“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Brad Power
July 20, 2022

“We get the fresh sample, we process it, we use what we need for drug testing, and we freeze them down. If we need to go back and test combinations, we're throwing those out fresh and testing within a few days.” - Diana Azzam

Meeting Summary

Presentation Highlights

Noah Berlow, PhD, CTO, First Ascent Biomedical, and Diana Azzam, PhD, Assistant Professor at Florida International University, led a discussion on their approach to functional drug testing and using AI/ML to guide complex treatment decisions for advanced cancer patients. Diana’s expertise is in functional precision medicine, and Noah’s is in AI/ML and bioinformatics. Together they have put together a pipeline that takes in fresh patient tissue and turns out treatment recommendations in two weeks. They use Diana’s functional drug testing protocols and send out some of the tumor tissue for DNA and RNA sequencing, then put the test results into Noah’s matching engine to report treatment options to patients.

Diana shared several examples of advanced cancer patients who had failed standard treatments and were desperate for treatment options, which were discovered by the drug testing she ran. Many of the treatment options were unexpected. Some were chemotherapy drugs that the patient had already seen and were assumed would be ineffective. One drug was an approved drug – for asthma. The drugs were delivered in combinations. All extended “progression free survival”, and one patient experienced a particularly durable response. These patients had urgent needs since they had failed their previous lines of treatment, and the analysis was completed within two weeks to give the patients timely treatment recommendations.

Noah described his work in integrating and interpreting the inputs of functional drug testing and sequencing data for individual patients with information about the drugs and their real world mechanisms to derive a personalized “tumor circuit” – a holistic view of tumor drivers and weaknesses to find the best combination of drugs for a patient. He shared his research in applying this analysis in mice, where he was able to find personalized drug combinations that performed better than a control or the individual drugs. He also showed how the same analysis that they built to find individualized combinations for patients can be applied to discover better biomarkers.

Discussion Highlights

We support administering multiple drugs together, instead of single drugs one-by-one, because you give the cancer cells the chance to adjust to every chemical you throw at them.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Noah Berlow: In many ways I'm in agreement. Some of the other work that I've done has been on showing the difference between sequential combinations or simultaneous combinations using the AI to find a combination and then testing that ex vivo on a couple of cell models derived from the same type of cancer, showing the combination can essentially stop tumor regrowth. But at the same time, we're showing in a practical setting, when you give the drugs at the same time, the effect is that the cancer cells go away and don't come back. Which of course is the key end goal.

We have heard concerns about toxicity from treating physicians regarding the kinds of drug combinations you are recommending. How did you overcome those concerns?

Diane Azzam: I have not really seen toxicity concerns because our patients haven’t had other options. We look at the drugs’ concentrations in the blood and use Cmax. (Cmax is the highest concentration of a drug in the blood after a dose is given.) In the case of the osteosarcoma patient, they administered the drugs in a rapid sequence - a few weeks. In the case of the rhabdomyosarcoma patient, we had seen in functional testing that one of the drugs (vincristine) was stronger as a single agent, so they administered that first, then the other two in a rapid sequence.

You're working off fresh tissue. Can you run one functional test, then come up with a new hypothesis, and run functional testing again without getting a new biopsy? Is the tissue still viable for testing after 48 hours?

Diana Azzam: We get a second shot. That's what happened in all the cases, because we get the fresh sample, we process it, we use what we need for drug testing, and we freeze them down. If we need to go back and test combinations, we're throwing those out fresh and testing within a few days. It's very helpful because sometimes doctors look at the single agent data, and say, “I want you to go back and test these combinations.”, and that's what we've done. It's been very helpful for the doctor.

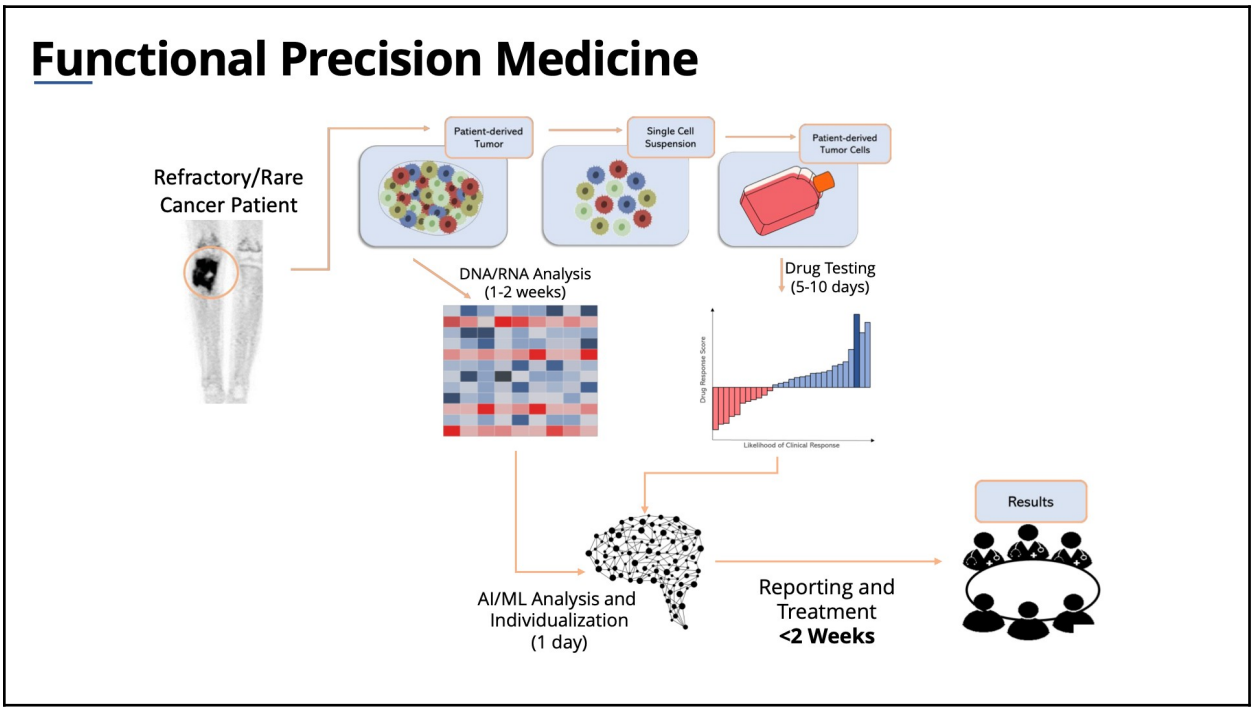
“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Meeting Notes

Diana Azzam: I'm an assistant professor at Florida International University. My PhD focused on ovarian cancer stem cells and then my postdoc focused on high throughput drug discovery. My research interests at FIU include implementation of functional precision medicine in clinical trials to guide individualized treatments in advanced cancer patients, and a major focus of my lab is to understand the resistance of cancer stem cells and how they play a role in metastasis.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Noah Berlow: I have a couple roles. Right now I'm most accurately serving as founder and CTO of First Ascent Biomedical. My background is AI engineering, computer science, and math. I did my PhD in electrical engineering. I got involved in pediatric cancer research using artificial intelligence machine learning to start solving some real big challenges in that space. I did my postdoc in bioinformatics and molecular biology. I eventually started getting more involved in the sort of day-to-day laboratory side of things. All of that was happening at the Children's Cancer Therapy and Development Institute, where I am an assistant member. My main focus is on First Ascent, which is bringing functional precision medicine and AI analysis for individualized treatment options into the clinic for patients in need.



Diana and I are collaborating to put together this entire pipeline to use functional precision medicine to guide individualized treatments. Together we are taking a biopsy sample from a patient's cancer, doing a rapid culture and drug sensitivity testing protocol, using Diana's technology that she's developed over the past decade.

Functional drug testing is side one.

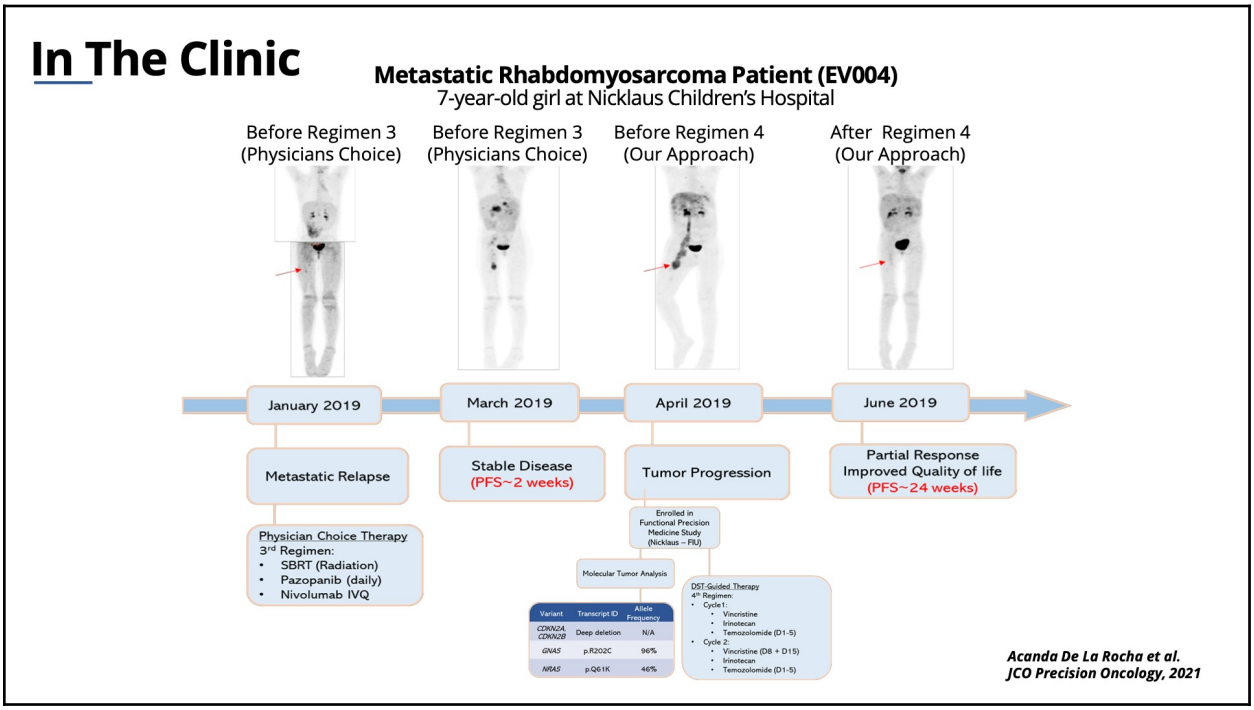
Side two is taking the same tissue and sending it for DNA and RNA sequencing analysis. Where I come in is taking both of those data sets and ingesting them into the AI/ML engine that I've also been researching and building over the past decade. From the drug testing data and the sequencing data, we can better understand the weaknesses underlying a patient's tumor. Another way of saying that is we take all the drugs that are working, and all the drugs that aren't, to understand the mechanisms that are really driving drug sensitivity. When we try to build combinations around the patient's key mechanistic targets, it becomes a lot easier since

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

we need to target this gene, and we can find the right drug already available in the clinic that best fits that need. Our goal is to deliver all of this data within two weeks, making this as clinically usable as possible because that turnaround is absolutely critical to meeting the needs of patients.

We've already done a lot of this work in the clinic already.

Diana Azzam: The goal is to be able to implement this in the clinic, and we have two feasibility studies, both in pediatric and adult cancer patients, in collaboration with Nicklaus Children's Hospital and Cleveland Clinic Florida, to test whether this is feasible. Can we recommend treatments in a clinically actionable timeframe? And if these treatments are recommended, how do the patients respond?



This is a seven-year-old girl with metastatic rhabdomyosarcoma, a particularly difficult-to-treat cancer. She has been through multiple treatments. None of them were effective. We received a piece of her tumor. We confirmed we had the right cells by looking at the different markers of rhabdomyosarcoma. We tested our panel of drugs and multiple drug combinations for the patient. We were able to deliver this data to the doctor and the molecular tumor board in about one week.

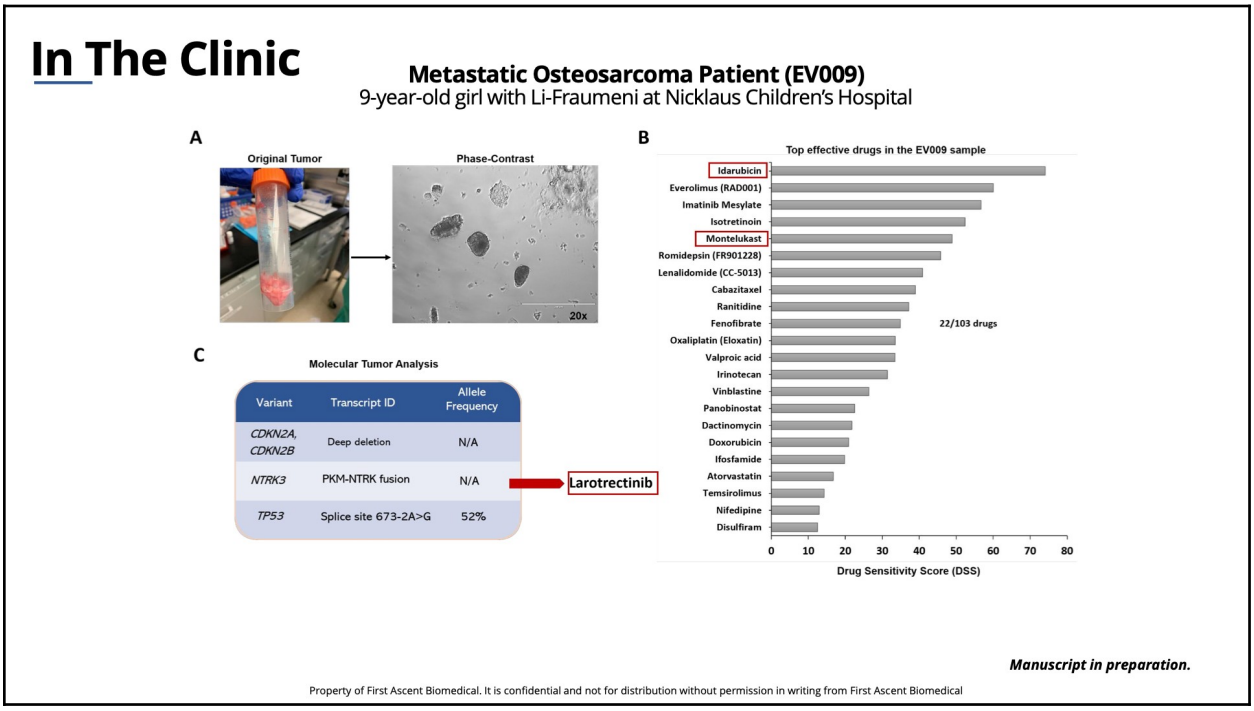
Brad Power: I helped Kasey Altman, a young woman with alveolar rhabdomyosarcoma, with her hackathon. I learned that rhabdomyosarcoma responds to chemo, but just about nothing else. So if you find more chemo drugs, you're probably going to delay progression, but you're not really going to come up with a durable response. Is that correct?

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Diana Azzam: We found chemotherapy drugs that were effective, and we also found targeted drugs. For example, for this patient we found:

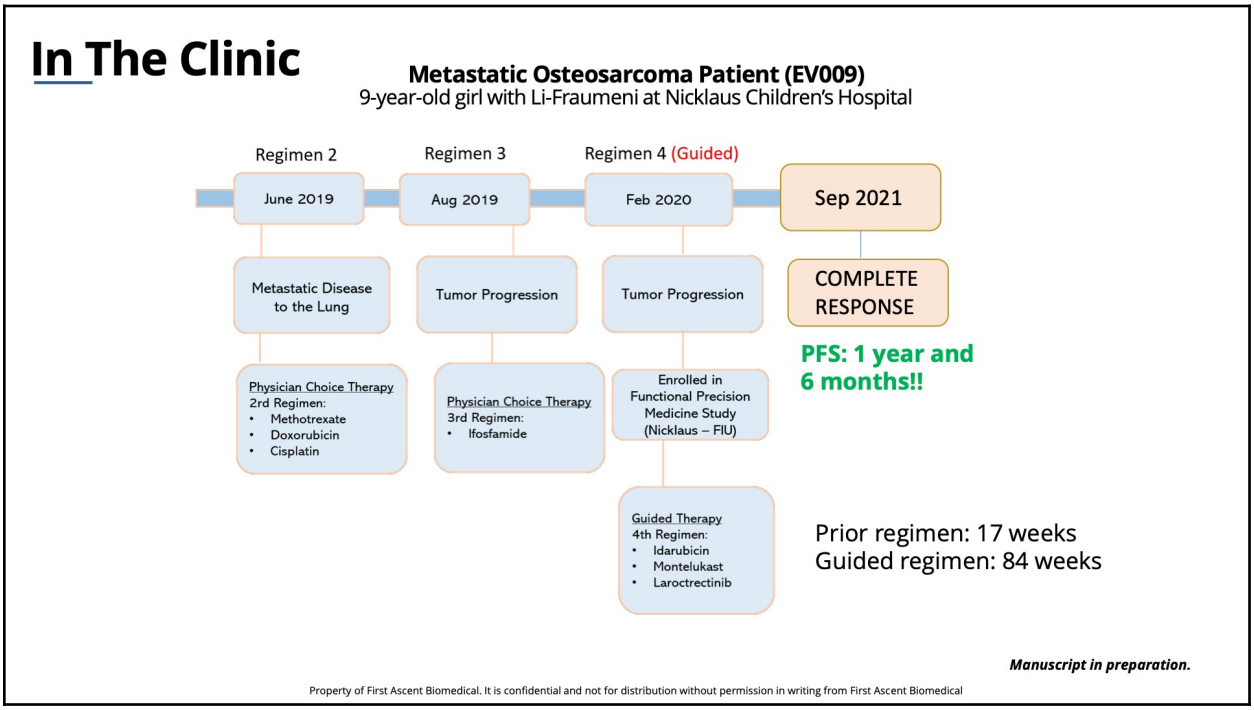
- dasatinib, (Dasatinib is in a class of medications called kinase inhibitors. It works by blocking the action of an abnormal protein that signals cancer cells to multiply.),
- HDAC inhibitors (Histone DeACetylase inhibitors are in a class of anti-cancer agents that play important roles in epigenetic or non-epigenetic regulation, inducing death, apoptosis, and cell cycle arrest in cancer cells.),
- lenalidomide (Lenalidomide is in a class of medications called immunomodulatory agents. It works by helping the bone marrow to produce normal blood cells and by killing abnormal cells in the bone marrow.), and
- an mTOR inhibitor. (mTOR inhibitors are a class of drugs that inhibit the mechanistic Target of Rapamycin.)

One of the challenges about recommending treatments is whether doctors have access to these drugs. With children we customize the library of drugs based on what's available in the pharmacy at Nicklaus Children's Hospital. In this case we wanted to treat the patient quickly. So they picked chemotherapy drugs (vincristine, irinotecan, and temozolomide), which they thought wouldn't be effective anymore, but we found were still sensitive on the tumor cells (from our functional drug testing). Before enrolling in our trial, she only had two weeks of progression-free survival, which wasn't really effective. Then she was enrolled in our clinical trial and the molecular tumor board recommended treatment based on the drug sensitivity testing. Her tumor decreased, and we observed that her progression-free survival was six months. She responded to the guided drug regimen. Unfortunately, the patient passed away because of metastasis in the lungs.



“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

This patient was a nine-year-old girl with metastatic osteosarcoma, a rare bone cancer which is incredibly difficult to treat, especially when it's metastatic. We received her tumor sample, and we did drug testing and sequencing. The results from the sequencing, molecular tumor analysis, and drug sensitivity testing were used to identify a combination that would be effective for her cancer. As you can see, for the NTRK fusion you have larotrectinib, which is a targeted drug (an inhibitor of tropomyosin kinase receptors), which showed up in the molecular tumor analysis. When we looked at the drug sensitivity testing, the most effective drug here was Idarubicin (a chemotherapy), which was recommended, and also Montelukast, which is an allergy medication.



This is a beautiful example of how results from both genomics and drug sensitivity testing were used in her treatment recommendation. When we looked at her clinical course before enrolling in our trial, her cancer kept growing despite multiple treatments. When she received a three-drug combination based on functional precision medicine (idarubicin, montelukast, and larotrectinib), she had a complete response, and her progression-free survival is one and a half years. If you compare the progression-free survival of the patient based on what was recommended by functional precision medicine versus her previous regimen, you can see that there's an improved survival benefit.

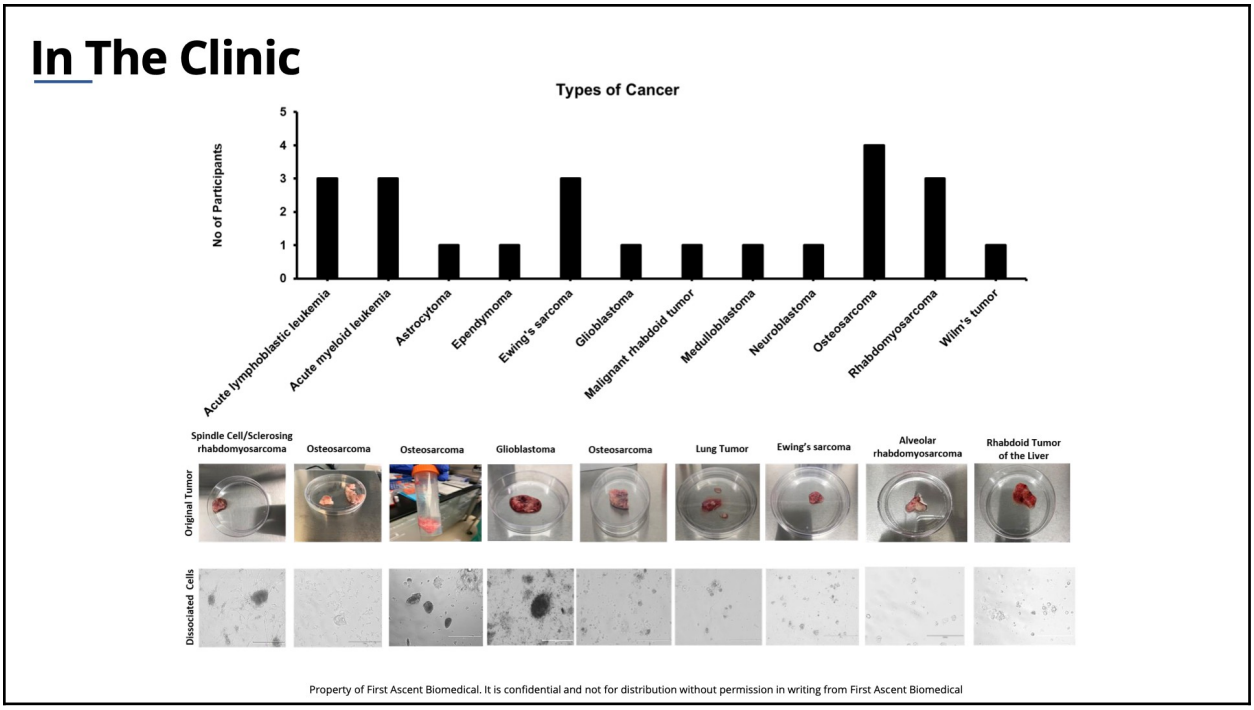
Brad Power: Was that a three-drug combination?

Diana Azzam: Yes: this was a combination of three drugs: Larotrectinib, idarubicin, and Montelukast.

Brad Power: Put a pin in that. Drug combinations have been one of our favorite topics.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

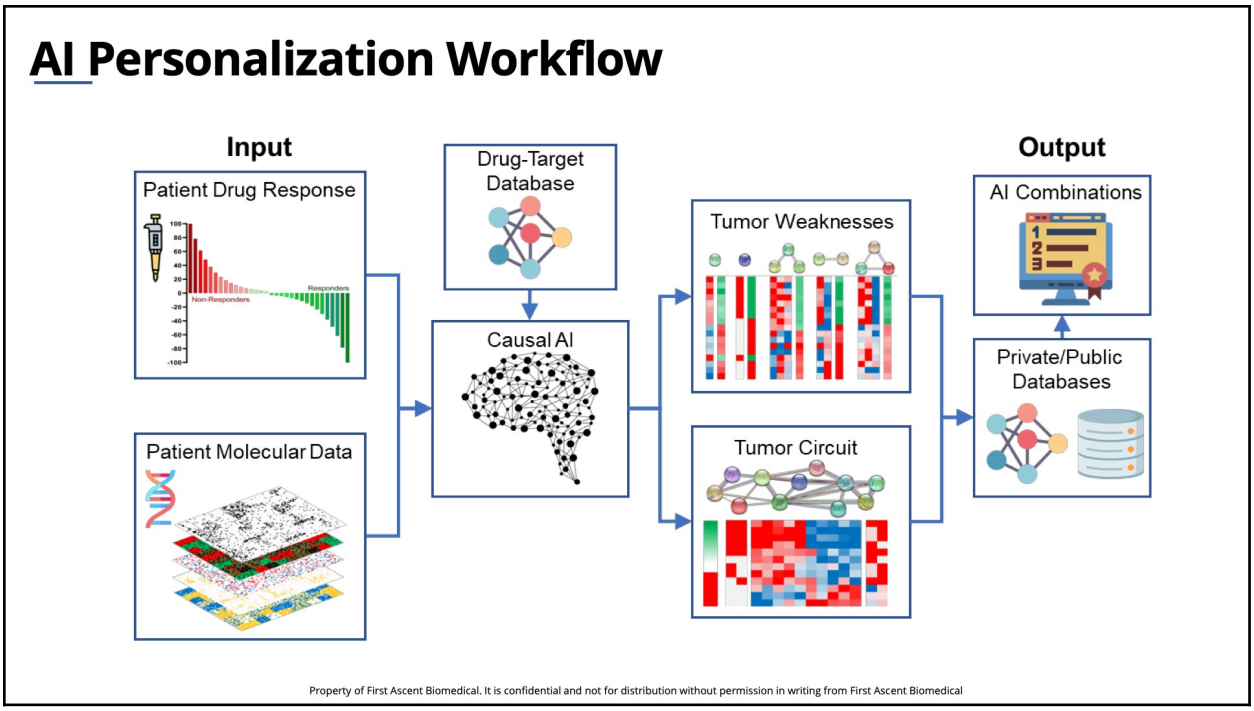
Diana Azzam: That's exactly where machine learning is going to be very important.



We've been able to optimize the drug sensitivity testing on at least 13 different tumor types. We can do this in liquid leukemias and in different types of solid tumors, as I've shown in a few case studies.

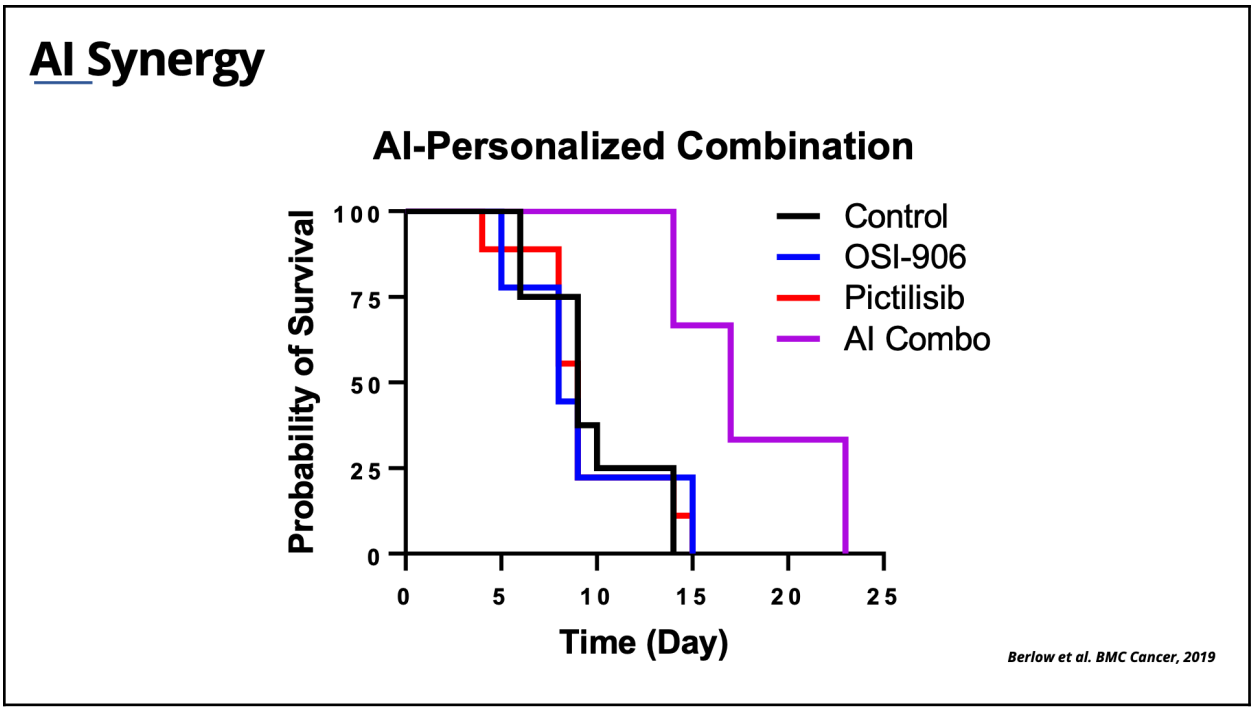
We are analyzing the data from a group of patients for their best overall responses. If you compare patients that were guided by our approach versus their previous regimen or standard of care, you can see that there's a huge improved overall response. We can recommend treatments within clinically actionable timeframes, and patients that were guided by our approach have improved overall responses compared to their previous regimen and compared to those that went through standard of care.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]



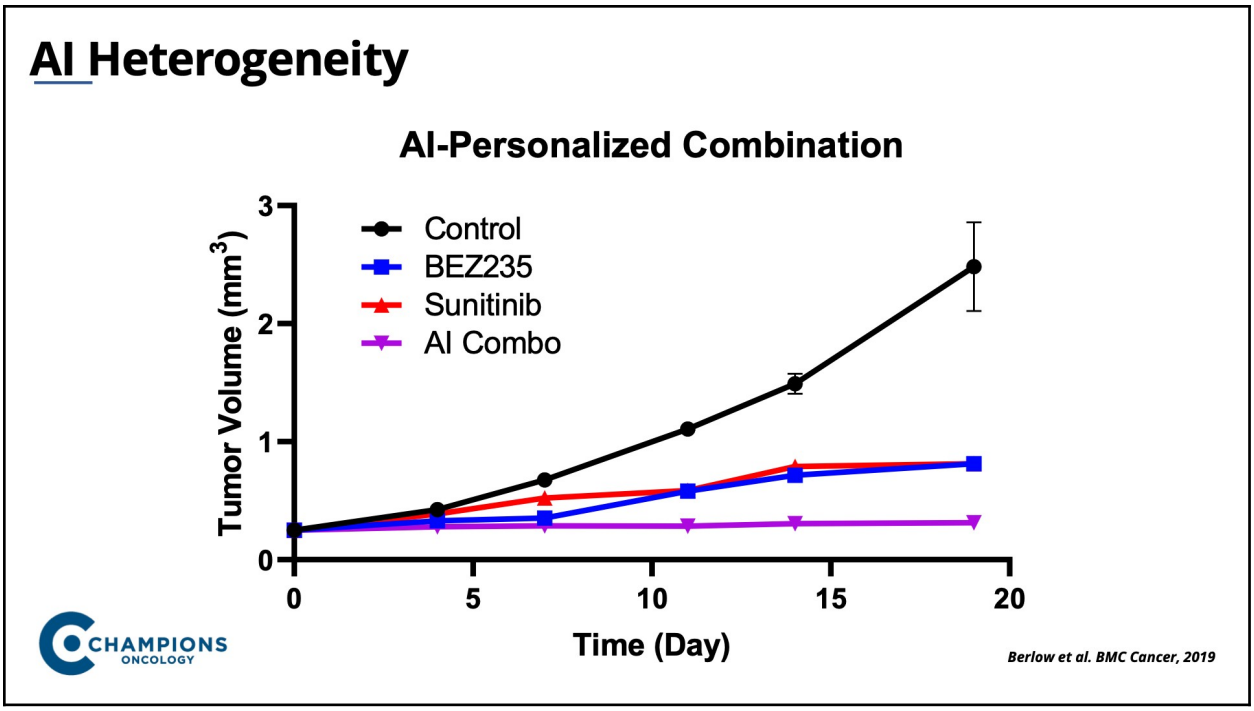
Noah Berlow: I have been focused on the AI and machine learning components – an AI engine that is able to pull in functional drug testing data and sequencing data integrated for an individual patient, and pulling in information about the drugs and their real world mechanisms, and putting all of this together to build out an understanding of the patient's tumor weaknesses and creating a tumor circuit. The idea with the tumor weaknesses is to find the multivariate genetic targets that are driving drug sensitivity, the main things we'd really want to hit with the drugs we would give to the patient. The tumor circuit is the holistic view of what this patient really looks like. When we put all of these individual weaknesses together, what can we learn about the patient, and what can we do to really drive forward individualized combinations? The goal of this has always been to find the right combination of drugs for the right patient. This work started at my beginning of grad school, since about 2012, so about a decade. We have validated this approach in multiple cancer types.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]



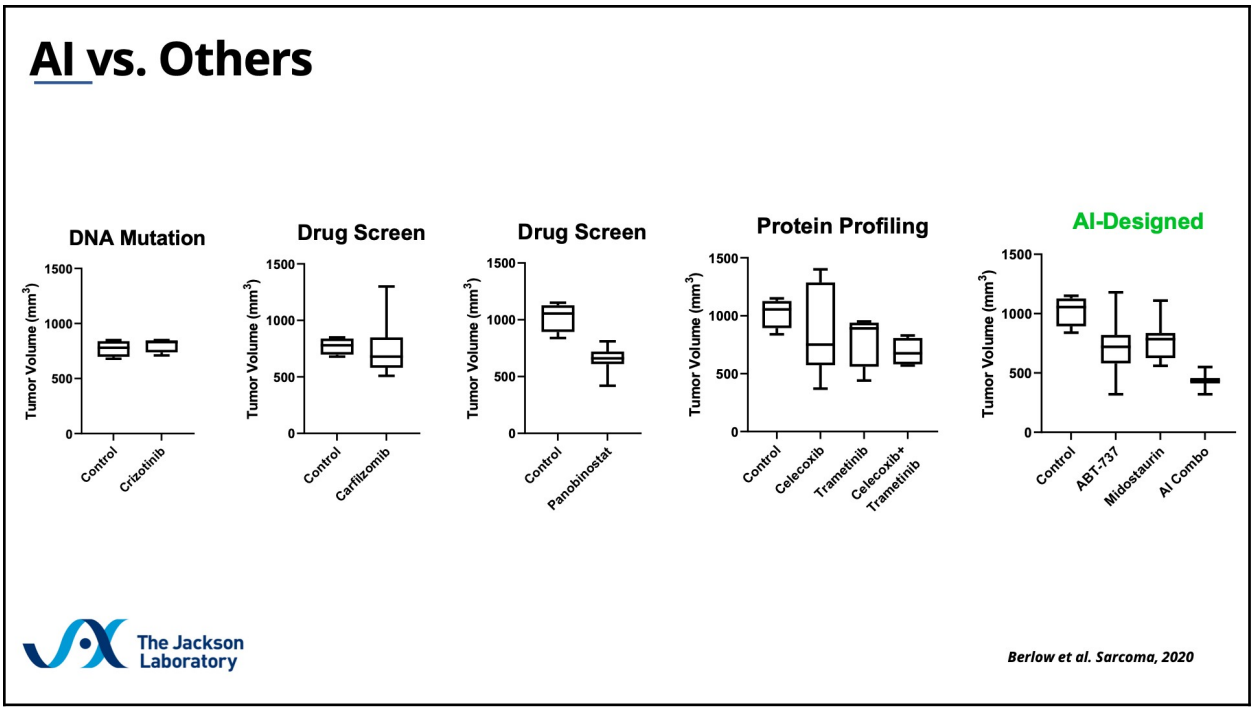
This is data from a publication from a couple years ago. This is in a genetically engineered mouse model of alveolar rhabdomyosarcoma. We followed the exact same pipeline that we've been showing. We took tumor tissue from a mouse, did drug testing using 60 agents, some of which were FDA approved, some of which were in phase two or phase three studies, and used all of that data, plus the genomics data, to build a circuit of the patient, finding that a combination of OSI-906 (linsitinib, a potent and selective oral inhibitor of dual IGF-1R/IR kinase) and pictilisib (a potent and selective oral inhibitor of PI3K - phosphatidylinositol 3 kinase) together seemed to have the kind of synergy that we would be targeting. Individual drugs may be less effective. There's a lot of data here. I'm not showing all of it as in the publication. But the point is when we did this analysis, what we found is that the targets of ISO-906 on their own were not critical nor were the targets of pictilisib. But when we put that combination together, we were finding the kind of synergy in vitro that we were hoping we would see. When we tried it out in vivo, the same sort of results replicated. We saw that the individual drugs are indistinguishable from a vehicle, but when we put them together, we see a far more profound effect.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]



We did the same thing in a cancer called epithelioid sarcoma, in this case using a human cancer, which we were able to engraft into PDX mice. (Patient-derived xenografts are models of cancer where the tissue or cells from a patient's tumor are implanted into an immune deficient or humanized mouse. PDX models simulate human tumor biology allowing for natural cancer progression, and offer a translational research model for evaluating efficacy.) We did the same approach of drug testing, sequencing, and feeding it into the AI engine to find a drug combination. One of the really cool things here was because the patient had a relatively large tumor, we were able to break it into different subregions of the tumor and do drug testing on each one of them, trying to account for intratumor heterogeneity, the property where one region of cancer can look a bit different from the other regions of the same tumor. When we put all of the data together we found this combination of BEZ235 (dactolisib, a dual phosphatidylinositol 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) inhibitor) and sunitinib (a protein kinase inhibitor, which blocks the action of the abnormal protein that signals cancer cells to multiply). We saw that combination outperformed the control, slowing tumor growth by 91%, but also outperformed the individual drugs alone. This is from N of three mice. Even in the minimum statistical sample size, you're still seeing a significant effect. We can take all of that data from that one patient and build a combination and do so quickly.

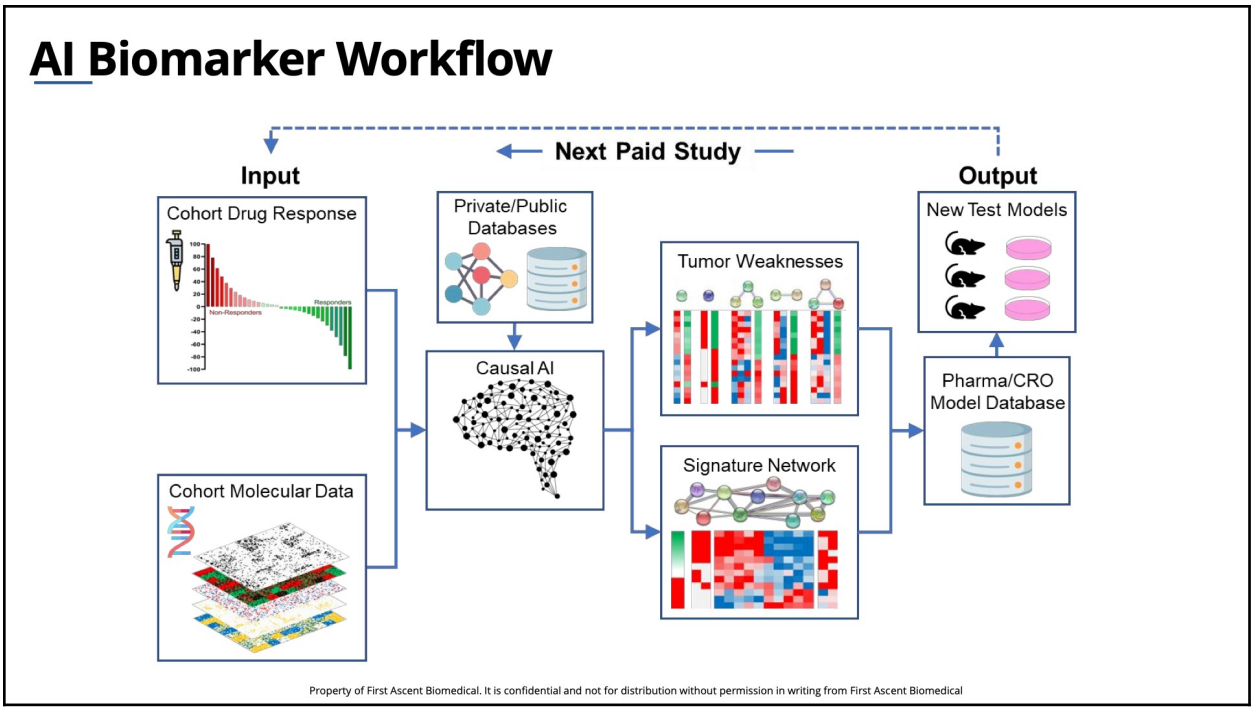
“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]



This third example was for a patient with an undifferentiated pleomorphic sarcoma (a rare type of cancer that begins mostly in the soft tissues of the body). We took the same approach. We were able to access and test on a PDX mouse model line. We did a full workup of this patient and their associated PDX doing DNA profiling to find druggable mutations, some drug screening to find possible candidates, even some IHC-based profiling, and used the AI approach to pull together the sequencing and drug screening data to find what we could find. We saw that there were some effective treatments, but the treatment that had the overall lowest tumor volume came from the AI-designed combination.

When we put all of the data together in the right way, we can find better insights for the individual patient and build individualized drug combinations.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]



The same AI that we built to find individualized combinations for patients can be just as easily applied to biomarker discovery for pharma companies. One of the things that we were asked to talk about was the business model. If the same AI can be used to work with pharma companies, it becomes a really easy way to monetize a lot of this. It's the same workflow. The only difference is a feedback loop that's based on working with pharma companies and CROs finding the next model for them to test the next PDX or set of cell lines. We have quite a bit of work in this.

We did a study on PARP inhibitors. PARP inhibitors are a relatively new type of drug. There is a lot of interest in them, and they have a gold standard biomarker – mutations in BRCA genes. But clinical studies have shown that there are some patients without BRCA mutations that will respond to PARP inhibitors, and there are some patients with BRCA mutations that won't. So it's really important to find better biomarkers that we can add to that decision-making. We took a data set of about 20 ovarian cancer PDX mice tested with a PARP inhibitor and used all the genomics data and the response data to find new biomarkers. We found that in the biomarker we built, that was a combination of a couple genes.

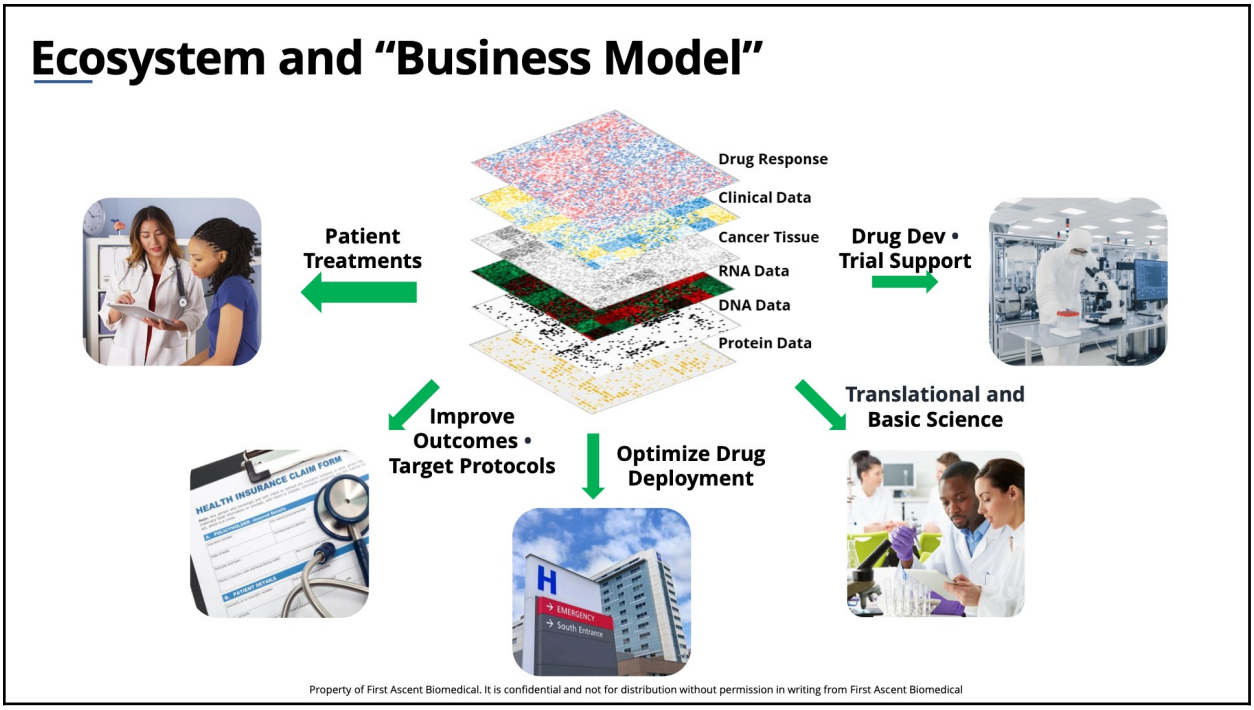
Regardless of how you look at the results, whether it's the actual distribution of tumor growth in the PDX, or if it's a variety of different test metrics, the biomarker we built using AI outperforms the BRCA1 tool or even the BRCA-associated genes.

This holds true even when we apply the same biomarkers to other types of cancer. We are doing significantly better in tumor growth inhibition distribution versus BRCA1, BRCA2, or BRCA. And when we look at accuracy, Matthew's correlation coefficient, F1 test, or the ROC curve, we're outperforming the biomarkers that exist today.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

The point is that when you put the right data together, you can start solving challenges in other fields as well. Building better biomarkers is critical to better assigning drugs to patients in need. There's an entire ecosystem that revolves around getting the right drugs to patients and building massive data sets.

The go-to-market hurdle that we have is getting CLIA certification for this drug testing, sequencing, and AI analysis, which we are in the process of doing. Once we have CLIA certification, we can work with any patient in the country from any hospital, but the bigger strategy here is building this massive data set of patient drug response and match sequencing data, and underneath that, a biobank of these tissues that can be used to do new studies that enables us to work with all of the players in the space, especially pharma companies.



From a business model perspective, Foundation Medicine, which has been one of the most successful companies in this space, built a process that anyone could use. And then they built the data set that pharma companies loved. We can do the same kind of thing when we put our technologies together.

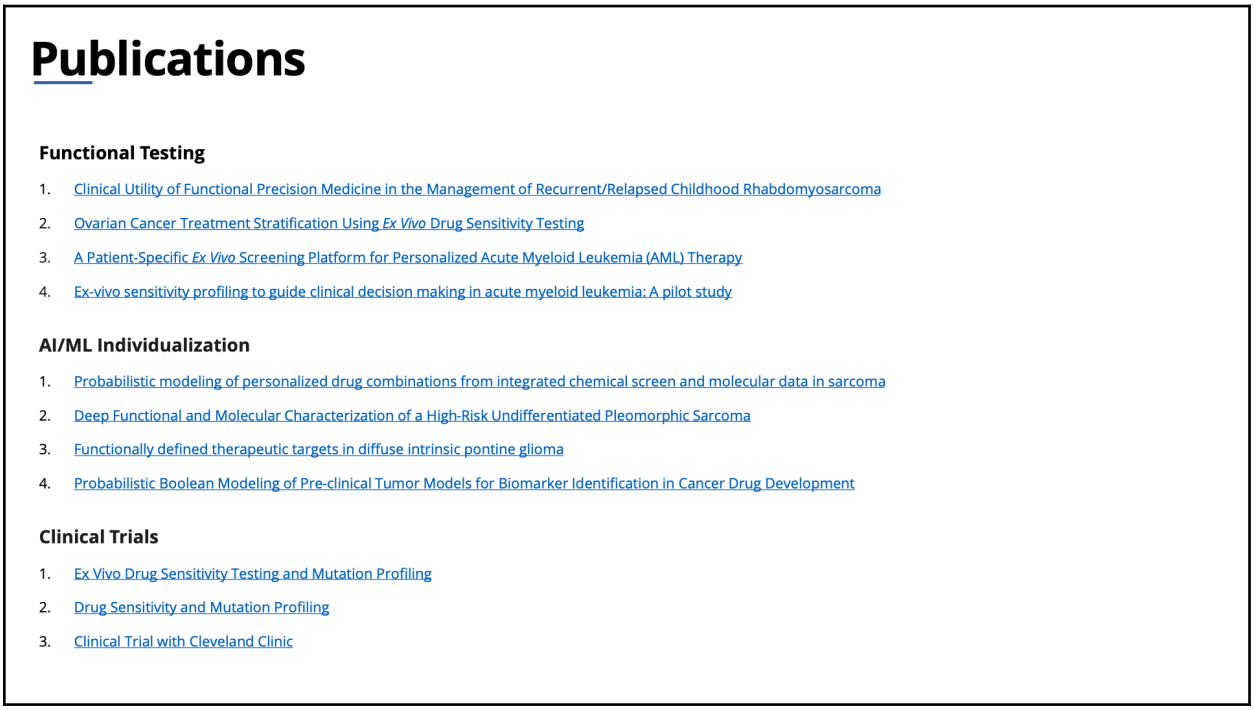
“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

We've worked with a variety of different institutions, including Stanford and Johns Hopkins.

We've worked with multiple pharma companies, including Roche, Genentech, and GSK all on the biomarker side.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

With Diana, we have done a lot of clinical work as well with Nicklaus Children’s and the Cleveland Clinic. And we're excited to say we're building relationships with a variety of different centers, including OHSU, Mayo Clinic, and so on.



Publications

Functional Testing

1. Clinical Utility of Functional Precision Medicine in the Management of Recurrent/Relapsed Childhood Rhabdomyosarcoma
2. Ovarian Cancer Treatment Stratification Using *Ex Vivo* Drug Sensitivity Testing
3. A Patient-Specific *Ex Vivo* Screening Platform for Personalized Acute Myeloid Leukemia (AML) Therapy
4. Ex-vivo sensitivity profiling to guide clinical decision making in acute myeloid leukemia: A pilot study

AI/ML Individualization

1. Probabilistic modeling of personalized drug combinations from integrated chemical screen and molecular data in sarcoma
2. Deep Functional and Molecular Characterization of a High-Risk Undifferentiated Pleomorphic Sarcoma
3. Functionally defined therapeutic targets in diffuse intrinsic pontine glioma
4. Probabilistic Boolean Modeling of Pre-clinical Tumor Models for Biomarker Identification in Cancer Drug Development

Clinical Trials

1. Ex Vivo Drug Sensitivity Testing and Mutation Profiling
2. Drug Sensitivity and Mutation Profiling
3. Clinical Trial with Cleveland Clinic

We have published a lot. Here are the top publications for the functional testing side, the AI and ML individualization side, as well as our active and completed clinical trials.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Diana and her team at FIU are incredible. And I have a ragtag group at the Children's Cancer Therapy Development Institute.

Brad Power: We have heard concerns from treating physicians that drug combinations might have toxicity issues and drug-drug interactions. How did you overcome those concerns? What were the arguments that you used? I assume that you were working with a treating physician in a collaboration with the insights you were coming up with. How did that work?

Diana Azzam: The main challenge is to make sure that the combinations of drugs are therapeutically relevant, and they've been used before.
We look at the drugs’ Cmax concentrations. (Cmax is a pharmacokinetic measure used to determine drug dosing. It is the highest concentration of a drug in the blood after a dose is given.) When we test those drugs, we test the doses based on the Cmax of the drug.
Regarding combinations, doctors are familiar with certain combinations of drugs, so they're more inclined to use those combinations.
In cases like this osteosarcoma patient, she didn't have any more options. They used the drugs in combination, but in a sequence. They gave the targeted drug (larotrectinib) first, and then they gave her montelukast, which is an allergy medication, so that's fine, and then they gave her idarubicin in a few cycles.

Brad Power: So it wasn't a cocktail all at once. It was staggered. How long were they waiting between administration of each next drug? They weren't waiting until resistance. They were consciously going in treating it with a combination, but in a rapid sequence.

Diana Azzam: I think it was over two months. In the rhabdomyosarcoma case they requested a test of the combination of the three chemotherapy drugs. When we tested the three drugs in combination, we found vincristine alone was more effective alone versus with the two other drugs. So they gave vincristine on day one, and then the two other drugs on another day. They followed the sequence of the combinations based on the drug sensitivity scores as well, but that was all given at the same time.

Brad Power: By sequencing the drugs, was that dealing with some of the toxicity concerns because they could monitor the response?

Diana Azzam: I have not really seen toxicity concerns because these patients don't have more options.

Brian McCloskey: Did they change the dosing?

Diana Azzam: For the patient who got vincristine and the other two drugs, they didn't change dosing. They gave the doses based on the Cmax, and they changed the sequence.

Brad Power: Standard dose, but spread out differently.

Diana Azzam: Yes. I have to emphasize that the Cmax is so important. When we test the drugs on the cells, we are using the Cmax. If we find that the IC50 (a quantitative measure that indicates how much of a drug is needed to inhibit, in vitro, a given biological process or biological component by 50%) of the drug is less than the Cmax, that's when we're using the drug. Otherwise they don't use the drug for treatment.
Cmax is the maximum concentration that the drug can achieve in the blood of patients. Because these are FDA approved drugs, we know this is the maximum concentration that the drug can attain on the blood. When we do the testing, we find that the drug is going to kill half of all the tumor cells, but at a concentration that's higher than what can be achieved in the blood, then we don't end up using the drug. It has to be within the Cmax concentration and what can be achieved in the blood.

Brad Power: Approved drugs only, no clinical trials?

Diana Azzam: So far we've been testing approved drugs only, but we can also test drugs that are in clinical trials. There is no reason why we cannot. I would actually push for this type of approach because we can guide patients to clinical trials, if we have access to these drugs. We can test them and also guide patients to clinical trials.

Noah Berlow: We're trying to open up as many options as possible.
Looking again at our business model, there are ways to build relationships with pharma companies around this. We're more than happy to de-risk some of these patients up front before they enroll in your study. This patient seems likely to respond well, as their cells ex vivo are responding to the drug that you're going to give them. That gives them a little bit more

confidence. Conversely, if they're seeing no response, it might help say this is not the right patient for this trial. They need to go somewhere else.

Brad Power: Would you consider the work you're doing more about functional testing or about AI/ML?

Noah Berlow: The functional testing is critical and it has immense power on its own. The AI also is really powerful and it has the ability to take the functional and make it better. It also has the ability to take the functional testing results and make it better for a lot of patients. We are still exploring all the ways that functional and AI mix and interplay, especially when you start layering in genomics. It's not that one is more powerful than the other, it's that the two of them are going to be synergistic and make each other stronger.

Diana Azzam: I've been working on functional drug testing for many years, but I always knew that there was something missing. We need to know why these drugs work and with the AI included, that integrates the whole exome sequencing and the transcriptomics. You are not only predicting combinations, but you are telling the patient why this is working. The power of integrating both is what we need to do in cancer treatment.

Brad Power: When you do the functional testing, you will have some hypotheses or maybe you have a standard set of drugs you test. You said you had something like 60 drugs you can test. DNA sequencing and RNA sequencing might give you some candidates you'd want to try in the functional testing. If you do the functional testing, you will get other hypotheses, and the AI can be telling you which ones to prioritize or which ones to combine. You could use the AI on both sides. Are you doing both?

Noah Berlow: Right now we're focusing on the AI on the end of it, because that's going to tell us why the drugs are working and help find what really is going to be the best combination for the patient. With all the data we have, if we know upfront that there's a PIK3CA mutation, that gives a good reason to add a few more PIK3CA targeted drugs into the screen. It'll help us better understand what that mutation means for that patient, because it might mean that PIK3CA inhibitors will be great, or it might mean that the right one will be really effective, or it might be completely unrelated. It'll help us better understand what that means. Right now, it's the end, but we're going to start layering it into the beginning as well.

Brad Power: You're working off fresh tissue. Does fresh tissue have a 24 or 48 hour life? Can you run one functional test, then come up with a new hypothesis, and run functional testing again without getting a new biopsy? Is the tissue still viable for testing after 48 hours?

Diana Azzam: We get a second shot. That's what happened in all the cases, because we get the fresh sample, we process it, we use what we need for drug testing, and we freeze them down. If we need to go back and test combinations, we're throwing those out fresh and testing within a few days. It's very helpful because sometimes doctors look at the single agent data,

and say, I want you to go back and test these combinations, and that's what we've done. It's been very helpful for the doctor.

Saed Sayad: First, we support multiple drugs instead of single drugs. Second, our simulation supports having drugs together instead of one by one, because you give the cancer cells the chance to adjust to every chemical you throw at them. And third, using the lowest amount of drugs, not the maximum amount, because when you increase the concentration, the side effects of drugs can affect the other drugs. That's our findings based on our simulation.

Noah Berlow: In many ways I'm in agreement. Some of the other work that I've done, but didn't present here, has been on showing the difference between sequential combinations or simultaneous combinations using the AI to find a combination and then testing that ex vivo on a couple of cell models derived from the same type of cancer, showing the combination can essentially stop tumor regrowth. But at the same time, we're showing in a practical setting, when you give the drugs at the same time, the effect is that the cancer cells go away and don't come back. Which of course is the key end goal. The place where the friction occurs is going to be giving those two drugs simultaneously may be a little bit much for a patient with less than optimal health. We're definitely focused on the right way to give the right combinations. In my mind, and I imagine Diana as well, we're looking towards that simultaneous combination as the right way to do it, but it's step by step. We have to get there.
Rebecca Driscoll: My experience with chemo sensitivity dates all the way back to 2004 with some of the original people that were working on chemo sensitivity. It's been around and evolving. I always saw that there was something to it. Now that we have genomics and more biomarker testing, I see a huge opportunity for this. What are your plans from a laboratory perspective? I come from Foundation Medicine, and collecting some of that clinical data can be very challenging. As we're combining these drugs and looking at the side effects and dosage and efficacy, what are you planning to do around acquiring some of that clinical data? I would say even broader in the community setting, because we can run these tests and studies a little bit more effectively at the academic level, but we also know that we need to reach patients more broadly in the community setting. Any commentary on that and collecting clinical data, to clinically validate this test?

Diana Azzam: The main challenge that we were working on, which we just resolved, is getting funding to get this up to CLIA standards. We just got state appropriations to set the lab up to be CLIA-certified. We can potentially expand this and really provide this type of service to work with any hospital and any clinical center because that's important for us as we know that we need that clinical data back. Nobody has validated that drug sensitivity testing or AI can actually improve survival in patients. That's what's missing. We don't have data in the field showing perspectively that we can potentially use this and it has clinical utility. We've been working on generating this data as you saw in the studies that I showed you, but these are small pilot studies with Nicklaus Children's and with Cleveland Clinic.
I've had very strong relationships with the doctors. They know that if they're going to work with me, I need data back. That was what made it successful in that we got the clinical data, but we're showing that it's feasible. There is clinical utility of this approach. The next step is in a

randomized setting in a large scale clinical trial. Does this approach really improve survival benefits in patients? The CLIA-certification is going to be a game changer here in terms of scaling this up and really getting more data from patients.

Rebecca Driscoll: That makes sense. From a stepwise approach, I would absolutely agree, obviously CLIA comes first. The other thing I was pleased to hear you say, just because I don't treat all testing as the same – you had mentioned whole exome sequencing and transcriptomics. Do you have a relationship with anybody running that or how are you going to work with that in your study?

Diana Azzam: On the clinical side we work with Sema4. They collect some sequencing and they're really great in terms of a CLIA-certified assay. For children, I've worked with UCSF and with OncoKids. We've been working with a few CLIA-certified labs that do this. It's been great, but we're open to other partners who would want to do this with us.

Noah Berlow: We're not planning to do this in-house because there are so many good assays out there, but what we're going to do in-house is get all that data and use our AI approach for analysis.

Brad Power: You're outsourcing the DNA sequencing and RNA sequencing. Are you also outsourcing the functional testing or are you doing that in-house?

Diana Azzam: No, functional testing is us. The lab that we want to set up for CLIA is my lab at FIU and First Ascent using that lab.

Rebecca Driscoll: What about proteomics?

Noah Berlow: Right now there's a lot more focus on the DNA side of precision medicine. There still is work to be done even in whole transcriptomics at a CLIA-certified level. We'll take the RNA we can get, but ideally we can eventually get to the point where the transcriptome is at a CLIA level and we can access that data routinely. Proteomics isn't fully ready for high throughput, whole proteomics analysis. It's not ready for prime time. There will come a point when that kind of data, when it's available, is going to be invaluable because right now RNA is a proxy for proteomics. The future I see is that we will integrate proteomics when it becomes readily available. It's going to be really valuable. It'll give us a direct idea of which genes are actively being expressed, and what we can reliably drug. We've already worked with proteomics on the AI side. It is incredibly easy to integrate because it is just quantified data. When we get to that point, it is going to be folded into the model. We'll do some studies, validating the proteomics. It will really help refine and strengthen our models. We are just waiting for that to be ready to go.

Rebecca Driscoll: You are using a lot of mouse models. What are your thoughts about the organoid models?

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Diana Azzam: Organoids are great models, but they take weeks to grow. In our experience with refractory patients, we don't have a lot of time. My approach is really more like a 2d, 3d model. What's important for us is to be able to do the drug testing and data within a week, if not less than that. The only problem with organoids is it just takes time, and you don't get the opportunity to test many drugs. You're limited by the number of drugs. In our approach, we can test hundreds of drugs within a few days. I believe that our approach is better in terms of delivering results in a clinically actionable timeframe.

Rebecca Driscoll: What's critical is to start getting the physicians to order sequencing upfront. That's the challenge. Not waiting for another analysis of weeks.

Diana Azzam: That's one of the challenges we had when we were going through all the feasibility studies. Sending these cells out and getting the data back from sequencing takes weeks. But it's still useful. With the AI, Noah has been able to take the drug sensitivity data and then slowly, when we get the sequencing, we can always modify the combinations, based on the sequencing data as well. We can recommend treatment, the patient is on treatment, and then we can go back and slightly modify the treatment based on that data. So we've been able to manage that.

Rick Stanton: Electrical engineers love electrical engineers, and I'm an electrical engineer. I think you're great. I have some detailed AI questions.
From the chat:

- What type of AI do you use? What exactly is the nature of the inputs? Genomic? What type of genomic? Somatic mutations I imagine? Other inputs?
- WES?
- Path segmentation - very gross segmentation I imagine from discussion? not high dimensional IF guided?
- Functional testing similar to SEngine?
- So many groups hand wave “we've got AI” !!! What exactly is the nature of the input (and N) to your AI, and what exactly is the nature of your AI?
- Who interprets the outsourced seq?
- Might nanostring disagree with your proteomics assessment of today?
- If not organoid assays - what type of functional testing?
- Any inclusion of immunotherapy AI? - don't see any?
- SVM? RF? Deep learning yet? transfer learning?
- One might think you are using a RF based approach given boolean treatment of data?

Noah Berlow: On NanoString might disagree: what I understand of NanoString right now is they can do some targeted proteomics, and that can be really valuable. What I'm thinking about is large scale, all of the genes that can be expressed, what is the protein activity level of all of those genes? That's when things are going to really transform, because we will be able to deeply understand where the activity is. How does the activity change in the presence of specific drugs? We don't really have a high throughput way of doing that yet. If you're looking at

specific proteins for specific treatment responses, there's a lot of potential, a lot of power there, but as an engineer, I'm always looking at the biggest data set I can get.
On who interprets the outsource sequencing: That's me, I've done so much bioinformatics work back at CCTDI (Children’s Cancer Therapy Development Institute). I actually built the pipelines from scratch for all of the sequencing analysis and we're publishing on it. That's my domain.

Rick Stanton: So you understand cancer signaling inside the tumor cell pretty well?

Noah Berlow: Well enough to be able to do what needs to be done. At a personal level where I don't always do well is if somebody throws 30 genes at me and says, “tell me what's going on here”. I need to look a lot of stuff up, but I know the way to put all the data together so that we can.

Rick Stanton: Do you use Qiagen tools?

Noah Berlow: Kind of. A lot of it is genomics databases because there's a heavy focus on the functional side. And drug-target interaction databases. A lot of ontology tools, GSCA (gene set co-expression analysis), pathway analysis tools.

Rick Stanton: On whole transcriptomics, how do you normalize your data?
Noah Berlow: It really heavily depends on the study. For the kinds of work we've been doing, the biomarker development, we normalize primarily using GeTMM (Gene length corrected trimmed mean of M-values). It does pool normalization across a cohort of samples. Your study in a way is self contained. What is the study telling you? The nice thing is when I've taken that normalized set and expanded out to include more. What I see is that the actual values post normalization don't really shift that much. Once you hit a certain number of samples, let's say 20 or 25, adding the 26th doesn't really significantly change the expression level for the individual patient. In the past I have used a pool of other samples, both normal and cancerous, from a variety of different databases, like GTEx (Genotype-Tissue Expression).

Rick Stanton: Isn’t GTEx normally captured with a different RNA captured technology?

Noah Berlow: Yes. In that sense, it's not ideal. With the RNA seq normalization, I would say it's kind of the best we can do. This is a debate I've had with Charles, who is in the middle of our team picture, about what is true normal in RNA seq because we focus on sarcoma. Is true normal damage that's actively regrowing but not cancerous? It is really difficult to establish a normal in RNA. What we're really looking for is trends that are overlapping between the functional response data and the sequencing data. We're not trying to make decisions from sequencing alone. We're putting it all together to really glean insights.

Rick Stanton: It's been a goal of mine as well.
When you say “AI”, what architectures are you talking about and the type of inputs? I see this hand wave of, “We use AI. Don't ask what’s behind the curtain of the great and powerful Oz.” Any clues would be absolutely appreciated.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Noah Berlow: First of all, I completely agree. There are so many groups that say, “We're doing AI.”, and it's just, “We're looking up stuff in an Excel table”. What I'm doing is mostly based on what's called probabilistic Boolean modeling, which actually puts it more towards causal machine learning than anything, but right now “AI” is a buzzword. So that's the word I default to, but the idea with probabilistic Boolean modeling is you have your response feature. In this case, that's drug sensitivity. You have your feature set, which in this case is going to be a variety of different things, including drug targets, sequencing data or RNA data, DNA data, copy number variation data, whatever kind of data you can build from your models, whether it's an individual patient, cell lines, or PDX models, et cetera. It's taking all of that data, finding ways to normalize it into Boolean variables, true, false, putting all of that together to start finding relations between all of those Boolean features and your response feature, drug response. Then take those top relationships, layering on additional data that's publicly available, or private data, to understand what all of those features are really telling you. When we find those features that are really strongly correlated with drug response, either to a specific drug or all the drug responses for a patient, and then put together a lot of additional data about what these targets really are, it starts creating that story and understanding, and gives us insight as to where to take a drug or what to give that patient. If you want to dive through all the publications, please do.

Brad Power: Let's suppose your AI is a black box, and you and Rick just had a conversation where you geeked out and went into the details. I'm now a treating physician, and your AI has spit out that these are the three drugs that I want to run in a combination. Why should I believe you? It's a question of trust in the predictive accuracy of whatever you've built, and if you haven't compared it to a randomized clinical trial, how do I know that your predictive model is worth anything?

Noah Berlow: The work we've been doing to validate it at the very least shows the potential that when you design a combination this way, you see a trend towards synergy and a better outcomes from your study. There's clinical work to be done. Absolutely. That is a threshold we'll overcome. We have our plan to do that. The reason that it's worth exploring is when we've built combinations this way for individual pseudo-patients, we see the outcome we were hoping for. The combination does better than the single drugs alone.
The second reason that it's worth trusting, at the very least to try out, is because there's a lot of black box AI systems that'll just say, “Here's your answer. Don't ask why we're very specifically focusing on the reasons why, because we're spitting out a combination.” But the reason for that combination is that this gene, this gene, and this gene are important. Here's all the data that's pointing towards these genes being important. In that sense, it is an explainable AI. The rationale behind your decision is actually pretty clear. It becomes a matter of if the physician wants to dive in that deep, do these genetic targets make sense from all the data we're seeing? These drugs are pointing towards this. These genes also happen to be mutated. This one also happens to be really highly overexpressed at the RNA level. It actually makes sense. That’s the rationale.

“Functional Drug Testing and AI/ML for Treatment Decisions” (Noah Berlow and Diana Azzam) [#18]

Brian McCloskey: Have you gotten it wrong? There are a lot of steps in this process and points of failure. It's very complex. This whole notion of a black box is real, it's a real issue because decisions are being made based upon this data. We've looked at my data many different ways, transcriptomic information, et cetera. We have gone back and questioned whether or not what we're seeing is actually accurate. Have you run into an instance where you actually got it wrong?

Noah Berlow: On the AI side, not yet. At every point there is going to be a situation where your outcomes are not what you expect it to be, and I'm fully aware that is going to happen. We haven't hit that yet. I know we will, but what we are going to be able to show is 70, 80% of the time, the combination recommended is going to be helpful, and it might be more helpful than any single drug alone. It may not be the perfect answer, but it's going to point you in a solid right direction.

Diana Azzam: When these patients were guided by functional drug testing, how did they respond to treatment? We're seeing so far that they are responding, which tells you that our assay has clinical utility. To be able to tell the doctor, “This is the randomized clinical trial. These are the patients that were guided by this approach versus patients that didn't, this is the data.” We're not there yet. And that's the goal.
When we get that data, we'll be able to resolve many of the unanswered questions. In our experiences, when we do the drug testing, what I've seen that's really helpful is we know drugs. We can tell which drugs are not going to work. I think that also is an important aspect because a huge part of the idea right now is to avoid the trial and error that physicians have to sometimes go through. That is critically important. This has been very helpful for physicians, that they can tell, “These drugs don't work. Let me look into other drugs.” Functional drug testing has been very helpful in that aspect.