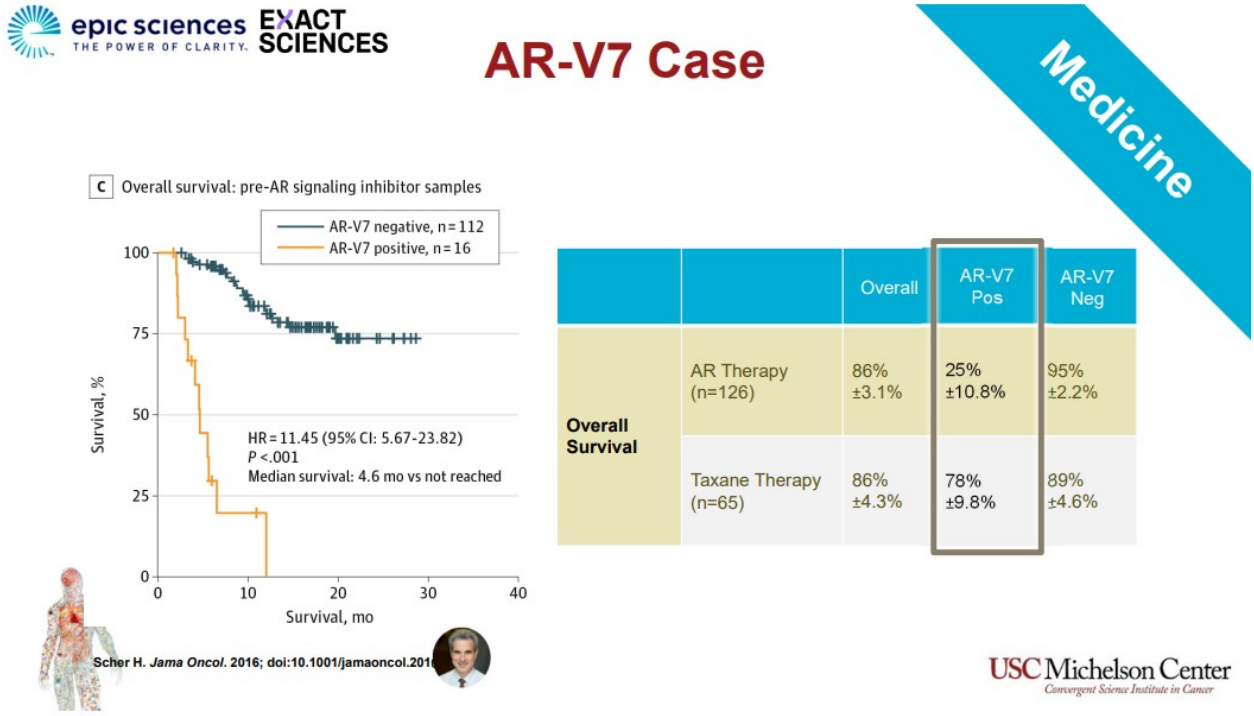
Rick Stanton 32:53

Yes. I was on androgen blocking and then I just failed the taxanes. So now I've started immunotherapy, PD-1 and adenosine inhibitors with no real evidence that it'll work. I just started so I'm a bit concerned that I got like about a 25% shot that will even modulate it. I'm starting to feel better because I'm not getting killed by taxanes right now. But I'd like to have a backup plan ASAP.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]



Peter Kuhn 33:40

The research conversations and the clinical conversations are always a little different. In a research conversation, I am trained to always say, "What's my next experiment?" Whereas a patient in a clinic setting is saying, "I need to make a decision today." This is where the two worlds must come together and figure out, “What is the strategy?” Part of that is recognizing where we are with the evidence we have built. What are the risks that we are taking at various points in time? This is super important with ARV-7 because Howard Scher really pushed that work forward. Look at the difference in survival. The yellowish line is patients who are positive for ARV-7. They have a much worse prognosis versus those who are ARV-7 negative, and they respond to antigen receptor targeted therapies. They do exceedingly well. That's why I surely want to know where I'm at on this spectrum. That was prognostic in the beginning. That is what that left hand side did. But does it make a difference? Is there an alternative? Is there a backup plan? What if we change therapies? On the right-hand side, it's really this understanding of, “What happens if we change therapy? What if we go from a targeted therapy, which is well tolerated, to a crummy chemotherapy? What do you get on average?” The ARV-7 positive patient gets that better outcome. This is really important. That is what we have been driving for these past six or seven years. It is a huge amount of drug development to move forward.

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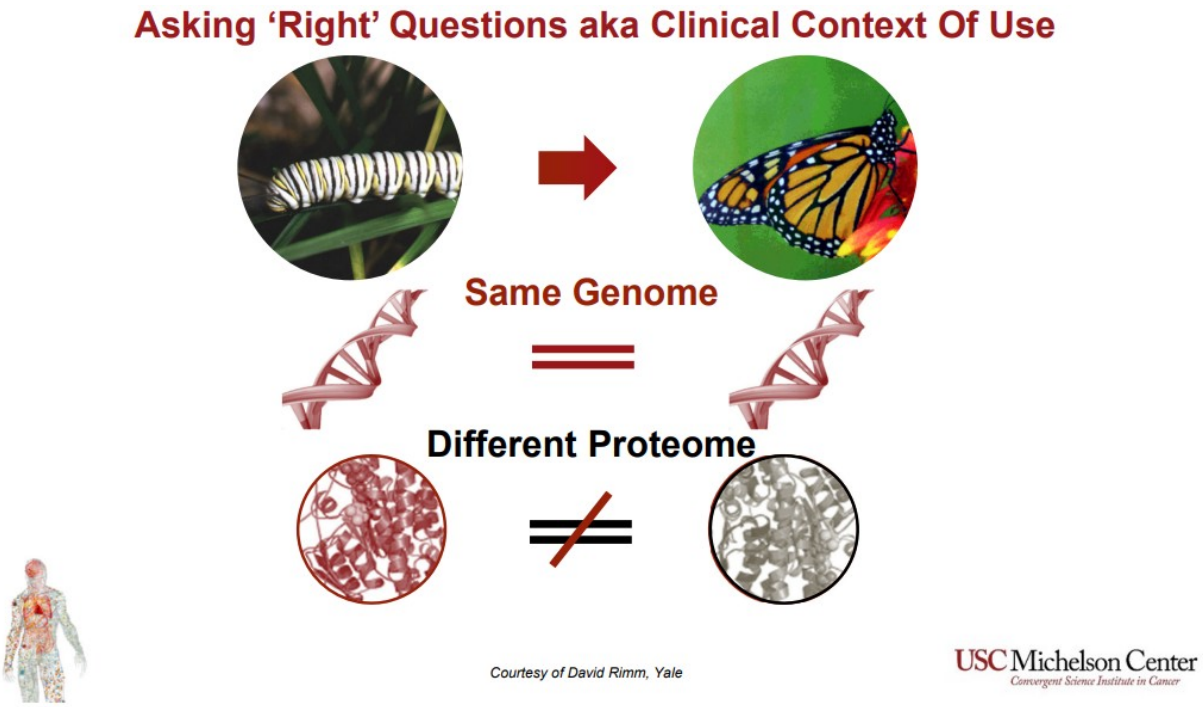

A lot of this comes down to asking the right question in the clinical context of use. Otherwise, we're just doing research for the sake of research. We must be thoughtful about this. I always like to know what a few of you have brought up regarding cell free DNA versus CTCs. “Are they competing? What is it? How do I wrap my head around them?” This has everything to do with the clinical context of use. If I have a caterpillar and a butterfly, and I ask the question, "Which one can fly?" If I sequence their genome, I will not get the answer because their genome is identical with the genome of the butterfly. I need to do this at the proteome level because that's where they are different. Luckily, there is no pathologist on this call because the pathologist would say, "Peter, you ding dong. Look at the bloody picture. The left is the caterpillar, the right is the butterfly." Always keep that in mind. Sometimes there are simple solutions, so don't forget that. But this is how to think our way through the clinical context of use.

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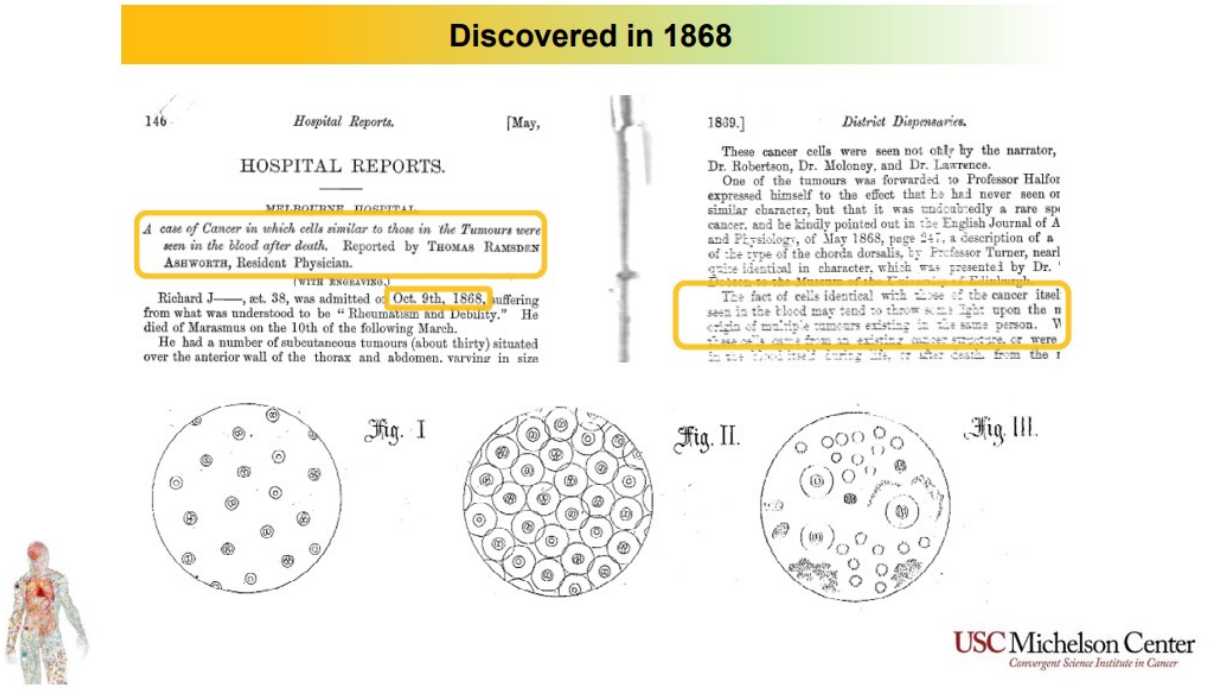
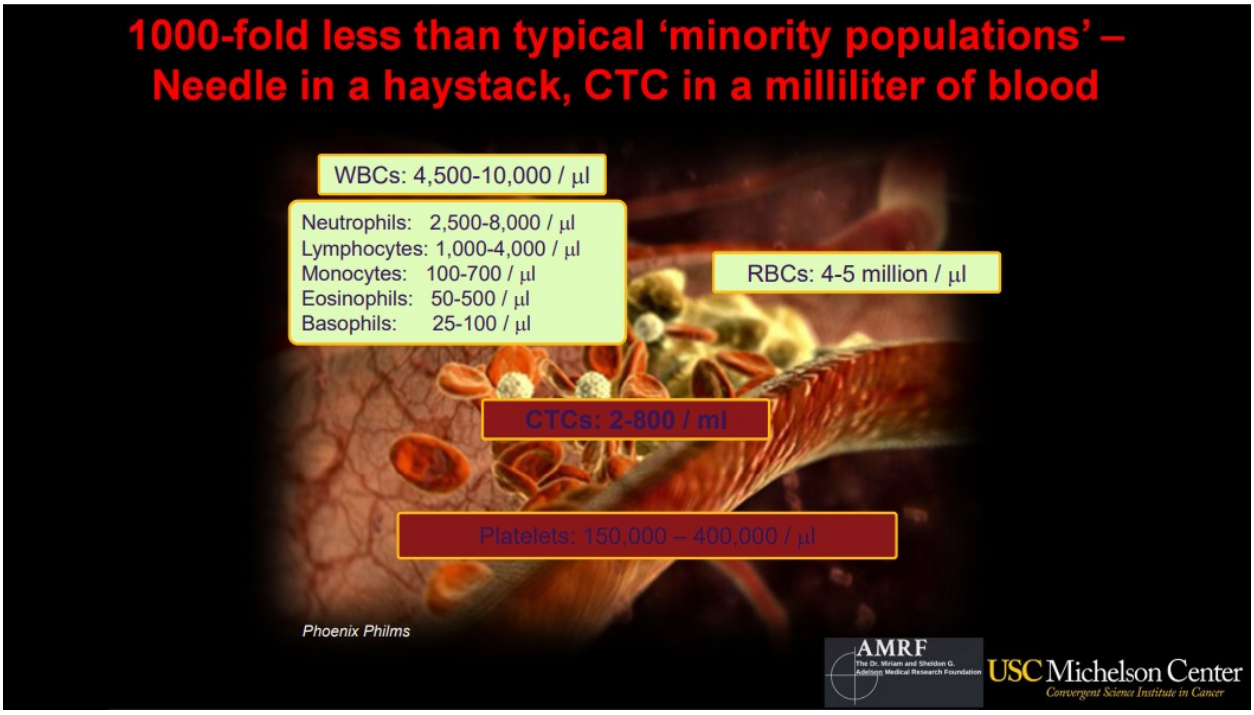
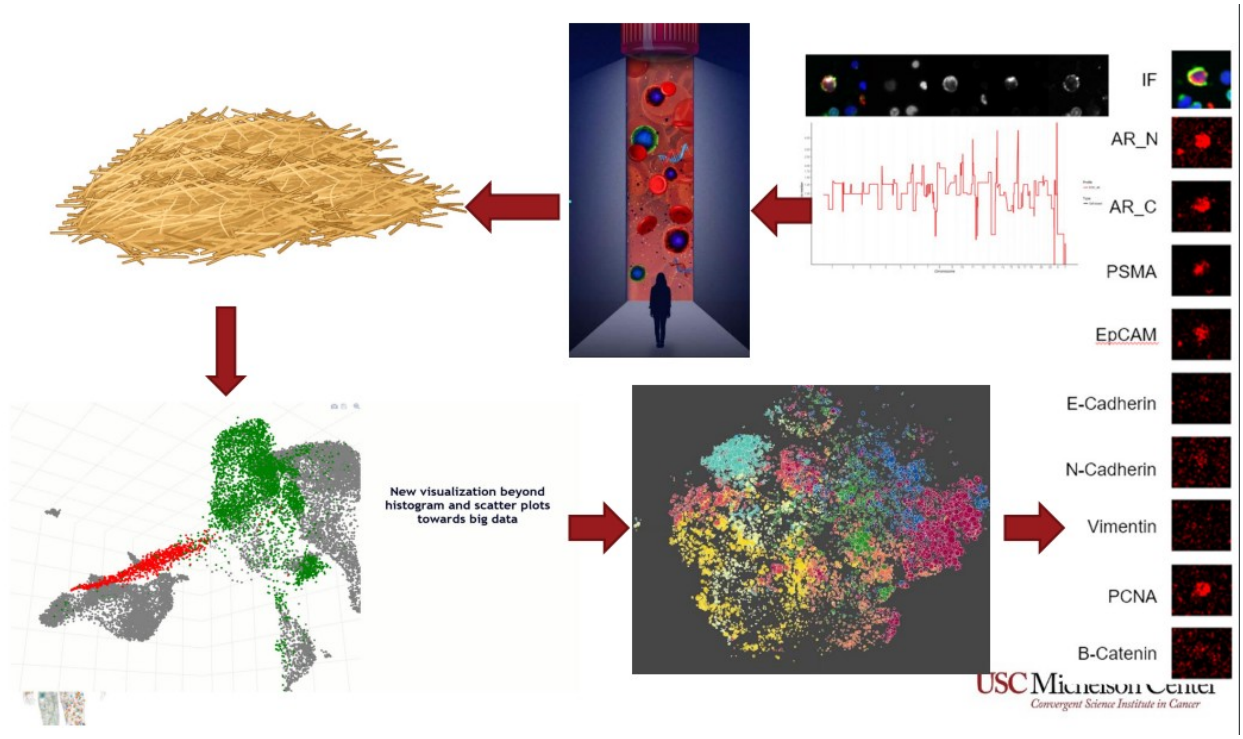


CTCs were around in 1868. A little while back. Neither me nor Mehmet Toner in Boston invented it. Just to be clear, it's been around for a while. So why are we still not using this daily?



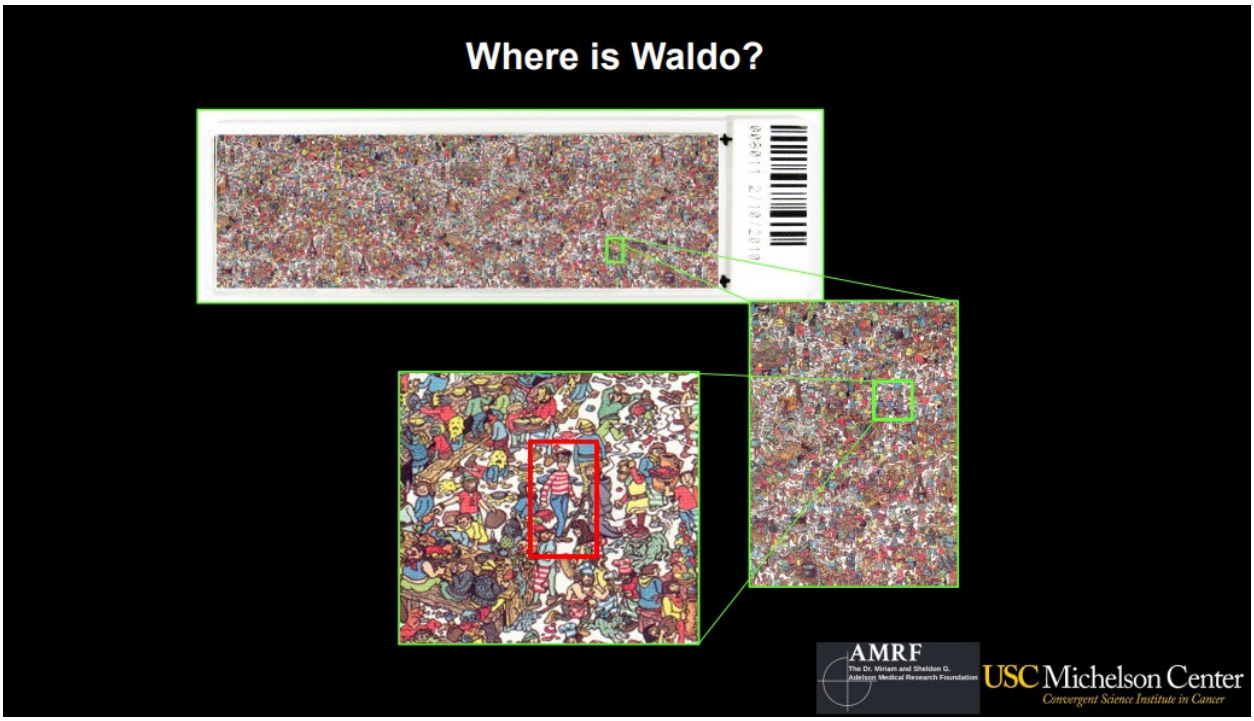
It's still the Wild West when we think about our immune system, our immune cells in the blood, the white blood cells. When you get your results back from your standard routine testing, you typically get your white count, or you even get that subsetting. Your white count is many 1000s up to 10,000 per microliter of blood. When we measure CTCs, we measure them in the single

digits per milliliter -- 1000-fold less. Anytime I'm trying to do something that's 1000-fold better, it gets really, really hard. That's why we must be careful about how we do this.

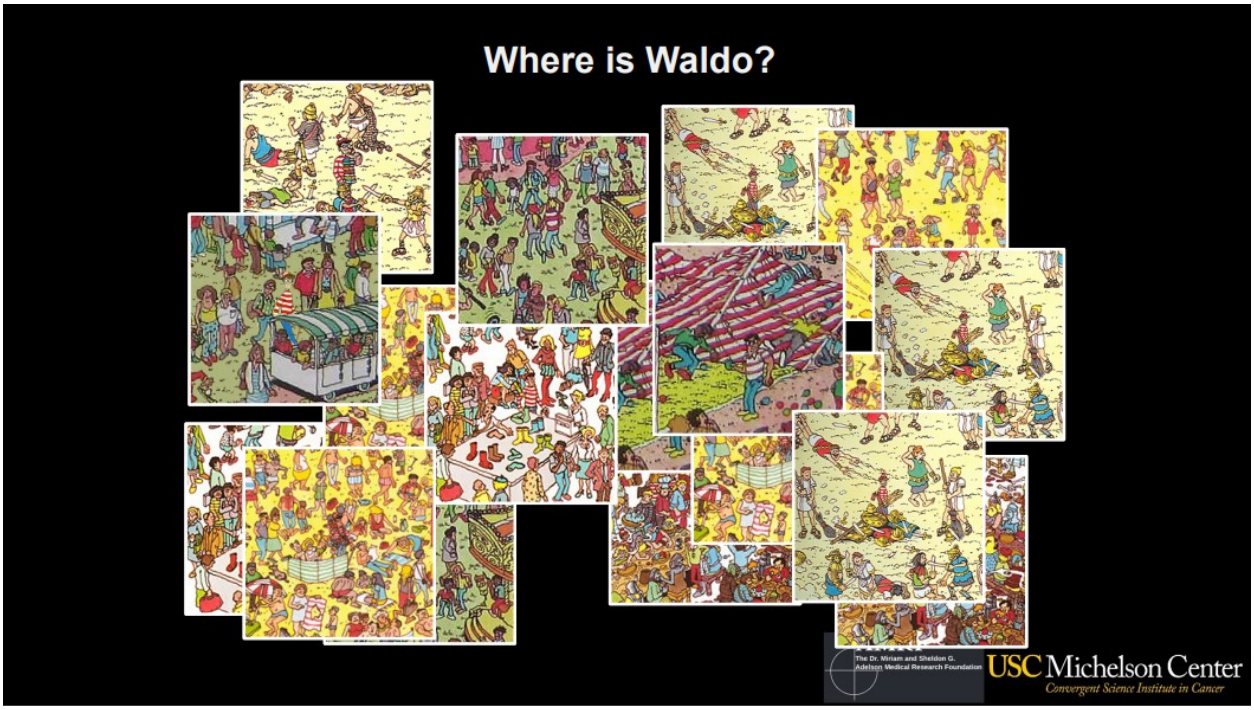


Oftentimes, we compare this to a “needle in a haystack” problem. Complicated, fair enough. This is where Catherine Alix-Panabieres and my team are different in our approach. I'm not saying one is better or worse. This was always about how these different groups complement each other. How do we make that bigger picture? She would take this whole haystack and push it through a filter and make the argument that the cancer hay is bigger than the regular hay. I'm like, "Ooh, that's complicated." We just blow the haystack over a very, very large area and then we just take one little bit at a time. We take a picture of it, and then we look at which one is more like one another. What you see in that lower left-hand corner is essentially one blood sample, and looking at which cells are most closely resembling cancer cells and which ones are white blood cells. We love that because that means we can build up these huge image libraries that we can start comparing against one another. We can start looking at the biology of the disease, the genomics of the disease, and we can bring it all back together into a big cycle of research going towards the product.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

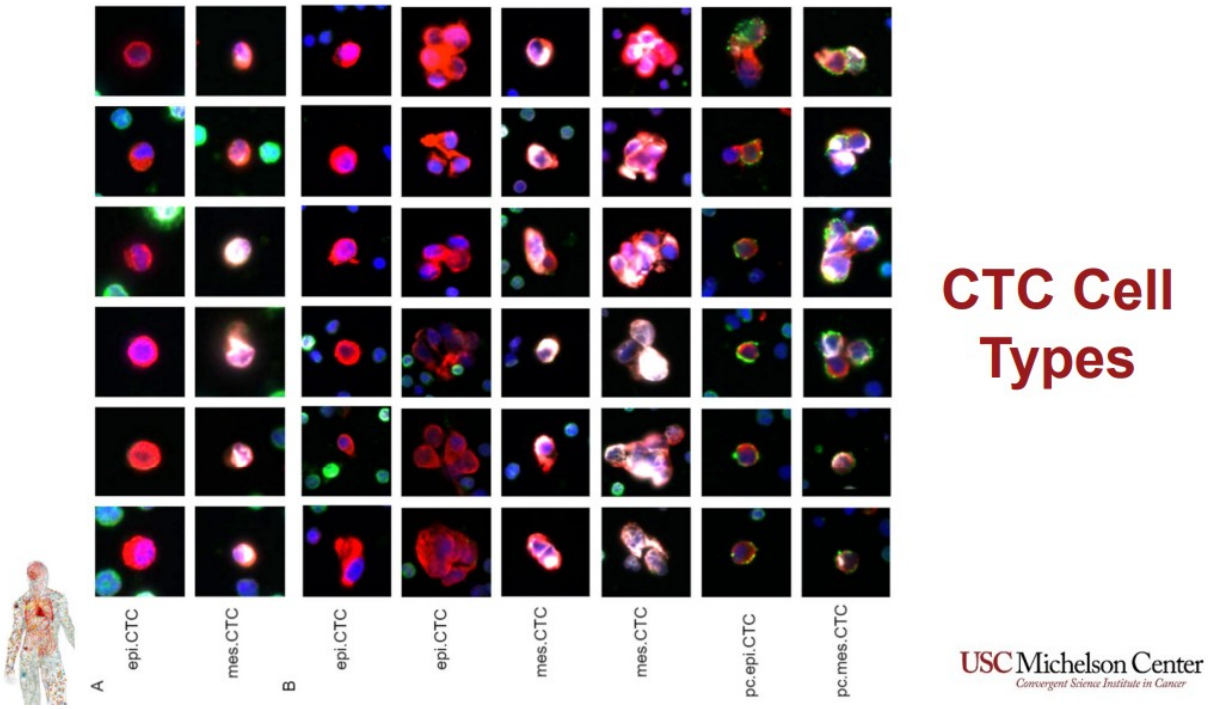


One way to think about this is for those of you who love “Where's Waldo?”. One piece of advice is to never ever play “Where's Waldo?" in a competitive way against a pathologist because you will lose. They're good at pattern recognition. This is the standard where once you see Waldo, you're like, "Oh, of course, this is really straightforward, Peter."



But can you do this super-fast? At very high speed? Where's Waldo? And that is when life gets a bit more complicated because there's lots of stuff that looks pretty darn like one another. We

need to figure out what is true, and what is not true. As you're training your own brain, when you play “Where's Waldo?”, your brain spends probably more time trying to separate out the false positives because the true positive is straightforward. It's filtering out the false positives, the ones where you're wondering. We play that exact same game with a computer science approach. Super high speed, fun, computation “Where's Waldo?”.

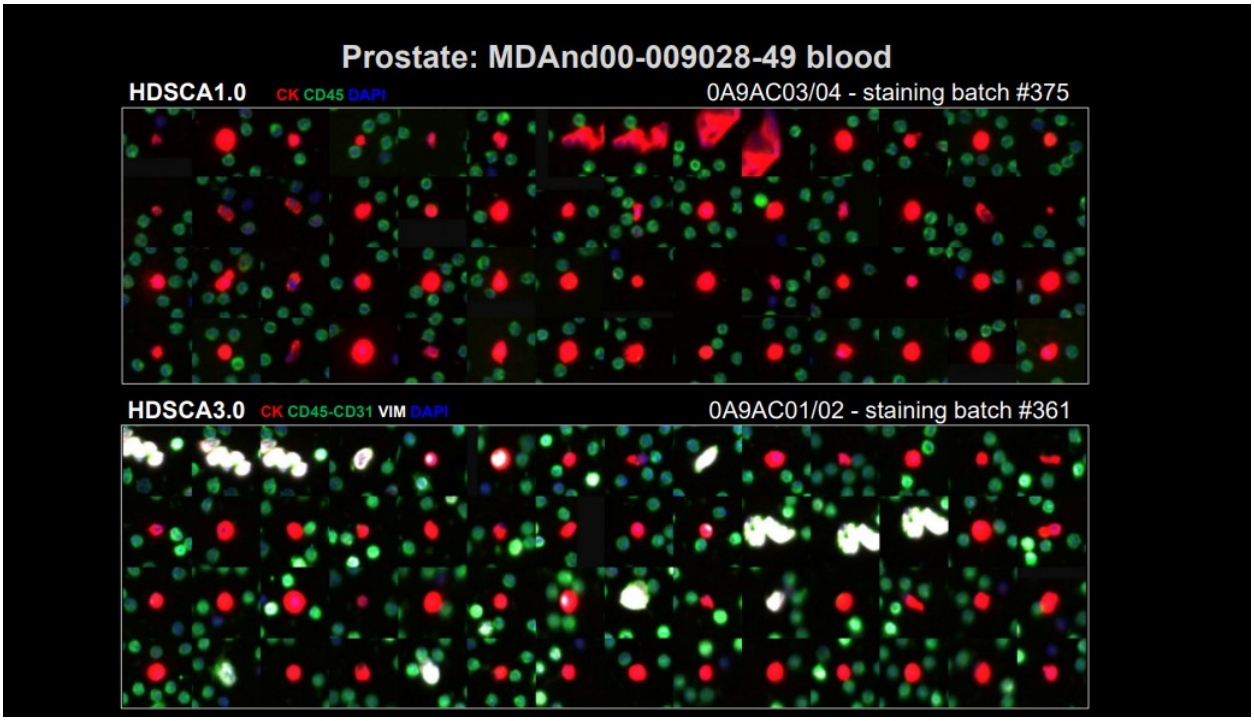


Peter Kuhn 40:53

The upper left-hand side is one cell that is a bright red unicolor, that is what we traditionally thought was the CTC. I, together with many others, (this was before Stephanie joined us) have spent a lot of time defining the CTCs until somebody slapped us left and right, and said, "What about the heterogeneity of the disease?" What about in the blood? Why would that not be represented in the blood? And that's, of course, very, very true. In the blood itself, we see the full heterogeneity of that cancer. All the cells that you're seeing in this image, they're all cancer cells in the blood, and the different colors, the different shapes, the different things that are attached to it. They're all representing what that cancer can look like. The color concept here is blue. Blue is something that we marked the DNA with. That's the genome, the nucleus of that cell. The green that you're seeing in the cells that are just blue, and green, those are your immune cells, or just white blood cells. The red that you're seeing is cytokeratin, which is a protein that marks tissue. So now, that means that a cell that is blue and red is a tissue cell. Tissue cells have no business being in circulation. White is a marker that is called Vimentin and it gives us a feel for what we think is a very aggressive disease because it has managed to figure out how to go through a transition that enables it to travel around the body. Then the green dots that you see on the right-hand side are essentially a protective layer that's sitting on top of these cancer cells and is probably allowing these cancer cells to be invisible. All of this is biology that we need to work out. We need to work our way through this very, very carefully.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

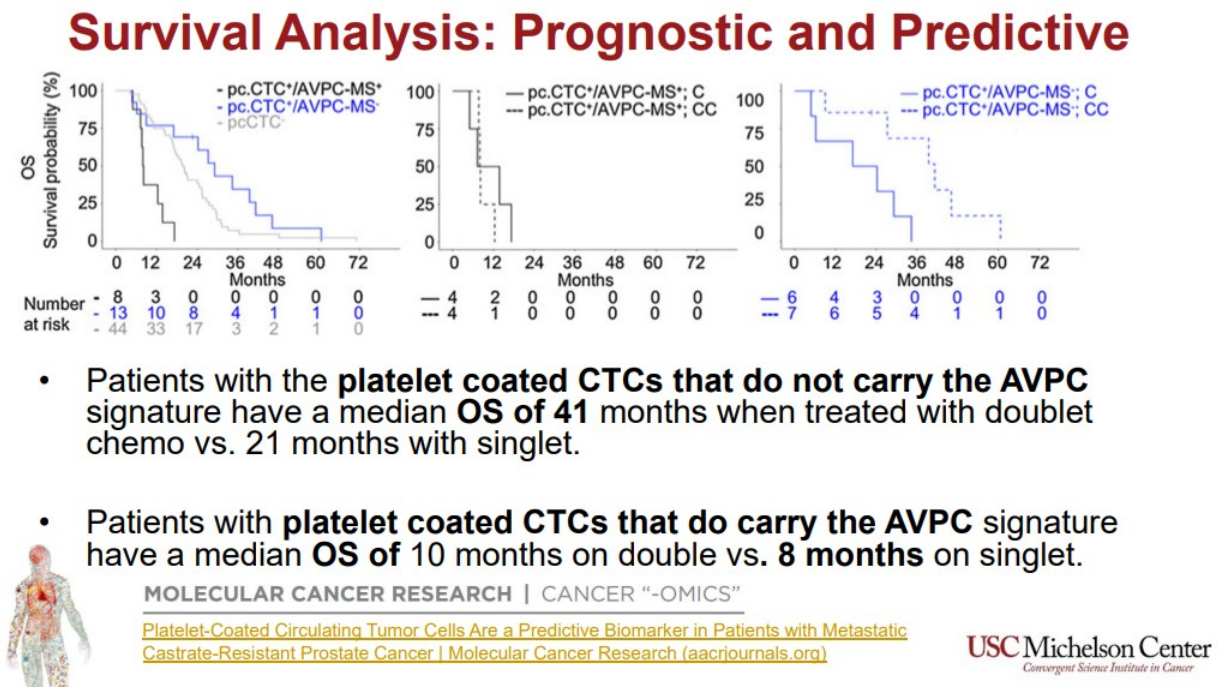
That's why we call this 3rd generation technology approach, which is what we have developed in the last couple of years. This is where all the new papers are now coming out.



Our first-generation technology with this upper left-hand side was all about looking for the red. In many ways now, we turn on the color channels. The third-generation technology is just better at delineating the different biologies of the cells.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Peter Kuhn 43:54



There is this concept of very aggressive prostate cancer and how you would deal with this. At some point, Rick asked about chemotherapy and whether he should get two chemotherapies at the same time. Well, two chemo therapies are actually double the shitty therapy. It gets really crummy at that point. But the question is, "Is it worth it? Does it lead to better outcomes?" If somebody could tell you, "Hey, this is going to lead to better outcomes", you'd be like, "All right, fine, I'll suffer my way through it." It’s complicated. This is the complexity that we're trying to figure out how to set that apart. That's the science around us. This is done with bone marrow aspirates. We can figure out whether this is on the far-right hand side. That dotted line is in this disease pattern, giving a patient with double chemotherapy real benefit. Whereas in some of these other settings, it doesn’t make a difference. Again, this is just on the research side. This is the kind of data that will then start triggering drug development. Blood and bone marrow won't talk about this, and chromosomes won't talk about it. That's for a future discussion.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

LEVs/Oncosomes: Hiding in plain sight

cancers

MDPI

Article

Large Extracellular Vesicle Characterization and Association with Circulating Tumor Cells in Metastatic Castrate Resistant Prostate Cancer

Anna S. Gerdtsson [1], Sonia M. Setayesh [1], Paymaneh D. Malihi [1], Carmen Ruiz [1], Anders Carlsson [1], Rafael Nevarez [1], Nicholas Matsumoto [1], Erik Gerdtsson [1], Amado Zurita [2], Christopher Logothetis [2], Paul G. Corn [2], Ana M. Aparicio [2], James Hicks [1] and Peter Kuhn [1,*]

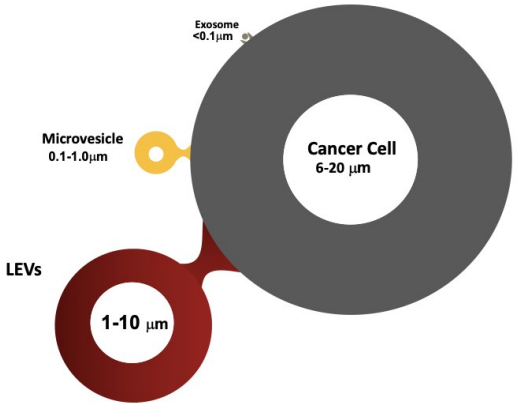
USC Michelson Center
Convergent Science Institute in Cancer

What are LEVs?

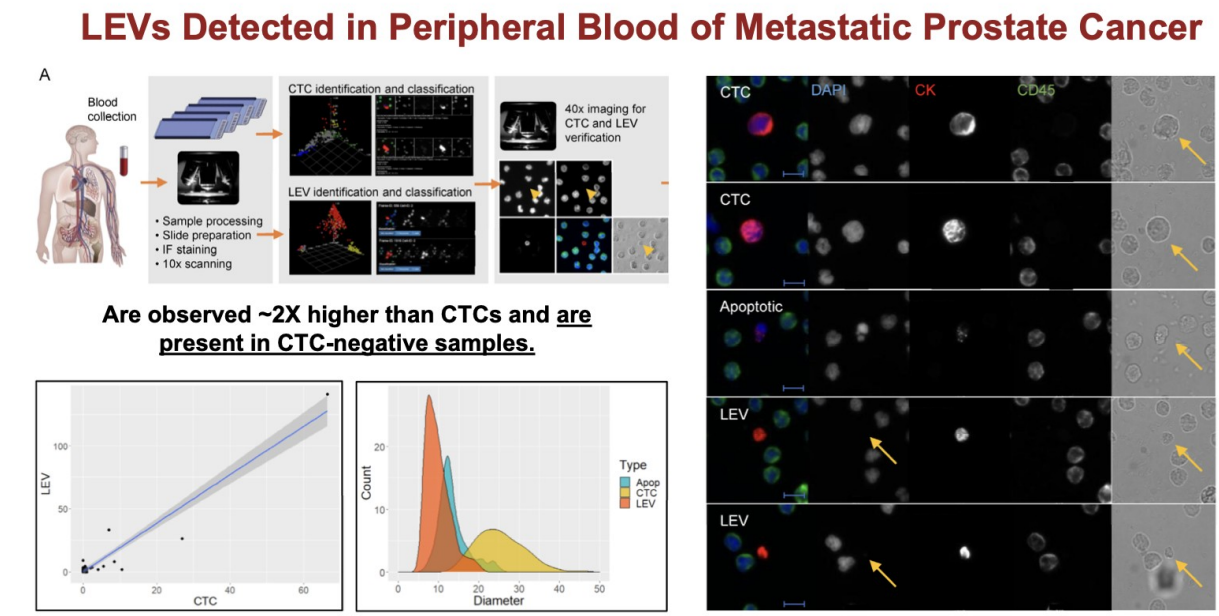
- **Large extracellular vesicles (LEVs) are shed from cells through active blebbing of the membrane. Tumor cells are known to shed a lot more LEVs than their non-tumor counterparts*.**

- **LEVs are exclusively shed by cancer cells in a size range from 1 µm up to 10 µm**

*Minciacchi et al, (2015)/ Cicardiello et al, (2017)/ Cicardiello et al, (2020)
*Gerdtsson et al., *Cancers*, (2012)



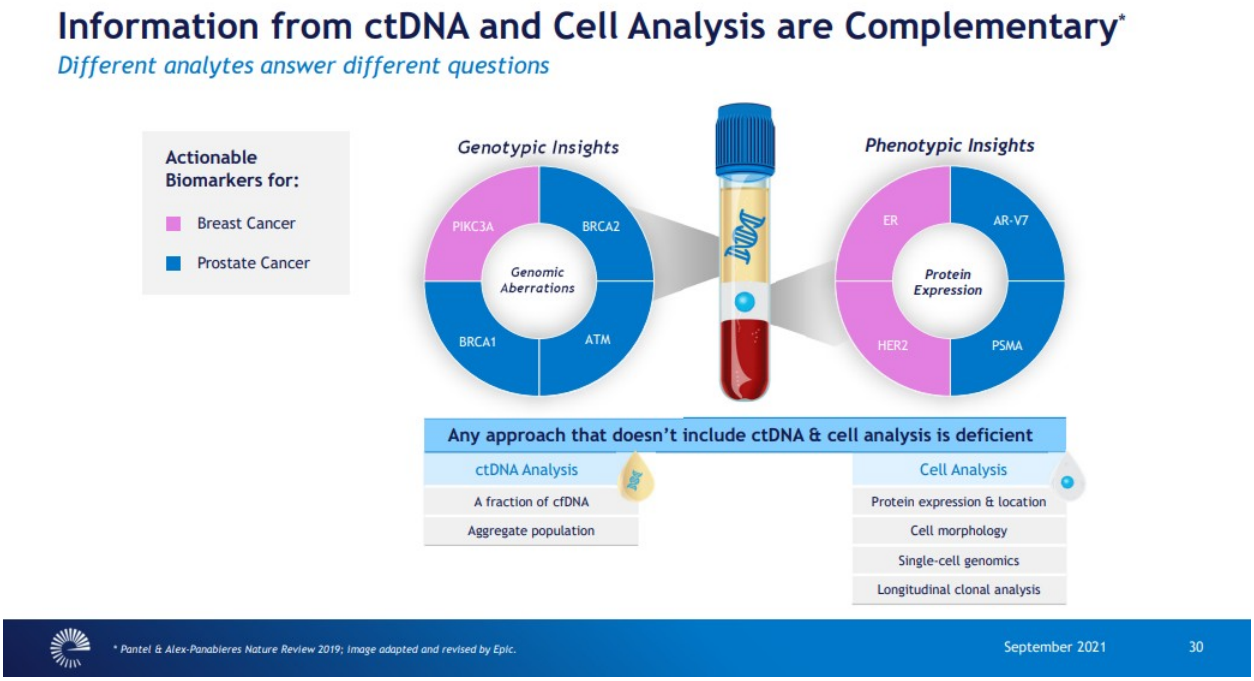
“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]



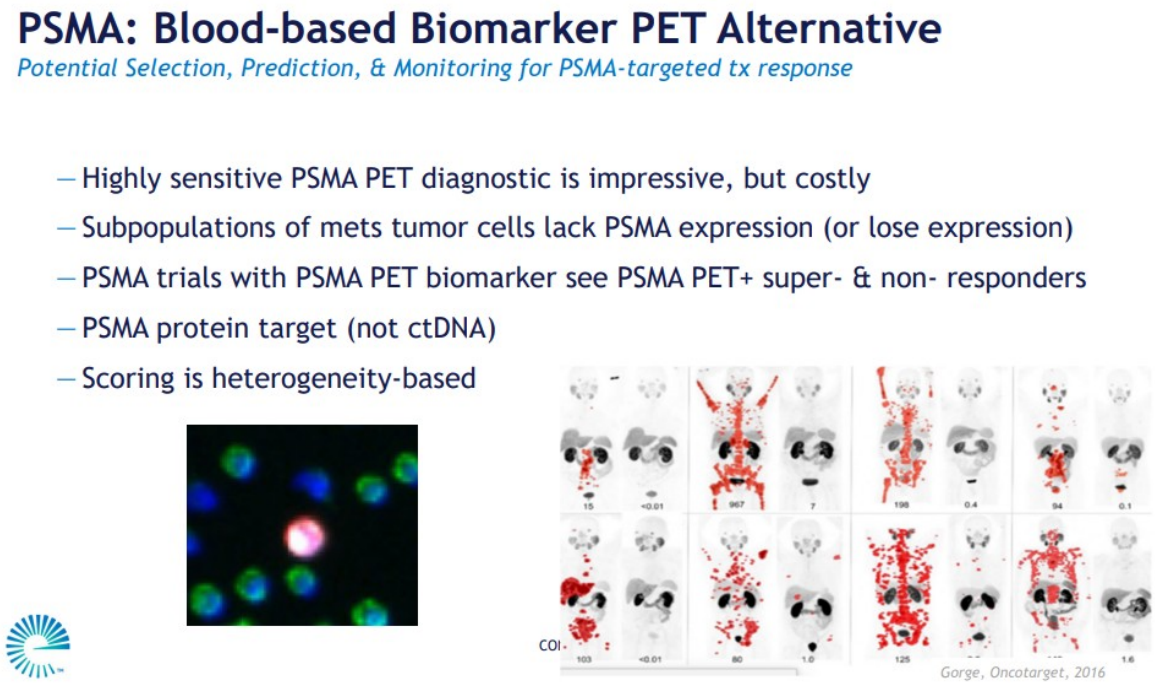
Oncosomes are “blebbles” that come from the tumor itself. They contain almost all the information except for the genome, as far as we can tell, and they're a big part of the communication path after disease itself. We just started this work a couple of years ago and we're making important progress. We have shown that they exist in prostate cancer and that they carry all the prostate cancer proteins that we would typically expect. We'll see how we make them useful over time.

Somebody asked about ctDNA and CTC overlay.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

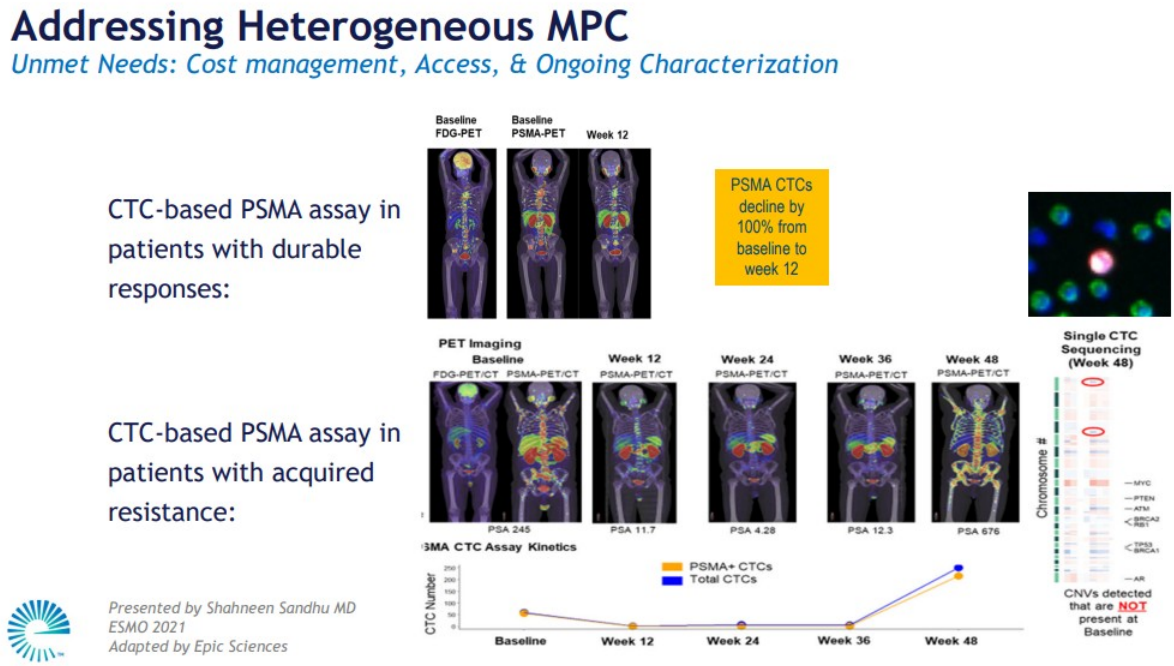


This is breast and prostate cancer together. This is a slide I stole from Epic Sciences. It's an adaptation from Catherine Alix-Panabieres paper that she put out. There is this combination between what we learned from the genome, which I can typically get from cell free DNA, and the data that I learned from the protein or the proteome, which is what I continue to get from the CTC. ARV-7 and PSMA, that's all measured on the cell itself. Whereas this combination of BRCA1 and BRCA2, and ATM for your PARP inhibitor response, that's measured in the cell free DNA. That's why these two go hand in hand.



“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Important new sets of drugs have shown some pretty stunning results, including currently, PSMA PET. There are limitations in the availability of PSMA PET, and we believe that we can complement that – maybe even replace that – with just a blood draw, which would give us much broader availability. The first set of experiments on those two slides I stole from Epic.



Trials are ongoing to see whether we can measure the equivalent of PSMA with just a blood draw. Our goal is to help patients participate. We need to learn across all patients. That's a big step that we must take forward in how we bring the data together.

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Mike Yancey 48:46

Maybe I should already know this, but when we talk about cell-free DNA, can you help me understand that?

Peter Kuhn 48:53

Absolutely. Think about your primary cancer in the prostate or metastatic disease somewhere else, perhaps in the bone. Then we know that cancer is unstable. We sometimes talk about the blood vessels being unstable because that is how the cancer feeds off of the nutrition that's coming from the blood by making this all a leaky system. That means the cancer leaks into the blood and can start traveling around the body. Now the question is, "What is it that leaks into the blood?" The two things that leak into the blood are the cancer cells themselves and associated cells, but also when cells die they fall apart and their nucleus which contains the entire genome falls apart as well. That trash all drops into the blood. If I measure it very, very carefully, I can measure the genome after cancer. That is very distinct from any sort of DNA that would come from healthy cells. We call that cell-free DNA. They didn't want to call it DNA from dead cells because that doesn't sound very attractive. Cell-free sounds a lot cooler.

Saed Sayad 50:28

What do you think about the Grail multi-cancer tests? I believe they have positive predictive values around 44%.

Peter Kuhn 50:40

We have to separate out the conversations that are in the setting of previously diagnosed with cancer, any prostate cancer, and the early detection problem, because the early detection problem is very different. There's a huge discussion around MCED tests, multi-cancer early

detection versus single cancer early detection. The MCED tests as they are designed by Grail and Thrive will be useful and impactful in identifying cancers that put those analytes in the blood. It's just like a PSA test is actually quite good at identifying a cancer or monitoring a cancer that is PSA- or androgen-driven.

Saed Sayad 50:44

Are you going to pay $1,000 to take that test?

Peter Kuhn 51:28

There are a lot of questions around not just the cost of it – I'm okay with the cost of it – but what is the consequence of it? We're trying to do what we call “downstaging”. We try to avoid patients ever being diagnosed with stage four disease. We want everybody to be diagnosed when they're still stage one so they can be treated with curative intent. I'm going to give a quick example of what's a bit complicated that I learned from being part of some legal issues around women with breast cancer. Every patient has been diagnosed with cancer and mammography is great to think our way through because typically women have that on an annual basis. When a woman gets diagnosed with breast cancer, if she pulls up her mammograms from the last 1234567 years, you can start seeing that cancer develop. So the obvious comment here is, "What the hell, I've got a bad doctor because even I can see this last year, a year ago, two years ago." Well, true for that patient. But at the time, there might have been 10 women who looked just like that, and none of the other nine developed an invasive cancer. That's the complexity of it.

Saed Sayad 53:33

It's like the lottery. If it's a six number, if you have five numbers, the probability of number six is almost 1 in 10 million.

Peter Kuhn 53:43

That's the complexity of it. I love it because what happens now next is that pharma and biotech companies are starting to work on early cancer drugs, but we call it early interception. It's a very different ballgame in what drug development needs to look like in the super early, maybe even precursor stages. In addition to the $1,000 question that you just asked, I would say, "Hey, what are you willing to do if your risk of developing that bad cancer is x?” That's a whole other fun conversation and maybe we should have that at some point because I think it's an important discussion.

Saed Sayad 54:30

On target.

Rick Stanton 54:35

Can I get access to your services and interpretations? I mean, it sounds to me like I've got decisions that would benefit, and I live in Southern California. Hang out with you guys and take my blood?

Peter Kuhn 54:55

This is all still at the research level. The question is, "What is actionable?" Back to John's comments on the Wild West, sometimes it's actually quite fun. If we know our way around the Wild West, we can navigate it. But do I feel pretty good about what we're doing? I do feel pretty good about it. But would I want to guide this process? Absolutely not. It's to be guided by the physician. Rick, in your particular case, I think the first step would be to have the conversation with your oncologist and play through the entire conversation, through all of the iterations of the what ifs. What is the potential state of the disease? How many alternatives are there right now? What is it that we might find and what kind of action would that trigger? I think that would be an important conversation to have. We are always happy to engage in that conversation, but we would never ever, ever, because we are Research Use Only. We would never say, "Oh, yes, I've got a solution for you" because that would be wrong.

Rick Stanton 56:24

I'm at UCLA, but I'm about to see Dr. T at Providence, St. John's...

Peter Kuhn 56:37

Who is your physician at UCLA?

Rick Stanton 56:40

Dr. Shen. I'm only his patient because I signed up for an Arcus PD-1 adenosine inhibitor. I'm on a clinical trial. The only reason is that he is running the clinical trial.

Peter Kuhn 57:03

We do quite a bit of work with UCLA. We just don't talk about it publicly.

Rick Stanton 57:17

My point is real quick. I failed the taxane and Dr. Shen said, "Okay, well, what do you want to do?" He's very much, "What do you want to do? You are a smart guy, Rick." I used to work at Amgen for 17 years, by the way. I said, "Well, I want to live. What do you think?" And he said, "If you go to 10 oncologists, you're going to get 10 different next therapy choices." So that wasn't very comforting. At the City of Hope Tanya Dorff said, "Well, you've got a lot of options on the table. You have a CDK12 driver that may be a PARP responder." It's a gray area, but she said in the event of a tie, I would take evidence that would point one way or another. That opened my mind. I've been working with Nik Schork in a PEOPLES protocol at TGen, where they're trying to open access to Research Use Only assays.

Peter Kuhn 58:32

Tanya is probably one of the most talented, super cool, young promising oncologists. That would be great. Great colleague and friend. We should engage there.

—

Recommended Reading

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Integration of Liquid Biopsies in Clinical Management of Metastatic Prostate Cancer - Beyond the Abstract

https://www.urotoday.com/recent-abstracts/urologic-oncology/prostate-cancer/138869-integration-of-liquid-biopsies-in-clinical-management-of-metastatic-prostate-cancer-beyond-the-abstract.html?utm_source=newsletter_10752&utm_medium=email&utm_campaign=prostate-cancer-daily

August 18, 2022

The term “liquid biopsy” encompasses circulating tumor cells (CTCs), cell-free nucleic acids (circulating tumor DNA or RNA), and extracellular vesicles (EVs) found in peripheral blood which is reflective of active metastatic sites. This minimally invasive technique throughout a treatment course would allow for sequential profiling to monitor clinical response in real time. We review the current data on the use of liquid biopsies in metastatic prostate cancer with a specific focus on evidence supporting their use in clinical decision-making.

CTC capture is accomplished using various methods including immunomagnetic enrichment, microfluidic sorting, and high content scanning.[1-4] The number of CTCs is representative of disease burden and serves as a biomarker for prognosis and response to therapy. In some studies, CTC kinetics on treatment were superior to PSA change in predicting overall survival.[5,6] CTC molecular phenotypes are also informative: For example, the AR-V7 splice variant of the androgen receptor, which is associated with therapy resistance, can be detected in CTCs by immunofluorescent staining using a monoclonal anti-AR-V7 antibody as well as by qPCR amplification of mRNA from immunomagnetically enriched CTCs.[7,8]

Cell-free DNA (cfDNA) analysis is accomplished through digital droplet PCR, beads, emulsions, amplification, and magnetics (BEAM), whole exome sequencing, or targeted

sequencing libraries.[9] Guardant360 CDx and FoundationOne Liquid CDx are FDA approved for comprehensive analysis of cfDNA.[10,11]

cfDNA can be used to detect clinically relevant DNA alterations in prostate cancer, such as homologous recombination deficiency (HRD) mutations. HRD mutations suggest a poor response to ARPIs as well as poor cancer-specific survival.[12] Conversely, they predict response to poly adenosine diphosphate-ribose polymerase inhibitor (PARPi) treatment; therefore determining HRD mutation status is clinically imperative in those with metastatic disease.[10] Serial cfDNA is also useful in monitoring disease response as well as development of resistant as HRD reversion mutations have been identified in patients with progression on PARPi treatment.[13]

Based on the evidence provided by a number of phase 3 trials supporting the clinical validity of liquid biopsies as biomarkers for prognosis and treatment response, we propose an algorithm for incorporating the use of liquid biopsies in the treatment of metastatic prostate cancer, summarized in the diagram below (Figure 1).

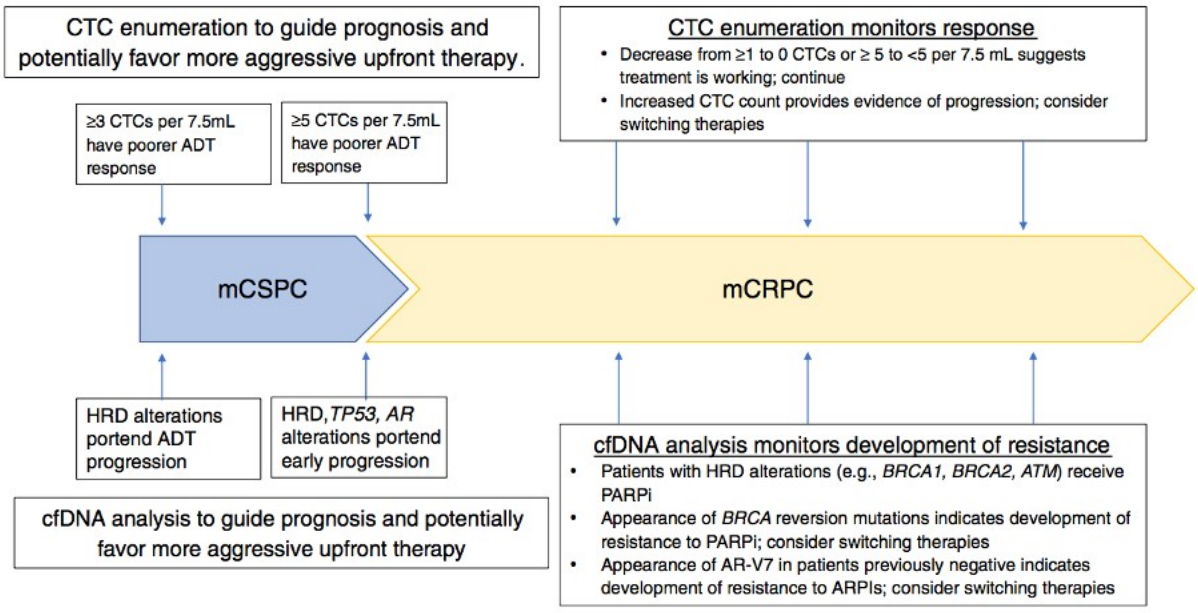


Fig. 1 Proposed model for incorporation of liquid biopsies into clinical management of metastatic prostate cancer

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Liquid biopsy approaches are rapidly evolving, with new capabilities such as highly multiplexed protein profiling enabled by technologies like single cell mass cytometry and evaluation of DNA methylation patterns by whole genome sequencing of bisulfite converted cfDNA.[14-16] Combination of multiple liquid biopsy techniques may provide a more comprehensive and nuanced molecular picture of an individual’s disease.[17] Our understanding of the potential utility of liquid biopsies continues to evolve, and with further clinical validation liquid biopsies will become an integral tool in the standard of care treatment of metastatic prostate adenocarcinoma.

Written by: Varsha Tulpule, Gareth J. Morrison, Mary Falcone, David I. Quinn, Amir Goldkorn

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NIHMSID: NIHMS837947
PMID: 27262168

Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer

Howard I. Scher, MD, David Lu, PhD, Nicole A. Schreiber, BA, Jessica Louw, BS, Ryon P. Graf, PhD, Hebert A. Vargas, MD, Ann Johnson, MS, Adam Jendrisak, MBA, Richard Bambury, MB, BCh, BAO, Daniel Danila, MD, Brigit McLaughlin, BS, Justin Wahl, BS, Stephanie B. Greene, PhD, Glenn Heller, PhD, Dena Marrinucci, PhD, Martin Fleisher, PhD, and Ryan Dittamore, MBA

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

—

https://www.nature.com/articles/s41586-022-04975-9

- Article
- Published: 20 July 2022

Deep whole-genome ctDNA chronology of treatment-resistant prostate cancer

- Cameron Herberts,
- Matti Annala,
- Joonatan Sipola,
- Sarah W. S. Ng,
- Xinyi E. Chen,
- Anssi Nurminen,
- Olga V. Korhonen,
- Aslı D. Munzur,
- Kevin Beja,
- Elena Schönlau,
- Cecily Q. Bernales,
- Elie Ritch,
- Jack V. W. Bacon,
- Nathan A. Lack,
- Matti Nykter,
- Rahul Aggarwal,
- Eric J. Small,
- Martin E. Gleave,
- SU2C/PCF West Coast Prostate Cancer Dream Team,
- David A. Quigley,
- Felix Y. Feng,
- Kim N. Chi &
- Alexander W. Wyatt

“Liquid Biopsies” (Peter Kuhn and Stephanie Shishido) [#23]

Nature

Abstract>

Circulating tumour DNA (ctDNA) in blood plasma is an emerging tool for clinical cancer genotyping and longitudinal disease monitoring[1]. However, owing to past emphasis on targeted and low-resolution profiling approaches, our understanding of the distinct populations that comprise bulk ctDNA is incomplete[2,3,4,5,6,7,8,9,10,11,12]. Here we perform deep whole-genome sequencing of serial plasma and synchronous metastases in patients with aggressive prostate cancer. We comprehensively assess all classes of genomic alterations and show that ctDNA contains multiple dominant populations, the evolutionary histories of which frequently indicate whole-genome doubling and shifts in mutational processes. Although tissue and ctDNA showed concordant clonally expanded cancer driver alterations, most individual metastases contributed only a minor share of total ctDNA. By comparing serial ctDNA before and after clinical progression on potent inhibitors of the androgen receptor (AR) pathway, we reveal population restructuring converging solely on *AR* augmentation as the dominant genomic driver of acquired treatment resistance. Finally, we leverage nucleosome footprints in ctDNA to infer mRNA expression in synchronously biopsied metastases, including treatment-induced changes in AR transcription factor signalling activity. Our results provide insights into cancer biology and show that liquid biopsy can be used as a tool for comprehensive multi-omic discovery.