“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]

Brad Power and Richard Anders
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“The idea of Travera is … narrow the list of those FDA-indicated drugs that are most likely to or have a higher likelihood of eliciting a clinical response in a specific patient.” – Dennis Watson

“We love to use the word ‘personalized’ in the genomic and genetic testing space. But the reality is, in most cases it is just smaller populations. If you're talking about a BRCA mutation for example, you're just part of a smaller population than you were before. That still isn't truly personalized therapy. What we're doing here at Travera is truly N-of-1 work.” – Dennis Watson

Meeting Summary

As an advanced cancer patient looking at your treatment options, how can you decide what will work for you? If you have multiple drug options, how do you decide which ones have the best potential to make an impact for you and your unique cancer?

The standard process for personalized cancer treatment is to sequence the DNA of your tumor, then identify mutations that point to a particular drug. But even with more and more highly-targeted drugs being developed, the odds of finding an “actionable” mutation are still low (depending on the cancer, perhaps in the single digits), and even drugs that work on these mutations may not provide the desired response rates. There is lots of room to improve.

Dennis Watson, Vice President of Business Development at Travera, is uniquely qualified to discuss one way to increase the range of personalized treatments you might consider: “functional testing”. Dennis has been working in genetics and genomics of cancer for over 15 years, and joined Travera in 2022. In addition, Rob Kimmerling, Travera’s chief technical officer, and Mark Stevens, Travera’s vice president of clinical development, were in the lab at MIT where Travera’s unique weighing technology was developed.

What is “functional testing”?

"Functional testing" is directly testing cancer drugs on your live cancer cells to see what the drugs do. This is in contrast to, say, a test which genomically profiles a tumor and then uses this information to predict how well drugs might work, based on the underlying mechanistic understanding of the drugs. It’s the difference between theorizing about something and trying it.

For more on functional testing, please see previous discussions we have had, including with Tony Letai of Dana Farber (#11), Payel Chaterjee of SEngine (#13), Noah Berlow and Diana Azzam of First Ascent (#18), and Robert Nagourney of the Nagourney Cancer Institute (#30).

What is the Travera functional test, and how does it work?

Travera’s process requires a tumor sample from the patient. This can be obtained from a blood sample (in the case of a liquid tumor), or a biopsy or tumor-surrounding fluid in the case of solid tumors. The malignant cells are separated using certain markers present only on one or the other cell type. The malignant cells are placed on a flat plate with multiple "wells" used as small test tubes. There are about 5000 cells per well. Travera then challenges the live cancer cells

against different FDA-approved drugs (a “functional test”) and measures how they respond. This is done by weighing the cells using an amazingly precise microscopic device, able to detect mass changes of 1 part in a billion (about one ten-thousandth of a gram in an average human) in cells as small as 5 nanometers, which is 100 times smaller than the wavelength of visible light. The company believes that a cancer cell which is being strongly affected by a cancer drug telegraphs that effect by a change in mass.

Unlike other functional tests where tumor samples are implanted in mice to grow tumors, or in organoids that are grown from the tumor sample, this test is extremely rapid – they claim an unprecedented two-day turnaround time. By getting results so quickly, they are less likely to be misled by the complex interactions of the tumor cells and the drugs in the artificial environment outside of the body.

Travera claims their test has a predictive accuracy of 85%. They provide a ranking of each tested drug’s predicted efficacy on a scale of 0-100. Scores above 50 are said to indicate a statistically significant likelihood of eliciting a clinical response.

They also have a test which they believe will measure immune cell response to checkpoint inhibitors. This is not measuring cancer cell kill rates, but rather the extent to which immune cells are activated by these drugs.

Why might you want to get a “functional test”?

- **Best drug for you**: Testing the response of your cancer cells to drugs is valuable because during your course of treatment you are only able to take a relatively small number of drugs, generally sequentially, with effects (such as side-effects on your body, or effects on the cancer itself) that may alter your body’s ability to tolerate other drugs or impact the cancer’s response to future drugs. Some drugs may not be generally used for your type of cancer, and locating such drugs from the myriad of possibilities is hard. In addition, a drug that is targeted at a mutation that you don’t have, such as EGFR, may hit more than one target, so you might respond unexpectedly. Or there may be seven EGFR inhibitor drugs, and one will work better, but not the others.
- **Complement to other tests**: Assuming that you do want to get a test, you should think about what type of test to get (although subject to cost and sample availability, you can often do more than one test). A standard test uses gene sequences taken from your cancer cells to make its predictions. But biology is complex – and much more than just genetics – and predicting a drug’s utility from the genetics of a cancer cell alone can be hard, particularly when the drug is not one designed specifically for a particular genetic aberration. Compounding that problem is the fact that a cancer population, like a region of garden overgrown by weeds, is often a heterogeneous collection of somewhat different cells. A genetic test might be able to give you information about how a drug will work in a particular cell type in your cancer, but may be less good at predicting the effect of a drug in the overall broader population of your cells. Other tests, such as RNA sequencing and proteomics arrive at their results using different techniques, can provide

different information, and they may not all agree. For example, a genomic test might tell you more about the makeup of your cancer and its course, which is information outside the scope of Travera’s test, which seeks to provide insights on specific drugs. If you are interested in obtaining a fuller picture of your cancer – and have sufficient samples and funds – it may be reasonable to use a variety of “standard” tests at the same time. You can repeat many of these tests, including Travera’s, multiple times (subject of course to tumor volume amounts and insurance) in the event your cancer was not adequately treated or it recurs. The tests may have different results and be of higher utility as the disease progresses. A functional test doesn’t seek to predict by theory what might work. Instead it just tries the drug on a group of cells to see what happens.

- **Large scale testing**: The Travera test enables the large-scale testing of many drugs, and even drug combinations, limited only by the number of tumor cells in your sample and your testing budget.

Why might you not want to get a “functional test”?

- **Cost**: Many functional tests (like Travera’s) are in a research phase at the time, so their tests are free so that they can gather data to support becoming standard. As tests move from research to commercial acceptance, the test could become expensive. This may pose a financial burden, particularly if it is not covered by insurance.
- **Sample availability**: It might be hard for you to obtain fresh tumor tissue, or sufficient volumes of tumor tissue to test as many drugs as you would like. Keep in mind that unlike many other test types, which can work with very small samples and can provide a comprehensive report based on that sample, the more drugs you wish to functionally test, the larger the sample you will need to provide.
- **Predictive accuracy**: While exciting and very sophisticated technically, there remain significant questions as to how well Travera’s test and functional testing in general works. The Travera test is not yet FDA-approved or cleared, and has not been extensively tested in clinical trials. It is thus hard to gauge how accurate their results may be. Specifically, they may give you suggestions that are less beneficial than the recommendations of your care team or other tests you may obtain. These recommendations may take you on costly detours from optimal treatment. Here are a few of the potential issues behind concerns about predictive accuracy:
    - **Creation of samples:** Travera separates malignant from non-malignant cells using certain markers present on the surface of the cells. But for particular patients with particular cancers, this sorting may be less complete or wholly ineffective. The result is that certain parts of the tumor may not be separated out as malignant and thus not tested. That can be a significant problem if, for example, these unsorted cells are particularly numerous or particularly aggressive.
    - **Ex Vivo (out of the body) vs. In Vivo (in the body):** Despite many attempts over many years, there is no way to mirror in ex-vivo wells the behavior of cells in their native environment in the body. So for example, an in-vivo cell has a complex environment with other cells near it that may provide nutrients, signals

or protection. Outside the body, such cells may be significantly weakened and much easier for certain drugs to kill.
    - **Drug effects:** Applying a drug directly to a cell ex-vivo is not the same as the drug getting to the cell through the in-vivo route of a patient swallowing a pill or via IV. To give just two examples, different drugs can be metabolized or excreted very differently by different patients and what might work well in one patient could have a dramatically different effect in another, even though the ex-vivo functional tests would not reveal this.

When should you get a functional test?

1. **Early (at initial diagnosis)**: In an ideal world getting this test as early as possible could have important benefits. First, at the initial biopsy it might be easier to obtain a large volume of tumor cells to test. Additionally, each round of treatment has effects on your body and effects on the cancer. So it seems plausible that using the most effective treatments earliest is the best strategy for fighting your cancer.
2. **At change points (advanced cancer)**: Assuming your cancer is advanced, the next best time to get a functional test is when you're planning to change your therapy. If there's a recurrence, this could mean the cells have changed from the original specimen, and there might be different drugs you could identify, and ones that were identified that are no longer actionable.
3. **To build on information from other tests**: The benefits of novel tests like functional testing must be weighed against the often well-validated vast corpus of accumulated oncology knowledge with more standard tests and the treatments associated with them. Especially if you have a treatable (or highly treatable) cancer with known effective early treatments, using a non-standard test which might recommend something that is not in the standard of care potentially exposes you to a level of risk that may be high compared to the benefits. But alternatively, if there are multiple possible known effective treatments and you are trying to select the best first one to try, you may want specific guidance. The risks and benefits are a tradeoff.

What are the barriers to getting “functional testing”?

- **Input**: You will need to get fresh tumor tissue or fluids on which to run the functional tests. If you have malignant fluids, you're commonly getting that drained at regular intervals, which is an easy point to get a specimen. When it comes to getting biopsies from a surgical procedure, it’s easiest to piggyback on biopsies that are being gathered for other tests.
- **Physician acceptance**: You will need your doctor’s order for most functional tests. Many oncologists won't accept the guidance from unvalidated functional tests such as these. The more open-minded oncologists may ask for clinical trial data and articles, but some might not be interested in anything but a product that has successfully been through the FDA regulatory process.You may well wish to discuss this with your clinician early in your journey.

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- **Reimbursement**: Tests which are not standard can be free if the test provider is gathering research evidence, but it can be quite difficult to obtain reimbursement from health insurers for non-standard tests, and if they won’t pay, you may have to. It is a long road for a test provider to have a product which is reimbursed by insurers. At a minimum, insurers, including Medicare, want to see evidence of both clinical validity (proof that the test does what it says) and clinical utility (proof that the test changes treatment and improves patient outcomes). For example, Travera is trying to prove their test works for a variety of different cancer types and stages, making their demonstration of validity and utility harder. Sometimes you can make arrangements with test providers for reduced payments, but the time to have that discussion is early, not after your claim has been refused.

How can you overcome the barriers to accessing functional tests?

Functional test providers can help you with accessing their services. For example, Travera has had good success in getting doctors to order their test, with only a few flatly refusing. They provide a draft email for you to send to your doctor to request the test. Dennis Watson will provide you with personalized support throughout the process, including connecting with your doctor and collecting follow-up data.

How can you access this technology and help Travera’s expanding validation efforts?

Travera offers a free genetic test for cancer patients through cancer patient groups like Cancer Commons, xCures, and Cancer Patient Lab. They plan to continue research and partnerships to improve the accuracy and validate the test. They are prioritizing the list of drugs based on patient and doctor input, focusing on gynecologic and lung malignancies to build data, but also accept patients with other cancers.

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Meeting Notes

SUMMARY KEYWORDS

drug, cells, work, patient, specimen, cancer cells, test, cancer, data, measuring, heterogeneity, CLIA, biopsy, response, assay, therapy, case, mass, call, process

SPEAKERS

Dennis Watson (66%), Rob Kimmerling (13%), Brian McCloskey (5%), Richard Anders (5%), Rick Stanton (3%), Gitte Pedersen (3%), Saed Sayad (2%), Alexis Hall (1%), Mark Stevens (1%)

OUTLINE

1. Cancer patient data and technology. (0:00)
2. Personalized cancer treatment using ex vivo drug testing. (2:14)
3. Improving ex vivo drug testing using a new technology. (6:35)
4. Using mass measurement to detect cancer drug effectiveness. (11:50)
5. Cancer treatment personalized with live cell testing. (16:45)
6. Biopsy testing for cancer treatment. (23:43)
7. Cancer treatment and testing with a new approach. (29:14)
8. Cancer diagnosis and treatment using single-cell analysis. (34:17)
9. Drug testing and tumor cell isolation. (39:02)
10. Cancer drug response analysis and genomics. (44:05)
11. Liquid biopsy technology for cancer diagnosis and treatment. (48:06)
12. Analyzing cancer cell responses to drugs. (53:21)

SUMMARY

- **Cancer patient data and technology.** 0:00
    - Dennis Watson introduces himself and Rob Kimmerling and Mark Stevens, and explains the technology they will be presenting.
- **Personalized cancer treatment using ex vivo drug testing.** 2:14
    - Challenges in finding effective cancer treatment due to limited success with current guidelines.
    - Dennis Watson highlights personalized drug testing for cancer patients, using a combination of single agents and drug combinations.
- **Improving ex vivo drug testing using a new technology.** 6:35
    - Travera has developed a novel approach to ex vivo drug testing that measures the drug's impact on patient cells without the need to keep them alive.
    - This approach is clinically available for the first time in 70+ years of research, allowing for more accurate testing of drug response in patient cells.
    - Dennis Watson explains how the SMR invention at MIT allows for accurate mass measurement of individual cells, with a precision of 10 parts in 10 billion.
    - The SMR technology can measure mass changes in cells as small as 5 nanometers, which is 100 times smaller than the wavelength of visible light.
- **Using mass measurement to detect cancer drug effectiveness.** 11:50

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- MIT researchers use precise measurement tool to detect cancer cell death in real-time, with potential to revolutionize cancer treatment.
- Dennis Watson discusses using a test kit to isolate cancer cells from a patient's tissue sample, then measuring the cells' response to a drug treatment.
- The predictive accuracy of the assay is currently 85%, but the Dennis Watson plans to continue research and partnerships to improve the validation.

- **Cancer treatment personalized with live cell testing.** 16:45
  - Dennis Watson highlights 80%+ positive and negative predictive value of their cancer drug test.
  - Dennis Watson explains that their company measures immune cell response to checkpoint inhibitors, not cancer cell response, in malignant fluids like pleural effusions.
  - Dennis Watson prioritizes the list of drugs based on patient and doctor input, focusing on gynecologic and lung malignancies to build data, but also accepting other cancers.
  - Dennis Watson emphasizes the importance of timing when it comes to getting the test done, ideally before starting therapy to ensure the most accurate results.
  - The test can be repeated multiple times if there's a recurrence of cancer, as the cells may have changed since the original test, and different drugs may be identified.
- **Biopsy testing for cancer treatment.** 23:43
  - Dennis Watson explains that their company provides a non-invasive liquid biopsy test for cancer patients, which requires a physician's order and can be done during existing procedures like SATs or pleural effusion draining.
  - Dennis Watson's success rate in getting doctors to order the test is high, with only a few doctors flatly refusing, and they provide a draft email for patients to send to their doctors to request the test.
  - Dennis Watson explains that the assay ranks drugs on a scale of 0-100 based on their likelihood of eliciting a response, with scores above 50 indicating a statistically significant response.
  - The report includes a plot of mass change and a binary assay line at 50, with anything above 50 having a higher likelihood of eliciting a clinical response.
- **Cancer treatment and testing with a new approach.** 29:14
  - Dennis Watson offers a free genetic test for cancer patients through their company, xCures, in collaboration with cancer patient groups.
  - Dennis Watson provides personalized support to patients throughout the process, including connecting them with their doctors and collecting follow-up data.
  - Dennis Watson highlights the potential risks associated with the test, including the procedures themselves and the lack of experience with the test.
  - Dennis Watson also mentions the possibility of the test not working, despite their team's efforts to improve accuracy.
- **Cancer diagnosis and treatment using single-cell analysis.** 34:17
  - Saed Sayad commends Travera on their progress and offers constructive feedback.
  - Saed Sayad discusses the problem of heterogeneity in cancer, mentioning that their assay works by lysing background cells and getting a single cell suspension, which inherently controls for heterogeneity.
  - Dennis Watson adds that they don't know which cells are responsive and non-responsive to drugs, so they use an iterative process to get at the heterogeneity problem, including mixture of the cells for follow-up testing.

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- **Drug testing and tumor cell isolation.** 39:02
  - Gitte Pedersen and Brian McCloskey discuss analyzing RNA to create hypotheses for drug development, with a focus on isolating tumor cells from tissue.
  - Rob Kimmerling explains that their team uses vehicle-treated control cells as an internal control to compare with drug-treated cells in their plating approach.
  - Rob Kimmerling's team historically did triplicate measurements but now prioritizes measuring a larger drug panel with fewer replicates to get more data.
- **Cancer drug response analysis and genomics.** 44:05
  - Researchers compare drug response in cancer cells from solid tumors and fluid surrounding tumors, finding inconsistent results.
  - Oncologists believe that targeted drugs may be more effective than previously thought, as some patients respond differently to various treatments despite having similar genetic profiles.
- **Liquid biopsy technology for cancer diagnosis and treatment.** 48:06
  - Brian McCloskey asks about correlations between spatial phenotyping and proteomics.
  - Rob Kimmerling mentions limited success with dissociating cancer cells from bone biopsies due to physical matrix.
  - Rob Kimmerling explains that 5000 cells are not a magic number for statistical robustness, but rather a sweet spot around 2500 clean measurements are needed for accurate analysis.
- **Analyzing cancer cell responses to drugs.** 53:21
  - Rob Kimmerling explains that the decision threshold for determining a response to therapy is not cell type specific, but rather a fixed threshold that is used for validation purposes.
  - Rob Kimmerling and Richard Anders discuss the potential for differentiated peaks in response to different drugs, which could lead to a more personalized approach to therapy.
  - Rob Kimmerling mentions that the company's CLIA certification constrains their ability to use patient-specific markers for sample prep.
  - Mark Stevens mentions resources for directing patients to research tools for processing their cancers, which have gone through research avenues.

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TRANSCRIPT

Brian McCloskey 0:00
I welcome Dennis and all who are participating. I know that we'll have a lively conversation.

Dennis Watson 0:55
Thank you very much, Brian. I want to start by thanking you and Brad for inviting us to this opportunity today. I have Rob Kimmerling and Mark Stevens with me as well. Rob is our chief technical officer, and Mark is our Vice President of clinical development. These are two of the gentlemen that were actually in the lab at MIT where this technology was developed. We're very lucky to have them on our team. They will be able to answer any questions that I'm unable to answer.

I'm going to go through a handful of slides, nothing too incredibly deep. I will share what our technology is, what's unique about it, and what data supports it as of today. Then I'll also take a few slides to walk through as a patient, how you might get access to our assay.

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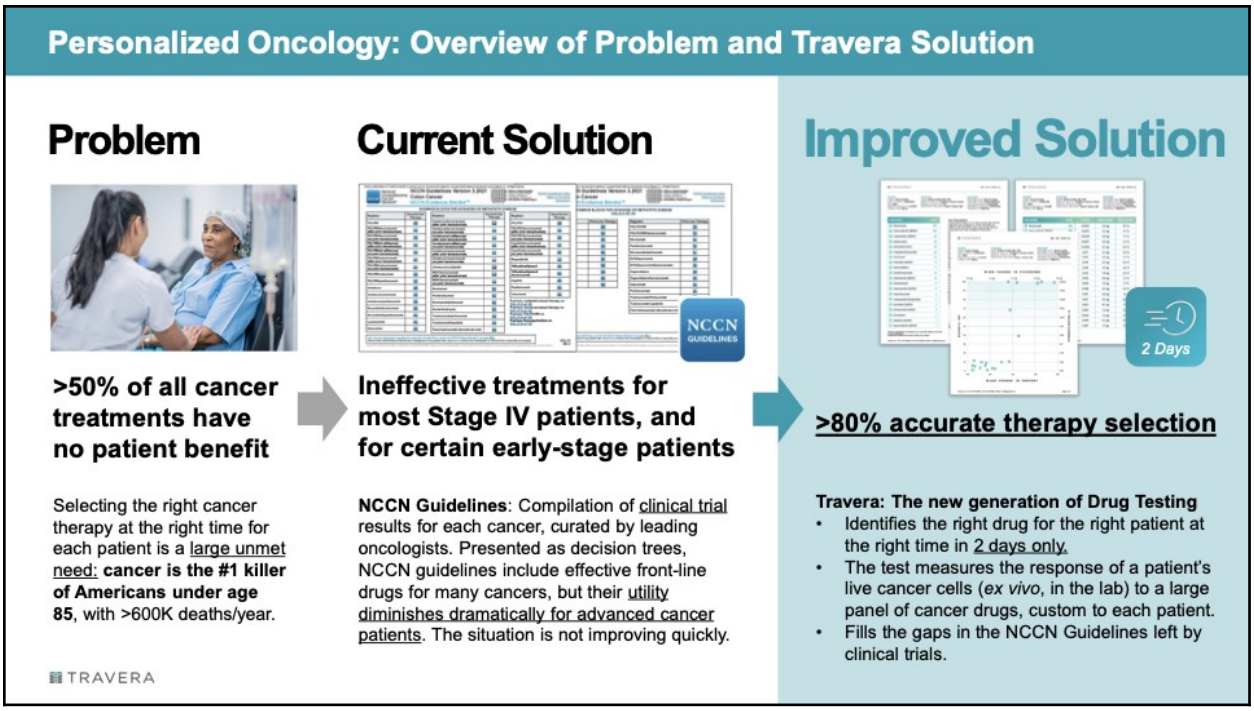


The first thing I'm going to start with is, there's a real challenge with finding the right drug in cancer treatment. This is even more true in later stage disease and in rare diseases. The reality is that the current solution is the NCCN guidelines. For early stage, for newly diagnosed patients, this is a pretty effective approach. The likelihood of finding an effective therapy for first line, early stage disease is relatively high in most cancers and most tumor types. But once you get to recurrent disease, once you get to second or third lines of therapy, the statistical likelihood of finding a drug that creates a clinical response is about 20%, at best, and it actually drops from there, depending on what specific disease you might be dealing with and what challenges you're facing. The reality is, though, that there are a lot of drugs available. There are hundreds of drugs listed in the NCCN guidelines. The statistical likelihood is that there's a drug in there that's going to create a clinical response. In a particular case it is probably a lot better than 20%. But it's the guesswork that we go through of trying to match the patient to the drug that is the real challenge.

I often share that I watched my mother-in-law go through this with late stage ovarian cancer and the toll of trying this next therapy. “Let’s see what happens.” It's an educated guess, as to what we're going to do next for you. It's not only physically demanding and exhausting to go through these extra additional lines of therapy, but it's emotionally exhausting too. I know with her, she just got to a point that she was like, “I'm good. I'm done.”

The idea of Travera is to help close that gap. It's to identify these drugs that are FDA-indicated that are appropriately-used drugs, and narrowing the list of drugs that are most likely or have a higher likelihood of eliciting a clinical response in a specific patient. It's truly personalized therapy. I stress the word “truly” because not all diagnostic assays we do are truly personalized. I've spent 15 years of my career in genetics and genomics in cancer, mostly in the breast

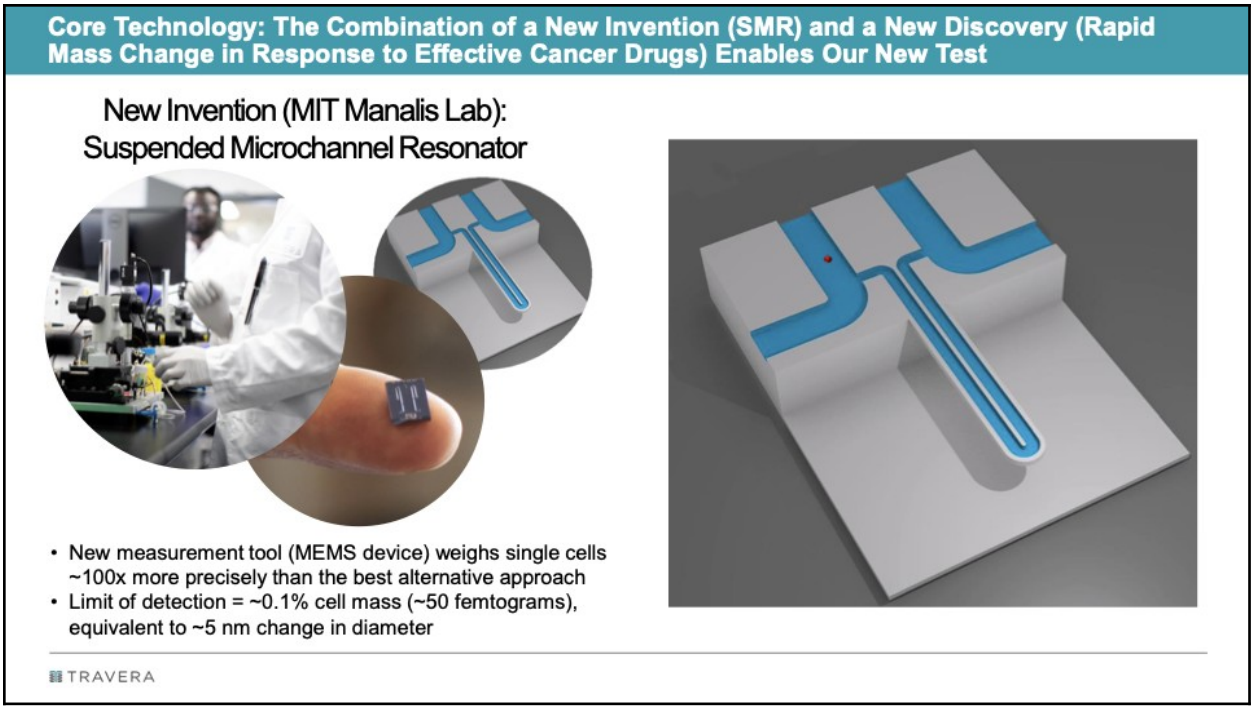
cancer and gynecologic cancer spaces. We loved to use the word “personalized”. But the reality is, it was really just smaller populations. If you're talking about a BRCA mutation, you're just part of a smaller population than you were before. That still isn't truly personalized therapy. What we're doing here at Travera is N-of-1 work. It really does separate it at that level.

Rick Stanton 5:38
Are you using your improved solution? Are you testing single agents only?

Dennis Watson 5:45
Most of what we do are single agent tests, which I will get into a little deeper. But we also do some combinations as well.

The idea of what we're doing is not really new. The idea of “ex vivo” drug testing comes from the infectious disease space originally looking at unusual infections. Your infectious disease doc doesn't go to their version of the NCCN guidelines. They take a biopsy, they culture those cells, they try 15 different antibiotics, and they give you what works. It's wildly effective. It's done every day across the US. It's the standard of care in complicated infections. We've been trying the same approach and cancer since the 1950s. There are a lot of companies that have gone by the wayside over the years trying to make this happen. Unfortunately, we've not had really consistently sustained success in ex vivo (outside of the body) drug testing approaches. One of the reasons that we believe this to be true is because when you take a cancer cell outside of the body, away from its life source, it dies pretty quickly on its own within a couple of days. But the average cancer therapy takes about a week to work. You have this really challenging conundrum in time management, of removing the cells, and measuring a drug’s ability to kill a cell when the drug is dying faster than the cell killed them, or when the cell is dying faster than

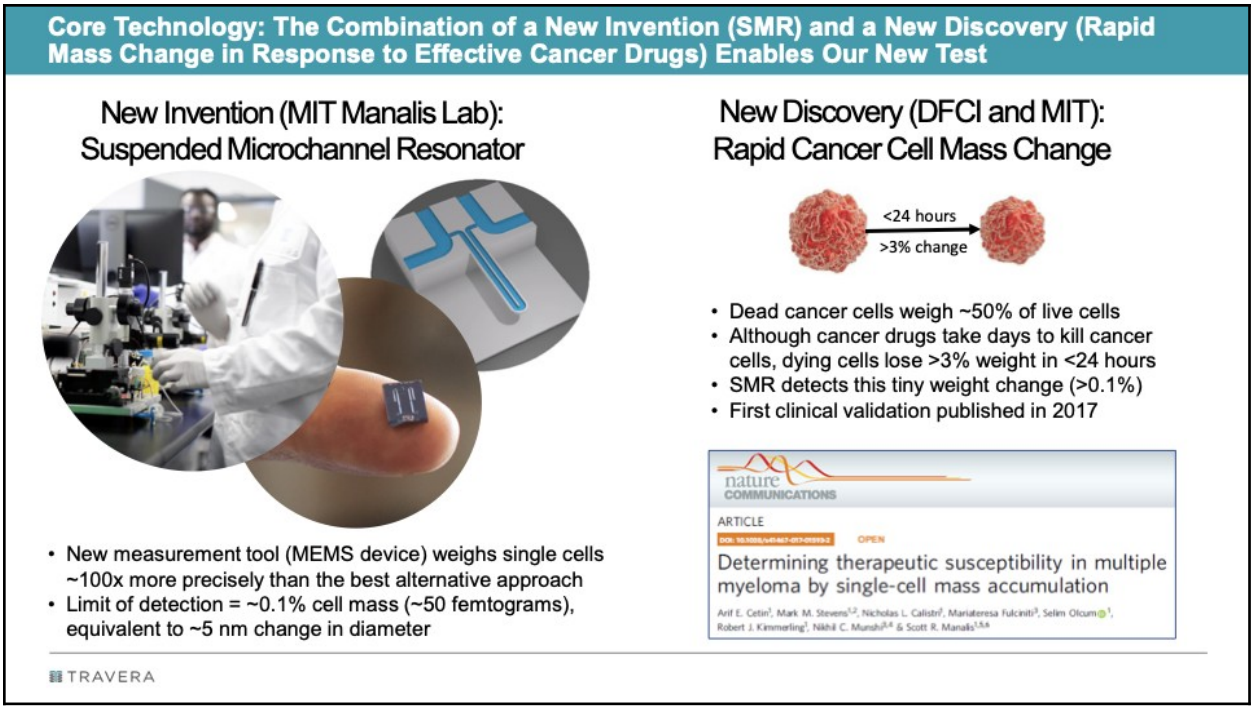
the drug can kill it. So we've had to intervene. This has been the typical approach. It's been cultures. It's been PDX (patient-derived xenograft, meaning that the models are initially created by implanting a fragment of a human tumor into a mouse). It's been more recently the organoid approach. We're improving the ways in which we do this over time. But the challenge that remains pretty consistent is that the biology over time inevitably changes. There's lots of published data on this. We see phenotypic drift in the cells. We see genotypic drift in the cells. We grow the wrong subclonal population because of heterogeneity and sample picking, etc. We ended up testing drugs against something that isn't necessarily representative of what was taken out of the patient. This has been the inherent challenge with “ex vivo” drug testing. What we do differently here at Travera is we have figured out how to work that clock backwards, rather than figuring out how to keep the cells alive long enough to measure their ability to kill cells – the drug’s ability to kill the cells. We have figured out how to measure the drug’s impact on the cells so fast that we don't have to do anything to keep them alive. This is the first “ex vivo” drug test that's clinically available, to my knowledge, in 70=plus years of research in this area, that is actually testing drug response in the patient's direct cells rather than a grown version of those cells. That is a really important paradigm shift in the way that we're approaching this.



This is how it works. It is because of an invention called the SMR: the suspended microchannel resonator, which is a physics device that was developed at a physics lab at MIT. This is a tiny little MEMS (microelectromechanical systems, a combination of mechanical and electrical engineering) chip. If you're not familiar with MEMS, I describe it as a microchip that has physical movement instead of circuits. The idea is this tiny little chip on the finger here, these are fluidics channels. I'll go through the exact process in just a moment of how that works. When we get a live cancer cell specimen we expose it to a drug, and we pass it through a single cell

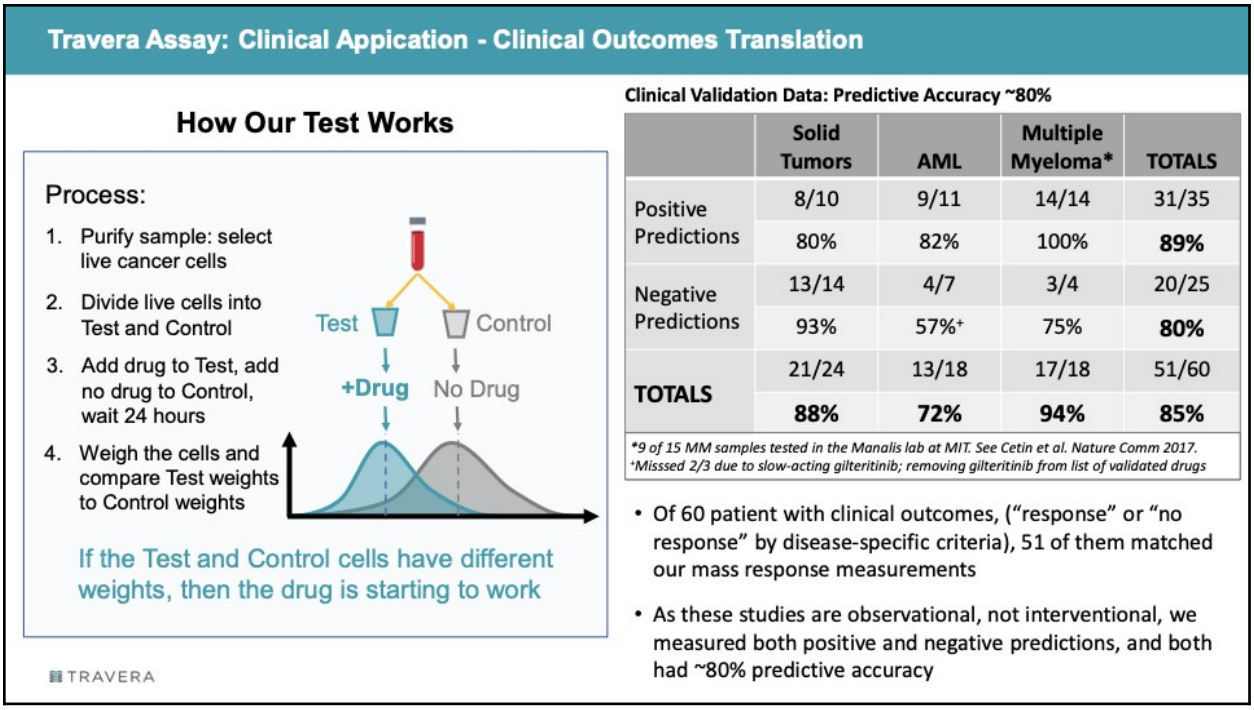
suspension over this chip. As it passes through here, it goes over this thing that looks like a diving board, this cantilever. It takes advantage of a really simple law of physics that says the resonant frequency of an oscillating cantilever is directly proportional to its mass. When you jump off of a diving board, you get a vibration after your jump. That vibration changes based on how much you weigh. If you can measure the actual frequency of that vibration, it is accurate to about 10 parts in a billion. It's an incredibly accurate measurement tool. We can measure the mass of an individual cell. That's about 100 times more precise than any other measurement technology available in the world today.

I always like to give this one little example. It gets a little deep, so bear with me. But we can recognize the change in mass of a cell that would be equivalent to that cell losing about five nanometers of diameter. Now, if any of you had freshman year physics classes in high school, you might remember that a single wavelength of visible light is between 400 and 700 nanometers. We can measure mass change down to about a five nanometer loss. So it's 100 times smaller than a wave of light. The reason that specific example is really important, is because the next best technology available today to weigh individual cells is a light microscope. But light can never be better than 400 nanometers. It's this incredibly precise measurement tool. Measurement tools change science: the microscope, X ray, MRI. New measurement tools create opportunities to do things that we thought were previously impossible. That's what this team has done.



The team at MIT, including the gentlemen on here, took this down the street to Dana Farber and played off of a really simple concept of biology, which was: we know that when a cell, really any cell, dies, on average, it loses about half of its mass. We know that when a cancer cell is responding to an effective cancer therapy, that the death process takes an average of about a

week. So the question is: “Would a cancer cell responding to an effective drug change its mass by a small amount in the first few hours or the first day of exposure?” The answer to that question has been a resounding “Yes.” I don't use this phrase lightly. But this is almost a universal biomarker. We've shown that this works across virtually all carcinomas, and many of the blood cancers. It works with just about every mechanism of action of a drug you can think of. It's incredibly broad, because, at the end of the day, if the drug is going to affect the cell, and eventually kill the cell, it's going to change the cell. When the cell changes, its mass changes. If you can measure the mass change to enough specificity, that's really where this comes in.



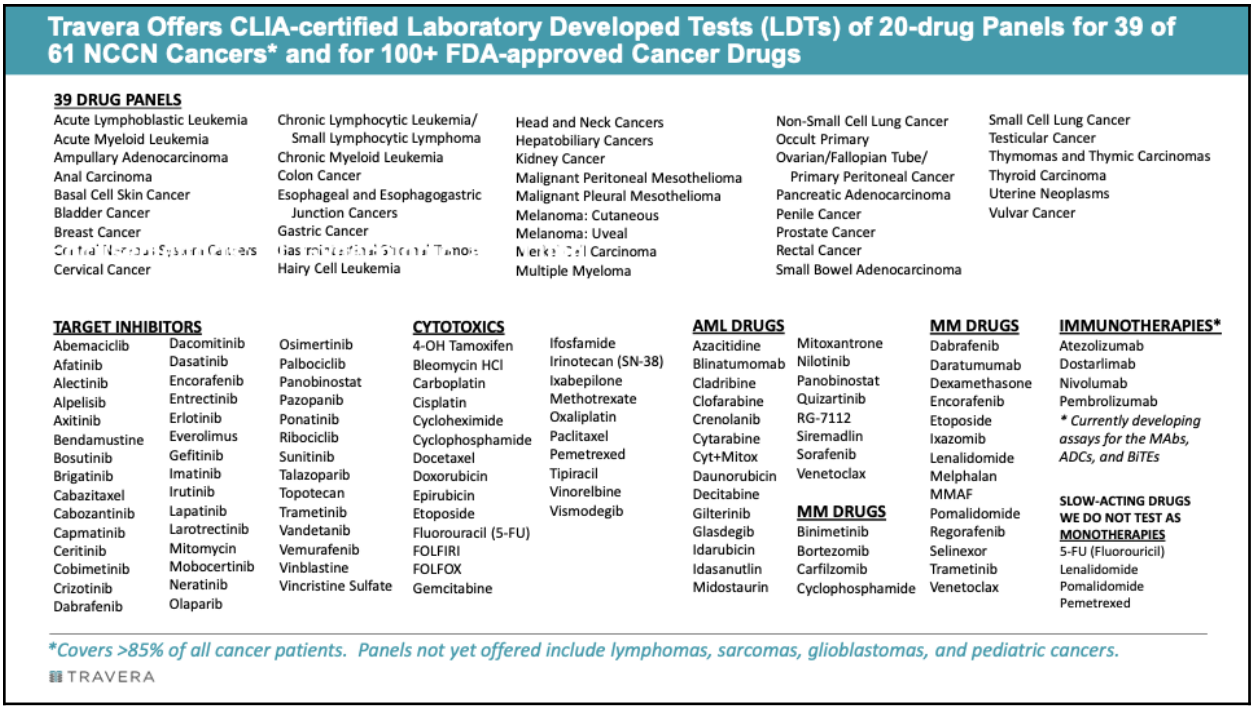
The concept in practice works like this: We get a lifestyle specimen – I'm going to go through the actual steps of what would be involved as a patient in just a minute – either a malignant fluid collection, if you have ascites (fluid in spaces within your abdomen), or a pleural effusion (fluid in the lining around your lungs). That's a really easy specimen for us to work from. It works great on our platform. It's probably our preference. But we also can work from solid tissue from CT-guided biopsies (a small tissue sample gathered through a needle, guided by a CT scanner), from fine needle aspiration, and from excisional or resections (surgery). We work with a lot of patients with peritoneal disease (cancer that begins in an organ within the abdomen – for example, the colon or the stomach – and spreads/metastasizes to the peritoneum/abodmenal lining), for example, who are having diagnostic laparoscopic ease (a type of surgical procedure that allows a surgeon to access the inside of the abdomen and pelvis without having to make large incisions in the skin). Those are really nice ways to get a nice tissue specimen. We don't need a lot. We provide our test kit ahead of time. The specimen is collected and placed into our kit, cold-shipped overnight by FedEx to our lab, we isolate out the cancer cells, and we separate them out into a 96 well plate in the lab, so about 5000 cancer cells per well. Then we put the drug in some of those wells and we leave others as controls. We incubate that for 24 hours,

then we go back and run our measurements. In the example you see on the slide here, you've got the blue cup, which would have 5000 cancer cells and an appropriate dose of drug for that amount of cells. Then in the grey cup, you've got 5000 cancer cells that are just sitting there with nothing really added to them. It's all about minimal change from when they were taken out of the patient, then incubate for 24 hours. We take 5000 individual measurements. We measure every single cell in that cup. From those 5000 individual measurements, we build these little mass diagrams that give a distribution of all of the different weights represented in that specimen. The goal being if the cells in the control, and the cells in the drug, are changing at the same rate, and those two curves overlap, or mirror each other, that drug was basically a placebo. It has done nothing in the cells. But if there's a shift, and the distribution of measurement from the one sample to the control, like you see on the slide, that's what tells us that this drug is beginning to take effect, and four or five days from now should kill the cells.

It all sounds really cool and interesting in a laboratory application. But how does it translate to outcomes? So far, we've done several 100 of these tests. But we have full clinical outcomes on about 60. This is spread across a lot of different tumor types, as you see on the slide here. The predictive accuracy of the assay right now is sitting at about 85%, which we're incredibly proud of. It's a great number, but it is a small n. So, we're continuing to look for opportunities to further our validation, our research, and our partnerships. I'll share in a minute about how you can actually get it if it makes sense for you and your case.

But two points I want to make on this: we have both positive predictive value here and negative predictive value, both sitting in the 80% plus range. What that means is that these are patients that received our test – we ran a panel of 20 drugs. Two days later, they got the results back and they went on one of the drugs we ran. A positive predictive value means that the patient

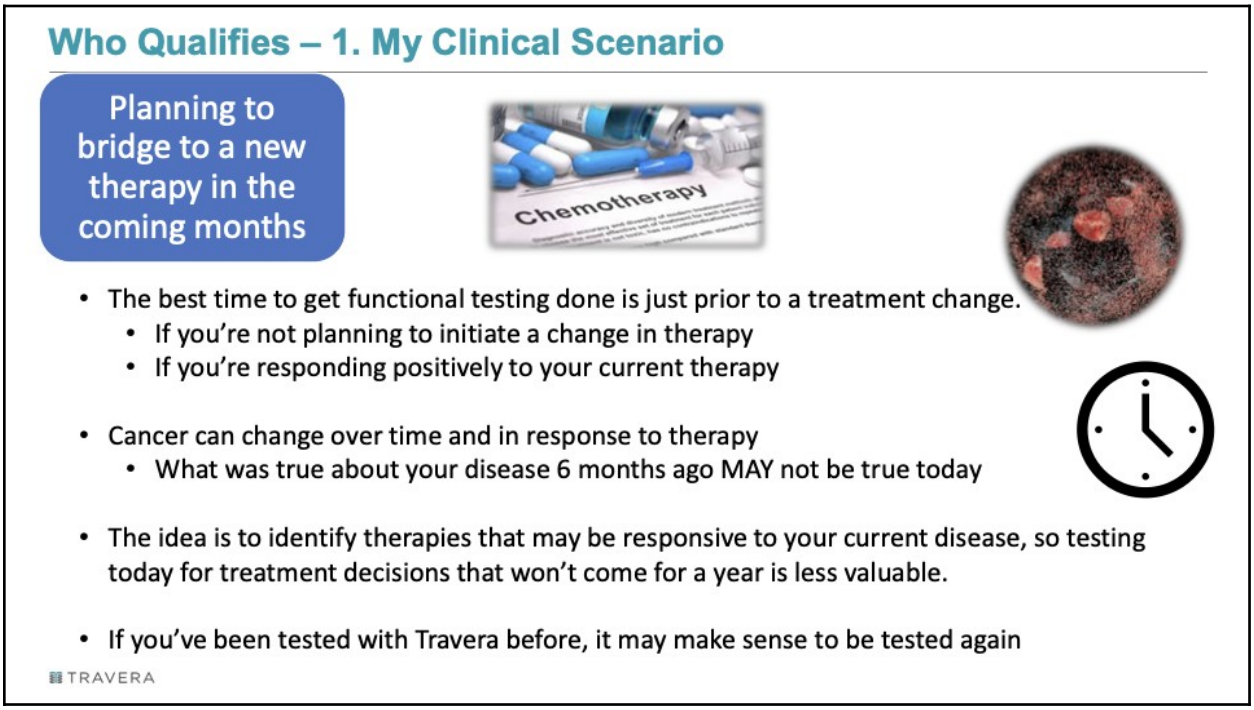
went on a drug that we said was going to work. Then some months later, we got clinical outcomes data from their physician as to whether or not they had a clinical response from that drug. When they did, that was an accurate call. So these are patients that got a drug we said was going to work, and the drug created a clinical response. Negative predictions: our patients that got a drug we said was not going to work, and those patients continued to progress on that drug. Those are the sort of differences between negative and positive predictive value. It's an important definition to understand because when we're running a list of 20 drugs, they're often or drugs, we say you're going to work. There are also a lot of the drugs that we say aren't going to work. There's the opportunity to not only rule in drugs that might have a higher likelihood of response, but there's also potentially the opportunity to rule out drugs that are unlikely to elicit a response.



This is the panel of tumor types, cancer types and drugs that we can do. I haven't mentioned it yet, but we also do immunotherapies. We have a checkpoint inhibitor panel. For those of you that understand how checkpoint inhibitors work, often I get the question, “How are you measuring cancer cell response to checkpoint inhibitors?” We are measuring immune cell response to the checkpoint inhibitors. We can't do that from tissue because we can't get enough immune cells out of the tissue. We're only doing that test right now from malignant fluids. When you have ascites or pleural effusions, there tend to be a lot of immune cells and, ideally, tumor-activated immune cells in that fluid. We're separating out those T-cells. We're measuring the ability for the checkpoint inhibitors to activate those immune cells. That's a separate test that we do. The technology, the process is the same, we're just measuring the cell response of the T-cell rather than the cancer cell. We do basically build 20 drugs, standard panels, depending on what the diagnosis is. Then we work with the doctor and the patient to edit and adjust that, on a case-by-case basis, as it makes sense. The other thing that becomes really important is that

we're not always able to get enough cells to run all 20 drugs. We also work with the patient and the doctor to prioritize the list because if I only have enough cells to run five drugs, I don't want to run five drugs that you already had and don't care about. We work with not only what drugs are on the list, but what priority is important to you in your care as well, which is why we've got a wide list of cancers we can do here.

We'll still take any and all cancers. We're obviously focused on building our data. We're specifically targeting right now gynecologic and lung-based malignancies. We're really very interested in building out our data set. We're doing a lot of other stuff. I know that there are definitely some patients in this community I've spoken with who have pancreatic cancer. We put that in the GI (gastro-intestinal) bucket. Anything that is in that peritoneal cavity, that GI and oncologic and gynecologic malignancies.

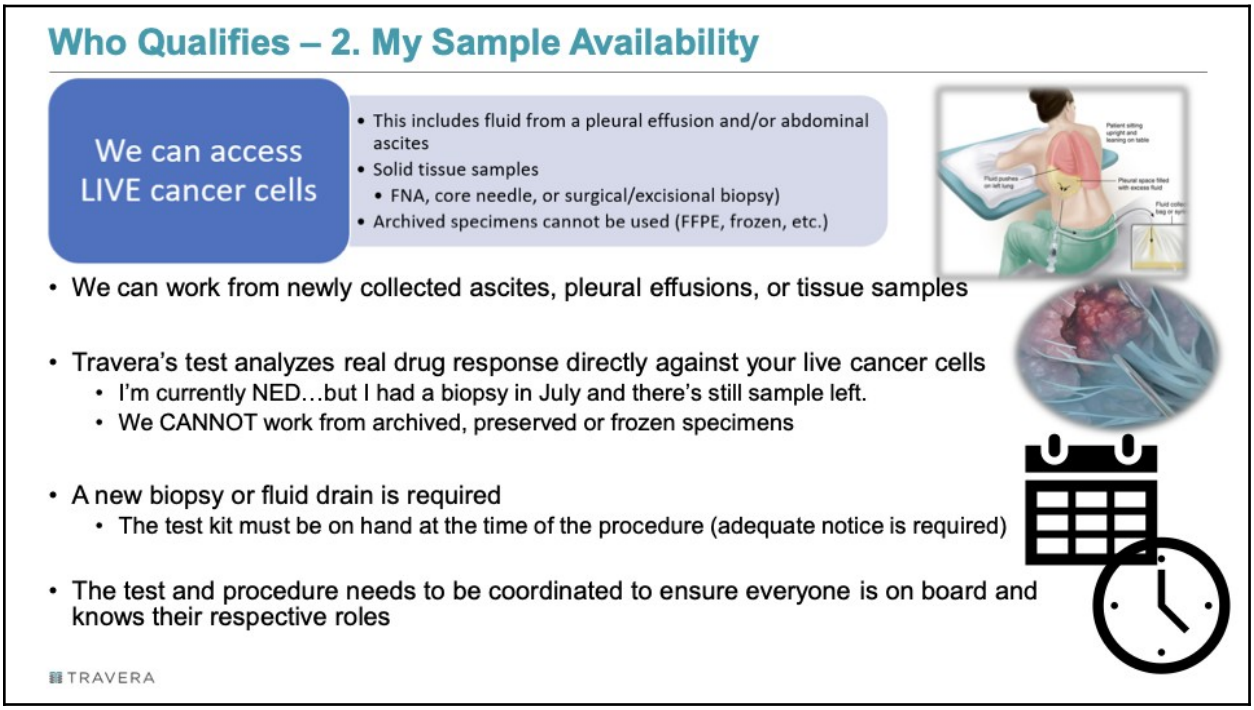


This is how it works. I'll give you a quick review.

Who qualifies for this? The first thing to be aware of is the timing. When should you get this specimen and this test done? It's really at a point that you're planning to change therapy. If you're on a therapy that's working, the likelihood is that even if you've got a biopsy, and you send us that specimen, there's a decent chance that we're going to get a specimen full of dead cells because the drug you're taking is working. I'm always of the mindset that, “Let's not break what's not broken.” The idea is to get this at a time that you're looking to make a change. Also, as I alluded to earlier, cancer is immune, by definition, and your cancer changes over time. It changes in response to environmental factors, and different drugs and treatments you've been exposed to, and just over time in general. Having this test done today, if you're not planning to go on therapy for the next year, might not be as valuable as it would be if you were getting it

done right before you initiate therapy. You want to catch this cancer in the cells – the most real-time way to make sure that what we're delivering is as specific to your current case as it possibly could be.

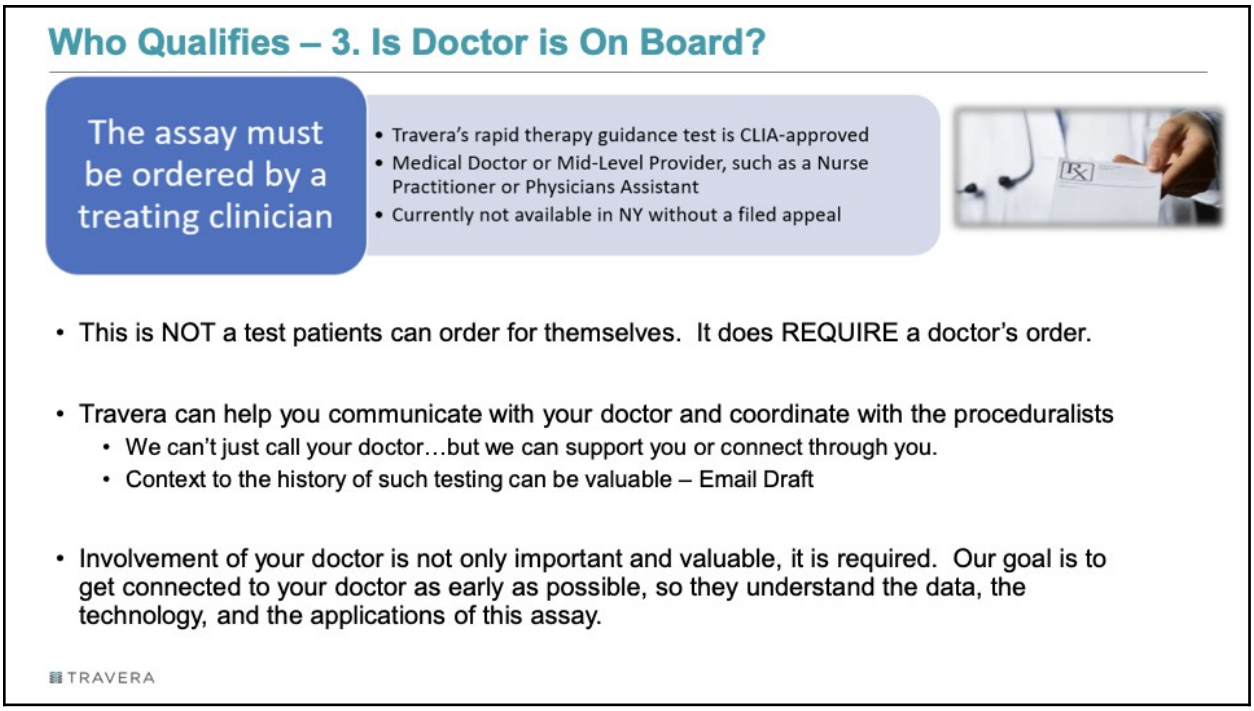
The other point here is that it can make sense to do the test more than once. If you have therapy, you may use this test to guide therapy, and then, say, at some point in the future, there's a recurrence. It might make sense to test again, with that recurrence, because again, this could be a recurrence where the cells have changed from the original specimen, and there might be different drugs identified.



We need live cancer cells. This is probably the most significant challenge that we face in this test. Live cell testing is hard. A lot of it is about timing. We need to get the word out, letting people know that this exists, and that it's available. Brian opened with, “I might be giving you a call in six months.” That's exactly the way to be thinking about this. If it doesn't apply to you today now that it exists, it might apply to you later.

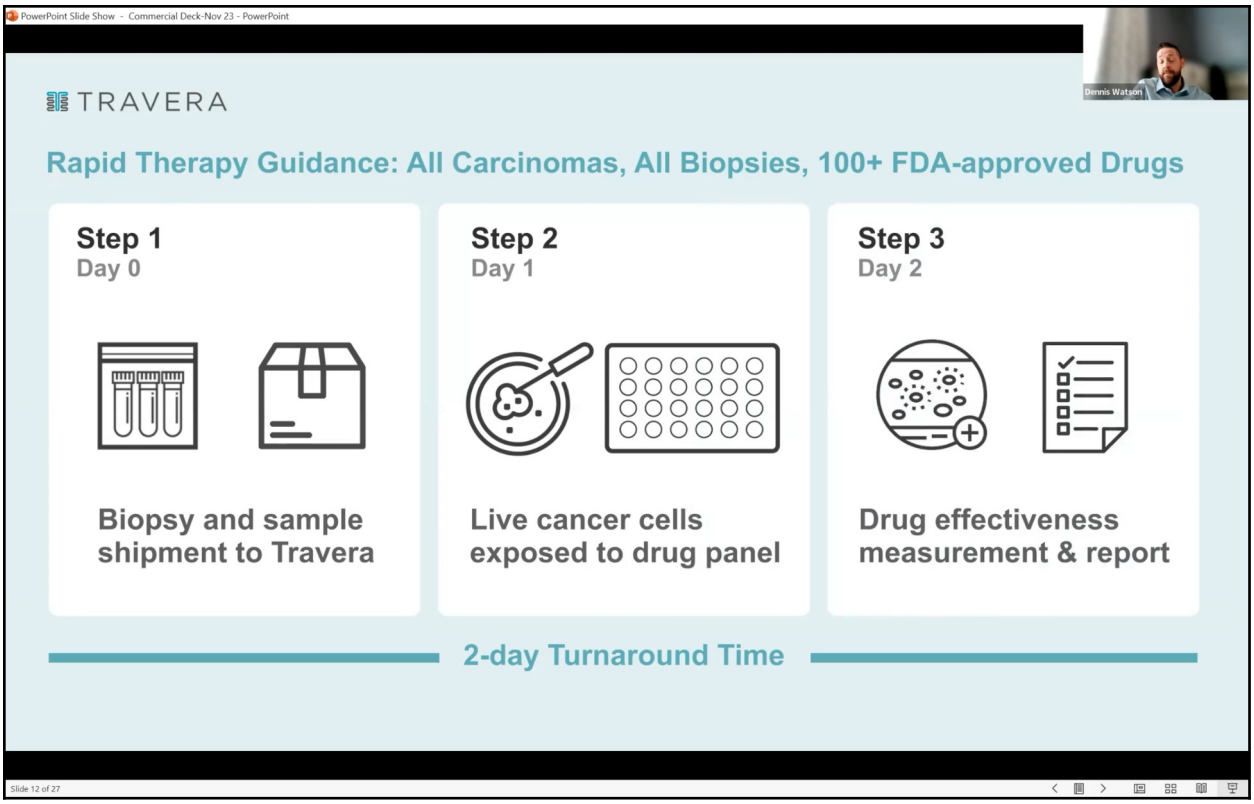
Because the test is not reimbursed by insurance right now, if you were to get a biopsy ordered specifically for this test, the biopsy isn't going to be covered by insurance either. Insurance companies don't like to pay for biopsies for tests that they don't pay for. So we typically just piggyback on existing procedures. Like I said, if you have ascites or a pleural effusion (malignant fluids), you're commonly getting that drained at regular intervals. So it's a really easy point for us to get a specimen. It's easy to work with. It's all the things. But when it comes to the biopsies or the surgical procedures, we just have to be conscious of the timing around that. I'll work with you closely to work through that process as much as I can.

“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]



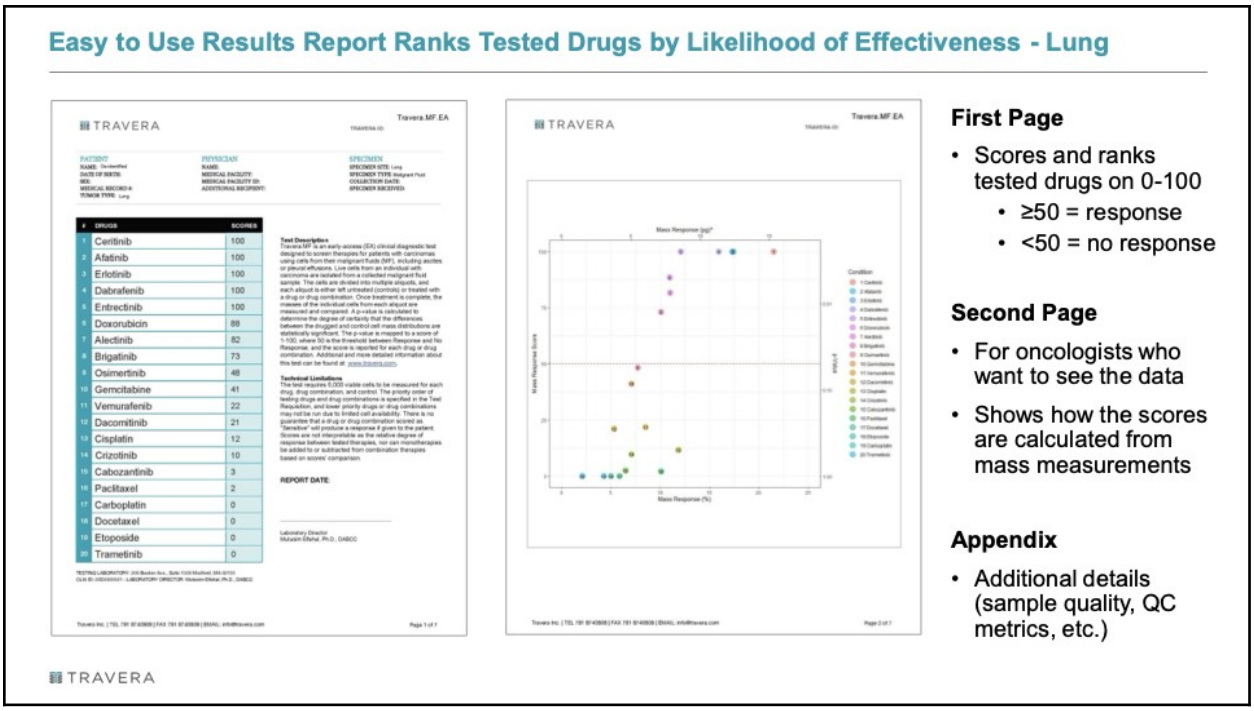
The third thing is that we are a CLIA pre-approved assay. That means we need a physician's order. This is not something that a patient can order themselves. We need your doctor’s support. We have a network of doctors that we can connect you to if that's something that makes sense to you or that you're interested in. We're always looking to expand that network. My success rate with getting doctors to order this test is pretty high. I've had contacts with somewhere in the neighborhood of 400 patients. In the last year, we tested more than 200 of them. Most of the ones we haven't tested have been patients that didn't have tissue or biopsy of a fluid available at this time, or they're putting it off for something in the future, et cetera. The vast majority of doctors that I talked to in those 200-plus cases, ended up being willing to order the test. We've only had about fewer than 10 doctors that have flat out said, “I won't do this.” What they do with the results when they get them, if it says something that they don't want it to say, is probably another discussion. But on the willingness to order the tests, we're able to work through. We always work with our patients as directly as we can. I even go as far as to provide a draft email that you can just make a couple of little edits to, to send to your doctor to say, “Hey, I've learned about this thing. I want it, and this is why I want it.”

“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]



The process is: once you've got a procedure coming up, we provide the kit ahead of time, so we need some notice. We can overnight it if we need to. But generally, at least two days' notice is ideal, really ideally more than that. Once we provide the kit, we coordinate with you, your oncologist, your surgeon, your interventional radiologist, whomever it is. They put the specimen in the kit. They ship it overnight to is, as I said, we get it in our lab, we run our process up to 20 drugs plus those for immunotherapies. If it's an immune test, if it's a fluid specimen that produces enough immune cells. And then basically the day after that, we returned results to your doc. So if you were to get a biopsy on a Monday morning, we're returning results to your doctor with analysis of those 20 drugs. Usually, by the end of the day, Wednesday or Thursday morning. The entire process only takes a couple of days, once we get the specimen. There's a lot of value in that when you're trying to make quick, quick clinical decisions. But it's also just an inherent feature of our assay. As I said, we're not doing anything to keep your cells alive. We're really minimally interfering with the cells. We have to work quickly. Because we don't want to mess with the cells, we want them to be as similar to what they were when they came out of your body as they possibly can be.

“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]



The report looks like this. We stack rank the drugs on a scale of zero to 100, where 100 is the highest likelihood of eliciting a response or seeing a response in our hands down to zero, meaning that we saw no response from these cells in our hands. Since this is a mass change assay, we plot the mass change on a chart as well. Some people like to see that. This is a pretty typical report. We usually will get a handful of drugs, five or six, that are up at the top, that are highly sensitive. We'll get a lot at the bottom, and there might be a few that are sitting somewhere in the middle. The line at 50 is the line that we've drawn to almost make this a binary assay. A score of 50 has a statistically significant P value, meaning that we were highly confident that the response we saw in these cells in our hands was due to the drug and not some other variable. That's how that works. But really anything with a score above 50 has a much higher likelihood of eliciting a clinical response and vice versa. There are always inevitably a couple that sit somewhere in the middle. The real thing with these drugs that would sit in the middle is just the comfidence level of what was creating that change is just not as high.

“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]

Logistics

What do I usually have to do as the patient?

- Contact Travera to express interest and we’ll walk you through the whole process
  - Connect us to your doctor and help to secure the order **and discuss the drug list**
  - Connect us to the proceduralist (IR/Surgery) to coordinate the process
  - Manage the test kit arrival and return

Logistical Challenges - Timing & Coordination

- Timing of the specimen (biopsy, fluid drain, etc)
  - Included adequate lead time
- Lack of “extra” sample, access, shipping delays, etc.
- Facility challenges with process and protocol
  - get us connected to surgeon or IR ahead of time

TRAVERA

What do you do as a patient? If you want this, we're offering this through groups like Cancer Patient Lab at no cost.

TraveraRTGx Clinical Trial

Unique prospective observational trial with xCures as the CRO and primary site, gives your patients the opportunity to enroll in a central-IRB approved trial, seeking to track patient outcomes and therapy selection.

Study Population

This pilot study will utilize samples from as many as 200 patients with a known carcinoma undergoing routine draining of malignant fluids for diagnostic or palliative standards of care (SOC).

Inclusion Criteria:

1. Patient is ≥18 years of age
2. Written Informed Consent provided by patient
3. Diagnosis of any kind of carcinoma
4. Malignant fluid (e.g. pleural effusion or ascites) drainage is clinically indicated as part of SOC
5. Proceeding onto therapy for treatment
6. Informed consent obtained for the XCELSIOR longitudinal outcomes registry (NCT03793088)

TRAVERA
xCures

Exclusion Criteria:

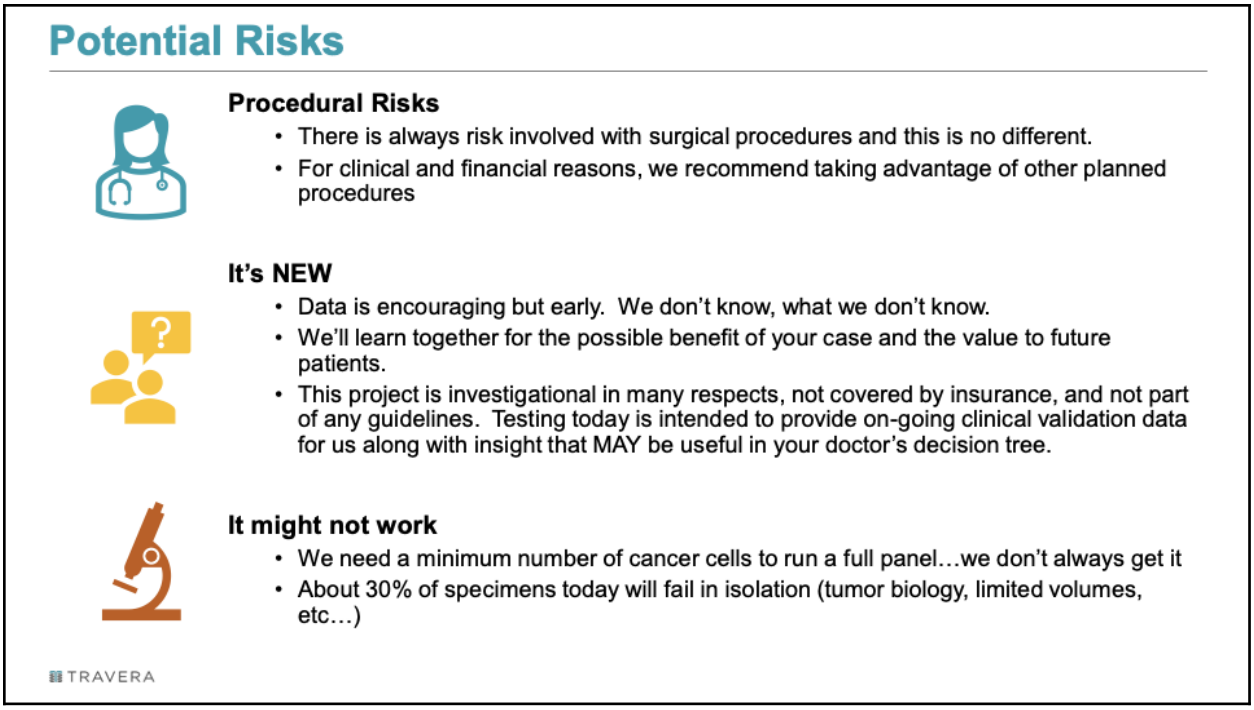
1. Lack of informed consent
2. Unable to obtain sufficient sample

TRAVERA

We're still doing clinical trials. We're working with a clinical trial with xCures. A lot of you are probably familiar with with xCures and Cancer Commons. I can show you how to enroll in that. For doctors that are a little more skeptical or hesitant to do the test through just the free early

access program, the xCures study just gives a little bit of protection. It gives something that the doctors feel more comfortable about if it's part of a clinical trial. They're often likely to do it that way. It also helps us with collecting the follow up data and the outcomes, which is really what we're looking for.

We're giving the test at no cost. The only ask that we have is that you let us know what happens. We're looking for real world case experience. We want to know, did you end up going on one of these drugs? And if so, what response did you see? We collect that data through a 15 question, HIPAA-compliant survey that I send a few months after the test is done. Occasionally, following that, we might follow up and say, “Hey, is it possible for you to get us a screenshot of these notes from from your medical chart?” Or something along those lines, just anything that we can get to create a little bit of validation data that we can fit into our real world evidence portfolio for future reimbursement. But basically, the process is that you contact me. I'm going to put my contact information on the last screen here in just a minute. You reach out to me. I'll walk you through the whole process, beginning to end. Every patient that comes through our lab comes through me as well, working with you to connect to your doctor, to communicate with your doctor, to get the order filled out, to get the test kit out, et cetera. It all works through me, and all you have to do is contact me directly to make that happen. I'll walk you through, helping you figure out if you're qualified. If you have questions, I'm happy to do all of that logistics. The biggest challenge is timing. With any scheduled biopsies and/or fluid drains. A patient, actually a nurse, called me yesterday morning at 8am and was like, “Hey, our patient is having a biopsy at 11. What do we do?” I'm like, “Nothing. Sorry, I wish I could help, but we need a little more lead time than that.”

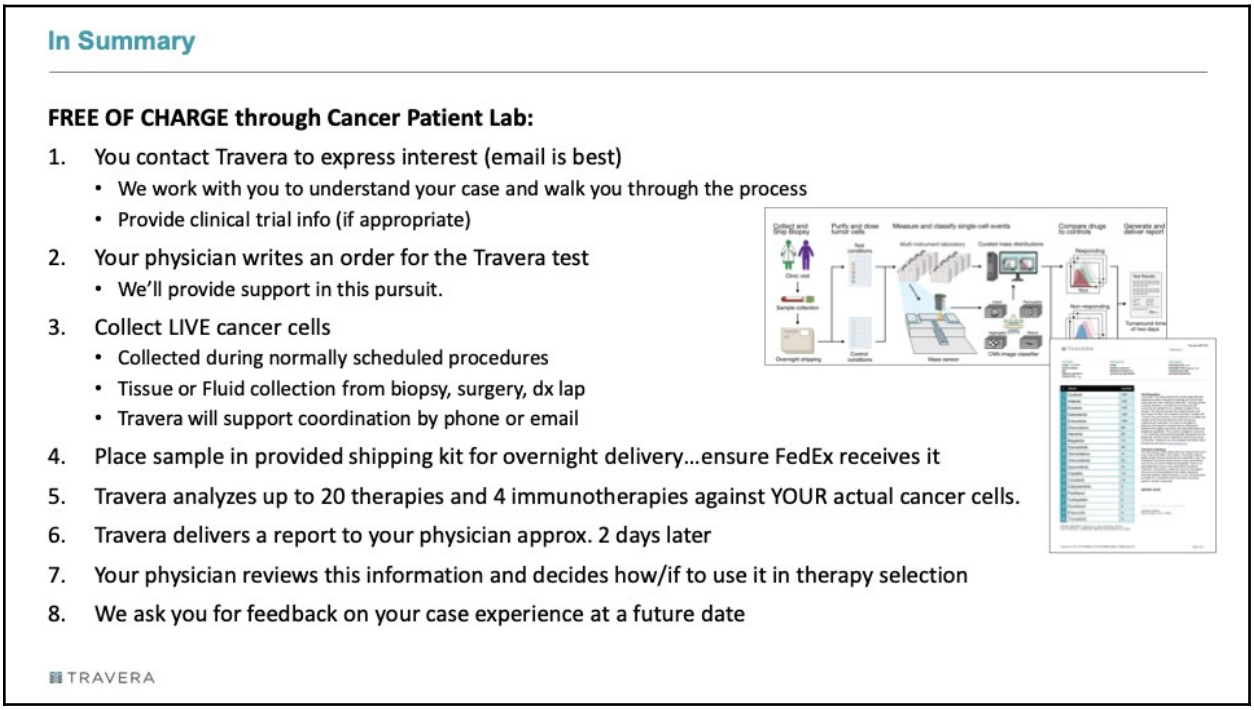


“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]

Potential risks. Obviously, anytime you're having a procedure done, a biopsy, a fluid drain, whatever it is, there are risks associated with that. Those really are the most significant risks associated with running this test. We're running a list of drugs that in the vast majority of cases are already in the purview of you or the doctor, already drugs that you're considering. All we're doing is giving some additional information and guidance on whether or not those drugs elicited a response in our hands. The biggest risk really is probably associated with the procedures themselves.

The second risk to be aware of is that this is new. The data looks really good. An 85% predictive value is an impressive number. We're seeing it hold true as we add patients to that data set. It’s a small number of cases. The reason we're willing to run the test at no cost in exchange for letting us know what happens is because we know that we need more cases. We need more experience. We need more data. There are just things that we don't yet know.

Finally, the other risk is that it just might not work. We got a really well collected specimen yesterday or earlier this week from an interventional radiologist that has worked really closely with us. He called to make sure there wasn't anything he could have done better. It was just a white marble specimen, really fibrous. There were like 500 cells or something. There just weren't any cancer cells in the specimen. Sometimes we just aren't able to get what we need. The majority of cases work. Rob and Mark and the team in our lab have done an amazing job of improving this accuracy or this success rate over time. As we get more cases we've upgraded our process, how we isolate the cells, and how we get rid of all of the background cells, etc. We're getting better and better at this all the time. But there are still some specimens that will just fail.



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Dennis Watson 34:16
This is the flow. There's nothing new here. This is what I already said. You let me know you're interested. I work with you to get your doc engaged and order the test. You get your cell specimen collection. They put it into our kit, ship it to us to run up to 20 therapy drugs and immunotherapies, and we return the report a couple of days later. At some future date we will ask for your feedback. This is the executors trial. I can give you the direct link. There's a landing page, or you can email me and here's my contact information.

Saed Sayad 35:36
In the last couple of years, I've been analyzing hundreds of thousands of the more than 5 million samples free of charge in the public domain. Your methods easily can have higher results than the other methods. I just want to say, “Congratulations.” You are on the right track. For sure, there are many things you need to resolve, but you are on the right path. I strongly suggest to everybody to try this method.

First is the problem of heterogeneity of the cancer. How do you manage it?

Dennis Watson 36:35
Heterogeneity is always an issue with anything that you do. We've got an interesting solution that was unintentional in the way our assay works, and that we work from a single cell suspension. We get that biopsy, or we get that fluid specimen, the first thing we do is lyse the background cells down to the cancer cells through existing technologies that we didn't develop. We get that single specimen down to a single cell suspension. It's been divided into 5000 cells per well. You end up almost tossing everything into a blender, in a really layman way to put it. We end up having a nice control for heterogeneity built into our natural processing.

Saed Sayad 37:39
Do you mix them together?

Dennis Watson 37:44
Yes. We do.

Saed Sayad
That is a challenge. But it's okay. I can show you the importance of single cell. That's what you want to do?

Rob Kimmerling 38:00
One quick point on the heterogeneity: to the point of whether or not all of the cells are responding in a population of a mixed population of cells. That's the question that we're not really parsing with these single cell readouts. We don't know which are the responsive and non-responsive cells. Instead, what we do is we take that population of single cells. And if we see a big difference, after 24 hours, we assume that the drug is affecting a large enough fraction of the clones in that population to induce your response. Then the idea there is, if there are resistant clones in that population of a tumor, if those come back, on this test can still be used for that follow up in the next case by mixture of it might once again affect a large fraction of those clones. That's an iterative process that is another way to get at the heterogeneity problem that you mentioned.

Dennis Watson 38:58
That's why I'm the business development guy, and not the CTO. Rob’s answer was much better than mine.

Saed Sayad 39:03
The future belongs to single cell. Your method is the right method. It's going to get improved. You have a bright future. There is no guarantee, but based on my many years of experience in this field, if I gave an “A,” your method is the only method I've seen so far that deserves it.

Gitte Pedersen 39:39
First of all, I'm on the same side as Saed. This is really interesting.

What we do (at Genomic Expression) is to analyze RNA and create hypotheses based on overexpressed targets for drugs that are already approved, outside of the standard of care. Not necessarily looking at or in no way limited to the standard recipe that doesn't work for most. So we are completely aligned. We don't have what you do, which is the functional piece. I could imagine that some of the processes you are talking about doing in the clinical study we would like to validate, if possible, and that's for Rob here, if we can get access to live tumor cells. I will follow up with you directly on that.

You're separating the tumor cells from the tissue? Is that correctly understood? And if that is correctly understood, how do you make sure you don't have normal tissue in the mix? How is that done?

Rob Kimmerling 41:19
On the purification arm: you are absolutely right, we really want to measure the tumor cells in isolation. We want to see specifically the drug effect on those tumor cells. We use commercial kits that essentially allow us to pull out all the background cells we're not interested in. This gets rid of some of the stromal cells, the immune cells, or we can use lysis to get rid of red blood cells. But then after that, part of our clear workflow is actually to verify the purity of the sample we're working with for drug plating. We do this with surface markers. We work primarily with carcinomas. We'll do flow cytometry for [epcan?], this is just an epithelial marker. We're in the process of bringing in another flow cytometry panel to just further verify the purity. It's exactly to that point, you just make sure that what we're measuring is actually the tumor cell population that we're interested in. That's part of the QC workflow for our CLIA pipeline. If we don't have a high enough purity, we actually can't proceed with the drug testing. That's definitely something that's important in R&D.

Alexis Hall 42:25
You've already addressed part of my question with that explanation about how you remove normal cells, because part of it was if you're looking at a heterogeneous population, maybe you have normal cells as part of your control. So I was a little curious about what your internal control was. Are these done in replicates? Because that's usually a standard practice when you're doing a 96-well plate. You usually have replicates, especially when you're applying one drug per well. Is that what's done?

Rob Kimmerling 42:58
For the plating approach, we use [vehicle?]-treated control cells as the reference population. These are cells that are treated with effectively no drug. That's what is incubated for the same amount of time as the drug. Then those two reference populations are compared for the statistical readout. In terms of the plating replicates, we found we had historically done triplicate measurements for all of these. We did a quite a bit of validation, where it turns out, because we're measuring 5000 cells from each of these populations, the single replicate actually does a really good job of capturing that response each time. After verifying that, we have now chosen to prioritize measuring a larger drug panel when we can. We still need 5000 cells per condition. But rather than measuring those in triplicate, we're trying to get more of a broader drug panel for a fixed number of cells. It's a great question that some people actually published last year, that validation data set that shows the replicates are very consistent with each other. That's the reason we started to prioritize more conditions and just get a bit more data on that front.

Alexis Hall 44:05
Have you done the comparison between cells harvested from the fluid versus a solid tumor when you're able to have both? Have you done that comparison?

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Rob Kimmerling 44:19
We have. We've had a handful of cases where we have, for example, abdominal ascites, at the same time as a peritoneal solid mass. What's interesting is we don't always see the exact same results in terms of drug response. So we'll see for the 20-drug panel, there might be a couple of drugs that are discordant between the tissue and the fluid. I don't think we have enough data to say whether or not that's truly biologically-related. It might be a fact that the cells in the fluid are in fact at a different sensitivity profile. We don't really have enough data to comment on that. But we do have a couple of those paired samples that show pretty interesting concordance a lot of the time, but also a few drugs that don't always agree, which is just biologically interesting, essentially.

Rick Stanton 45:04
Is there any correlation of results with genomics and transcriptomics?

Rob Kimmerling 45:24
Yes. This is another case where we have a handful of specimens, we've run them, we know the reference genomic panel for them either by Foundation or Tempus. What's interesting is that, again, those aren't always concordant. We actually view this as a pretty big strength of the platform that we're working with is that we're not relying on the genomic panel and assuming a biological response. We're just measuring cells and seeing if there's a response. What we found is we've seen cases where this confirms a mutation, that the sample does not respond to the drug. And it turned out to be true for that patient. Unfortunately, we've also had cases where we saw that there was resistance to a confirmed mutation that, again, that patient didn't see a response. There's a mix between them. We have a fair amount of information now that it doesn't always overlap with the information you get from genomics and transcriptomics, which, from our perspective, we would feel like that's what we're excited about. We're offering a bit more information to layer on top of those existing clinical readouts.

Dennis Watson 46:22
The other thing that's important to add is that most drugs are marketed as for example, an EGFR inhibitor. In reality, it's really common that those drugs hit more than one target. There's plenty of reason to believe in some of the data that we've seen, just because somebody doesn't have an EGFR inhibitor, as an example, doesn't mean that they might not be responsive to one of those drugs that are maybe hitting that pathway, but maybe hitting some other pathways as well.

I've been in the oncology space for the better part of 15 years, and the general consensus among oncologists with the checkpoint inhibitors and with a lot of other targeted drugs is: if one checkpoint inhibitor doesn't work, then probably none of them will, and if one EGFR inhibitor doesn't work, then probably none of them will. But the reality is, that's not necessarily the case. We show differential responses. We get lung patients, and there are seven different EGFR inhibitors approved on the market. What if you just picked the wrong one, doc? Every molecule is different, and every patient processes every molecule in different ways. Finding the right drug among a class can be a really valuable thing that is maybe often overlooked.

Brian McCloskey 48:06
What about proteomics and spatial phenotyping, where you're getting at a single cell view? Are you seeing any correlation to that? Are you looking at spatial phenotyping or proteomics?

Rob Kimmerling 48:21
Yeah, so for our our side, the only real comparator we have that's semi-spatial, would be the PD-L1 readouts for some of the checkpoint histology that's done. We really don't do much on the spatial side, mostly because, as Dennis mentioned before, we're dissociating these tissues down to a single cell suspension, and we're running it in a fluid through our machine. So we lose that spatial context. The real main reference point we do have is for the PD-L1, where we have found that, again, for our checkpoint readout, there's not always a correlation with that sort of spatial PD-L1 readout from the histology slides for that mass. We don't have a huge amount of data on that front, though, to be fair, so that comparison isn't very deep, but that's the main reference point we have so far from some of the work we're doing.

Dennis Watson 49:04
I noticed that Rick had another question in the chat about a ctDNA (circulating tumor) liquid biopsy. That is probably not in the in the near future for us. The number of cells we need is about 100,000 cancer cells to run a full panel. The volume of blood you would need to get 100,000 cancer cells out of a blood specimen is that you would die of blood loss. It loses its value at that point. Now, that said, we do have early research happening. This isn't available in our CLIA pipeline, but we are looking down the research road to do this. Rob has absolutely done work with the immune checkpoint panel and getting immune cells from peripheral blood. That shows some promise, but being able to get enough cancer cells from the blood to do what we're doing right now isn't, at least in the near term, realistic.

Rick Stanton 50:07
Have you had any success with cancer in the bone, which would be me? My prostate cancer has spread to my bone. Is that something that can work?

Rob Kimmerling 50:24
We've had only a handful of bone biopsies that we've tried. They tend to be really difficult to dissociate just to get the single cells out of them. It's mostly just because of the physical matrix that that sample was collected in. But it depends on the type of mass. We've had a couple of soft tissue masses within the bone that we've had some success with. But it really is very context dependent in terms of what that mass looks like in the tissue we're able to get.

Rick Stanton 50:52
Who would make that call? A pathologist?

Rob Kimmerling 50:55
Typically, yeah, that's not something we would specialize in. We don't know the composition before it gets to us. Typically it would be in consultation with the oncologist and the pathologist.

Rick Stanton 51:08
I think you guys are great. The reason I asked about the correlation of results was not about where you are today, with your low sample numbers, but the hope you offer for the near future. You could really change so much understanding, and that's why I mentioned Gitte (Pederson of Genomic Expression). This could really open up a validation avenue on things that you see with transcriptomics, that you may loosely think is something. And then your fantastic measurement technology can help tease apart the answers to those tough questions.

Richard Anders 52:09
I echo everyone else. I met Scott Manalis (MIT researcher) when he just had the resonant tuning fork, probably in about 2005. I've been hearing about it for many, many years, as he's been looking for applications. My angel group (Mass Med Angels) meets at the Koch Institute. I've been following this and didn't know you guys were doing this, but it's great work.

I have a couple of questions about sample prep. First of all, the 5000 cells is not a magic number, right? It's just you're looking for statistical robustness. It could be 2000, or it could be 3000, as long as you get the distribution that you want, and can measure the band shift. It's good enough, right? I just want to be sure I'm not misunderstanding that.

Rob Kimmerling 52:57
That's absolutely right. The main thing is that we reasonably shoot for 5000 cells. It's actually the sweet spots, right around 2500 clean measurements, that we need. But when we collect 5000 cells that will often lose some of those two image curation or background debris. The 2500 number is really the sweet spot for our statistical model to make sure we can actually parse a reasonable response between them. But you're absolutely right. There's no magic to that number.

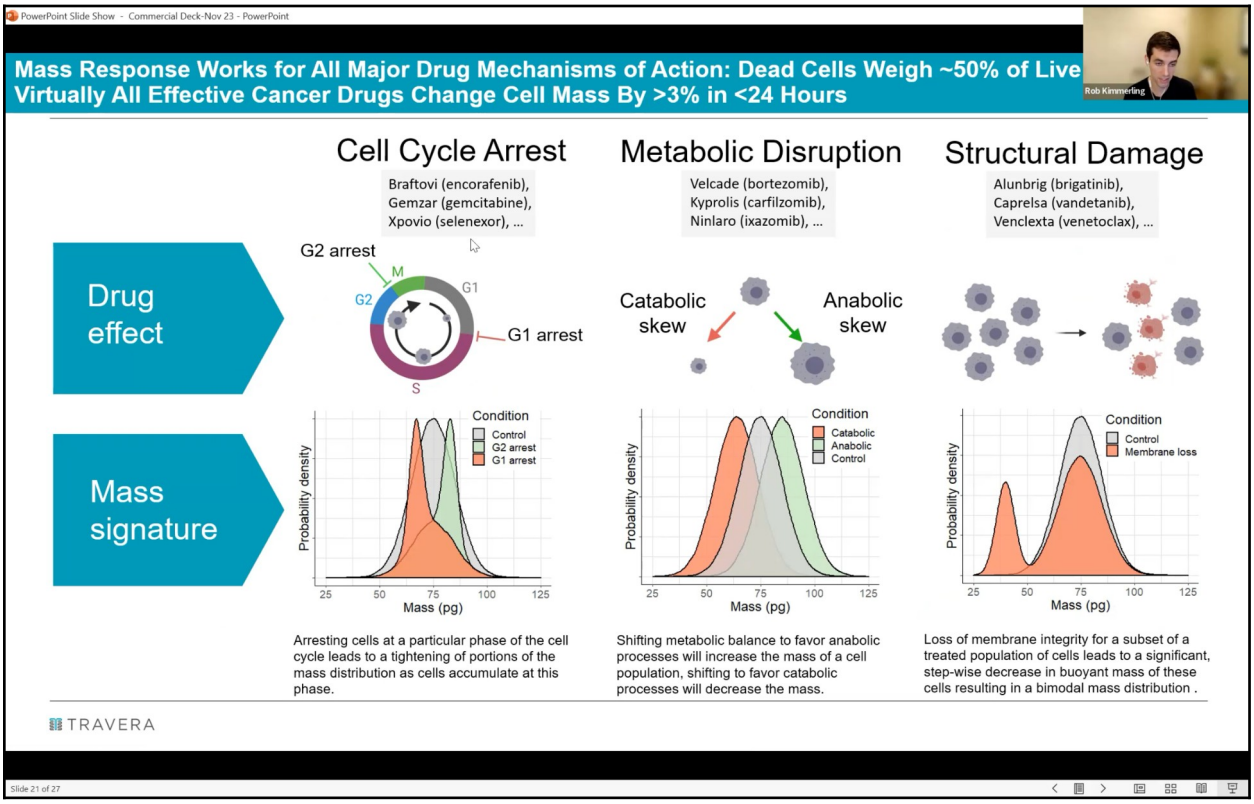
Richard Anders 53:24
There’s probably quite a large heterogeneity around the cells. I mean, not whether they're heterogeneous cancer cells, but just cells, I mean, cell size and cell mass. So you have to deconvolute that issue in your statistics as well, I assume.

Rob Kimmerling 53:42
Yes, exactly. That's one thing that's baked into the decision threshold for how large of a shift corresponds to what we call “a response”. It's certainly true that different cell types will affect what that decision threshold might look like. But also different drug mechanisms that affect that as well. Some drugs might induce a smaller shift than other very cytotoxic drugs that essentially blow up cells when they're working. As of now we don't take a cell type and drug-specific approach to it. We have one fixed threshold that we're using. This is primarily for the sake of gathering validation data. We can use that threshold and see how well we're performing for calling it a “response” or “non-response”. The idea for the long term is that as we have larger cohorts of drug- and cell-type-specific datasets, we can start to refine those thresholds and see

if there's more meaningful ones that can be used for single drug bases or single tumor type bases.

Richard Anders 54:36
What I was starting to wonder, and the heterogeneity is a little bit of a problem for this, but if you have a drug, if you have a heterogeneous sample, heterogeneous relative to cancer, so you have two different or three different cancer sets, genetic or epigenetic, sitting in the same well, you might have two or three peaks where the drug is working on some of those cells, partly working on some of those cells, and not working on other of those cells. I don't know what the statistics are for that. But that'd be very interesting if you started to get some differentiated peaks and could somehow analyze that. You might have this drug that is useful, but only for some percentage of the cells. And that other drug is useful for some other percentage of the cells. That might lead a clinician to want to prescribe a combination therapy or something.

Rob Kimmerling 55:27
That is absolutely a really interesting use case. It's part of the publication I mentioned earlier, where we dove into some of the mechanism, parsing for different drug mechanisms. Like you said, there will be different mass peaks that shift in different directions based on the drug mechanism. You can use that to back out a signature of what's happening with the cells. As of now for the clinical test, we're really just looking at a distance, whether or not there is some change that was induced.



“Finding Personalized Cancer Treatments Beyond the Standard through a Unique Test” (Travera) [#77]

Here's the slide that shows the different types of drug mechanism effects we might see. When cells get locked up in a late cell cycle, they become bigger. You'll see that mesh shift to the larger end of the fraction. You can start to have these fingerprints for different drug mechanisms. Then, like you said, for different subclones within a population of cells, that's something we may also be able to parse. It's not something we really return data on because we don't exactly know how to interpret it. It's more just a general readout of “response” and “no response” right now. But you're absolutely right, there's quite a bit of richness to that data when you start thinking about the commonality in those populations.

Richard Anders 56:31
You could theoretically see in one of these slides two or three peaks in one well, and you could gather that you're seeing one thing there.

Dennis Watson 56:47
I always love this slide, because it shows how incredibly sensitive this assay really is. I've always thought this was really cool.

Richard Anders 56:57
I assume that the reason you work on different cancer types is you've isolated signatures to pull out different types of cancers, or whatever markers you use. If for some reason a patient had a belief that there was a certain marker that would distinguish something on their cancer cells, and they wanted you to use that as part of the sample prep, would you be willing and able to pull out cells using that marker?

Rob Kimmerling 57:32
It's primarily a constraint of our CLIA certification. Our protocols are fairly stringent in terms of how we have to validate. If it goes through a research avenue it is something we're happy to do. It's just our CLIA SOPs are pretty darn constrained in terms of what we can do.

Mark Stevens 57:51
We have worked with organizations that help direct patients to research tools to look at their cancers, and those have all gone through research avenues for processing these samples in ways that are not purely in our CLIA pipeline. There are resources like that we can connect people to.