“Functional Precision Testing” (Tony Letai, MD, PhD) [#11]

June 1, 2022
Brad Power

Meeting Summary

Tony Letai, MD, PhD, Professor at Dana Farber Cancer Institute, and President, Society for Functional Precision Medicine, led a discussion on "Functional Precision Medicine for Advanced Cancer". As we look at novel, personalized therapies, how can we have confidence that they will work? Tony wants to increase testing of drugs on patients' tissue, using the better tools available today, to predict outcomes and guide treatment decisions.

Tony first critiqued the standard process for personalized cancer treatment, which is to take the patient's tumor, sequence it, and identify a mutation that tells us to use a particular drug. But how often does that happen and the patient gets a response? It’s much less than most of us would think, probably a percentage in the low single digits. There is lots of room for improvement.

He then proposed a different approach to personalize cancer treatments: “functional precision medicine”. You take a drug, you put it on a bunch of tumor cells, and then you use a smart assay to see what happens. This was tried for chemosensitivity about 30 years ago. But there were very few drugs, it was very difficult to culture the tumors in a way that was informative, and we didn't have good assays. Today, all of those process problems have gotten much better. We have many, many drugs; we have much better ways of culturing tumor cells; and we have much better ways of analyzing them. Tony cited several examples where his assay (“BH3 profiling”), which measures how close a cell is to dying (apoptosis), can be used to measure drug effectiveness. In several studies actual patient outcomes were blinded, yet the assay accurately predicted how patients would respond to drugs. The number of such functional testing studies and the number of patients in each study, while providing good evidence, are not as many as we might expect or hope for such a valuable service.

Why isn’t adoption of functional precision testing faster?

One of the biggest challenges is getting fresh tumor tissue on which to run the tests. There is a “Catch 22”: it requires extra effort to do the biopsy to get the tissue, and without evidence that it will accurately predict outcomes, patients and clinicians are loath to expend the extra effort and risk.

One of Tony’s initiatives is to raise awareness of the benefits of functional testing through a society he founded, the Society for Functional Precision Medicine, where experience and successes are shared monthly. He encourages those doing functional tests to accumulate and publish their results, and he also encourages patients, patient advocates, and patient foundations to learn more and be involved.

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Laura Kleiman of Reboot Rx and Ally Perlina of CureMatch both agreed that combining functional precision testing with their services is an opportunity worth exploring.

Requests

- Do you have any comments on functional precision testing?
- The value of functional precision testing seems intuitive, yet it’s not widely practiced. What are the barriers or objections to it?

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Meeting Notes

Brad Power: We're very honored to have Tony Letai of Dana Farber with us. This is very timely. We've been having a conversation over the last weeks about combination therapies with Ally Perlina of CureMatch and Bob Gatenby of Moffitt Cancer Center. Bob Gatenby adds other treatment strategy considerations that combinatorially make having evidence from randomized clinical trials pretty problematic for some of the more personalized strategies.Tony has ideas about functional testing that might provide a way out of this conundrum of personalized treatments that include dosing and sequencing of treatments and so little or no evidence from the batches of patients that are required for randomized clinical trials.

Tony Letai: I'm a clinician, but I mainly run a cancer biology lab at Dana Farber. I've been at Dana Farber since roughly 1995 when I was an intern, and I have been there ever since. I'm going to talk about an alternative way of personalizing therapy or choosing the right drug for the right patient. We can have a very open-ended conversation, which can certainly include how you can incorporate this into making novel therapy combinations. There's always going to be a definite tension between personalizing therapy and assembling novel combinations, which we know are going to be important for long term disease-free survival in a lot of cases on the one hand, and on the other hand verifying safety ahead of time. We can't solve everything, but I acknowledge this tension.

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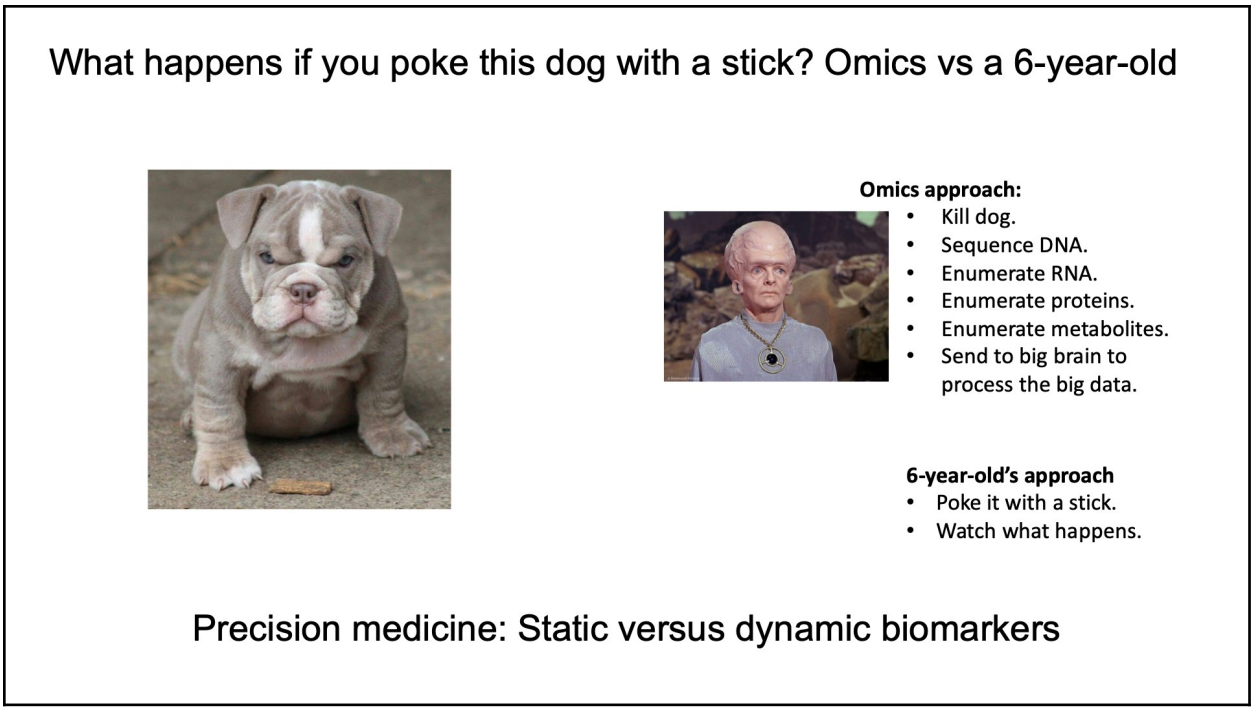


This is usually how this goes. This is all about getting information to choose a therapy for the right patient. I like this metaphor, so I'm going to present it to you. I find it analogous to this question. Say you're walking along the street with a six-year-old kid. Maybe it's your niece, your nephew, or your grandkid. I see this little dog, and you want to know, what would happen if I poked that dog with a stick? How would the dog respond? Would it run away? Would it bark? Would it wet itself? Would it bite me? What on earth would it do? How do we answer that question?

If you're a conventional omic person in today's cancer biology world, it's an analogous problem where they're trying to figure out, using genomics, how a complicated system – in their case a tumor, in this case a little dog – how it's going to respond to a perturbation – that's treatment with a drug or some other sort of treatment. The way omics or modern cancer genomics addresses this problem is: the first thing you do is you kill the dog. Then you sequence the DNA. Or now we're getting more mature: you're sequencing the RNA. You're looking at proteins, metabolites, all these, what I would call static phenomena associated with the tumor at time equals zero – initial condition measurements. You get big, big data sets.

These enormous data sets that people like this gentleman live to analyze. They're often housed at big universities and they take these enormous data sets and do all sorts of classifications and whatnot. And every now and then you actually spit out a suggestion for whether or not a drug works or not; not always, but sometimes you do.

What is an alternative to this approach? I'll summarize what I think the success of this kind of approach is in a little bit, but an alternative approach, if you want to know what happens, if you poke this dog with a stick, how would a six-year-old solve this problem? The six-year-old would

poke the dog with a stick and then watch what happens. I am a firm believer that we do not do enough of that in cancer biology.

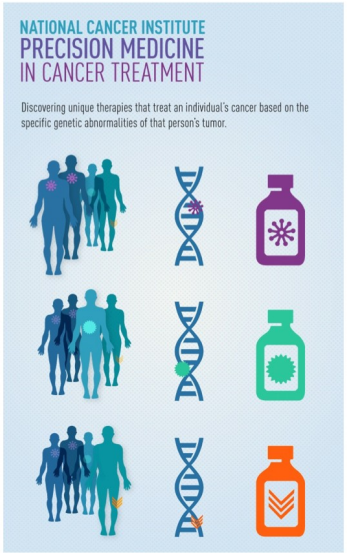
Of course, what I'm going to get to with regard to poking it with a stick is actually taking the patient's living tumor cells, exposing them to drugs, and seeing what happens.

Why don't we do more of that?

You might ask why we call this *functional* precision medicine. The omics approach looks purely at static biomarkers. I think of this as looking at dynamic biomarkers.

I was originally trained as a physicist. One thing we know is that you can learn a lot from a complicated system by making perturbations to the system, rather than just measuring initial conditions.

But there's already a way to solve it, right? If you've listened to anything from the NCI, from big cancer centers around the world, including my own at Dana Farber, this problem of choosing drugs for patients, we've kind of got it licked, right? We know that we do precision medicine: we take the patient's tumor, we sequence it, and it's going to spit out a mutation that tells us to use a particular drug.

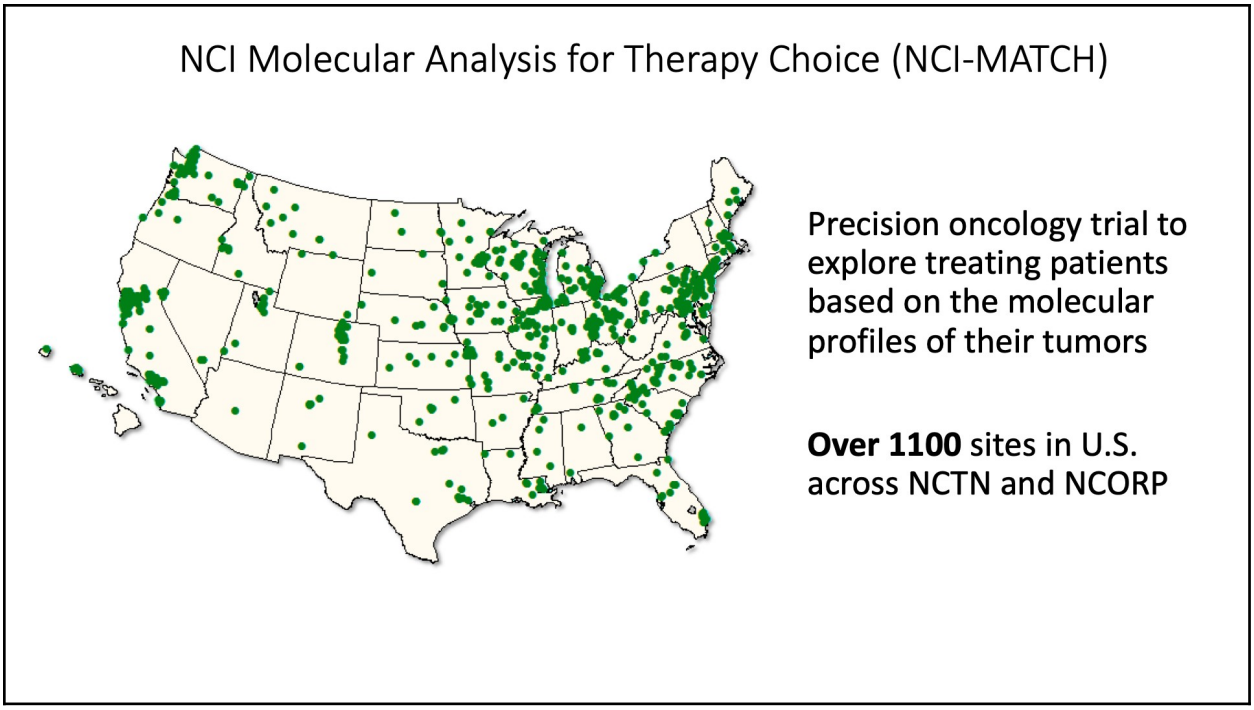


What patients will respond to what drug?

- Drug perspective – how do I choose the right patients?
- Patient perspective – how do I choose the right drug?
- The realm of genomics, right?

As you can see, in this famous graphic the NCI uses, this is how you do precision medicine. You see it's very persuasive because there's this DNA chain. You can see for these different patients, there's a blue asterisk or purple asterisk there, then a green dot there, and an orange dot there, all these different parts on a DNA double helix. You could see if you look at all the

patients, they're all served by this. Everyone gets a drug out of all this. This approach was tested in the NCI-MATCH trial.



You may have heard that they took people with advanced, solid tumors, all across the US, and did a genomic test, some kind of sequencing, to find mutations, then assigned them to different treatment arms. It started out with around 10 treatment arms, and then grew to something like 30 as new knowledge increased.

It's worth considering: How well did this work?

This was the National Cancer Institute using a general approach for advanced cancer patients doing the sequencing that we've been told is the way to do precision medicine. Let's talk about what we mean by “work". What I mean by “work" is: how many patients got a drug that helped them? I could tell you if you look at a lot of the publicly available information from the NCI-MATCH trial – and I encourage you to look into this yourselves – you won't find a lot of information about how many drugs were found that actually helped patients. You find a lot of information about logistics, how many people were sequenced, how many sequences there were, and so forth, but not so much about patient response. But I'll show you what I know and what I could find in the publicly available information.

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Status of NCI MATCH: Enrollment as of May 31st 2020

	Central Screening Cohort	Designated Laboratory Referral Cohort	Total	
Enrolled for Screening	6391	592	6983	
Screened	5548	592	6140	
Assigned to Rx	987 (18%)	504 (85%)	1491	
Enrolled on Rx Arm	686 (70%)	423 (85%)	**1109**	**16%!**

As you see, they enroll big numbers. They were able to sequence like 7,000 people. But what's really important to recognize is how many of those patients after sequencing made it to a treatment arm. That is how many patients had a mutation identified that actually was relatable to an arm that had a drug assigned to it. Only 16% of the patients even made it to a treatment arm of these 30 treatment arms. How did those patients do?

Table 3

Select NCI MATCH Treatment Arms with Findings

Out of the 16% that got onto a treatment arm!!

Drug	Target	Arm	ORR	Publication/Status
Ado-trastuzumab emtansine	HER2 amplifications	Q	8%	Jhaveri KL, *Ann Onc,* online ahead of print 08-27-19
Afatinib	HER2 activating mutations	B	2.7%	Bedard PL, AACR 2019 Annual Mtg
AZD1775	BRCA1 or BRCA2 mutations	Z1I	3.2%	Kummar S, AACR 2019 Annual Mtg
AZD4547	FGFR pathway aberrations	W	8%	Chae YK, *JCO,* ASCO 2018 Annual Mtg
Capivasertib	AKT mutations	Y	23% ☆	Kalinsky KM, EORTC-NCI-AACR 2018 Mtg
GSK2636771	PTEN expression or loss by IHC PTEN mutations/deletions	P N	0% 5%	Janku FM, *Ann Oncol*, ESMO 2018 Mtg
Nivolumab	dMMR status	Z1D	24% ☆	Azad NS, J Clin Oncol, 38 (3), 214-222 2020 Jan 20
Palbociclib	CCND1, 2, or 3 amplifications and Rb protein expression by IHC	Z1B	0%	Clark AS, AACR 2019 Annual Mtg
Taselisib	PIK3CA mutations	I	0%	Krop IE, *JCO*, ASCO 2018 Annual Mtg
Trametinib + Dabrafenib	BRAF V600E or V600K mutations	H	33% ☆	Salama AKS, ASCO 2019 Annual Mtg

☆ Studies that met the Primary endpoint

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Well? It depends on the treatment arm, and for the most part, there's no publicly available information on what I would consider the most important result of this trial. Maybe it'll come out eventually, but here's what we do know. Here's maybe about 10 different treatment arms. You can see they have different letters as they sort of advanced through the alphabet and then needed more.

The “ORR” is the overall response rate. That's an objective response rate based on shrinkage of the patient's tumors. This means a patient went to a group of patients who had a mutation identified, went to a treatment arm, and then got a drug. If you look at these numbers, you might have anticipated that number would be a lot higher, based on what we know about genomically-directed precision medicine. Recognize that this overall response rate is only for the 16% that even made it to a treatment arm.

My point in presenting this is not to tell you that genomics is useless. In a lot of patients it can be very helpful, but the ceiling on how many patients actually benefit from this is lower than I think many of us consider. And if you take in all cancer patients getting genomic tests, it's not that nobody benefits. Some people certainly benefit. I can list EGFR-mutant lung cancers, or BRAF-mutant tumors, whether it's melanoma or colon cancer or something like that, or patients with TRK mutations in a wide variety of diseases, that certainly benefit, but that's not most cancer patients. In fact, we're probably down in the single digits percents for patients who actually benefit from these genomic analyses.

I'm saying this just to indicate that, yes, there is room for improvement. There is a need for additional methods to assign drugs to patients. If we rely purely on genomics, we're tying one hand behind our back. It's not a useless test, but it definitely needs help.
So what's my proposal?

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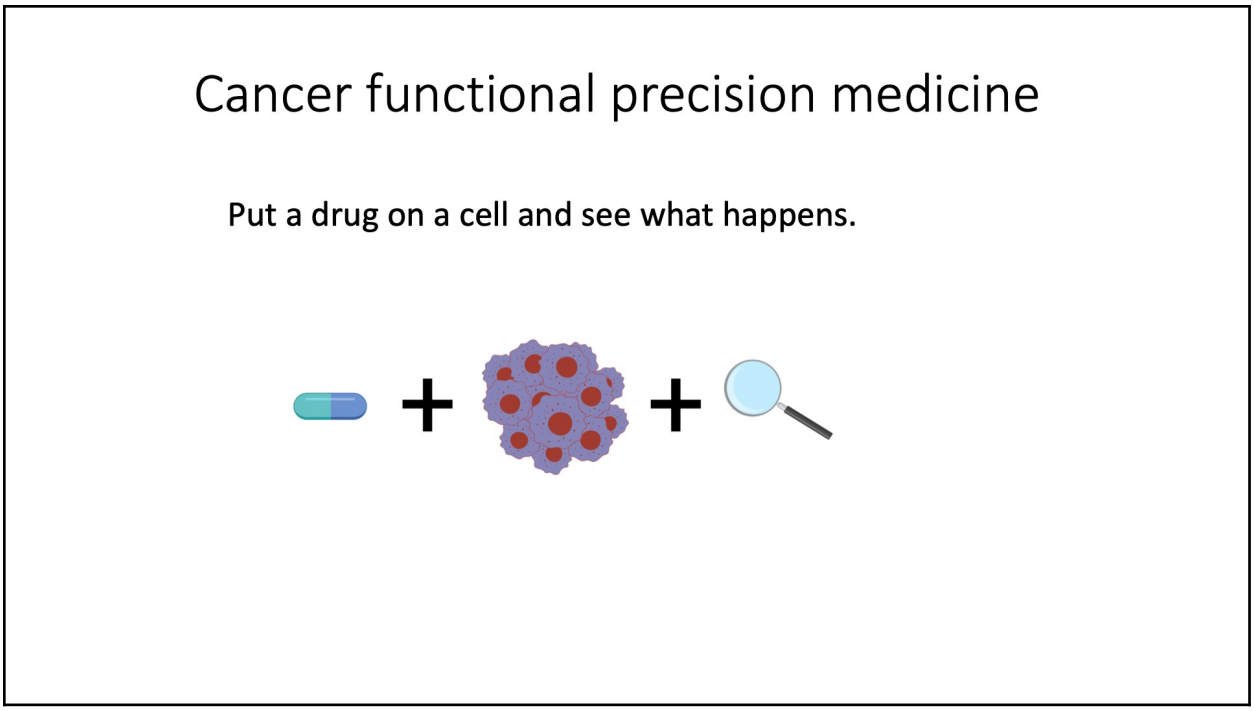


Something that we talk about called cancer functional precision medicine. It's a very simple idea. You take a drug, you put it on a bunch of tumor cells, and then you figure out a smart assay to see what happens. This is what we use even today as the gold standard in microbiology, except for in that case, you are usually trying to kill a bacterium. If you have a blood infection,urine infection, or pneumonia, your doctor will try to culture the bacteria causing that infection. We'll grow it in a Petri dish, really fast, treat it with all the drugs, and let's see which one works best. And that's how we choose. That's still the gold standard for what the best antibiotic is.

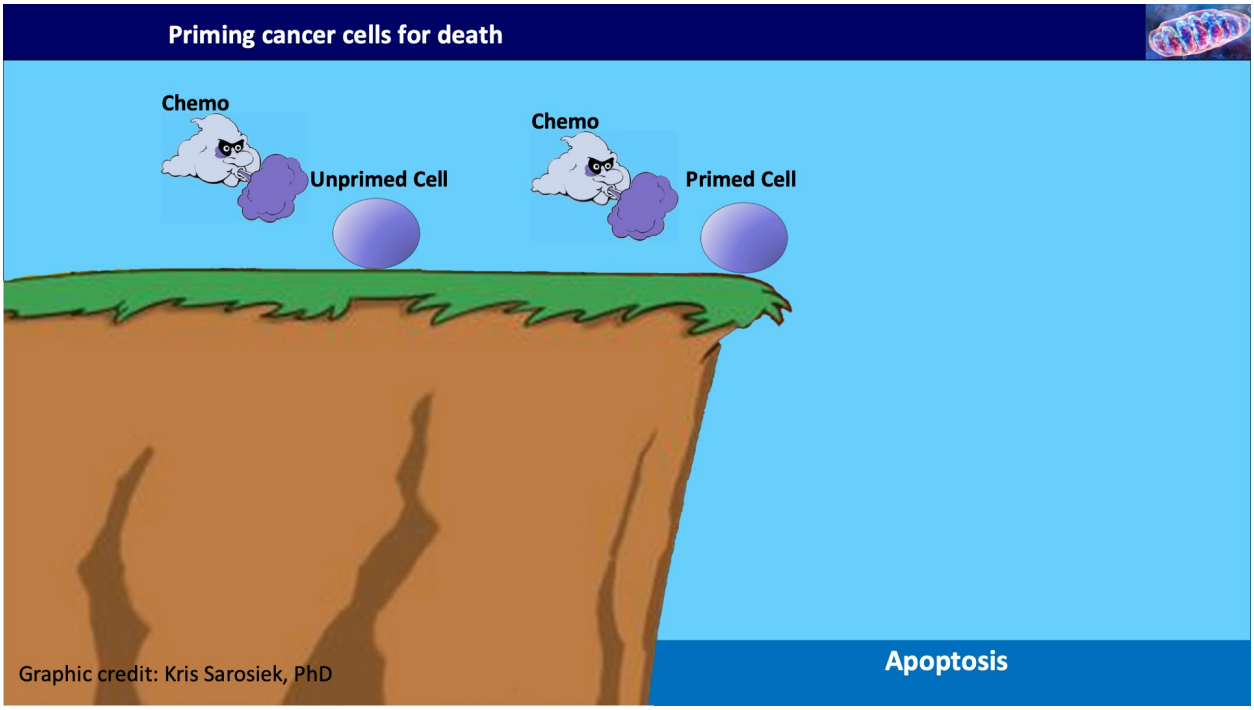
Today, in 2022, you might ask, this is so common sense: Why don't we do this in cancer? People did try ideas like this. The heyday was something like 30 years ago. There were so-called ex-vivo chemosensitivity assays, and most of the leaders in the cancer biology world decided they weren't ready for prime time. Back then there were very few drugs to choose among. We had maybe cisplatin and something else. It wasn't very useful information to know whether or not you could receive cisplatin if there wasn't a great alternative available to you. Culture conditions by today's standards were exceedingly rudimentary. Very often what happened over these extended cultures, the tumors were overgrown with normal fibroblasts and the assay provided no information. All the assays available back then, what we call bulk assays, meaning there was no single cell information obtained. You had this heterogeneous mix of God knows what, and you'd get some readout that had some metabolic value that wasn't clearly associated with tumor cell death, maybe vaguely correlated. There were a lot of problems with every step here. We had very few drugs, it was very difficult to culture the tumors in a way that was informative, and we didn't have good assays.

30 years have passed, and all of those things have gotten way better. We have many, many drugs, we have much better ways of culturing tumor cells, and we have much better ways of analyzing them.

Brad Power: What is the correlation between test results and clinical results? How has that been progressing? As you said, maybe it wasn't so good before, and maybe it's better now?

Tony Letai: I'll give you some examples, and also some challenges. It is very hard to answer sometimes.

There is a property of cells called “apoptosis”. Every cell in our body has built within it a suicide mechanism so that that cell can decide to kill itself. The cells are programmed to do this. If they go bad, if they're doing something they shouldn't, like growing in a way they shouldn't, or metabolizing something in a way they shouldn't, they can activate apoptosis to eliminate themselves. It's good for the body as a whole and virtually every multicellular organism that's around that it has this process in itself. It's a threshold effect. Once it gets triggered, the cell rapidly commits to cell death within minutes.



I like to use a cliff metaphor to model it. You can imagine that there are some cells that are really close to the edge of the cliff. And there are other cells that are really far away from the cliff. It turns out we've shown that the cells that are really close to the edge of the cliff are really sensitive to chemotherapy and are easy to kill by our treatments. And the ones that are further from the edge of the cliff are harder to kill. They might endure the chemotherapy and then live to fight another day.

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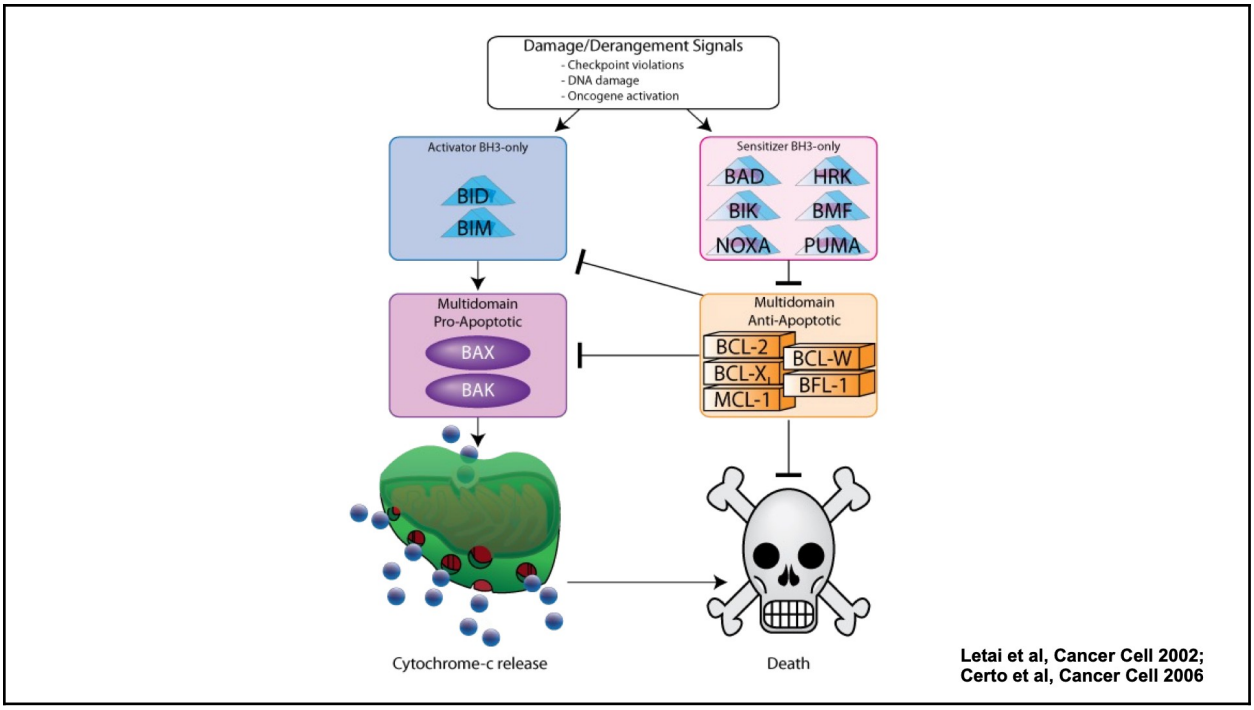
We wanted to figure out ways that we could selectively provoke this type of signaling in cancer cells to move cells from far from the edge of the cliff to close to the edge of the cliff, what we call “priming them for apoptosis”. That required an understanding of the apoptotic pathways. All of your cells have mitochondria. You may be familiar with them as power plants of the cell. For our purposes, they exist as the decision mechanism around whether or not a cell progresses to cell death. And that involves the membrane around the mitochondria. And if that permeabilizes, it releases a bunch of factors that causes the cell to chop up its DNA, chop up its protein, and even tag itself with signals so that it gets eaten by neighboring cells. That's regulated by this complicated protein family called the BCL2 family. There are some proteins in that family that cause this permeabilization event. Those are the ones we call “pro-apoptotic”.

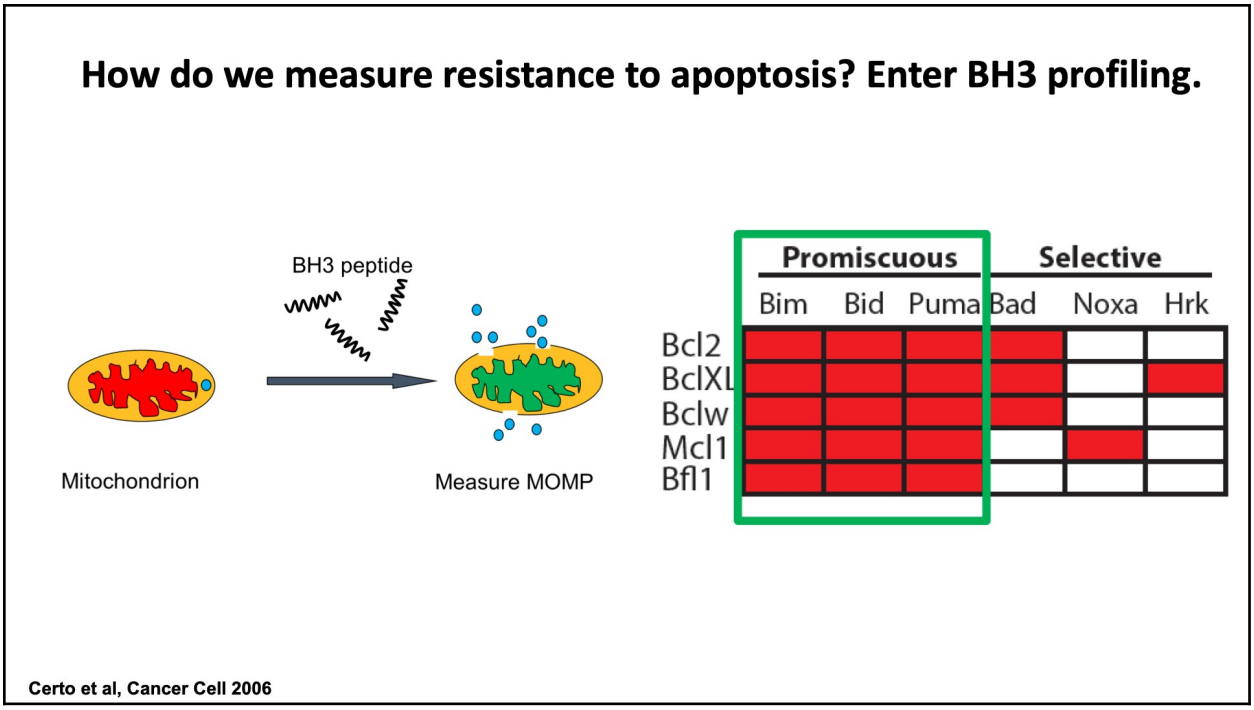
And there are other proteins that oppose this permeabilization event. Those are the ones that we call “anti-apoptotic". They function by binding up the pro-apoptotic ones so that they can't make the holes in the membrane.

Using knowledge of this pathway, we developed a way to measure how close the cell is to the threshold of apoptosis. We call it “BH3 profiling”.



The way we do this all takes place at the mitochondria. We probe the mitochondria of the cells, usually cancer cells, but you can do it on normal cells, any cells you want. We probe them with these synthetic things we call “BH3 peptides”. These are parts of the pro-apoptotic molecules I just told you about. If we need to put a lot of these BH3 peptides on a mitochondrion, then we know it's very far from the cliff's edge, and it's very unprimed for apoptosis, in order to make it permealizable. If we only need to add a little bit of the BH3 peptides to make it permeabilized, then we know it's very close to the threshold of apoptosis. I may use this term “MOMP” once or

twice. That's “mitochondrial outer membrane permeabilization”. That's what we measure in our assay. If you need a little bit of BH3 peptide to cause permeabilization, then it's pretty primed, if you don't need a lot, it's pretty unprimed. We have a good assay to tell you whether this cell, the cancer cell we're studying, is close to or far away from this commitment to programmed cell death.



These are the peptides that we use for it, and this binding chart, as you can see here. Once we have this measure of how close or how far it is from the threshold of apoptosis, then we can ask the question. If we add a drug, does it push it closer or further away from that threshold? We want to see the drugs that push it close to the threshold.

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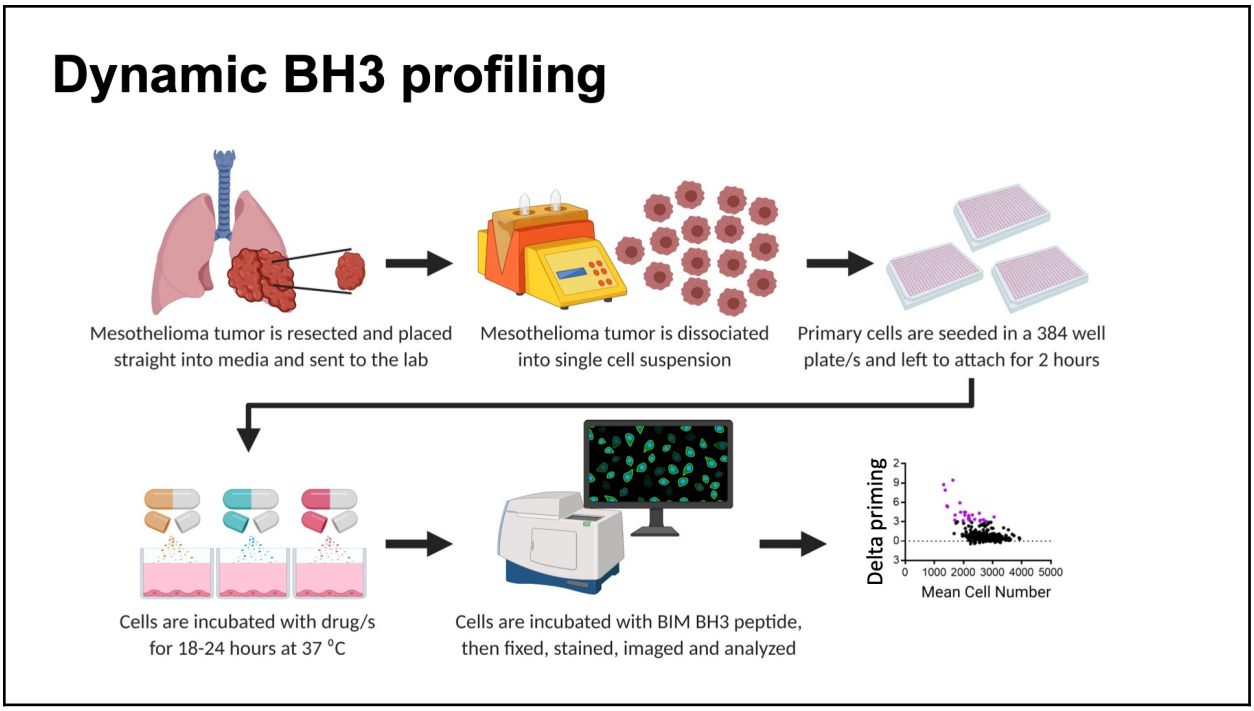

This is a process we call “dynamic BH3 profiling”. I'm showing it for a particular kind of cancer called mesothelioma, but they're all the same. We take the tumor cell, and we dissociate it so that it's in a suspension that we can then distribute to our platform, which is a 384 well plate. We can do a lot of this with robotics and automated handling of liquids and cells and so forth, so that you can take humans out of the equation and make it relatively high throughput. Once it's in this 384 well plate, we can then add a bunch of drugs, and we can add single drugs, combination drugs, really anything you want that we can add to these wells. Then we let them incubate.
A big advantage of our method of analysis is that in order to see if the cell is moving towards the threshold, after being exposed to the drug, we don't need to wait for it to go all the way over the edge, which can sometimes take days. We just need to see it starting in the right direction. Our assay is sensitive enough to measure that. So after only like 20 hours in culture, we can then perform our assay and ask if the drug is moving the cell closer to the threshold of apoptosis.
If it does, it turns out that's an identification of a good drug, and I'm going to show you a bunch of in vivo comparisons. Most of them are in humans, but there's also some mouse data.

We've developed a lot of data over the last 10 years. I'm just going to show you a few examples to whet your whistle.

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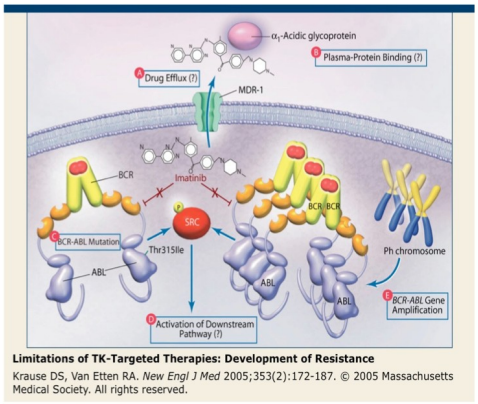
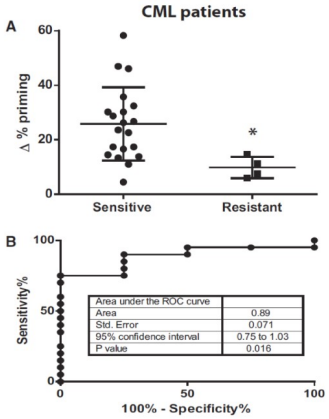
Imatinib and CML



Limitations of TK-Targeted Therapies: Development of Resistance

Krause DS, Van Etten RA. *New Engl J Med* 2005;353(2):172-187. © 2005 Massachusetts Medical Society. All rights reserved.



Montero et al, Cell 2015

The hallmark of this kind of positive control of rational personalized therapy in medicine was imatinib and CML. This kicked off a revolution in cancer, where people identified with chronic myelogenous leukemia had a translocation that caused the formation of a new protein. And this new protein was a particular kinase that then you could develop a specific kinase inhibitor – the first one of which was imatinib – to inhibit that special kinase activated by this translocation mutation. A CML patient should then respond to imatinib. Most do, some don't. We asked ourselves if we studied samples from CML patients, before they got the drug, using this method, if we could predict who would respond. We took these CML cells, put them in culture, added imatinib, and then measured what we call “delta priming”.

This is what spits out of our assay. It is the measure of whether it moves closer is becoming more primed for apoptosis. Then we ask, can we predict the responders more than the non-responders? And what you see here is we could do a very good job of predicting responders and non-responders to imatinib. You could see the sensitive ones have a higher signal than the resistant ones. That's what tells us that it's a good predictor.

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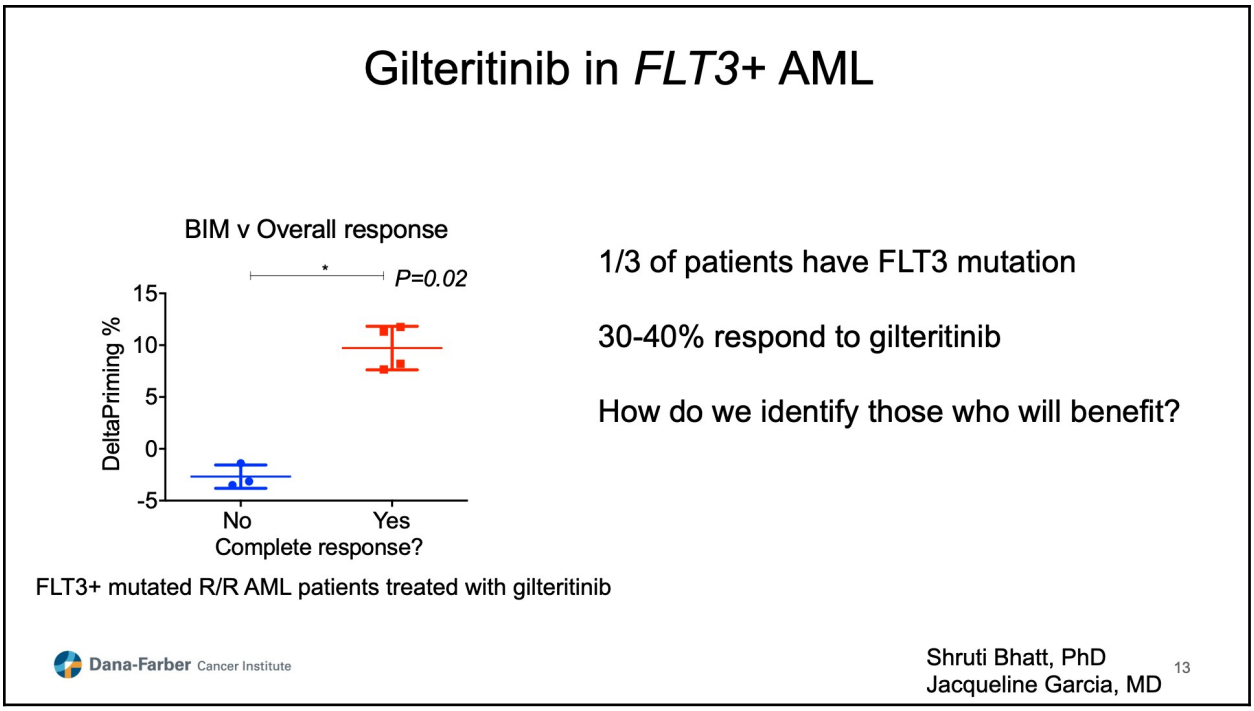

There are plenty of other targeted therapies that have come along. One of these is a drug called “gilteritinib" that inhibits FLT3 in acute myeloid leukemia. It's generally only patients with FLT3 mutations that get this drug. But even among these patients with FLT3 mutations, which are sensitive to gilteritinib, only about 40% have a response.

Can we do better? We took a small set of patients, only 7 or 8, all of whom had this FLT3 mutation, and we were able to look at the ones that responded and the ones that didn't respond. The ones that did respond had a higher Delta priming score – the output of our assay. We were able to very neatly discriminate between patients who responded and those who didn't respond to this fancy targeted agent gilteritinib, without any prior knowledge of the patient, just by studying the sample.

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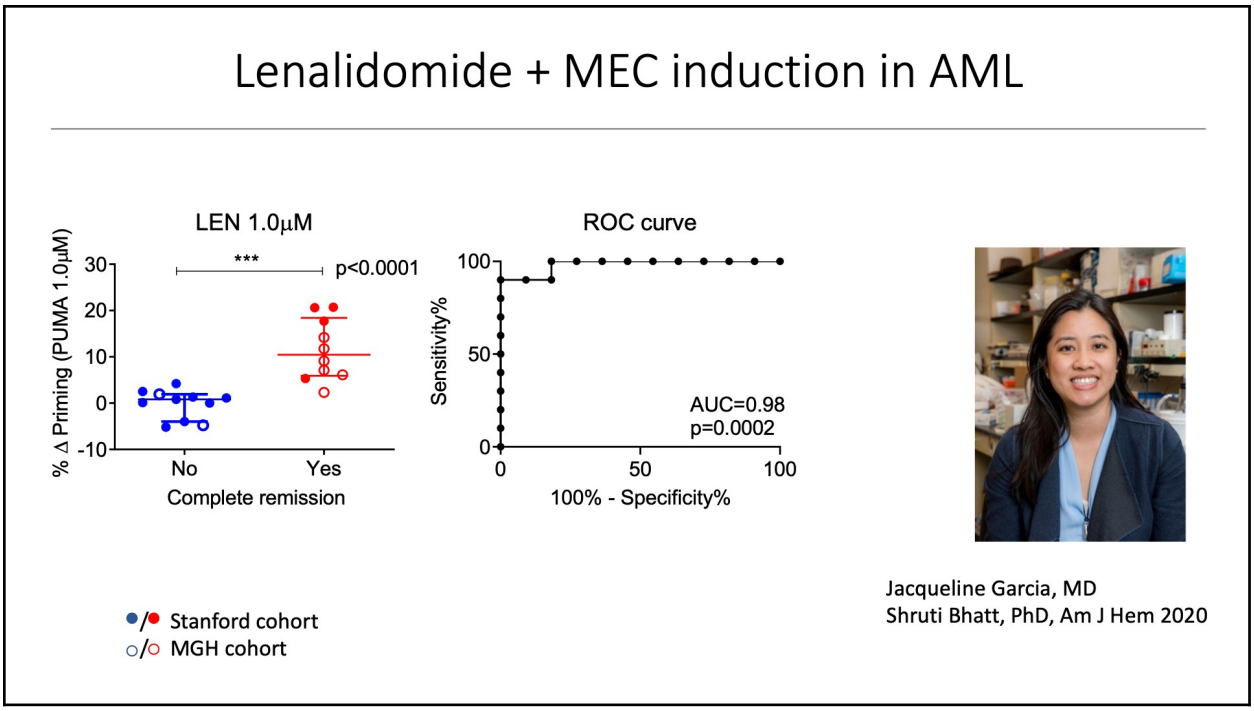

Here's a case of a more complicated regimen for AML, which is this drug called lenalidomide, used for a bunch of things more often in myeloma. But here they were adding it to conventional chemotherapy in AML. We performed this in completely blinded fashion, where we didn't know the clinical results, where we just performed our assay on patient samples prior to their getting this complicated regimen and looked at our ability to distinguish between patients who had a complete remission and those who didn't.

You can see here on the left, the ones who had a complete remission had a higher score than the ones who had a poor remission. For those of you who are into the statistics of binary predictors, there's a thing called a receiver operating curve, where the absolute best you can get is an AUC of 1.0. You can see in this relatively modest number of samples, maybe about 20, we had an AUC that was nearly perfect. We were able to nearly perfectly predict those who were going to respond, compared to those who were not going to respond; again, with no prior knowledge. We were blinded as to the outcome when we performed our assay, but we could still distinguish, purely based on whether this drug induced an apoptotic signal in our assay system. Other people have used other assays to do similar work, and this is an example.

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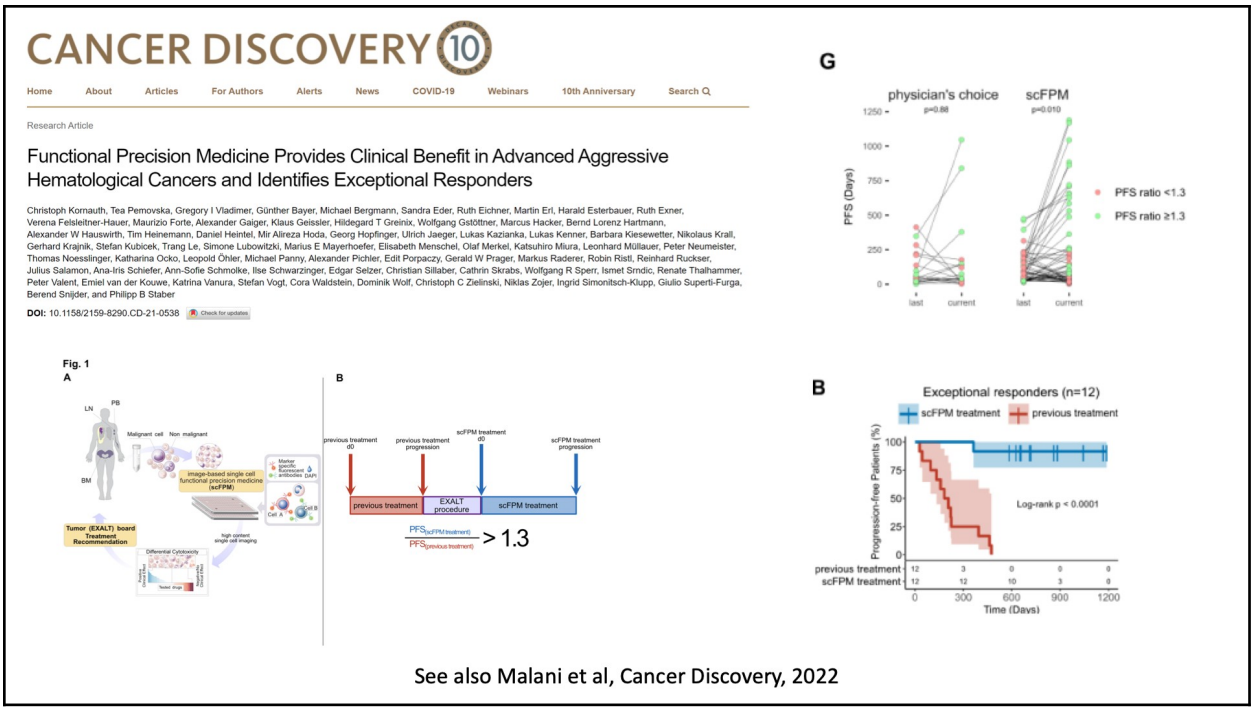

See also Malani et al, Cancer Discovery, 2022

I'm very gratified to say that we're building a lot of momentum in the field as a whole. You may be aware that Cancer Discovery is probably the top journal in cancer research in the world nowadays. Back in February, there were a couple of very nice studies in it. They both happened to be European studies which took blood cancers and did these very straightforward tests. I'm not going to march through all the metrics of success, but they did very well. Perhaps it's easiest to see down in the lower right that the people who they could identify as really good responders did much better in terms of progression free survival than if you compare them to whatever they got before.

In cancer, almost always people respond worse to a subsequent therapy than they did to a prior therapy. The fact that they were able to improve therapy in patients who'd received multiple prior regimens is an indication of their ability to actually identify active regimens. In this case, they didn't use the complicated BH3 profiling that we did. They used a different assay, but it was a very simple culture system where they had to read out in just a few days.

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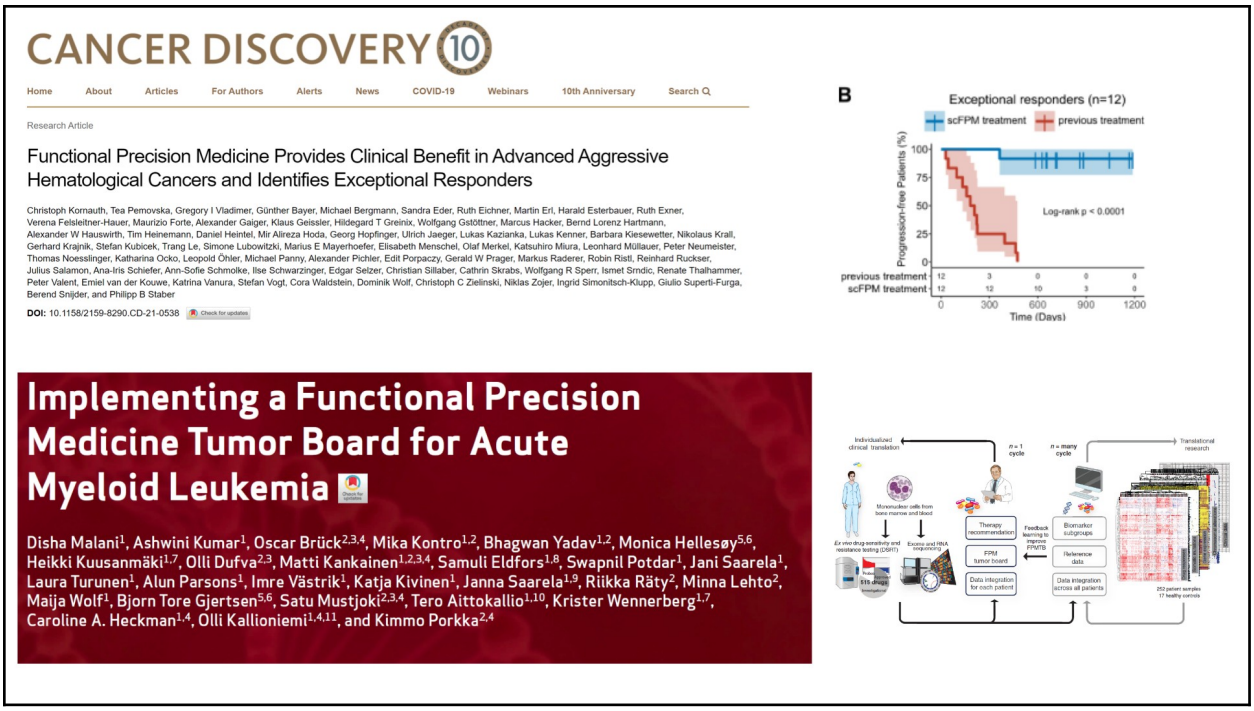
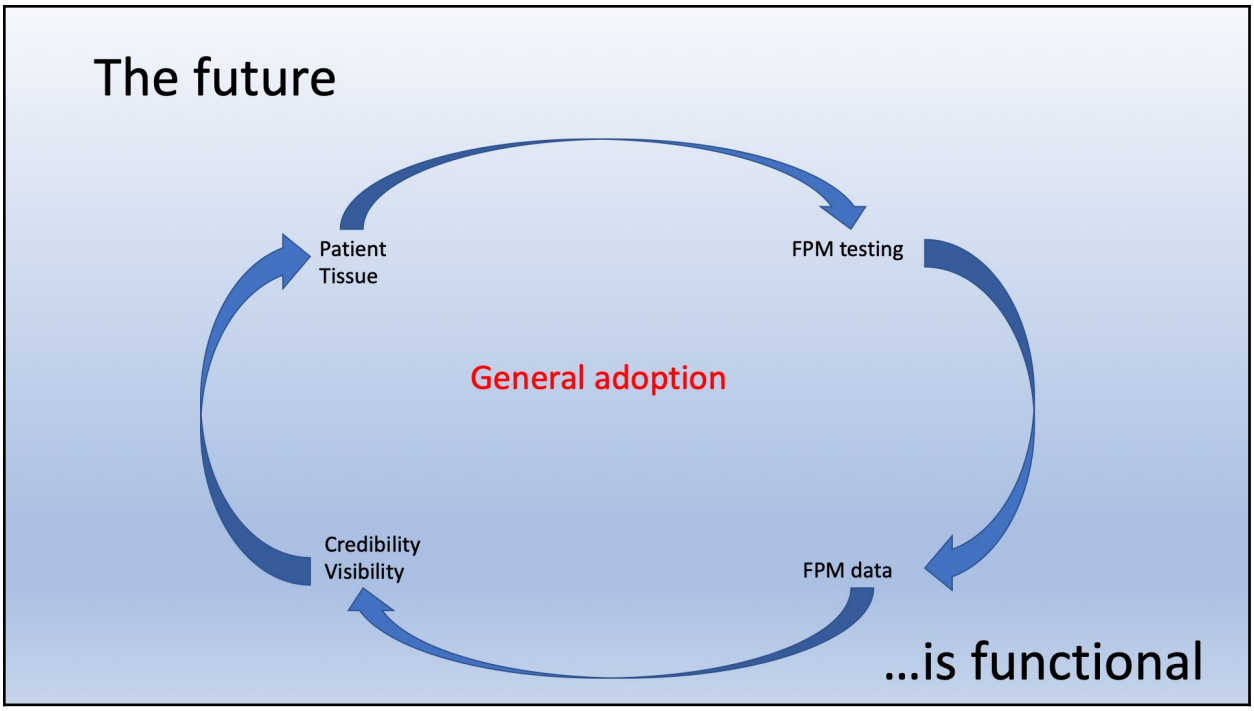


This related study was done by another group of people in the Vienna - Zurich axis, a combination of academics, as well as a commercial company. They showed very similarly that they could identify active therapies in patients who had this approach to identifying good drugs for these patients.

Now I want to get to the very hard question you were asking, Brad: in all of these cases there was correlation between clinical response in patients and performance of our assay. The sort of numbers we're talking about here is dozens to hundreds of patients. All of us would prefer to see thousands of patients, and given the number of cancer patients in the world, you might ask, why don't we see more of this?

It largely comes down to great difficulty in getting the tissue in the first place. We’re in this difficult cycle, where in order to do our functional precision medicine testing, we need to get patient tissue. In order to get patient tissue, we need to convince the patients, as well as the clinicians, that we have a good assay that merits doing this extra biopsy so that we can get that tissue. We need to have the credibility and the visibility of our approach, and in order to do that, we need to have the hard data to back up that we can predict things. In order to do that, we need the patient's tissue. We're caught in this vicious cycle where we need tissue to do the assay, to develop the evidence, to get the tissue in the first place.

“Functional Precision Testing” (Tony Letai, MD, PhD) [#11]



The future is going to bring – and we're already starting to see – a virtuous cycle, where we're going to see more data. It's already appearing in high profile journals that will allow us to have more credibility, and gradually there will be general adoption. What I foresee is it'll be somewhat non-linear, which is to stay once a few of these chips fall, we're going to see a lot of patients adopting this approach because it's so common sense. It seems to me that it's very hard to get around the simplicity of: if you want to know if this drug works on this tumor, put this drug on this tumor and measure it out. There is definite difficulty, and there's definite logistical challenges in getting viable tissue to the labs that do this. But in my opinion, the quality of the information is so great, that it's going to be worth it to do that. In the long run, there will be well established assays in CLIA-certified laboratories in the United States and other regulatory bodies worldwide, where this will be a standard in the treatment of cancer patients. I just want the day when that happens to come fast.

“Functional Precision Testing” (Tony Letai, MD, PhD) [#11]

Society for Functional Precision Medicine

- Uniting scientists, clinicians, pharma, regulators and patients to speed the day when functional diagnostics are everyday tools in cancer treatment. http://sfpm.io/
- @theSFPM
- anthony_letai@dfci.harvard.edu; @DrTonyLetai

SfPM

Meeting April 9 in New Orleans,
Next to AACR meeting.

I founded a society (The Society for Functional Precision Medicine) to accelerate the day by unifying different people doing this around the world. Now that we've accumulated a bunch of scientists and companies that are doing this, I'm very interested in getting patients as well as patient support groups and foundations more involved in spreading the word, as well as getting this done faster.

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If you're interested, visit our website (https://participatorymedicine.org/), and check us out on Twitter (@TheSFPM). We have monthly meetings where people present the latest in what we're doing. This whole field benefits from greater involvement of patients and greater exposure. I'd love to hear your ideas about how we can make this happen faster.

Brian McCloskey: Regarding the tissue requirement, I presume that you require live or live fresh frozen tissue. You can't work with FFPE (Formalin-fixed paraffin-embedded) or anything like that?

Tony Letai: That's right. FFPE is dead tissue, and that is a challenge. I would love to validate my assay on banks of tissue that are where you already have a correlation between the patient tissue and the response in the clinic. But it's very rare – almost unheard of – for people to store those viably. There are certainly ways to store them viably. Some of you may be familiar that you can store it in 10% DMSO, and then you can often get sufficient viability to do these sorts of assays. But even that is rarely done. It's almost always FFPE, and that turns a living cell to a nonfunctional cell. A principle I want to get across is a lot of this just has to do with how we do things. All biopsies start out viable, and it's us who kill them. We go out of our way to immediately preserve them, so that we can do these static assays. While these static assays have some benefit, what I've tried to convince you of is probably the benefit Isn't so great that we want to be putting all our eggs in that basket, and these functional approaches have actual, tremendous potential to them. We need to focus on making the tissue available for those types of assays too.

Brad Power: In Brian's case, he had a primary site, and there's some tissue from that, but it's not live tissue, and now he has some metastases in the peritoneum. I guess there's a “Catch 22”: if people are under treatment, as Brian is, then they don't have enough tumor tissue to run tests on. Either you're in a very serious situation where you have lots of cancer tissue, or you're in Brian's case under treatment and maybe your PSA is low, and there's no accessible live tissue at the moment.

Tony Letai: If there's nothing there at the moment, that's probably good clinically. The time to do a biopsy is if there was a progression or a recurrence. One of the nice things about these assays is once the tissue is available, the turnaround can be quite quick. It's not like other models, like PDX models, that take weeks to months to get enough tissue to put in a mouse and expand and so forth. Organoid models are a little bit longer than what I was telling you about. Those are often on the order of a week to weeks to generate an organoid model and get information from that. I chose to share with you some assays that are very simple, two dimensional assays, where the information can be available really in something more like days.

Saed Sayad: So far we have processed maybe close to 1000, all mixed data, and the number one issue we have seen is the reproducibility of the omic data: anything related to the genes, RNA, micro RNA. If you repeat this, if for example we have a data set, and we find the biomarkers for cancers, then when we repeat the test on a different group, the list of biomarkers is different.

“Functional Precision Testing” (Tony Letai, MD, PhD) [#11]

What has been your experience?

Tony Letai: I'm not a practitioner really. You need to choose how you spend your time. There are plenty of people doing that type of omics research. My impression is that the sort of predicted biomarkers that are very reproducible and do very well are DNA-based biomarkers, where there is a single mutation that indicates response to a drug that targets the exact protein that bears that mutation. Examples would be EGFR-mutant lung cancer, which is a good biomarker for response to an EGFR inhibitor. BRAF mutations in melanoma are a good biomarker for response to a BRAF inhibitor. My impression is where that starts to fall apart is where people are constructing more complex, perhaps RNA-based predictors, where you are maybe taking a vector or a set of gene expression data, and developing a more complicated signature and showing that that correlates in some way with response. That might be a little more difficult to reproduce.

One of the challenges that I've seen in that is often what they present it as is a correlation with response. In a lot of the early testing, they don't force themselves to turn it into a binary biomarker. They don't force it into a “yes” or “no” decision, where if they did that, sometimes they would find that it doesn't have great clinical utility because it's very soft in its ability to actually predict “yes” or “no”, even if there is a statistical difference.
The difference between two sets of responders and non-responders in the presence of this signature isn't great enough to give you the kind of stark difference to make a binary prediction.

Saed Sayad: For the last 20 years, we have created a huge amount of data, such as The DepMap (Cancer Dependency Map of the Broad Institute) database. It is a huge database. But we haven't had that much success in finding biomarkers, not only for prediction, for prognosis, for predicting the result of the treatment. This isn't the biggest obstacle right now, because for sure we have a huge amount of data.

Tony Letai: That’s a different issue. The DepMap has generated a lot of interesting, useful information. However, it's all cell lines, and cell lines are weirdos. Any cell line like the cancer cell line encyclopedias that people use for these sorts of things is a very weird cell that has been selected by many generations of culture to divide often – on the rate of once per day. They grow in the presence of 20% oxygen, instead of 1% oxygen, and in the setting of very high glucose. There's no end of different metabolic and proliferation abnormalities in it, just as in comparison, cancer cells in our body don't divide once a day, even in relatively aggressive tumors. That would be extremely rare. They're more on the order of once per month.

Studying in cells that have such different characteristics is part of the problem. We're trying to draw lessons from zebras about horses. Their cell lines are very different. That's why in my functional precision medicine community, we're not trying to generate information from cell lines. We're trying to generate information directly from patient tissue immediately, so that we're not setting up models. We're just using the patient tissue. It shouldn't have that weakness.

“Functional Precision Testing” (Tony Letai, MD, PhD) [#11]

Anonymous Caregiver: You're speaking my language. I should have mentioned earlier when I left the software industry to move into healthcare, I did my training at Cancer Support Community. So I've been listening to prostate cancer patients for over 10 years, and I can absolutely validate that they feel that “Catch 22” as well.

I have two ideas for you on how to move this forward. One of them is: could you arm patients with a statement that they can bring to their physicians, anybody who's treating them who gives pushback on testing. They know how to not threaten their physicians, ask for what will help them feel like they have another weapon in their battle.

The other idea is from several years ago when Chuck Ryan was at UCSF, and he was one of the leaders of the west coast “Dream Team”. They used that very same language about expecting a lion and seeing a tiger, in terms of the profiles of treated cancer. So I'm wondering if you have access to, or you could get access to, data from the results where they were collecting plenty of biopsies up and down the west coast and trying to look for how cancer changes when it's been treated over time and mutates into things they weren't expecting. I'm not sure. That's downstream, and you're trying to catch the problem upstream, but I wonder if there's a way to reverse engineer from their discoveries to help you with yours.

Tony Letai: Let me take the first part of what you said, and then the second part.
On the first part, it's a great idea to arm patients with those letters. What I see as the main obstacle right now is getting the word out to patients. If you surveyed a hundred patients, I would be surprised if five of them knew about what I'm talking about, the availability of functional precision medicine. We're trying to get the word out, but we're a small, relatively academic society. Preparing a letter is relatively easy. How do we let patients know that we exist?

Anonymous Caregiver: There are many organizations and foundations that serve patients. Obviously they're all going to have their own economic interests, but Brian and I, with our relationship to CPCC (California Prostate Cancer Coalition) and NASPCC (National Association of State Prostate Cancer Coalitions), and their mission of patient education, is one way to distribute. I have connections in The Cancer Support Community. These are conversations that Brian and I continue to have offline. My thought is those of us with direct inroads can do that at a grassroots level and hopefully higher.

Tony Letai: I would very much appreciate any help you could give us. I, or some other member of my board, would be very happy to get the message out.

Anonymous Caregiver: The problem maybe needs to be solved at two different levels. There's the idea of, do you use an institution to spread the message, or do you try to reach patients from the ground up? For instance, I wouldn't distribute this through UCSF, but we could do it through other affiliations, which are patients coming together for support.

Tony Letai: I completely agree that it wouldn't work through academic institutions. There are a lot of reasons, but part of it is that the people who would be doing this sort of work have

commercial interests, and academic centers don't want to advertise commercial interests. They may see these sorts of assays as directly competitive with other things that they're doing. Patient advocacy groups and patients themselves are great candidates for getting the message out.

This is something that we have been trying to focus on more in the past year, and why I'm actually on this call with you guys is to do a better job of that, to learn how to do it here.

Anonymous Caregiver: Do you have thoughts on how to harvest learning from the west coast Dream Team project?

Tony Letai: I can't tell you about that project specifically. The only way that we'd be able to get information from them would be if they had viably stored patient samples. I would bet you that they don't. No one seems to be forward thinking enough to do that right now, because they're not aware enough of us. If they were, then it would be a simple thing. There'd be academics like me waiting in line to answer those sorts of questions.

Anonymous Caregiver: Fortunately that project was funded by the Prostate Cancer Foundation. Brian, maybe that's an avenue to explore with them.

Brian McCloskey: Tony, have you ever connected with anybody from the Prostate Cancer Foundation?

Tony Letai: I don’t think so.

Brian McCloskey: That could be an opportunity that we could help with.
The question I had going back to the process is, as Brad mentioned, I do have three metastatic tumors in my peritoneum, and I've had one scientist physician recommend not to “poke the bear”. What are your thoughts on that barrier? Because I'd have to “poke the bear” to take advantage of your assays, right?

Tony Letai: By “poke the bear”, you mean sticking a needle in the tumor? If a tumor is completely dormant and not causing you any problems, It might not be of any immediate clinical benefit to do that. There's a wide range of how prostate cancer can behave, and some of it is relatively indolent. Maybe you would want to wait until it's a problem. I'm more affiliated with lymphomas, and there are lymphomas that are very indolent, and you just leave them alone. However, the idea that by poking a tumor, you're going to make it grow? I don't know that there's a lot of support for that. If there were a case where someone did need treatment and was missing information to guide that treatment, that “poke the bear” concept wouldn't stop me from doing a biopsy.

Glenn Sabin (via the chat): Based on where we are with dynamic BH3 profiling, can we move this to more use in patient treatment decisions, as opposed to drug discovery research?

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Tony Letai: There are companies that right now are doing this work. It is just a matter of contacting the company, providing the biopsy, paying the money, and having it done. There are also academic laboratories like mine and several others, members of the Society for Functional Precision Medicine around this country, Europe, and other places, that are very happy and ready to do this if you’re nearby. I urge my community to accumulate your data and publish it. Some companies are slow to publish. They're hiding their secrets, or I don't know what it is, but some companies are a little slow to publish. I try to convince everyone that a rising tide is going to raise all ships, and there is plenty of work for everybody to do in this. I don't have a great fix other than just volume. We just need to do more of exactly what we're doing. I'm confident that we're going to show benefits to patients. The more we do it, the more we demonstrate benefit.

Brad Power: As an example, when you and I spoke, there is Robert Nagourney at the Nagourney Cancer Institute in Long Beach, and you said you wished he published more about his results.

Tony Letai: I do. He is public about his overall ideas about this. What's less public is the specific assays that they use and the success of those assays. He's been at it for longer than I have. He's one of the guys who was doing it back in the 90s, if I recall correctly. I would love to see him take his 30 years of practice and publish it and show what he's done. Hopefully he would show some very positive results that would help his company, but also help the field as a whole. It's very hard to know what he is doing, and how successful it is. That's what I try to push everyone who's working in this to do.

Brad Power: Laura Kleiman is with Reboot Rx, which is trying to repurpose drugs, and this would seem to be a good way to take some tissue and then try some drug options that come out of their engine. Laura, what do you think about what Tony might offer as an added step once you've identified some potential off-label uses or other uses of approved drugs?

Laura Kleiman: Yes, I am definitely very excited about pursuing this opportunity. I've been following your work, Tony, for a long time since I was in Peter Sorger's lab (Harvard Medical School Systems Pharmacology) many years ago. I am excited to see the progress you've made.

Tony Letai: I love Peter. It probably means we were sitting in the same room a lot back when they had those meetings in the Warren Alpert Building. He's been a great help. He's given us our space in his Laboratory of Systems Pharmacology, where we have a little section that we call the Laboratory of Functional Precision Medicine. He's never had any trouble seeing the benefit of what we're doing. He's very far seeing in that respect.
There are three aspects of repurposing and functional testing that are worth considering:

- Patient-by-patient, every now and then you'll get lucky and find a place to use your HDAC inhibitor, or whatever.
- From functional assays, you may discover a very broad vulnerability across an entire tumor. Let's say it's prostate or mesothelioma or whatever, that was previously unrecognized because it bears no genetic hallmark. I'll give you an example of exactly that using this process, venetoclax in AML (acute myeloid leukemia) or in CLL (chronic

lymphocytic leukemia). There are no mutations in BCL2. That's the target of venetoclax in CLL or AML. Venetoclax is nearly universally active in both of those diseases. It rapidly went from testing a hypothesis to FDA approval. Now there are several FDA approvals in those diseases. What was the hypothesis that was tested? It was my lab, and a couple others like me, who did purely functional studies showing that these tumors were addicted to BCL2. We did it by doing BH3 profiling. That was what got the AML program started. There was no genetic information. That was repurposing a drug using purely functional information all the way to an FDA approval. That's identifying something out of the blue that's brand new.

- You could actually make this commercially very viable if a company has a biologically active drug. They go into their phase two trial, and they have a real response rate of, say, 20% or 25%. That's great for that 20 or 25% of the patients, but that is often the kind of response rate that Wall Street doesn't get that interested in. People will call that a failed drug, but what makes it a huge success, and takes an asset worth $5 million to one worth $1 billion is a predictive biomarker that turns that 20% response rate to an 80% response rate. And that's what a good companion diagnostic does. These functional assays have tremendous promise in doing that. That's going to happen in the next few years where some drug company is going to realize the value of these, and they'll go through the logistic problems because it's the huge leverage at taking a drug from an unapproved status to an approved status using a companion diagnostic.

There's ample opportunity in those three methods of repurposing a drug.

Brad Power: Ally Perlina of CureMatch has advocated treating patients with a combination of drugs that better fit the patient’s biomarker profile, rather than a single drug. We've been discussing the challenges of getting drug combinations accepted when there may not be a clinical trial that will have tried those drugs in combination before. They may be off label. It would seem that your approach would give patients and physicians confidence to try a novel combination that hasn't been proven with a clinical trial.

Ally Perlina: We have some patients who once in a while have fortunate connections where a clinical academic center lab can test the tissue against certain drugs. Although that's not standard practice, any kind of assay that could be offered more routinely would definitely help to see if some of the top matched candidates, whether combination or monotherapies, can be tested. Unless it takes a lot of extra time, and time is of the essence, it should definitely be offered and practiced more.

We score the match of any drug combination to the molecular profile of the patient, and that includes chemotherapies, immunotherapies, and all kinds of targeted therapy. We match it for not just NGS data, but also markers deemed clinically significant at an RNA or protein level. We then come up with a two-drug therapy or a three-drug therapy. Then we want to know experimental evidence to show that it is efficacious against this patient's cancer. What some of the people have done, and this is really rare, actually I just know of one case, and, Brad, you know who I'm talking about, is to get mouse models. That takes a lot of time.

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There's also another company that does fly models but with some restrictions on the cancer types and the types of drugs, and then there are organoids. I have seen organoids being done through various collaborations on clinical patient cases some years ago. It's not a common practice. It also takes time, maybe not as much as getting a whole mouse colony.

This would be great because it doesn't take a lot more time to do these experiments. If there's a way that you can route certain types of patients, it would be wonderful.

I just wanted to double check what kind of therapies, or if there are any restrictions on the types of candidates that you'd run the assay for? For instance, if it's more about the synthetic lethality approach, which we recognize is really important, then maybe it's a bit closer to saying if somebody's going to be sensitive to chemotherapy, or assessing apoptosis levels of DNA damage in the cell already sensitizing to chemotherapy in combination with anything else. Would you say that this can be a way to test any type of combination, say a combination of two targeted drugs, and there's no chemo in the mix?

Tony Letai: You can test anything you can pipette into a well. That can be single agents or combination agents.

It's an open question, in designing novel combinations, whether you want to use purely single agents, given that you're usually limited by the amount of tumor provided and how many wells you have to learn from. You can use up all those wells in just single agent testing. Alternatively, you can test fewer drugs, but do them in two-by-two combinatorials, or something like that. You could do like 20 drugs in all twofold combinations, or you could test like 400 drugs as single agents.

It's funny: it comes back to work coming out of Peter Sorger, that if you look at the successful combinations that are out there, the ones that have stood the test of time, they very rarely depend upon synergistic interaction between the different drugs. Instead, they depend on tolerability and targeting different targets and having different mechanisms of resistance so that it's very hard to be resistant to all three drugs or all four drugs at once. And that seems to have evolved as more significant than actual mechanistic synergy. All of those sound like good things.
If you think this is a good idea, one of the things that you and I might want to do is have you refer patients. I can give you information on where you could refer patients in a more standardized way, rather than the “Hail Mary” pass, set up a mouse model or fly model, or something like that. Have them arrange ahead of time where their tissue would be sent to one of these places.

Has your company ever considered more explicit partnering with these other functional precision medicine companies?

It would be mutually beneficial. I don't own one, so I'm impartial in this. I'm just trying to get the ball rolling.

Ally Perlina: That sounds like a great idea. We should try.
On the synergistic effects of drug combinations, we’re saying the same thing. It's not that the multiple drugs would work better because they have synergies mechanistically, like one potentiates the other, it's just that they independently target the heterogeneous subpopulations of cancer in a given case in such a way that together they produce better effects. It's not because one makes the other more potent mechanistically. It's really just that you attack as many birds as you can with as few stones as you can.