“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Brad Power and Brian McCloskey
October 5, 2022

“If we step on Starship Enterprise to the future, the diagnostics that are going to combine these (attributes) are, in essence, spatial phenomics. These (diagnostics) will combine multi-marker cell phenotypes with tissue contexts.” – Keith Wharton

“This technology is at that stage where there's a lot of promise. There's this mountain of evidence that suggests the immune system is doing something (to influence cancer), but nobody's ready to place the bets on (assay) cut-offs to generate the evidence.” – Keith Wharton

Meeting Summary

Engaged cancer patients want access to new testing technologies that might help guide their treatment. These patients and diagnostic companies developing the new tests have a shared interest in accelerating adoption. But the diagnostic companies face a number of challenges, including modeling, calibration, regulation, and collaboration.

Keith Wharton, MD, PhD, FCAP, and VP, Medical Director, Ultivue, led a discussion on "Bringing Novel Immune System Tests from Research to Clinical Use". His experience in research and industry gives him a wide range of experience in seeing how new medical technologies come to market.

What does the future hold for diagnostics guiding cancer treatment? For example, how will cutting-edge technologies help cancer patients better understand their tumor microenvironment and identify drugs that will better target their cancer?

In the future, tissue images will be integrated with many diagnostic modalities (gene sequencing, RNA sequencing, proteomics, spatial transcriptomics, and single cell analysis) in a pathology workflow leading directly to treatment guidance. Biomarkers will be identified which will select which targeted treatments might work best, and predict patient outcomes. Multiple fluorescence stains will be applied to tissue and stacked to visualize the tumor and its microenvironment. Artificial intelligence will be applied to the images and do a better job than a pathologist at interpreting them and predicting patient prognosis and drug response.

What is the current state of analysis of the tumor microenvironment?

Most analysis of the tumor microenvironment (spatial analysis) is in an early stage of research. It is seldom being used to guide decisions for individual patients. Fluorescence imaging technologies (a sample is labeled and then emits a distinctive light) are being used today to find good and bad cells in retrospective analyses of cohorts of patients. With current technology, they are expensive. Spatial analysis of the tumor microenvironment is rarely being combined with genetic targeting of drugs.

What are the challenges for patients wishing to access a future vision of integrated testing?

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

- **Modeling**: Testing can reveal that there are differences between different types of immune cells that otherwise look alike in the H&E tissue stain, but we don't know what they're doing. The stains are telling us something about the patient's tumor and immune system that needs to be understood for future therapies to be designed and to know whether they're going to work.
- **Calibration**: It’s hard enough to identify biomarkers which predict disease progression or drug response, but that’s not the end of the story. Once you have a potential biomarker, or measurement of one or a combination of different markers, you also need to define what the cutoff is. For example, it may be easy to identify a biomarker which helps you see the extreme cases of whether a tumor is hot (with a brisk host immune response) or cold (with the relative lack of a host immune response), but the area in the middle between these extremes is where false positive and false negative diagnoses may occur, leading to errors in either recommending or denying treatment, and potentially causing harm (and professional liability). Another example is HER2, which for a couple decades was measured as positive or negative to predict if a particular drug would be effective. Then a new drug came along (“Enhertu”) that works for lower levels of HER2, and the diagnostic needs to either be redeveloped, or at least recalibrated.
- **Regulation**: There are many regulators which review various aspects of new tests coming into standard clinical use. The FDA regulates the testing devices. If there's a clinical trial, IRBs must sign off on it. ISO 13485 is a quality standard for manufacturers. If a lab buys stuff from a manufacturer, they want to have good manufacturing processes (GMP) and have an international certification. The patient signs documents such as an informed consent. Typically, labs are regulated by CAP/CLIA. CMS regulates reimbursements, and you don't get reimbursed if you're not accredited. Joint commissions regulate hospitals and other health care facilities. Practitioners must have a medical license and are regulated by boards and licensed by the Federation of State Medical Boards. All of these regulatory bodies should have the same goal: they want patients to be safely treated, but they have narrow, specialized roles and responsibilities.
- **Collaboration**: An integrated analytical testing workflow for spatial phenomics requires multiple steps with modular components that in general are not standardized or easily interoperable. In contrast, diagnostic tests require system lockdown, standardization, strict and expensive testing on reproducibility, and robust performance. No one company, lab or manufacturer, can do all the tests and analyses, and all of their workflows are different. Different companies have different strengths, different business models, and different pressures to survive and grow. And there's no incentive to standardize. If you're an academic doing research, your job is to carve a moat around you so that your findings are novel, and nobody else does what you do. You're the world leader. It's the same thing in biotech. You need market exclusivity to enhance your business model. You don't want to have any competitors. Thus, all the incentives for all the players are against collaboration. Pharma companies pursuing a new drug target don't agree on biomarkers: one company thinks that this marker is better to identify this cell type, and then you go to another company, they think a different marker is better. Standardization of marker profiles to identify particular good or bad cell types is lacking and sorely needed to drive standardization of methods to identify them.

What can be done to overcome the barriers to rapid adoption of diagnostics of the tumor microenvironment?

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

There is a huge opportunity in real world evidence studies because the vast majority of people who get cancer don't participate in clinical trials. It's from these real world patients we have the opportunity to learn from, but we are not properly investing or studying them.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Meeting Notes

> *The information and opinions expressed on this website or platform, or during discussions and presentations (both verbal and written) are not intended as health care recommendations or medical advice by Cancer Patient Lab/Prostate Cancer Lab, its principals, presenters, participants, or representatives for any medical treatment, product, or course of action. You should always consult a doctor about your specific situation before pursuing any health care program, treatment, product or other course of action that might affect your health.*

SUMMARY KEYWORDS

tumor, technology, patients, cells, called, pathologist, lab, diagnostics, tissue, biopsy, phenotypes, cd8 cells, spatial, her2, tests, marker, stain, drug, fda, study

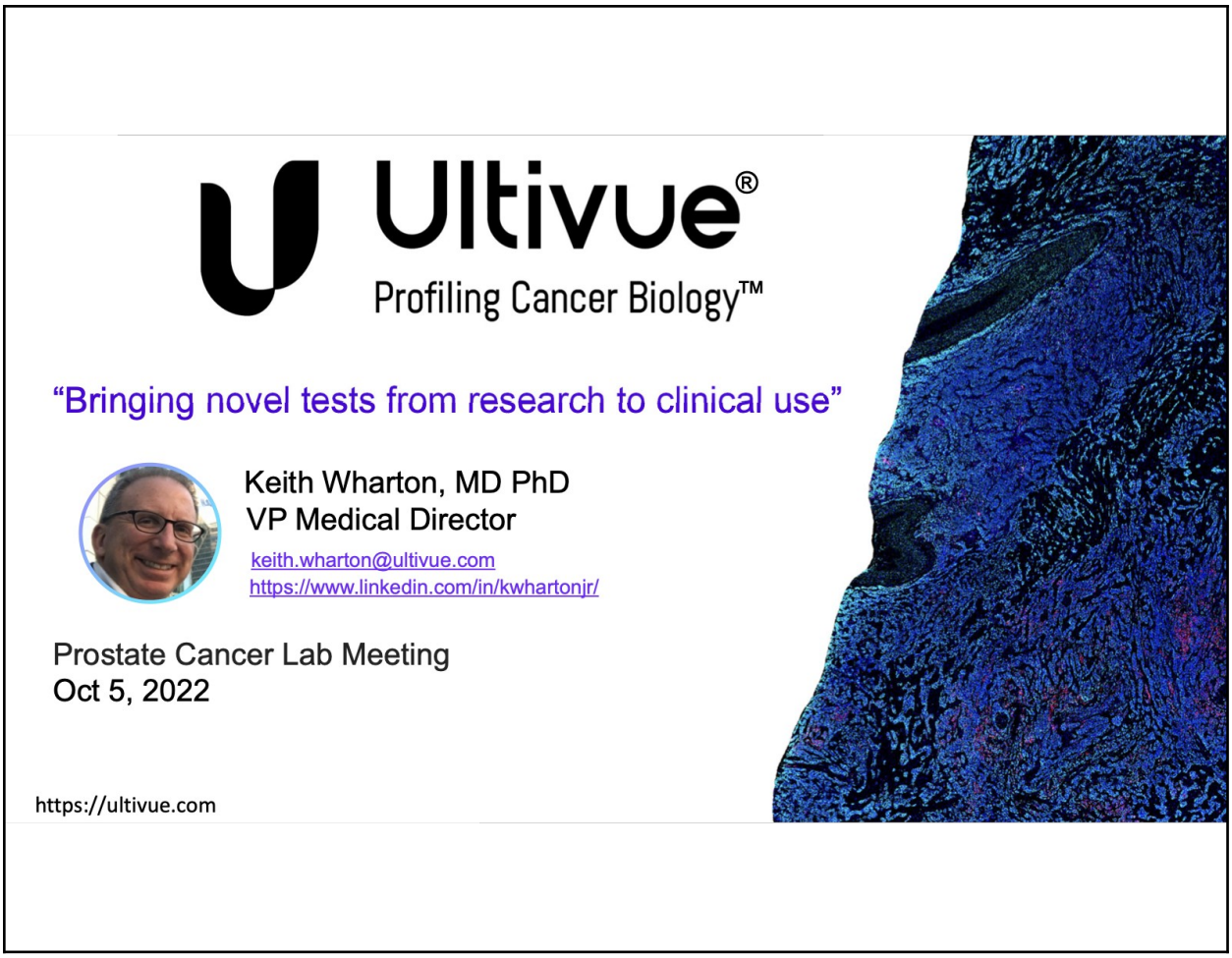
SPEAKERS

Keith (78%), Brian (17%), Rick (3%), Anonymous Caregiver (2%)

Brian McCloskey:
Welcome everyone to the Prostate Cancer Lab. Today we're honored to have Keith Wharton with us. Keith is the VP and medical director of Ultivue. Ultivue discovers, manufactures, and uses highly sensitive DNA barcode technology-based tissue multiplex immunofluorescence staining solutions, immuno-oncology research, and biopharma therapy development, in anatomic pathology laboratories.

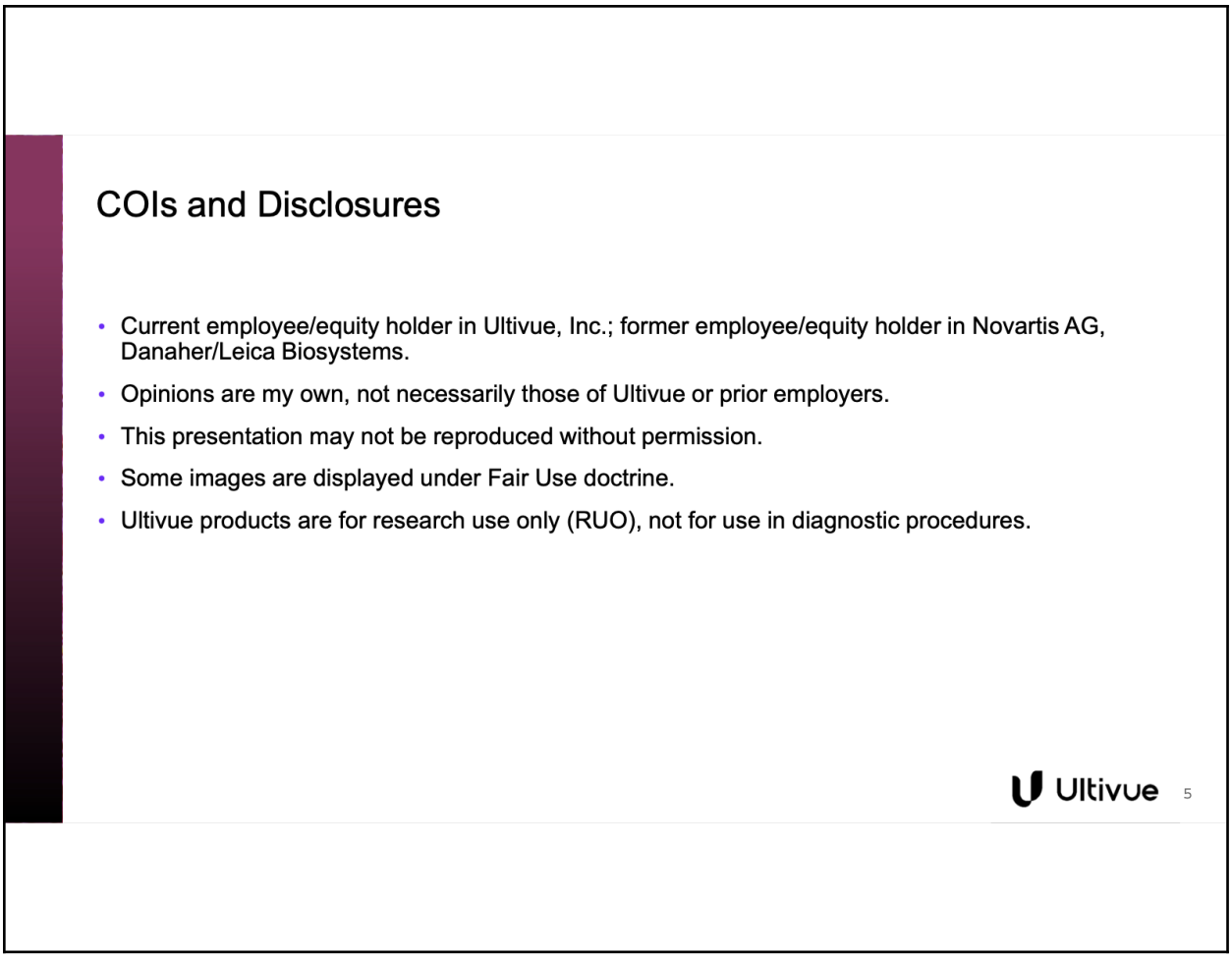
Keith, we're going to have to start off with a translation of that description of Ultivue.
We're also going to talk about what goes on behind the scenes at companies like Ultivue in developing these new testing technologies, what's the process by which these new technologies go from “Research Use Only” to being available for clinical guidance, and how patients like us can get access to these technologies to support our clinical guidance.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



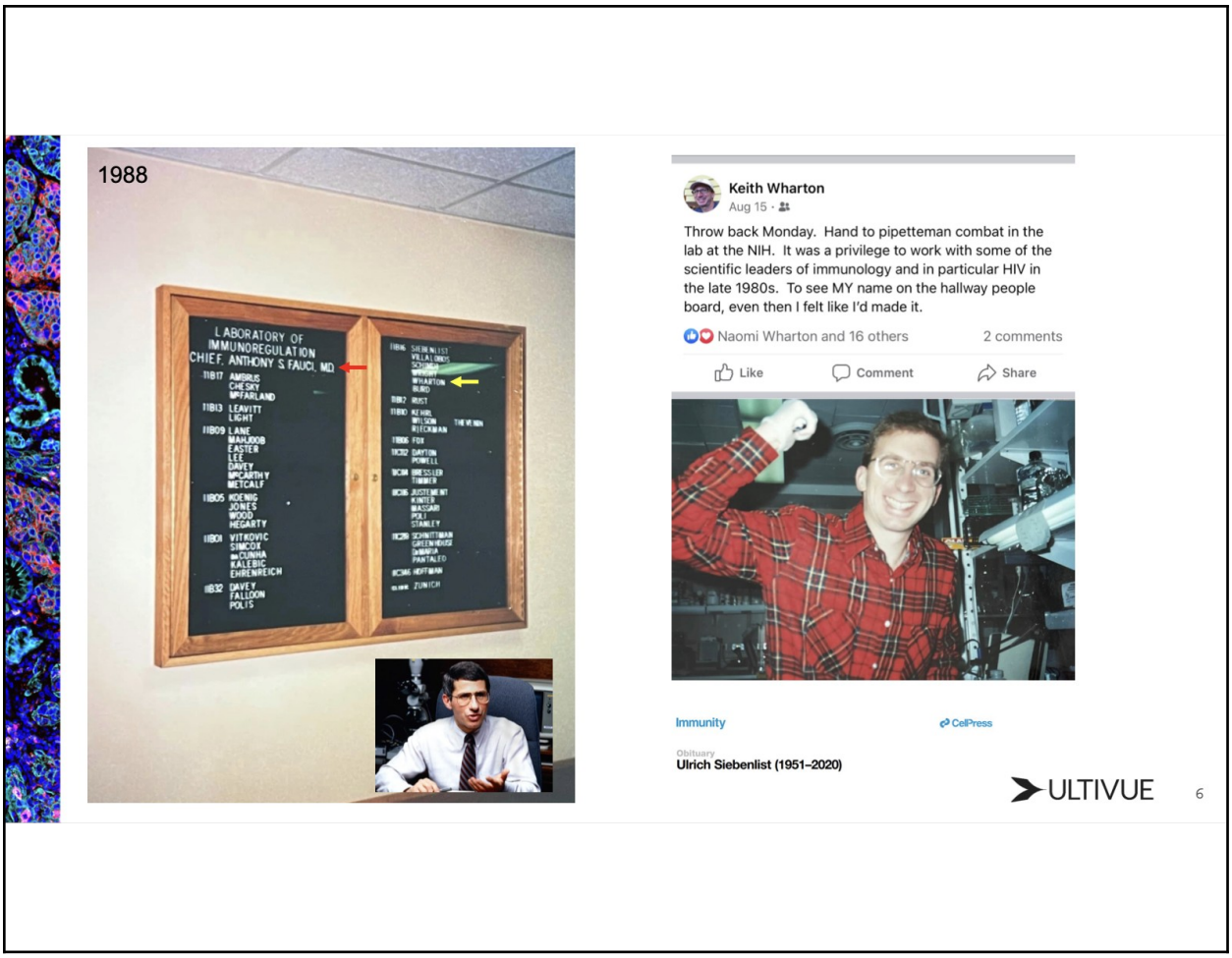
Keith Wharton:
I consider it a privilege to speak to a group like this. I know you have tremendous personal needs.
I’d like to share the experiences I've had, and the decisions I've made going through my training in the field, starting at the beginning,

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



First some disclosures: the main one which you need to be aware of is that everything we do is research; it's not producing diagnostic procedures.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



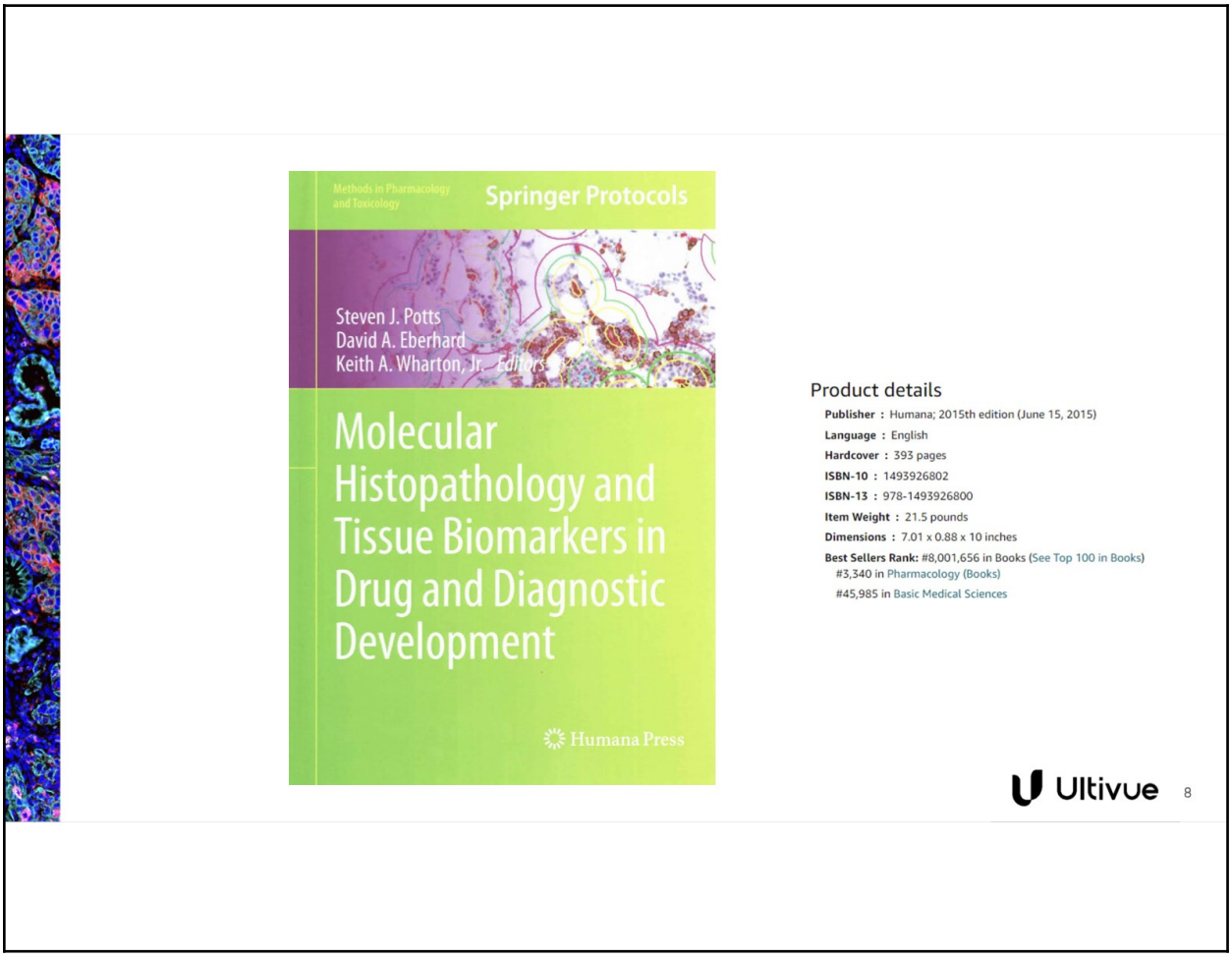
I wanted to tell you a little bit about my background, before I go into what we do.
I know we all have a journey through life. We are all confronted with decisions to make at various points in our life as to which way we go. I was recently thumbing through some old photographs and found a photograph I took when I worked at the NIH. This was after I started med school, and before I started a PhD, 34 years ago. I had an opportunity to work at the NIH in the Fauci lab, and you can see what he looked like back then, and what I looked like back then. This was a personal post to some Facebook friends. I look back and think, “Wow, what a real privilege it was to work in this environment with these people at this stage in history.” I took a picture of the wall board. To see my name on that board, I felt like I had to made it. The person I worked with the most, Ulrich Siebenlist, passed away a couple of years ago. In his obituary, he was termed “the scientists’ scientist.” He's one of the people who solidified my love of molecular biology, reductionist approaches to biology and disease, and influenced the choices I made.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

A couple more personal slides around my journey, talking about the epic game of Life. We go through life, make decisions, and try to make the best decisions. I started out studying engineering. I did a lot of computer programming. That strange thing in the upper left corner is a punch card. I decided to go to med school, but really got frustrated with writing Fortran (on punch cards). I got fascinated with molecular biology and ended up, believe it or not, after working in the Fauci lab, studying fruit flies. You might think, “He's in med school and studying fruit flies? What the hell's wrong?” This is what my family was asking me. “What the hell are you doing?” It turns out that that system was the only way at that point in history you could study the function of genes in entire organisms, and then you would have some hope or promise that what you're studying in fruit flies actually works the same in mice and maybe humans. I chose to study pathology, which you can see by the picture of the old time pathologist, Quincy on TV, and I ran a mouse lab eventually.

I show a toolkit because the work I chose to study at that point was before the genome was cloned. It was really foundational. There was a set of genes, and I picked one of them to study for 12 years of my life that was evolved almost a billion years ago in the history of life, and has been present in some form or another and is distributed in all animal life on the planet. Among our 18,000 to 20,000 genes, there's a subset that are really old, really critical, and have been modified in evolution to create a human versus a mouse versus a fly. These are the toolkit

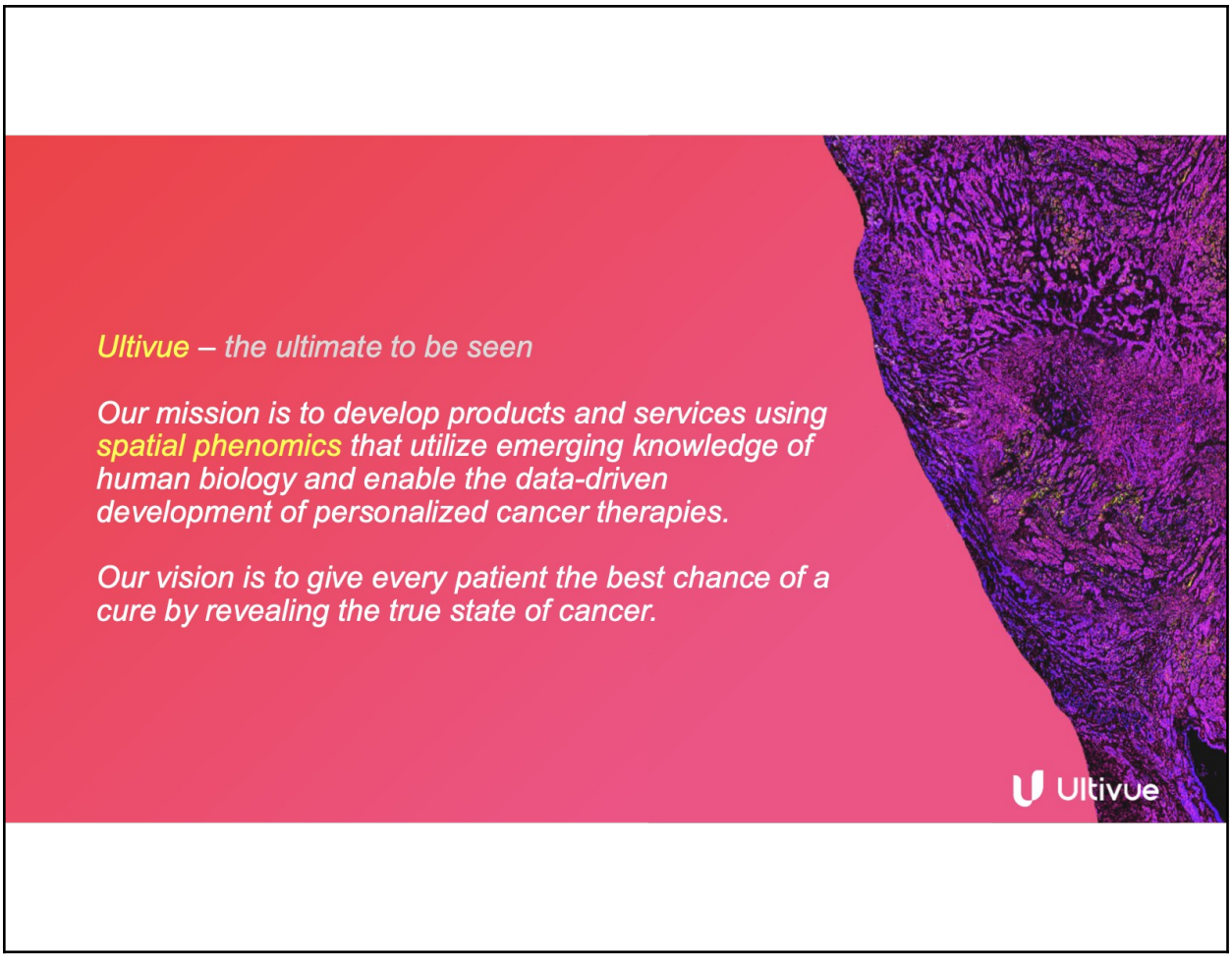
genes. That to me was the coolest thing to do. I felt that however you studied that it would have relevance to disease downstream. That worked for a while, but then I jumped over to the other side of the board on the right, to industry. I've been fortunate to work at drug development companies Biogen and Novartis, and at Leica, which is an anatomic pathology lab provider. I was a representative to a couple of organizations and then joined Ultivue a couple of years ago. That's my journey. As I reflect on my life in industry, I've really focused both on pathology in drugs and diagnostics.



I wrote a book with some colleagues on drugs and diagnostics, published six or seven years ago. Another mouthful: “Molecular Histopathology and Tissue Biomarkers in Drug and Diagnostic Development.” The idea with tissue biomarkers is that the information about the disease comes from the tissue. To a pathologist that is germane and obvious, but it's not obvious to the world.

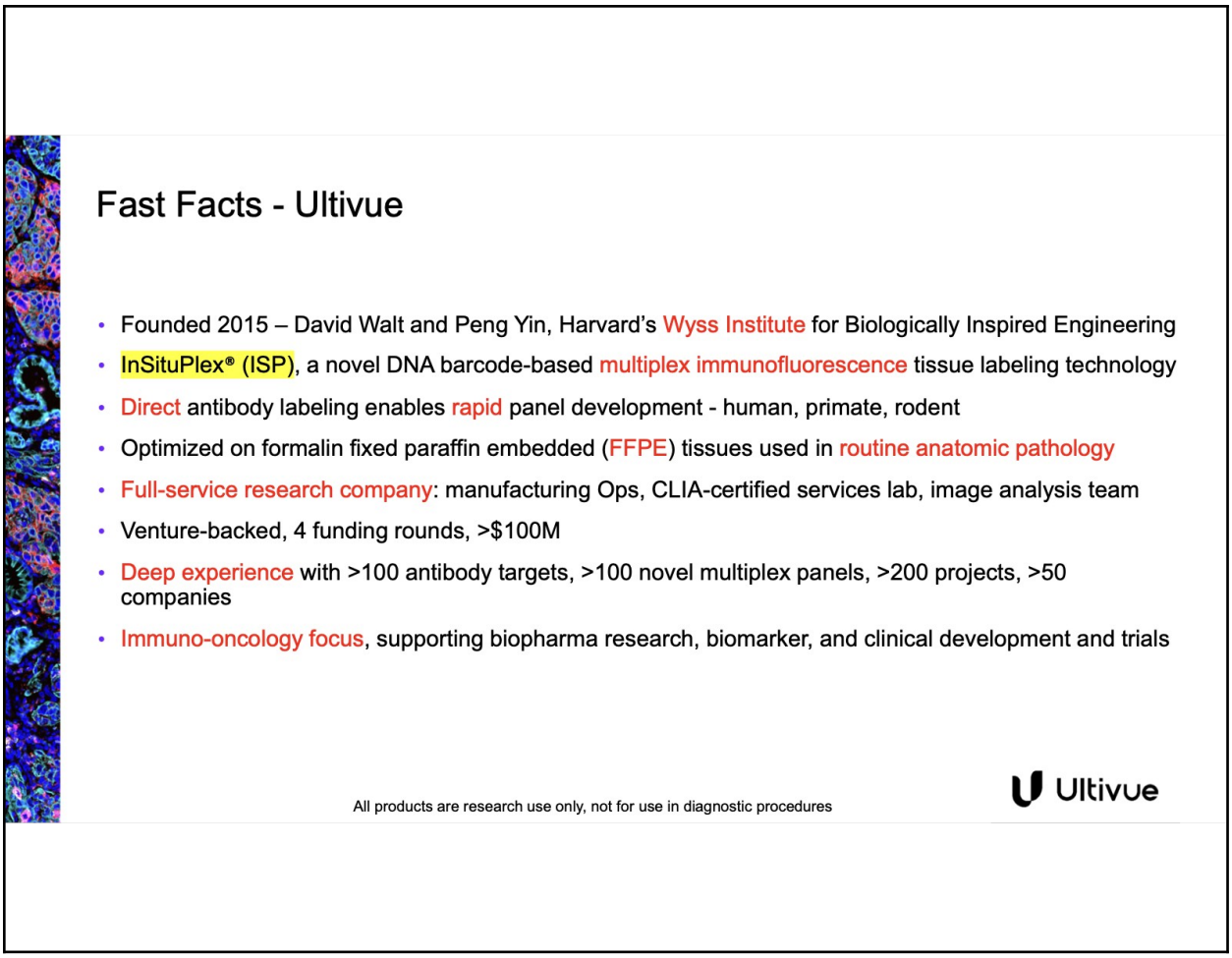
This is an important part of disease diagnosis, and to work with a few dozen folks to aggregate knowledge and publish it was a real privilege.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



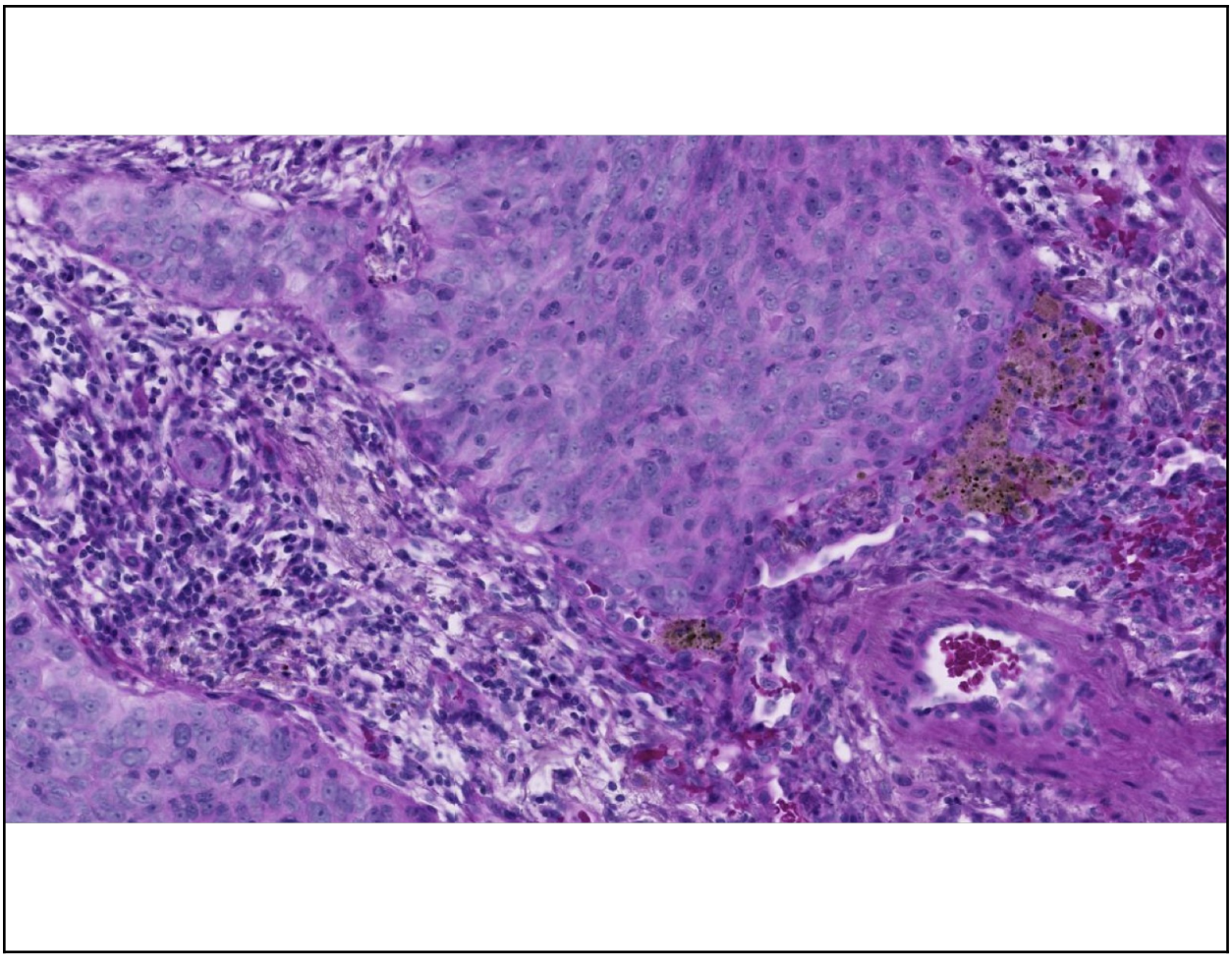
Ultivue has a mission, if you look at our name, "vue" is French for “to be seen”. It's the ultimate “to be seen”. Our mission is to develop products and services using spatial genomics and the emerging knowledge of biology to enable data-driven development of personalized cancer therapies. Not tomorrow, of course, but down the road. Our vision is to give every patient the best chance at a cure by revealing the true state of their cancer. Our vision is in the future. It's not there yet.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



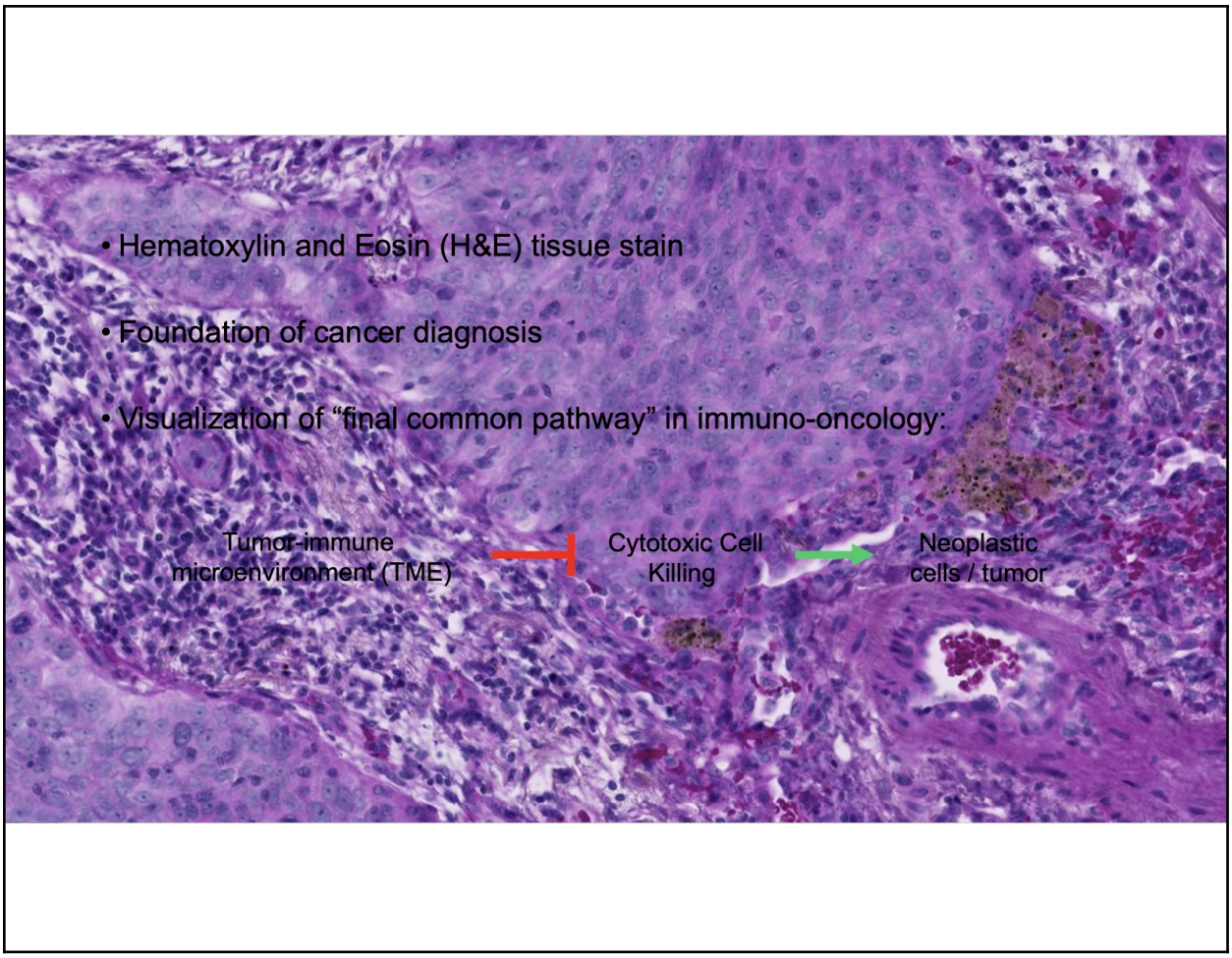
Some fast facts: We're a spinout of Harvard's Wyss Institute. We have InSituPplex, which is a labeling technology. I'll show you some pictures of that. It has some advantages. Our primary customer right now is a pharma company that wants a custom panel made. We do all of this work in house. We have experience with lots of different protein targets, and we've made lots of panels, and we've done lots of projects. We focus on immuno-oncology. There are lots of applications of this technology to biopharma in fixed tissue analysis.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



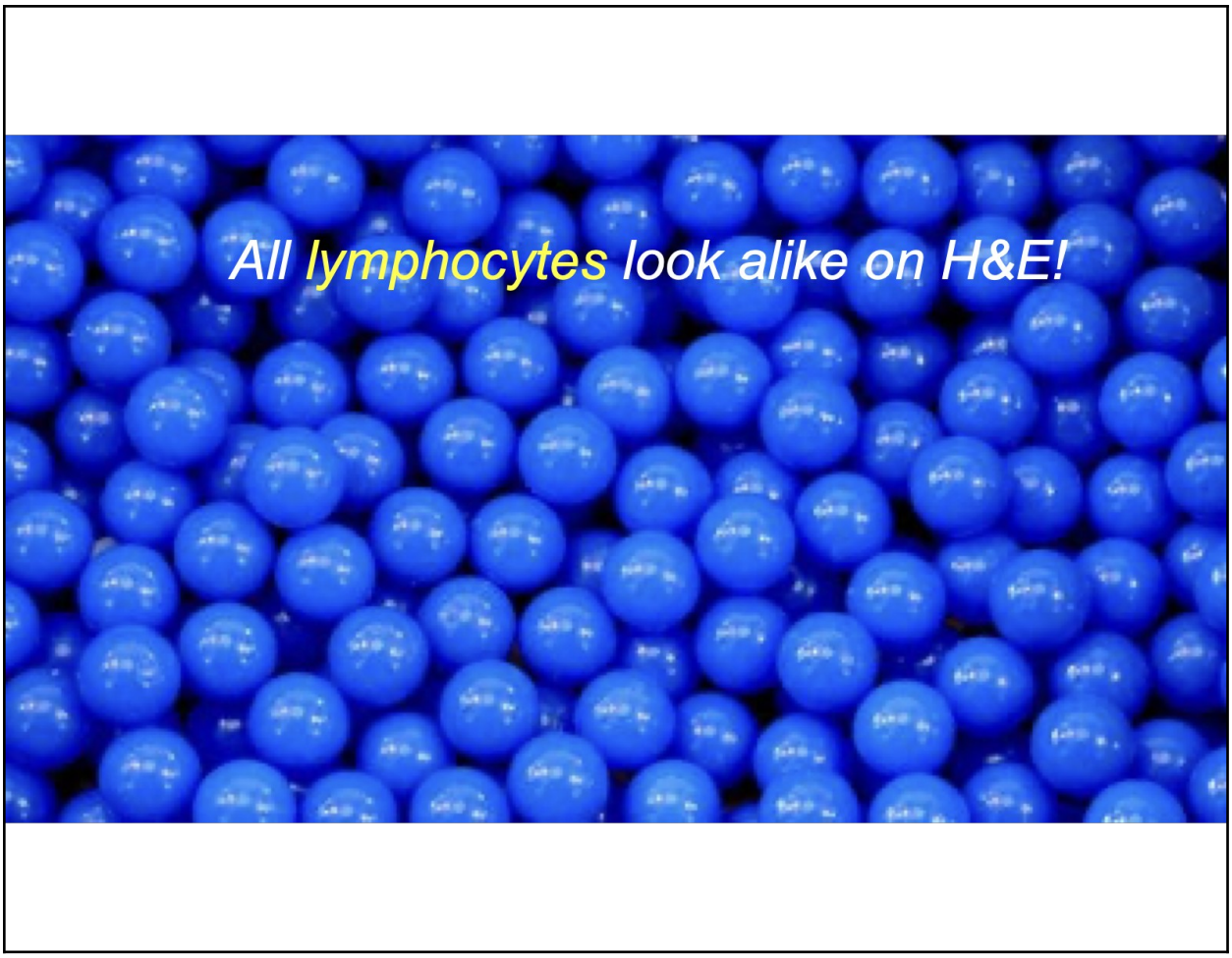
I'd like to start out by showing what some might consider beautiful, but maybe a pathologist would consider ugly. This is an H&E section of a typical cancer. This is lung cancer, in which you can see an aggregate of tumor cells. Between the aggregates of tumor cells is a huge number of little blue cells and so called stroma. There's a blood vessel, and there's a hemorrhage. As a pathologist, I can look at this and see what's going on. There is tremendous complexity inside every tumor, whether it's from the prostate or any site in the body. For scale, these little blue balls are about 5 to 10 microns in diameter. Which means that if you line up a 100 of these next to each other, it would be about a millimeter. The scale is super small. It's incredible how small cells are. You can only see them in the microscope.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



This is an H&E tissue stain – the foundation of cancer diagnosis. This is what a pathologist looks at from the biopsy. What's becoming clear is that it's almost like a canvas to visualize a pathway to treatment in oncology. The idea here is that as the host, you have the ability in your immune system to kill the tumor. But through the tumor's evolution, in this environment, these cells off to the side, the whole area where the tumor grows, has evolved mechanisms to block your host immune system from killing the tumor. This is a super simplified view of cancer, but I think about it this way. We're doing things to make it easier to visualize cancer.

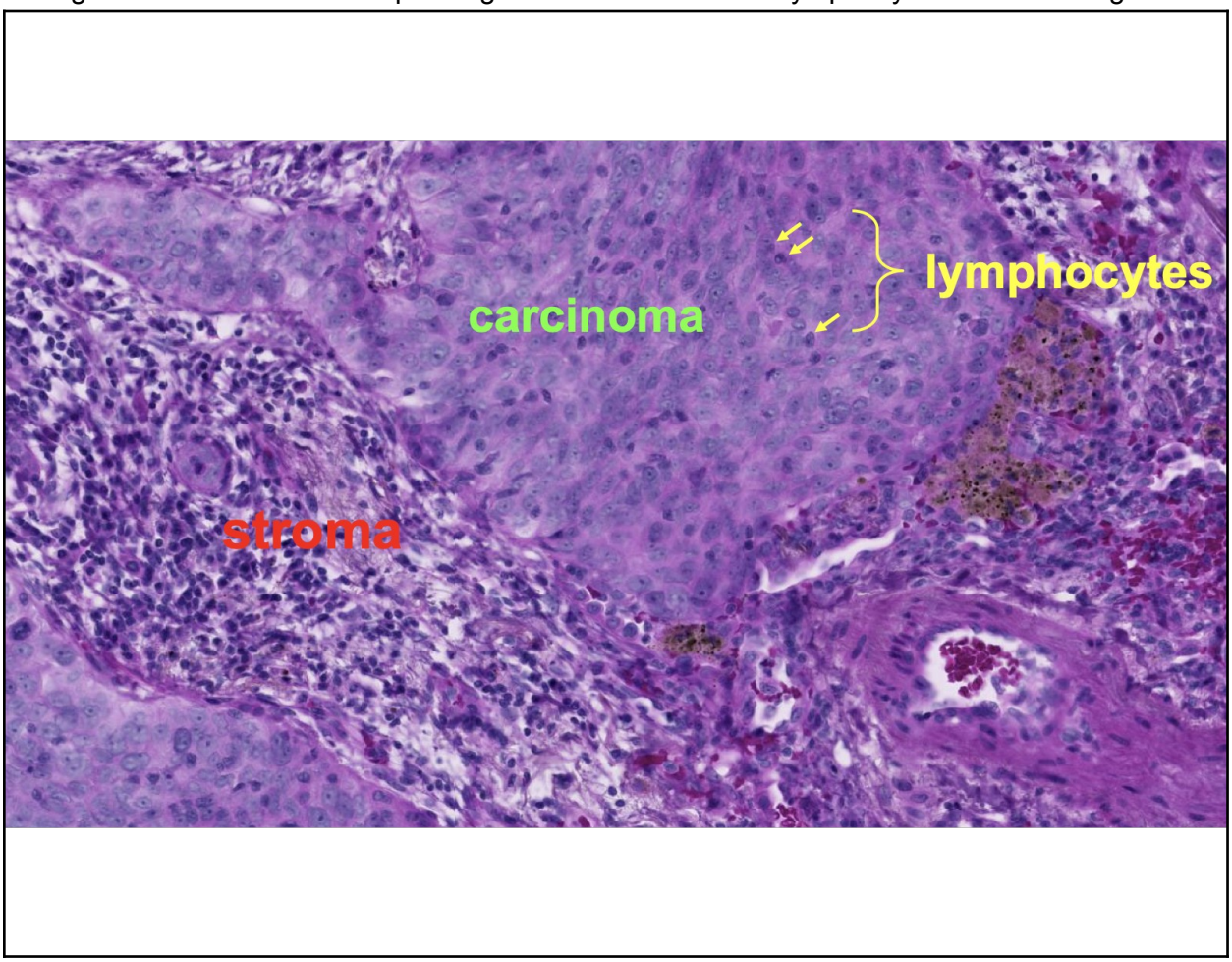
“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



The problem is, if you look at the little round blue cells you see throughout, lymphocytes, a subset of cells in the immune system, the darn things all look alike. You have probably heard of B cells versus T cells, and activated cells versus exhausted cells. These are all different identities and phenotypes. Remember I said spatial phenomics are all phenotypes of lymphocytes. The problem is with the standard stains, the standard ways we look at these tumor biopsies, the damn things all look alike. That's why I show all the little blue squishy balls.

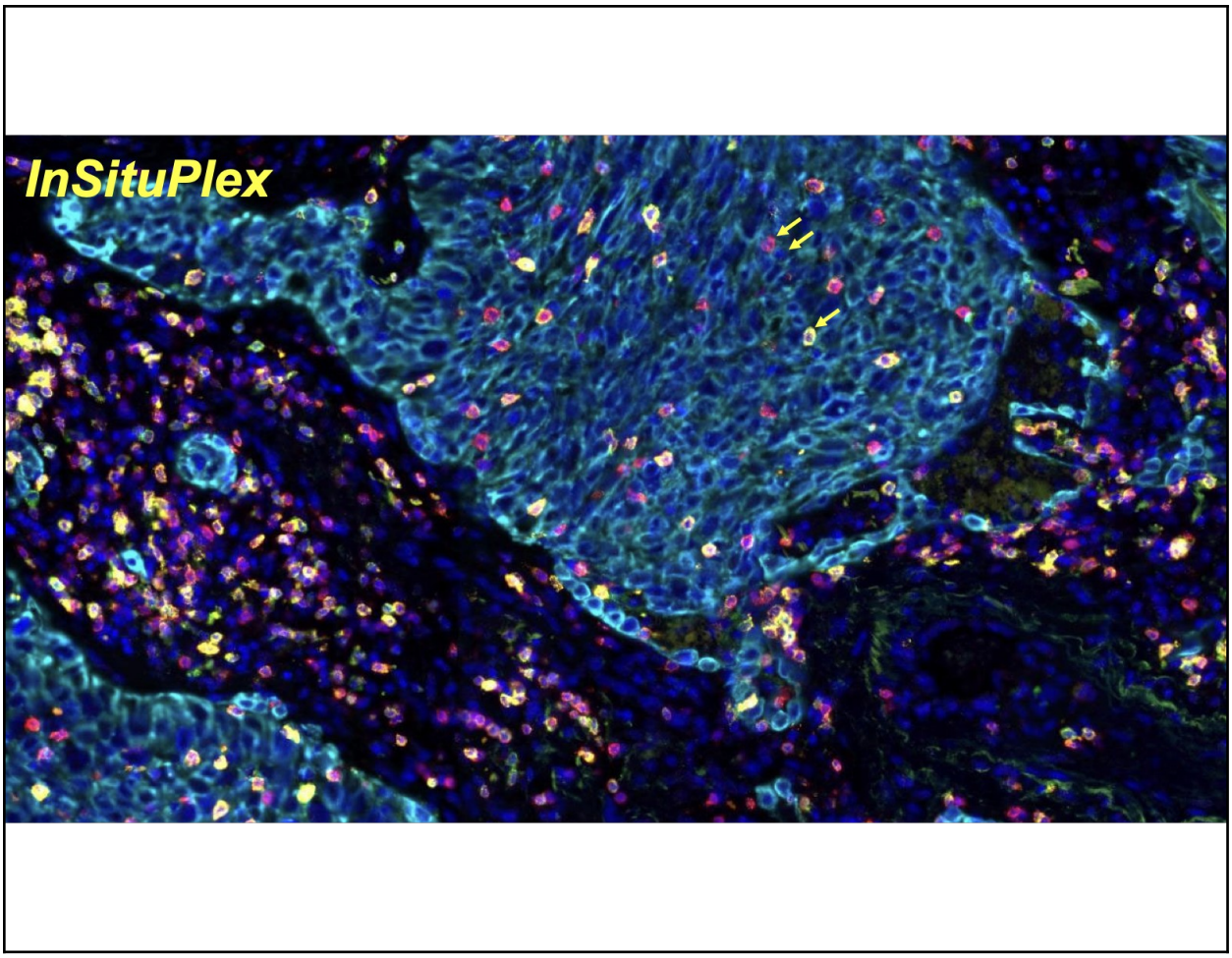
“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Let's go back to the tumor. I'm pointing at three little blue ball lymphocytes that have migrated



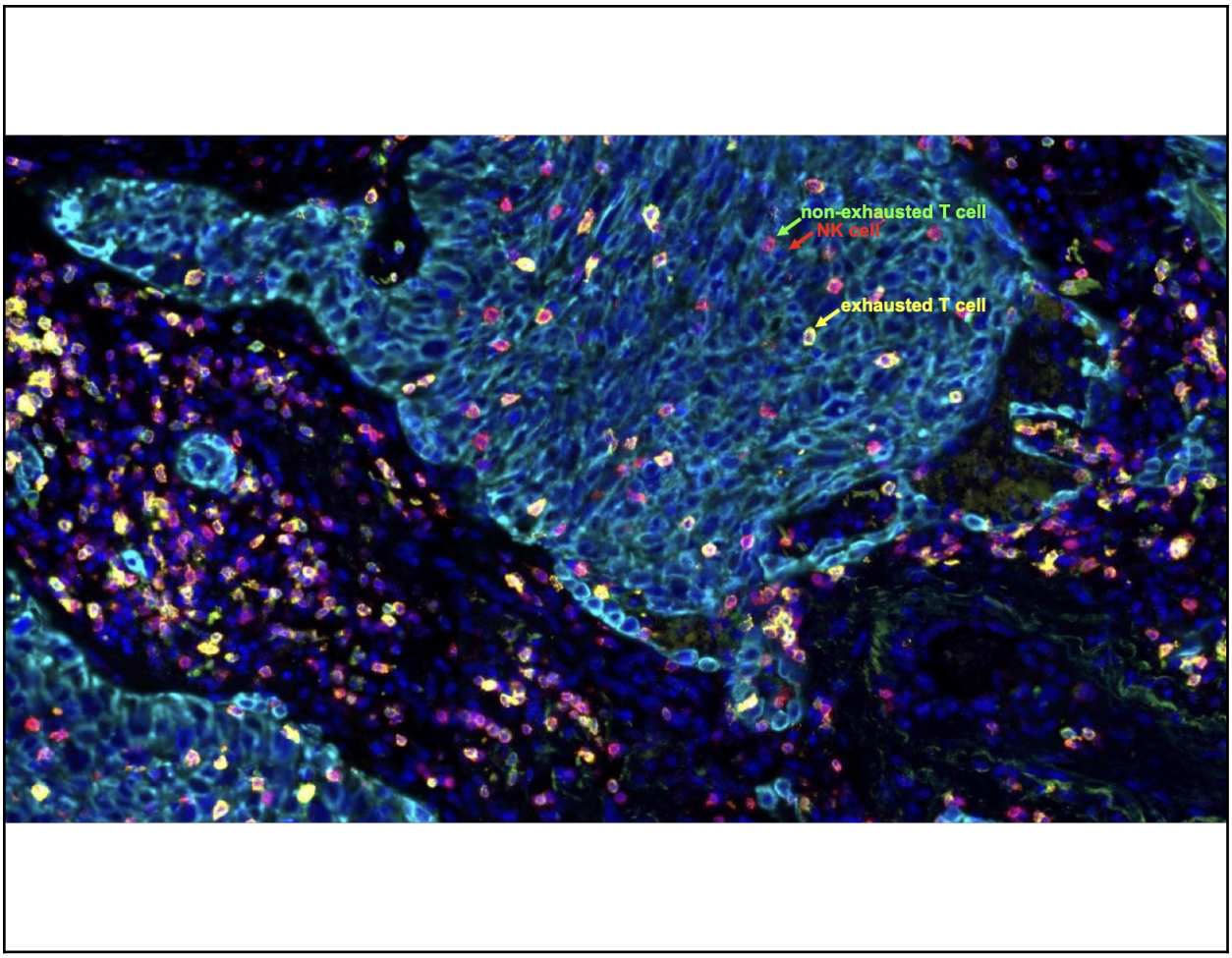
their way into the tumor. You don't know what they're doing, but they're there. I can see that they're there. Their nuclei look different from the other cancer cells. But the H&E doesn't really tell us what's going on.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



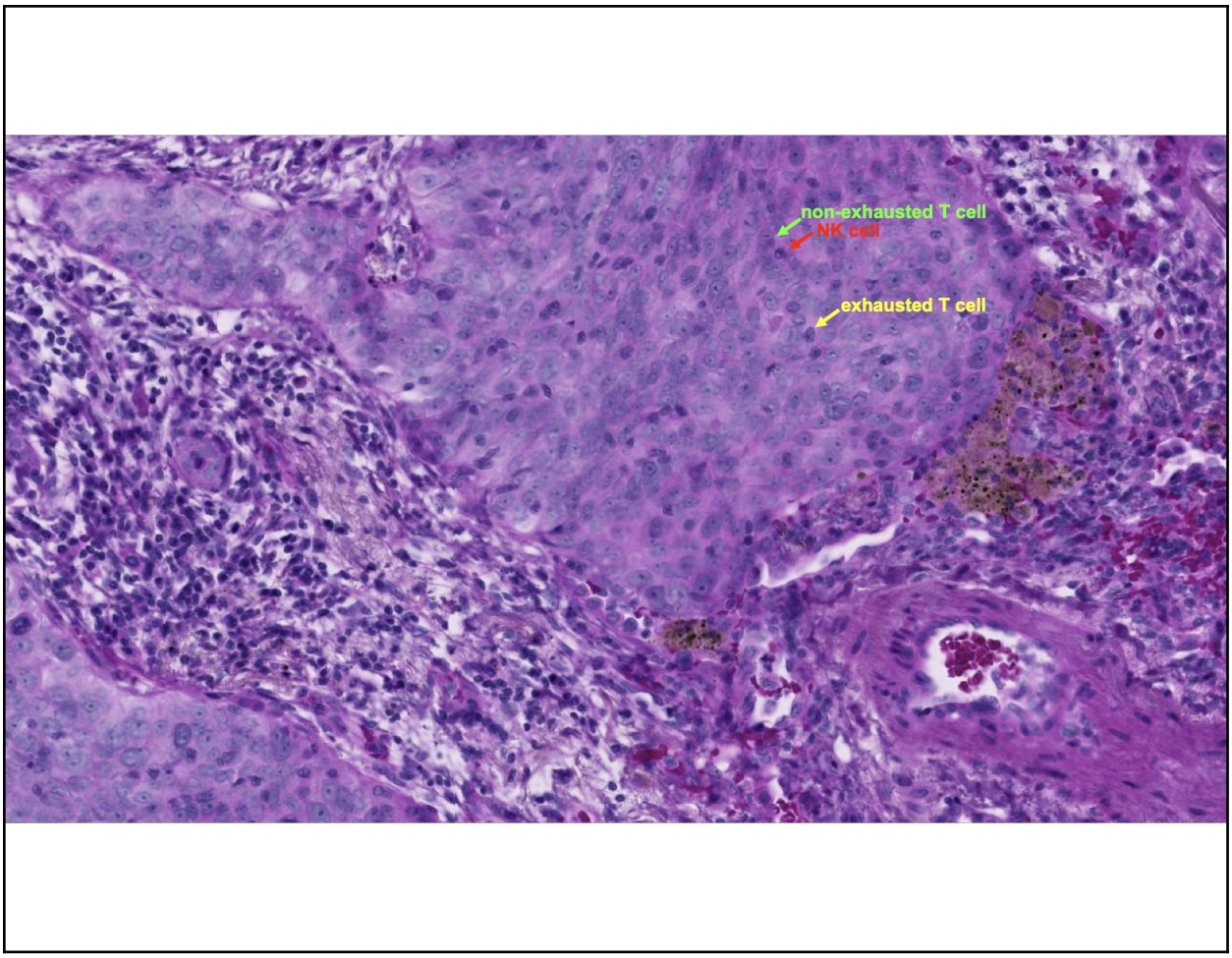
When we use our technology InSituPlex to label these cells with different markers, what we see is that they each have a slightly different color, or at least there are groups of them that have the same color and other groups that have a different color. One is labeled in red, another one is labeled in yellow, and another one's not labeled at all. It doesn't really matter what the markers are. Even though they look alike with the H&E stain, they're not alike.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



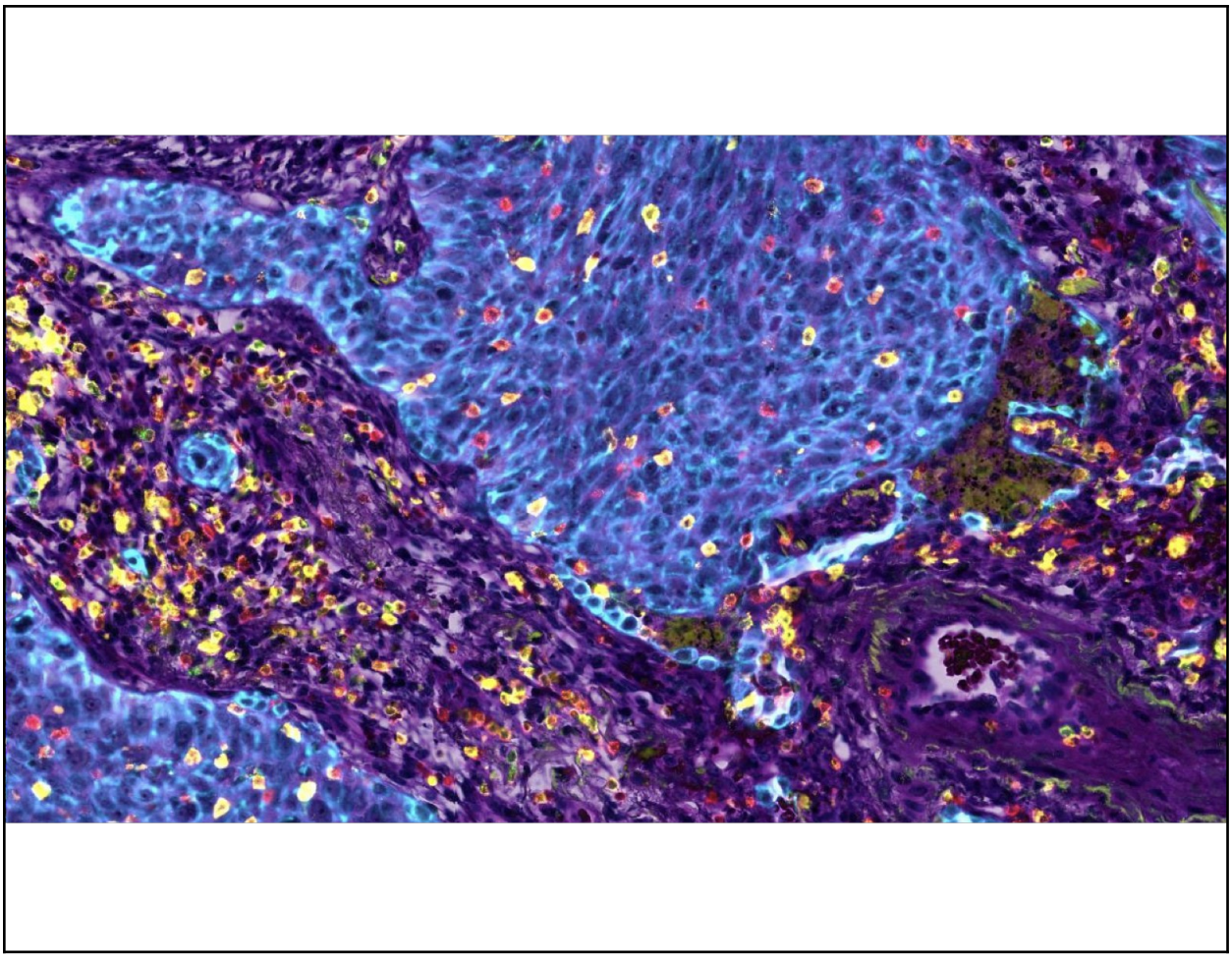
I can conclude from these markers that one is an exhausted cell. It’s not going to be effective at killing the tumor. Another one is not exhausted; it might be effective. Another one is a different type of cell altogether; it doesn't express this marker.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



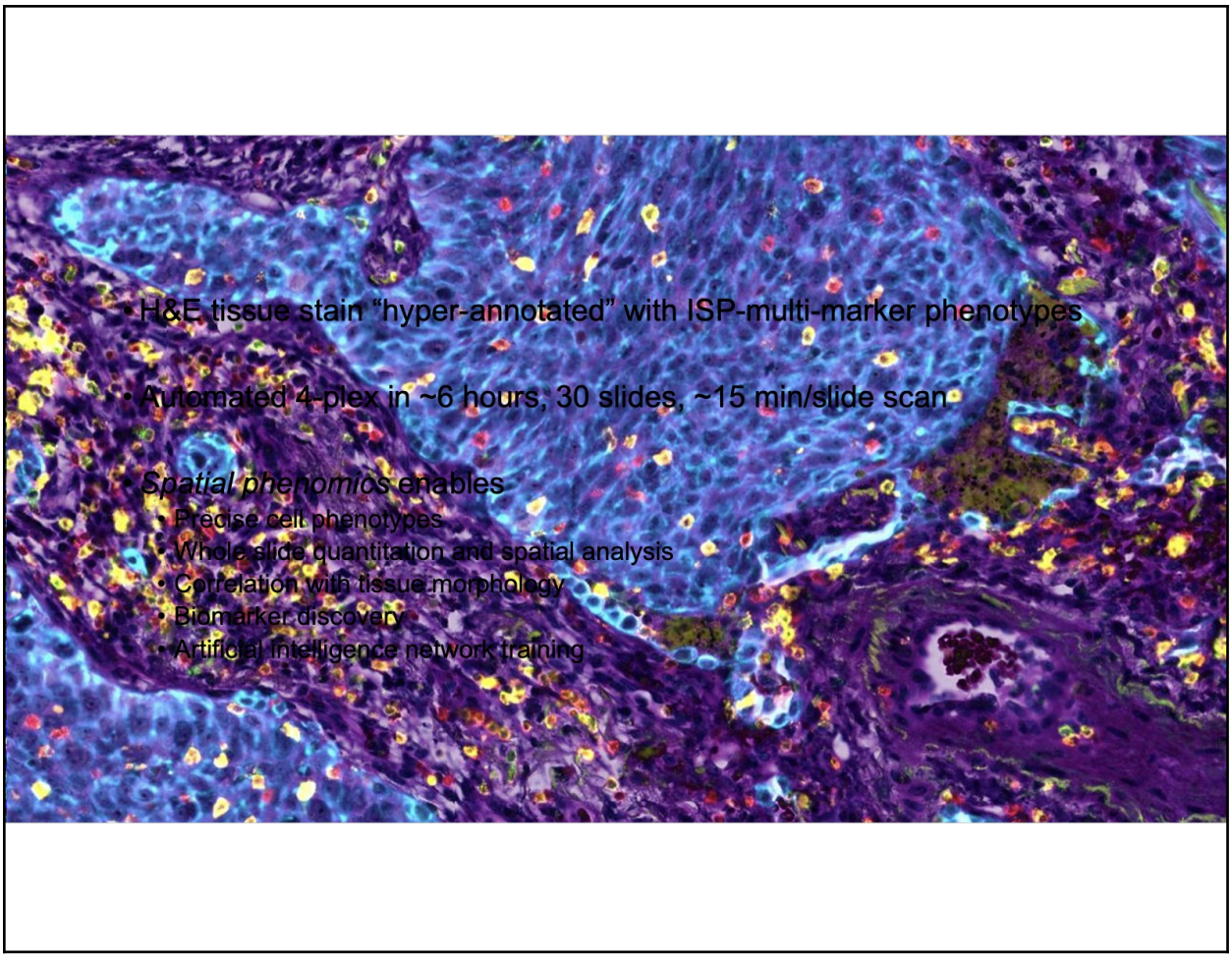
So then I go back to the H&E. We know that one is an exhausted cell, one is not exhausted, and another is an NK cell, but this image per se doesn't give the pathologist that information. We have software that stacks these images on top of each other, which gives us a beautiful data set. You could send it to the Museum of Fine Arts, and they probably would put it on the wall.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



It's telling us something about the patient's tumor that needs to be known for future therapies to be designed and to know whether they're going to work. This is a novel data set. I call this hyper-annotated. It’s an H&E tissue stain that is hyper-annotated with these multi-marker phenotypes for spatial phenomics. We're looking at things in space, two dimensions at least. That's really what spatial phenomics is.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

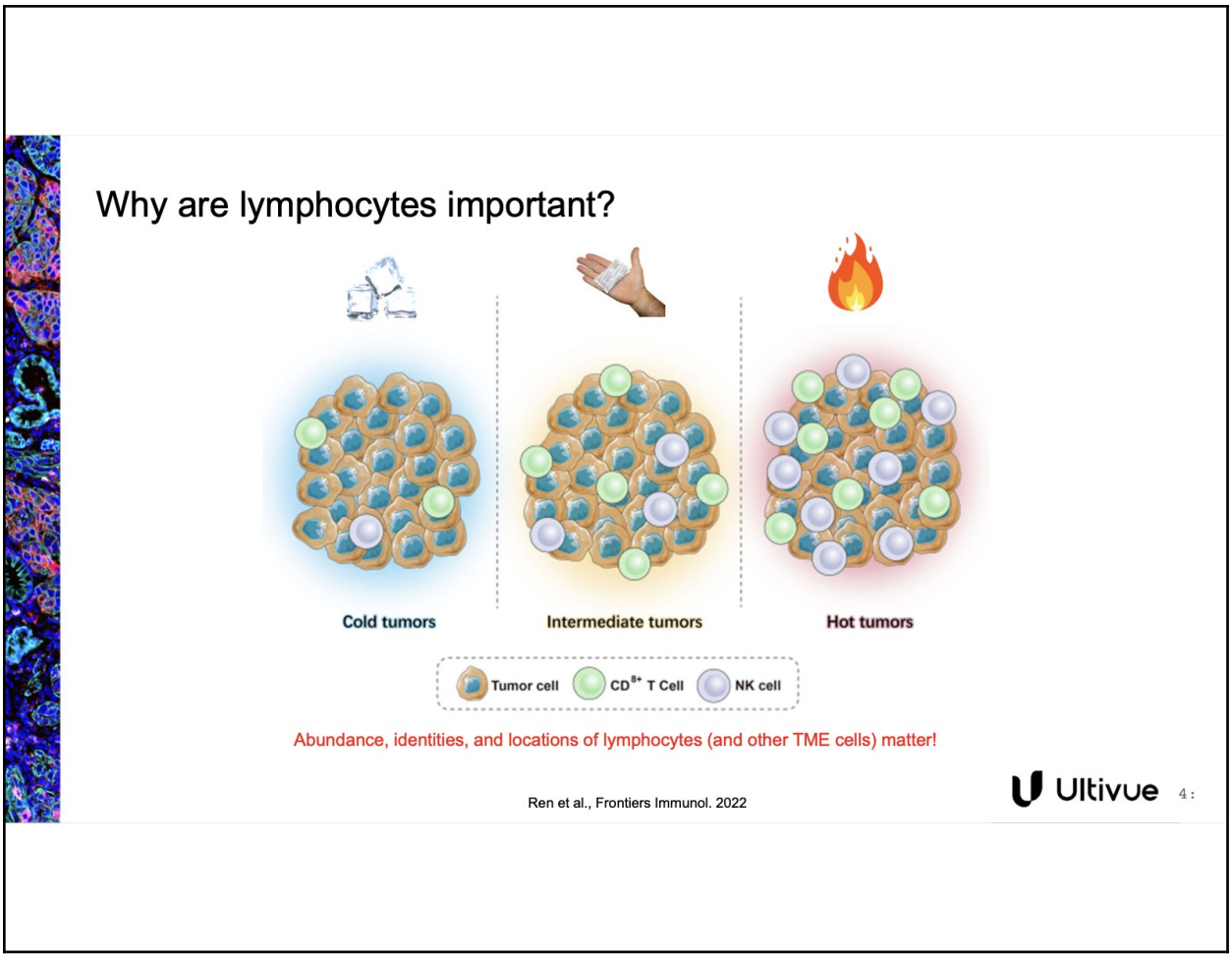


As a bit of a sales pitch, this fits in a pathology lab. You can do fourplex (four stains) in about a day with 30 slides. These have to be scanned on the scanner. It's about 15 minutes per scan. A lab could get through a few dozen of these a day if it needed to. It’s not high throughput, but it’s not super low throughput either.

What can you do with this? You can look at phenotypes. There's a whole field of computer vision that looks at these images and counts the cells, and makes decisions about how to classify them. And now there's AI, artificial intelligence algorithms, that look at this as well. You can correlate these phenotypes with morphology for discovering new biomarkers.

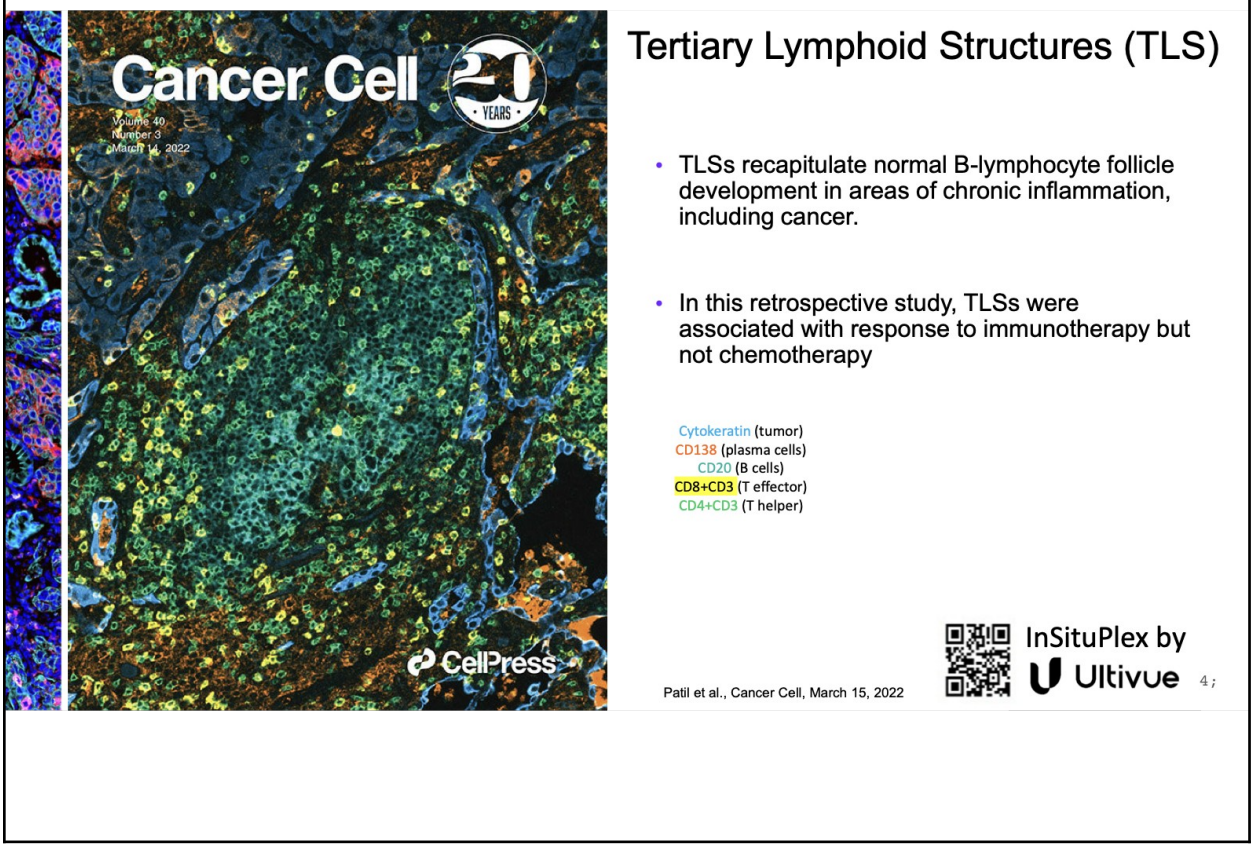
We're really excited about in the future is a whole field of pathology that's looking at H&E slide collections of tumors, applying so-called Deep Learning to the slides, and then showing in some ways that they're actually doing better than a pathologist at reading the slides and predicting the future. This field of artificial intelligence and deep learning is really exciting. We think this technology and many others feed into it, and they are going to make diagnoses better in the future.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



You may have heard that tumors have been likened to a temperature scale with respect to the immune response. There are so-called “cold tumors”, “intermediate tumors", and “hot tumors”. Cold tumors have been called immune deserts. The key thing is that each patient has a different interaction between lymphocytes and the tumor. Even different parts of the same tumor could have different interactions with the host immune response. This is not really used in diagnosis at all right now. But it's very clear that it's important. Other than the obvious, which is the hot ones, is there stuff going on in the cold ones? Maybe there is, but it’s at the research stage. The abundance, identities, and locations of these cells matter.

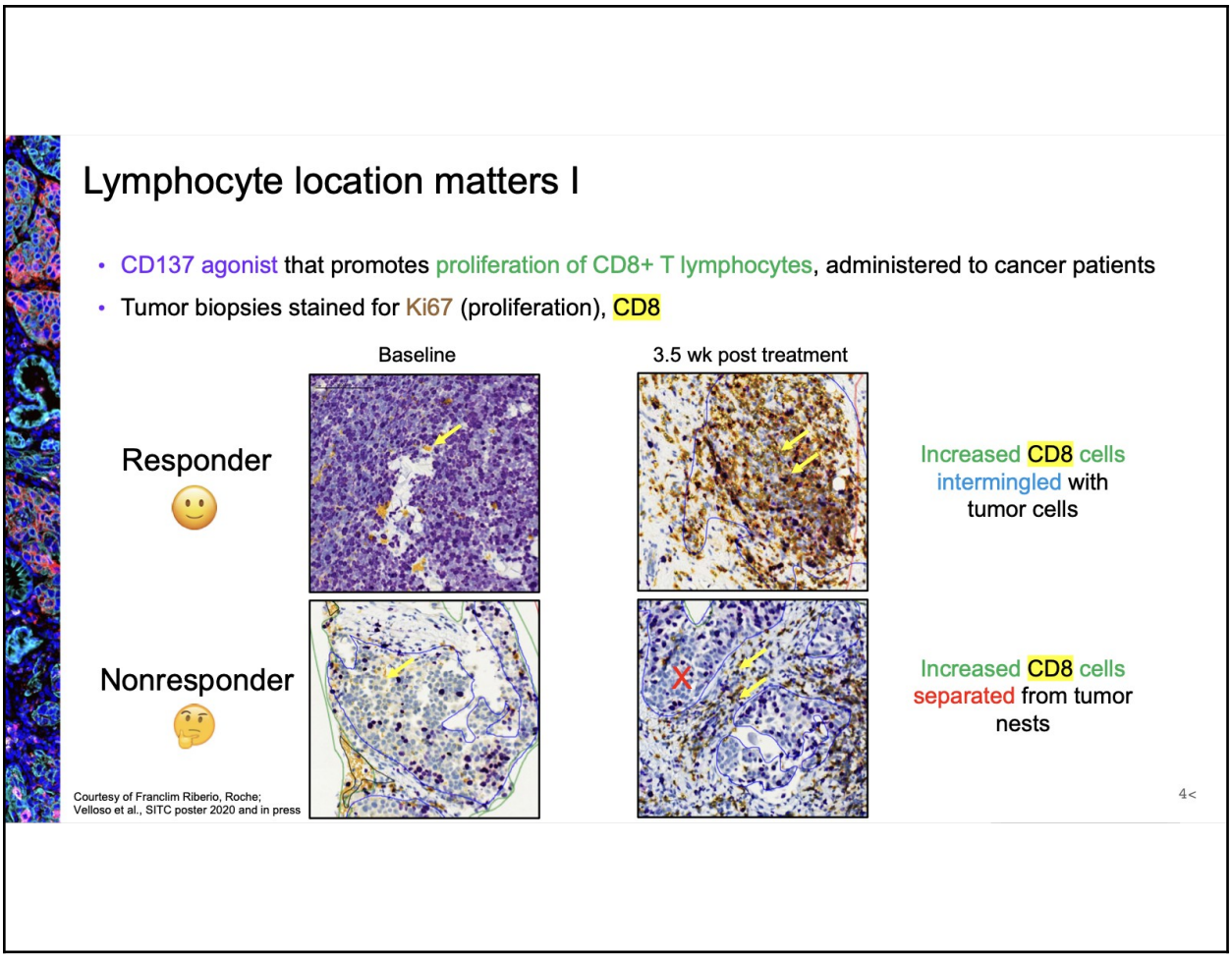
“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



One of the studies our lab did was in collaboration with Genentech, published in this prestigious journal Cancer Cell. Our technology made the cover. This is a tertiary lymphoid structure. In your lymph nodes and your tonsils, you've got these little factories that make lots of B cells, and they make better antibodies. It turns out one feature of so-called “hot tumors” is the presence of these structures.

This was from a cohort of lung cancer. It was a really interesting study and produce pretty picture showing these tertiary lymphoid structures. But the idea here is that the presence of these were associated with risk in this cohort response to immunotherapy, but didn't really have an effect on chemotherapy, which is really interesting. You can see this was just published this year as a research study. It's going to take a while for this information to potentially make it into diagnostics.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

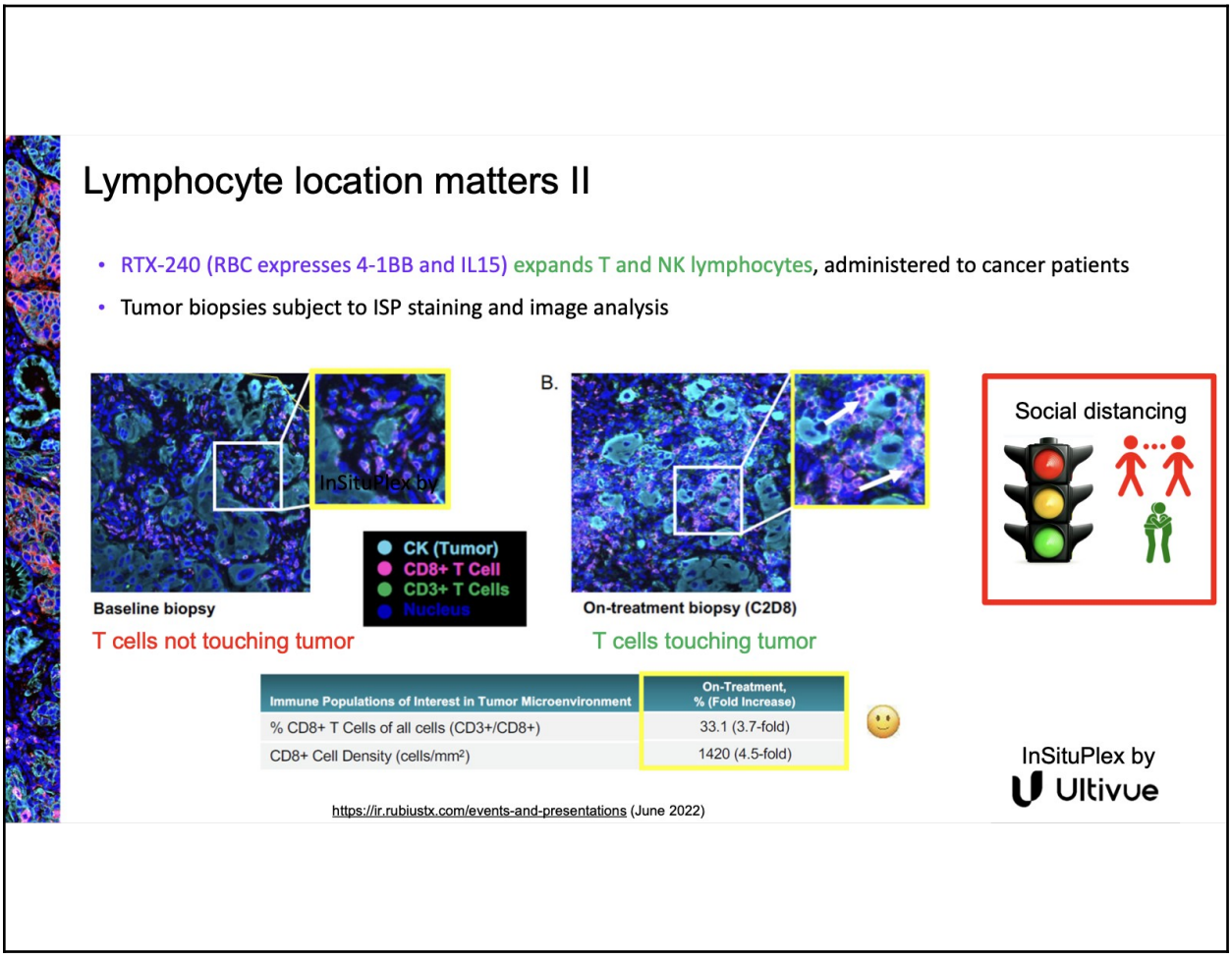


We're privileged to support clinical trials. These are histology biopsies from different types of patients. I'll try to explain what's going on here. This is not our technology. This was from a customer who shared this with me. The idea here is to bump up the number of CD8 positive T cells, because those are the effective ones that will kill the tumor, or can kill the tumor. They stain for T cells with CD8. In this case we looked at two representative patients. Here's a good responder, and here's a non-responder, and you can see the CD8 cells are staining kind of an orange color. In both cases, the tumor looks a little bit different. Don't worry about that. But at baseline, there aren't many CD8 cells. You give the drug, and it increases the number of CD8 cells. Lo and behold, there's a lot more CD8 cells, which we can see. The drug is doing something. But in the few patients that responded, the CD8 cells are migrating into the tumor, probably knocking them down in some way. The CD8 cells are intermingled with the tumor cells. In these other cases, there are more CD8 cells, but they can't get in the gates. There's something going on in the tumor or stroma that's blocking them from getting them close to the tumor cells. We can only document that there's something different. We don't know why. But this represents an opportunity. We're looking at these tissues from patients, and we hope it benefits the patients, but this is an opportunity to learn and then apply the data potentially to other patients in downstream trials.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Brian McCloskey:
The big challenge that prostate cancer patients have is that our tumors are cold. The tumor microenvironment has built up all of these walls that prevent these tumor infiltrating lymphocytes from attacking our cancer cells. Maybe if you can just expand on that a little bit.

Keith Wharton:
So two things. One is that it's called a desert. When you look at a desert, what do you see? A bunch of sand. The implication is that there's not much going on. Maybe there's a scorpion, some snakes, and some dirt tracks. But there's not much there. Now there's a technology we'll talk about a little later called single cell sequencing. It turns out, there's a lot of expression of immunosuppressive molecules in these desert tumors that you don't see when you look at H&E. But they're there, and they're doing something. I haven't studied prostate cancer specifically, but the idea that the desert is just the absence of something is patently false. There are going to be targets in there that are preventing the immune activity from coming in. And those could be drug targets. I can't share any details, because it's confidential, but I'm looking to do some consulting in the future, and I talked to a company that wants to make cold tumors hot, basically, as a generic strategy. I'm sure it's not a secret. This is a common strategy to look at and understand what's going on in cold tumors, to amp them up, and make them hot.



“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Here's another example from another customer. This is InSituPlex. It was a poster they presented at a recent cancer meeting, again, not really looking at responders versus non-responders, but just looking at baseline versus on treatment. This is probably a month into treatment. There is the tumor on the left, with the tumor cells in this kind of pale blue color, the nuclei are dark blue, and the CD8 CD3 cells are labeled green. There are a fair number of CD8 cells here, but once again, they're close to, but not on the tumor. In this particular patient, after treatment, what you see is a couple of things. One is that the tumor cells are dispersing. They're getting pulled apart, but we don't know why. There's more of these lymphocytes, and in contrast to baseline, at least a subset of them are sticking on to the tumor. We don't know if these are in the process of killing the tumor, but that's the implication. The cells are at baseline doing some “social distancing” that we don't understand, that the drug might overcome.

Anonymous Caregiver:
When I think about workflow, I'm reminded that in 2012, I referred a patient for C 11-acetate, a whole body scan that is metabolic. They got an injection, and you'd be able to differentiate and see where the patient is showing prostate-specific characteristics. I remember hearing that acetate is something that's even present in potato chips, for example.
Prior to receiving a biopsy, are you aware of any things that either can be done clinically or digestively to activate prostate cancer cells to be more lucent in some fashion for these scans and tests that will ultimately follow?

Keith Wharton:
What I see from following the literature is that now that we have these profiling capabilities, I think we're starting to cash in on all that biochemistry knowledge we forgot about when we took it in medical school. Turns out that cells in response to the oxygen gradients and invasive programs radically alter their metabolism. It's kind of obvious that a cell that's replicating needs lots of ATP to make more mass. That's the basis of FDG PET and other types of tests that look at the metabolic activity of cells. But there are some types of brain tumors that have mutations in Krebs Cycle components and other metabolic components that imply that it's actually a switch in the metabolism. It's not just compensatory, but it's driving the tumor. IDH, succinate dehydrogenase and other metabolic enzymes have mutations that are found in tumors.
I can’t answer your question directly. What I can say is that we're getting closer to having tools that allow us to assess the metabolic state of the cancer cells as part of the diagnostic workup from biopsies, for example. That will eventually be of use in this space.

Anonymous Caregiver:
The opportunity here for a novel intervention in the workflow sense would be how to give these things to patients prior to biopsy as opposed to prior to scan. I've not heard of anybody doing that.

Keith Wharton 25:02

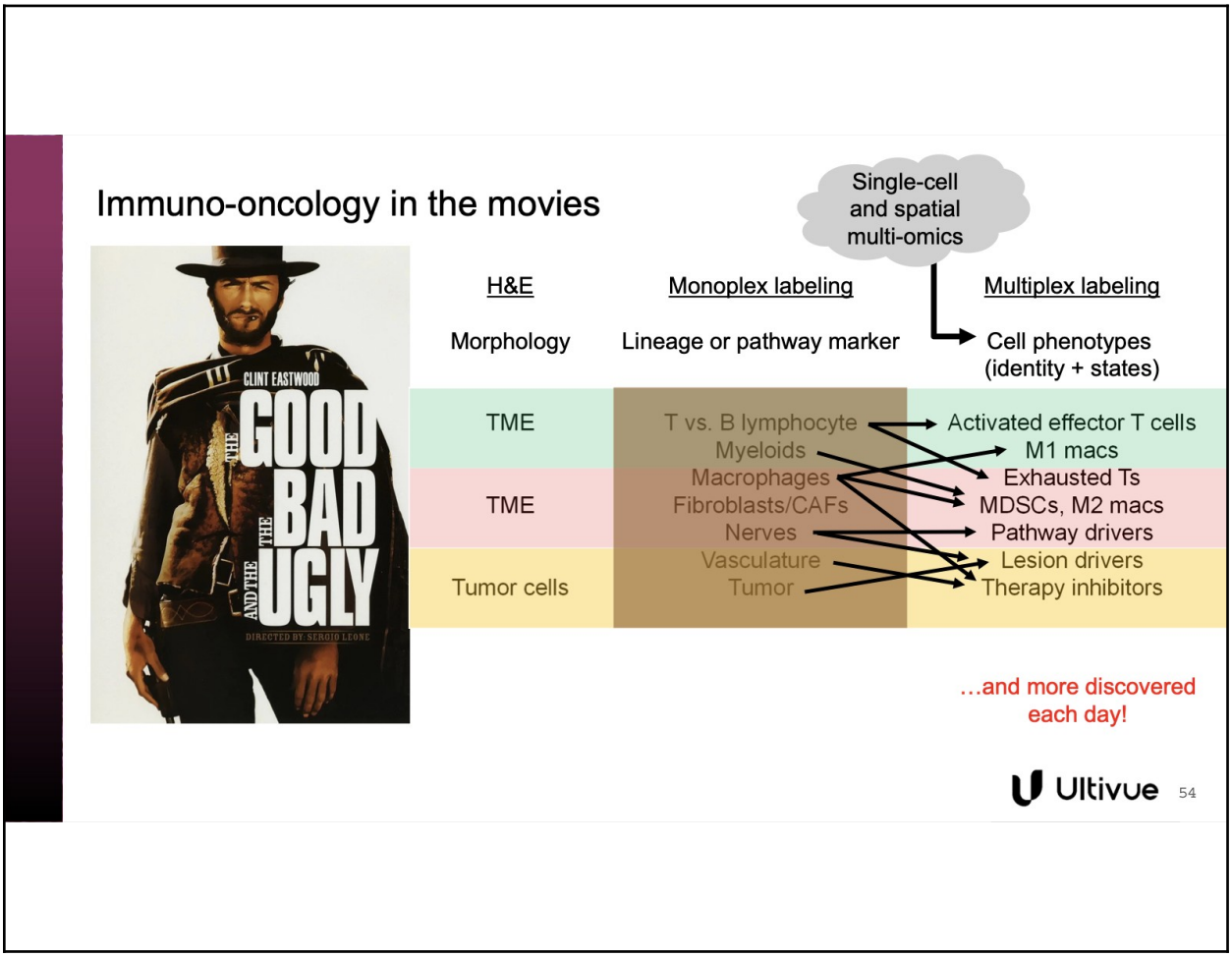
Before you treat a patient with an antibiotic, in principle, you'd like to have a culture and understand what the cause of the organism is. The same is somewhat true in cancer. You want

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

to have a baseline picture of the disease before any intervention so you can establish the right diagnosis. I don't imagine that's usually a problem in prostate cancer since the morphologic spectrum is well defined. But I can tell you there's some cancers of unknown origin. There are poorly differentiated cancers where you don't know the primary origin. Interventions (prior to accurate diagnosis) can impact the speed at which the accurate diagnosis is obtained. You'll have to ask the question of the real goal of doing an intervention before you biopsy.

Another thing related to that are new adjuvant therapies, where you give therapy before you do a resection, for example, which is more common in breast cancer than other types of cancer. One of the goals of the resection after new adjuvant therapy is to find residual disease. You're trying to understand if it's a pathologically a complete response or partial response. That turns out to be prognostic. That's a case where you must be competent in the diagnosis up front because it's done by needle. You put them through three months of treatment, you do a resection, then you have to take 100 sections to examine the tumor bed to make sure that there's no residual tumor. The pathology lab does this. Surgeons and radiologists take the biopsies, but you have to think carefully about what biomarkers you want from each stage before you change things.

Keith Wharton 26:56



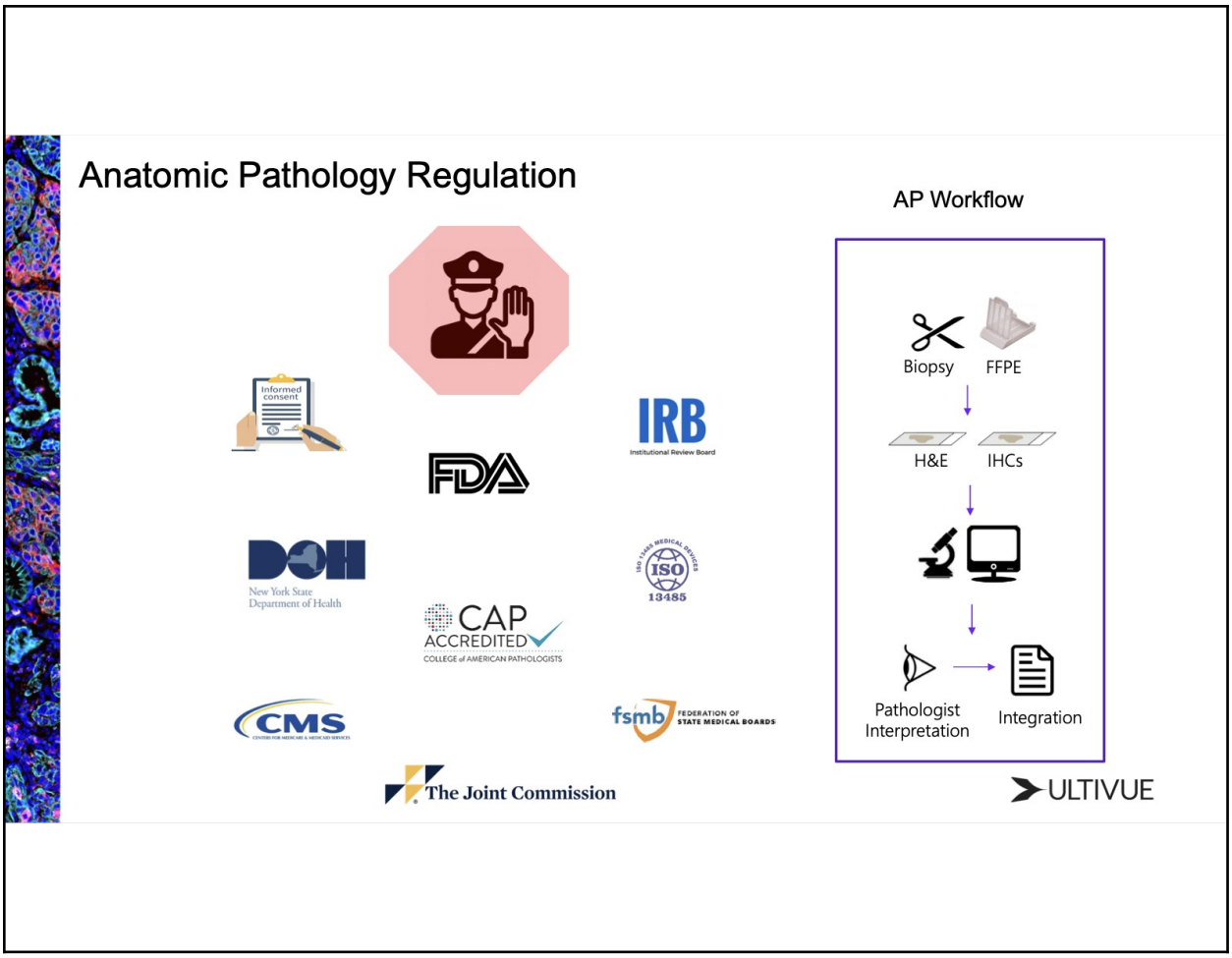
“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

I've been watching a lot of Westerns lately, and one of them was called, “The Good, the Bad, and the Ugly.” If I just draw an analogy to the cells in the tumor that I showed you, I can tell you that there are good cells and bad cells in the tumor microenvironment. But we can't really tell which are which from morphology. If we look at lineage or pathway markers with immunohistochemistry, we can label these cell types, but we still really don't know which ones are good or bad. It's this idea that in lymphocyte populations, macrophage populations, and maybe in some of these other populations, that there are good and bad cells, but we can't see them. What we need is multiplex labeling to see them. We can sort, for example, the macrophages in the M1s and M2s, and we can look at therapy inhibitors. I can tell you that these definitions are plastic. They're changing every day. There's a huge amount of information being generated largely through the single cells, spatial, multi-omics space, RNAseq and others. We're just now learning the actual good states and the bad states, the good phenotypes, and the bad phenotypes. This technology has just exploded in the last decade, really in the last couple of years. Yet there's no agreement on what the important ones are from a diagnostic standpoint.

Keith Wharton 28:35

I'm not going to tell you the entire process of how something goes from a twinkle in somebody's eye research idea to a diagnostic test. I have been exposed to it - and it would be a whole lecture. Suffice to say that the labs that work with these specimens, anatomic pathology labs, are regulated by a variety of bodies and at a variety of levels.

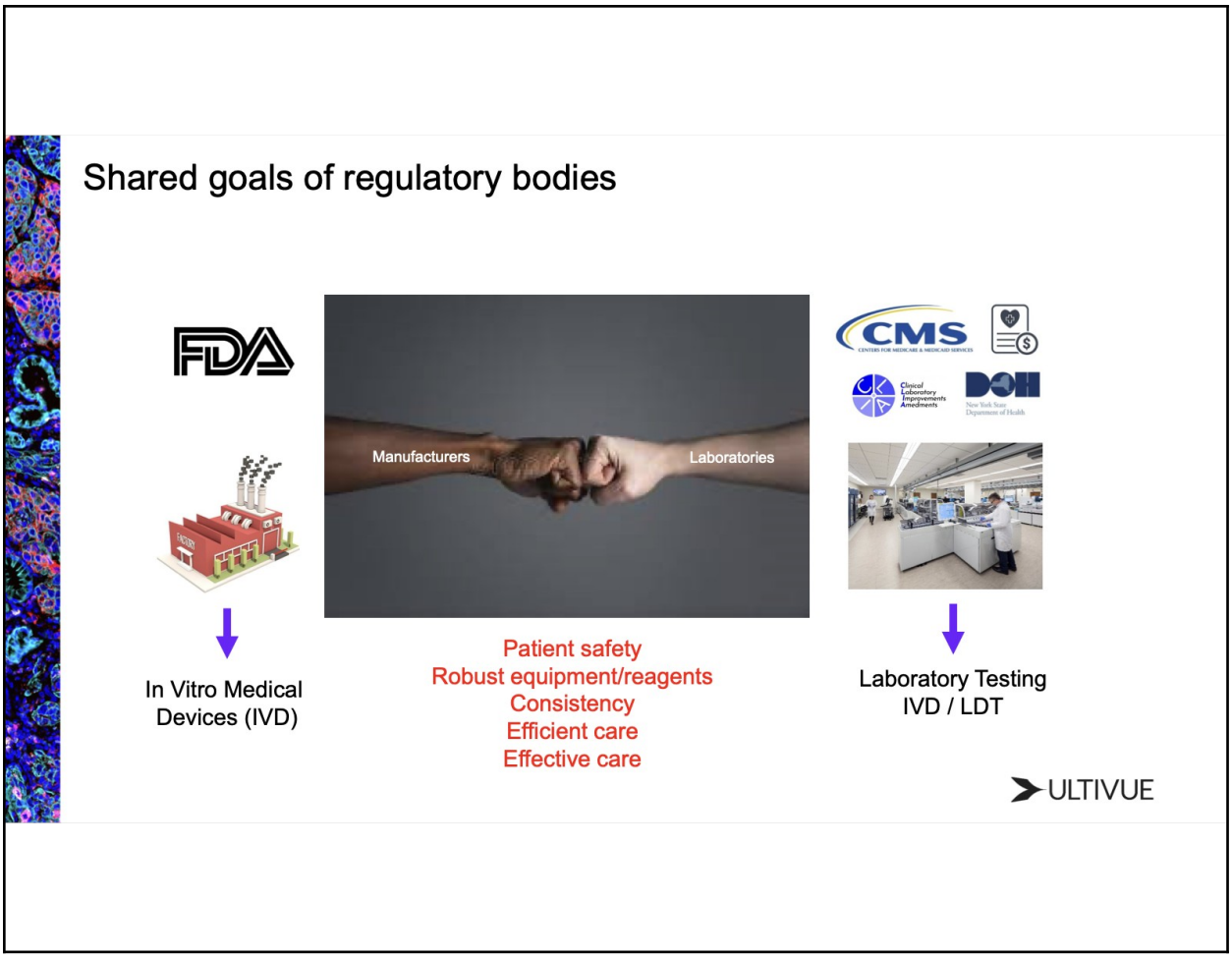
“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



The FDA regulates devices, but they don't regulate the practice of medicine. If there's a clinical trial, IRBs must sign off on it. ISO 13485 is a quality standard for manufacturers. If a lab buys stuff from a manufacturer, they want to have good manufacturing processes and have this international certification. A patient signs a document such as an informed consent. Typically, labs are regulated by CAP CLIA. CMS regulates reimbursements, and you don't get reimbursed if you're not accredited. Joint commissions regulate hospitals and other health care facilities. Of course, the practitioners like me, although I'm not actually doing this actively, have a medical license. We're regulated by state boards and licensed by the Federation of State Medical Boards.

The overall process of a biopsy journey from your prostate to a report within an archive of data looks like the flow on the right. First you need a biopsy, then make FFPE sections, then make sections where there's stain, then analyze by a pathologist, and then they integrate the data and put it into a report. This is just a reminder of the workflow, it's not just to press a button and get an answer. It is a multi-step workflow, complicated, regulated at a variety of levels.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



All of these regulatory bodies should have the same goal. They want patients to be safely treated. The lab should have robust equipment and reagents. The results of the test should be consistent and reproducible, reliable, etc. The goal is efficient care and care that's effective, not just a boondoggle for a lab because they can bill for it, but something that makes a difference clinically. There are different sorts of stakeholders here. The FDA regulates manufacturers that make IVDs, in vitro medical devices, but their labs typically don't do the tests. These other bodies regulate labs, and labs can set up tests that can be done either under IVD, so they'll purchase the device from the medical device company, or this concept called LDT, laboratory-developed tests. Most tests that are done in pathology labs are laboratory-developed tests.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

We should all have shared goals, but there is this scenario that's been set up over time. I don't know if you remember the Rock em, Sock em Robots, but they were fun. I like to think sometimes that the regulators and practitioners are in this Rockem Sockem Robot duel. What do I mean here? The regulators look at FDA approvals and clearances, and then there is this concept called enforcement discretion. In other words, the FDA might claim that they oversee a lot of things, and there's some debate around some of the things they oversee, but they choose not to enforce a practice because it's not causing a problem, or they don't have the bandwidth, or it's not a public safety issue. That's a mechanism that was used around the pandemic. This is an important thing because it doesn't mean that you're off the hook with respect to being looked at by the FDA. It just means that if you try to do things that are going to hurt people, they're going to pay attention to you. In Europe, lots of the in vitro devices are going through reassessment of safety risks to patients involving so-called “IVDR”. In the US, we have the Valid Act, which claims that all tests are “in vitro clinical tests”. These bills have gone through revisions. They either propose more or less regulation of LDTs. This is all tied up with resources that labs have, and ultimately reimbursement. I've worked on this side, and the things you think about here are those regulations: quality systems, what can you do that's legal, compliance, certification, and some of these are checkboxes. It's really important to build quality into your system. But let's face it, some of them are checkboxes, and ultimately, a lot of the checkboxes are linked to liability or avoidance of liability. You have liability lawsuits and patient issues, samples lost, and people misdiagnosed. These are problems nobody wants. It's embarrassing. It's not what I think anybody wants to do. You need quality systems built in.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Brian McCloskey 34:10

What strikes me, Keith, is that there are a lot of breaks or walls in the process. There is no organization here that is responsible for accelerating the integration of safe new technologies into patient care. Unless I'm missing it, it's up to the individual companies to break through all these barriers so that they can get their technology integrated.

I'm about ready to have surgery in a month. I want to plan on how my tissue is going to be used, and I know pathology is the first step in the process. I know that there are technologies that are available that can make a difference in my treatment decisions. You've talked a little bit about spatial, spatial transcriptomics and spatial proteomics. These are cutting-edge technologies that can help me better understand my tumor microenvironment, particularly proteomics. Drugs target proteins, and through proteomics you can identify drugs that will better target my cancer.

Keith Wharton 35:31

In the future we will have most of the targets and many of the pathways covered, so that you could apply these techniques to tissues in discovery mode. What I haven't talked about is that most of the diagnostics have an intended purpose to fulfill. A group sponsored by the Foundation for NIH called “CIMAC” is an attempt to cross sites to standardize immuno-oncology biomarker assessments, mostly as part of clinical trials, but starting with retrospective collections. Your point is a good one in that there are efforts in a herd mentality, in a population sense, to bring a modality to an indication, but how that's done with individual patients is a problem today. There's a gap. It's a missed opportunity.

Keith Wharton 36:45

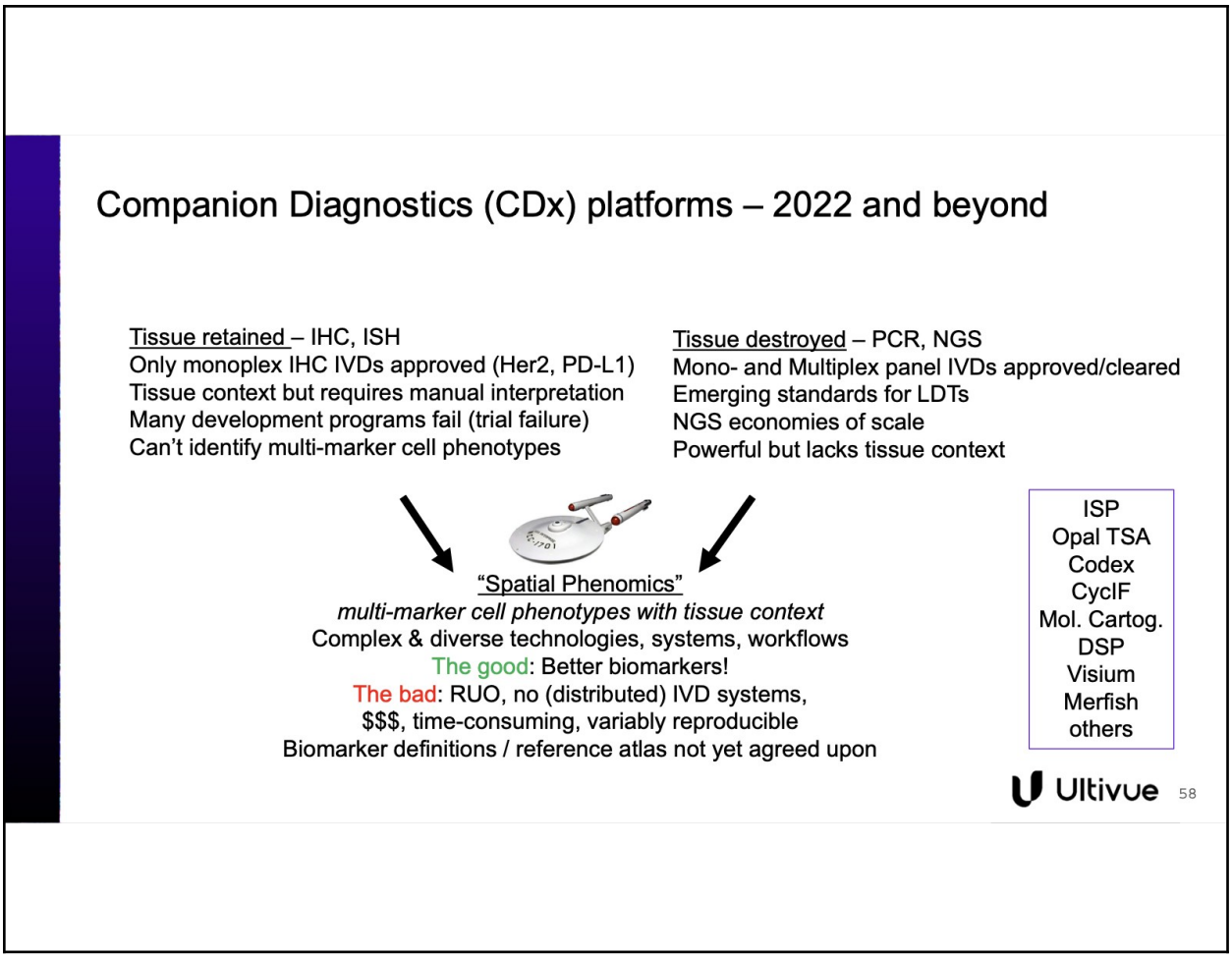
Practitioners like autonomy. There's this concept of the practice of medicine, where you trust the doctor. You trust the training. They must make the best decision for the patient. They deal with real world evidence, and they deal with good and bad real-world evidence. I remember reading a book several years ago called “How Doctors Think”, and a lot of this is not evidence-based. You must consider the fellowship, the training, the journey of the provider, the tutelage that you get as part of the practice of medicine. You learn a lot just from being with an experienced practitioner. Heuristics are used quite a bit now.

Keith Wharton 37:45

When I was in training, a lot of my colleagues went into their own practices and started their own businesses. Very few doctors are employed independently now. They're often part of large practices, large healthcare organizations. This potentially affects the freedom with which they can practice. Then of course, it's linked to reimbursements and efficiency. No answers here, but just recognizing that there's a tussle going on here.

Keith Wharton 38:19

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]



I just went to the World CDx meeting that's in its 12th year, regarding clinical biomarkers and companion diagnostics. The official companion diagnostics are called “capital C” companion diagnostics, which are those that are cleared by the FDA or approved by the FDA to have a particular role in patient care. In 2022, there are two types. One is where tissues are retained and the other is where tissues are destroyed. The only ones here that are cleared are these monoplex IHC: HER2 and PDL1. They're manually interpreted by a pathologist. I was involved in some of these as new tests, and they all failed. The diagnostics were fine, but they didn't have clinical utility related to a drug. As I mentioned, they can't identify these novel good and bad cell phenotypes. The last FDA guidance around this was in 1998. This is a field that's been stuck in time, decades of time.

On the left, even though PDL1 came out seven years ago, there's been fantastic advancements on the clinical application of next generation sequencing panels. These are historically LDTs, but other multiplex panels that are IVD cleared: Foundation Medicine, Guardant, and Memorial Sloan Kettering. There's a lot of LDTs out there. Every cancer center has its own panel. There are economies of scale here because the early molecular tests used a PCR to measure one marker at a time. You could do serial PCRs looking for a targetable mutation for three months and still not find the target. NGS kind of does everything at once, which from an efficiency standpoint is fantastic for patients because the data from hundereds of targets is identified simultaneously. It's powerful, but lacks tissue context.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

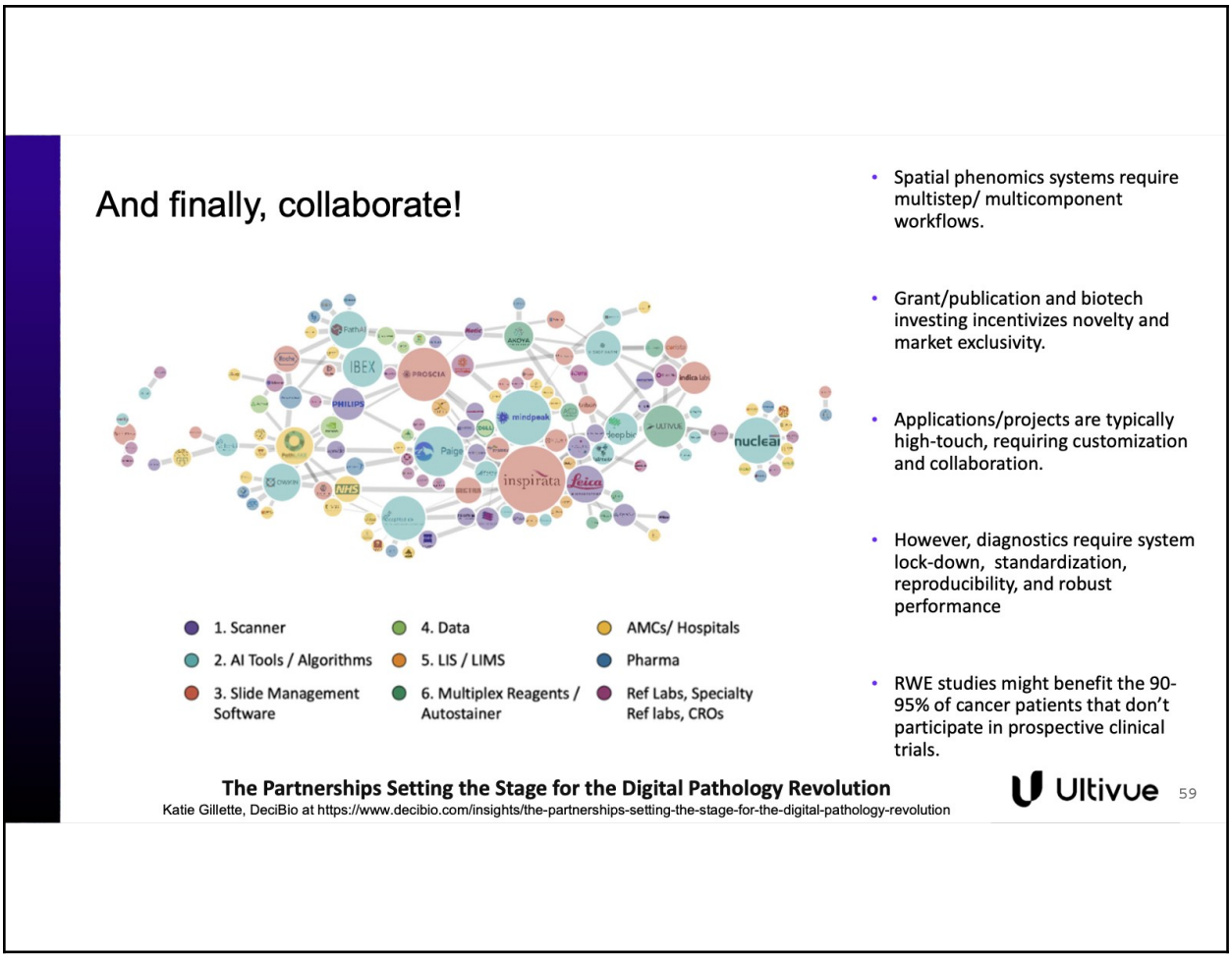
If we step on Starship Enterprise in the future, the diagnostics that are going to combine these are, in essence, spatial phenomics. These will have multi-marker cell phenotypes with tissue contexts. Right now, we have complex and diverse technology systems and workflows. They are not standardized. When we set up our company, we got our labeling technology into a pathology workflow. It's something that is adaptable to what they are already doing in the lab.

Off to the right are the names of some of the single cell technologies, which means the good is that they're better at identifying biomarkers, but we don't know exactly what they are. The bad thing is they’re “research use only”. They're not distributable yet as easily as IVDs. They're expensive and time consuming. Reproducibility is an issue for some of them. The key is how we define what the biomarker is, what the cutoff is with which combination of markers. We don't know yet. For example, Brian, you could say you have a whiz bang technology applied to your tissue. I would say it'd be easy to see at the very ends of the spectrum of what you could see. It'd be pretty easy to say whether your tumor is hot or cold. But it's this area in the middle that relates to the existence of false positive and false negative diagnoses that the FDA really does care about. If you are declared to have something positive and you are false positive, you either get treatment or denied treatment, and there's harm and liability. There's a landscape in place that prevents you from being harmed. The FDA does look at risk vs. benefit and tends to take novel technologies of any kind to improve diseases when there aren't existing therapies that are effective, or when the patients are desperate. Patient advocacy organizations like yours are critical for banging the drum to get things moving in particular directions.

Keith Wharton 42:37

I mentioned the workflows are complex. What I found is that no one company can do all of this well from idea to clinical diagnostic with the whole workflow. It turns out different companies have different strengths. They have different business models and different pressures.

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

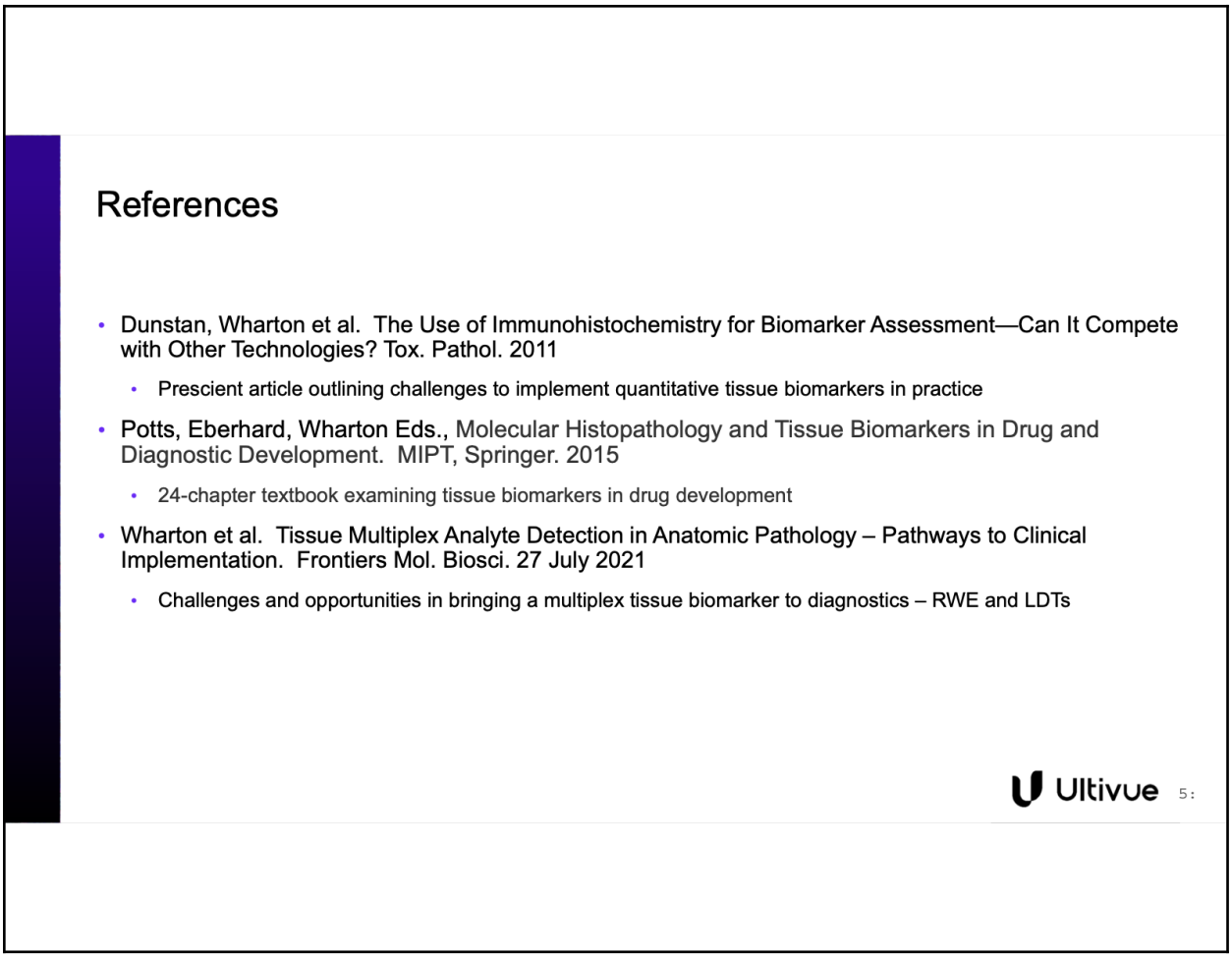


A consulting firm went through all the publicly available co-marketing agreements and press releases and publications, and then made this web of collaborations which are happening. We are in here, and you can see we've set up some collaborations with AI companies because we think we need help in the AI area to understand what our scans and stains mean. I can tell you, there's not an incentive to standardize. If you're an academic, and you're doing academic research, your job is to carve a moat around you so that you are novel, nobody else does what you do. You're the world leader. It's the same thing in biotech. You need market exclusivity to enhance your business model, and we're no different. We don't think we have any competitors. But there are other technologies that can do somewhat similar things. All the incentives for all the players are not towards collaboration. Working with dozens of pharma companies, we find they don't agree on anything. Everybody thinks that this type of marker is better for this cell type and then you must go to another company for something else. It's a bit of the “Tastes great, less filling” analogy if you remember those early commercials for a light beer. People just don't agree. But the concept of a diagnostic requires system lockdown, standardization, strict and expensive testing on reproducibility, and robust performance. I wish these technology workflows were here, but I'd say today, most of them are not. What I believe is the opportunity are real world evidence studies because the vast majority of people who get cancer don't participate in clinical trials. The figure I got is 95%, and maybe 90%. I don't know. But it's these patients who get standard of care, we can learn from. We do have technologies to learn from them. I do believe there are huge opportunities from folks like yourself, and we've had some discussions like this simply to learn from you. It's just hard to learn from you right now. If you're not part of an

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

official double blinded, placebo controlled, randomized multicenter bla bla, bla, trial that generates the highest level of evidence, that design of trial does not consider the unique nature of each person's disease.

Keith Wharton 46:03



Here are some references.

When I started in industry, I worked with a veterinarian, who was a visionary in this field, and we were able to look hard at some of the issues that prevented immunohistochemistry from being a robust quantitative biomarker. I re-read this article recently and realized some things really haven't changed, while others have dramatically. Then coming to Ultivue, I was really excited about exploring the landscape and what it's really going to take to bring this multiplex detection in a digital pathology environment to diagnostics. I think this so-called “real-world evidence" is a real opportunity.

Rick Stanton 47:32

Keith and I chatted five months ago. At the end of our discussions, it was kind of a roadblock to access this neat stuff. How can this (technology) help me on a decision? A huge hope for us prostate cancer patients is to develop a prerequisite that will change us from a cold to a hot

tumor that will allow a response. But boiling it all down is if somebody like Brian, who's about to get a biopsy and has a massive amount of metastatic tissue, can he use Ultivue yet? And if not, is there anything we can do to help?

Keith Wharton 48:47

First of all, I mentioned we have a CLIA certified lab for our research customers. But, we don't do diagnostic tests in our lab right now. Our CLIA license is based on a CLIA validation we did with one of our kits in one tumor indication, but we don't sell that, and we don't use that for any diagnostic purposes. Our lab needed a quality system so that when pharma came to us, they audited us and could say, "All right, we can send them their tissue", but we don't physically do diagnostic tests on the tissue. Everything we do is research and needs to be labeled as research. All the research we do is typically contracted by a sponsor.

We've discussed whether you need to be part of a protocol, or some study, or be affiliated with somebody who has a chunk of money that they could send us, or we could use that as a basis of negotiation. I'm not on the business side. I'm not the one to quote a cost. I'll just say, it's probably between $500 and $1,000 just for the raw reagents, and then you add the cost of the lab and then the cost to keep the lights on. We need a sponsor, and we've been in discussions about that.

The other side is that no standards exist. I could look at it and make a statement, like, "Oh, there's lots of lymphocytes there.” I can say, “Oh, it's a desert." You actually just need the H&E, you don't need our technology to say that. Our technology is probably better for the warm and hot tumors that we want to know where the lymphocytes are, and what the cell identities are. There are other technologies that are better for cold tumors. Maybe there are other reasons or other therapeutic possibilities that could be applicable in individuals. Sadly, there are roadblocks that are related mostly to the maturity of the technology. If we felt that technology was more mature, and we could define cut offs, then we would be pursuing this very model. We do these tests in house, but those cut offs are not agreed upon - there's not good evidence. I will say that the cutoffs where determined are very specific to specific treatments.

I'll give you an example: breast cancer with HER2. HER2 is the poster child for a personalized medicine drug called trastuzumab that targets HER2, which has been made for 20 years. I think it's generic now. HER2 testing with IHC was really set up to divide those patients who had massive amounts of the HER2 target in their tumor from everybody else who didn’t. It was like 10 to 20% that have massive amounts of HER2 and respond to trastuzumab, and everybody else doesn't. Now, there's a new drug now called Enhertu. You may not have heard of it, but it is better. It works well in patients that have low amounts of HER2. The diagnostic test wasn't really designed to distinguish those that have low amounts from high amounts. It's really forcing a reassessment of not an assay per se, but how pathologists read it. I expect our technology will be similar. Some company A will come to us and say, we hypothesize that patients with high levels of markers A, B, and C are going to be responsive to our drug, and maybe down the road, we'll all be successful. We'll have a diagnostic that gets approved. But then Company B comes around and says, “We think it's high levels of A and B, but low levels of C, so use the same test, and use it off label. The labs and doctors will do what they want to do, becuase this is the practice of medicine. Different labs have different levels of sophistication or trust in IVD labeling. In other words, some labs really want to do everything as an LDT because they believe they're better than the manufacturer in setting up the tests, or they can do it cheaper, or whatever. Other labs do everything by the book. Some institutions say, "If it's not FDA approved, we're not going to touch it." It's a risk tolerance. It's a culture issue, its risk tolerance, business model, its

related to competence. And this was very true in COVID. There were a lot of labs that had to scramble. They wanted technology that worked. They didn't know which among the 69 kits that were being put out under Research Use Only enforcement discretion, which would actually work. A bunch of tests eventually got yanked from the market because they weren't working, and were causing harm. This technology is just at that stage where there's a lot of promise. There's this mountain of evidence that suggests it's doing something, but nobody's really ready to place the bets on the cut offs to generate the evidence.

Rick Stanton 54:24

You mentioned other technologies that might be helpful in making decisions. Would that be IHC with a CD3, CD8, or PDL1 stain? What are those other technologies that we can access today? What would you recommend to Brian for example?

Keith Wharton 54:44

It depends on whether Brian's goal is to help Brian or to help 10,000 people.

Rick Stanton 54:49

It's to help Brian.

Brian McCloskey 54:53

Hopefully it would help me, and by helping me it's going to open the door for others. Keith, if I can just be a bit more specific. I know that UCSD, where I'm being treated, has instantiated Akoya spatial phenotyping machines. Yet, when I asked my medical oncologist about it, she knew nothing about it. And there are other providers that don't know either. I finally got a hold of a couple pathologists and they don't know anything about it. I'm trying to connect dots so that I can leverage this technology. My question isn't so much about connecting the dots, but let's just assume that I'm going to connect the dots, and I'm going to get access to this technology. Should I use this technology to identify treatments?

Keith Wharton 56:00

From my knowledge, there's no organization that's offering a pure diagnostic with this technology with any of the fluorescence technologies. Some of the cancer centers will be looking at retrospective data from cohorts. Some of the pharma companies have biopsies from patients who've been in their trials. The major use of this technology, any of the fluorescence technologies today, is in retrospective analyses of cohorts to pull out these good and bad cells. In terms of potential clinical benefit, these next generation sequencing panels are probably the next thing that is being done on more and more patients and they are not cheap. But they look at hundreds of genes. The problem with them is that if you find mutations in a target for which there's a mutation selective drug or another drug that targets a pathway that they can help you, but they don't use spatial technology. They don't look at spatial attributes of the lesion. I know, for example, Tempus is doing whole genome transcriptomics with all the tumors that come in the door. You can learn things from these types of bulk technologies. It's not spatial, but you can learn things about driver pathways and certain cell types that are present, but they don't provide the spatial context enough to lead to specific hypotheses.

Brian McCloskey 58:00

“Bringing Novel Immune System Tests from Research to Clinical Use” (Keith Wharton) [#28]

Tempus does a lot of WES and WGS, and they are getting into spatial. It's behind the scenes. Maybe there's an opportunity to do a one stop shop.

Keith Wharton 58:26

Caris is another one who is doing transcriptomics I think on all tumors there. There are forward-thinking large, private CRO-type companies that are doing this.

Brian McCloskey 58:37

Eric Hall, who's on this call, sent us a note just prior to this that he has whole exome sequencing which is phenomenal.