“Proteomics and Clinical Decisions” (Panel Discussion) [#19]

Brian McCloskey and Brad Power
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“Genes don't reliably predict response to treatment, and the gene mutations or expression in cells don't reliably predict the expression of proteins in our cancer cells… If we ever want to achieve precision oncology, we must figure out both on the science side and on the institutional side how to embrace diagnostics of many proteins, coupled with some genes or gene expression, and patient data.” Amanda Paulovich

Meeting Summary

A panel of experts in proteomics: Karin Rodland, PhD, (Pacific Northwest National Lab and OHSU), Amanda (“Mandy”) Paulovich, MD, PhD, (Fred Hutch), Kristina Beeler, PhD, (Biognosys, which provides proteomics services to biopharma), and Marlon Ruiz and Michael Förster, PhD, (Olink, which offers a proteomics platform to scientists), discussed the role of proteomics in clinical decisions.

1. ***Where are proteomics as an emerging technology?***

    Amanda Paulovich: Mass spectrometry has proven to be a reliable measure of proteins, which can be combined with genomics to offer clinical insights through laboratory-developed tests. The challenges now are (a) clinical translation from a laboratory environment to a regulatory environment meeting FDA approval, such that pharma will begin to adopt it in its late phase trials, potentially leading to companion diagnostics; (b) increased reimbursement for diagnostics in general, and for mass spectrometry specifically, so that laboratories that provide the service can stay afloat; and (c) increased physician uptake.

    Kristina Beeler: The applications of our proteomics technology when we started a decade ago were in small scale studies answering basic research questions for our biopharma customers. In the last seven or eight years, we've seen a push for the technology to be adopted much later down the drug discovery pipeline. We've seen the adoption of the technology in preclinical settings to understand the mechanism of action in drugs to identify novel drug targets. Now, clients are waiting for the data to make decisions on the next cohort enrollment. This is something that we could never have imagined a few years ago. This is still in a clinical trial setting. Taking that to the next step is still quite a way down the road.

    Karin Rodland: We're at the stage of developing tools to combine genomic, mRNA, and proteomic data to improve the selection of likely therapeutic drugs that you will respond to.

2. ***How are proteomics helping guide treatment decisions - what is distinctive?***

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Michael Forster: Proteomics have the ability to monitor proteins and biology and cancer-related changes in real time. The biomarker information you derive in real time is closer to the therapeutic intervention, enabling you to gear therapies better to specific patients.

3. ***How should proteomics be integrated with other diagnostic tests to guide clinical decisions?***

Karin Rodland: If I was doing an N-of-1 research experiment on Brian, I would take his RNA-seq data and the biological processes that had been implicated, and I would use Mandy's targeted proteomic assays, and I would verify which kinases in that pathway are actually upregulated and driving the abnormal behavior in the pathway.

4. ***What's next?***

Kristina Beeler: Complex biological processes are characterized not by just one level of -omics. They need the multi-omics level and current status. Proteins provide essential functional information to understand the true phenotype.

Karin Rodland: Looking at Brian as a research project, how do we make proteomics available to him as a research subject? He has to find a physician and a precision oncology clinical trial to look at all these different molecular measurements and integrate them and get enrolled. Then we will need to address the question that Mandy is trying to focus on: “how do we turn that into the standard of care?”

Amanda Paulovich: You really need partners in large academic centers that are willing to go out on a limb, and centers that are legally adventurous enough and willing to go down that path with you. Unfortunately, it's just hard to do. As Karin said, you're pushing the envelope beyond what standard of care is.
We need better diagnostics. 80% of healthcare decisions are based on diagnostic tests, but they account for less than 20% of healthcare costs. Somehow, diagnostics must be valued in the same way that blockbuster drugs are, and they're way way undervalued right now.

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Meeting Notes

SUMMARY KEYWORDS

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SPEAKERS

Karin Rodland, Amanda (Mandy) Paulovich, Kristina Beeler, Saed Sayad, Michael Förster, Marlon Ruiz, Brian McCloskey, Sheeno Thyparambil, Brad Power

Brad Power 00:03

This is the Prostate Cancer Lab where we're learning about novel tests that can help personalize cancer treatment decisions. Today we have a panel, which is the first time we've done this. I want to first introduce Karin Rodland, who introduced me to a number of the panel members, so she's really the network organizer and helped to bring the panel together today. I'll let each of the panelists introduce themselves. Please provide your personal background, your organization and its role, and the topic you will cover including where you are in the evolution of this emerging technology. Karin, why don't you start, please, and then we'll move to Mandy, Kristina, and then Michael and Marlon.

Karin Rodland 01:17

I am a cancer cell biologist who has been studying signal transduction in cancer for close to 50 years. I use mass spectrometry and mass spec-based proteomics very heavily. I am a joint appointee at Pacific Northwest National Lab, which is a major site of technology development in the field of mass spectrometry. I also have a joint appointment in Oregon Health Sciences University, which is a leader in precision oncology and SMART trials. Also, I do know a lot of people, and I tried to get them involved.

Brad Power 01:54

Karin was featured in a previous session we had for which we have a recording, a transcript, and her slides.

Amanda Paulovich 02:20

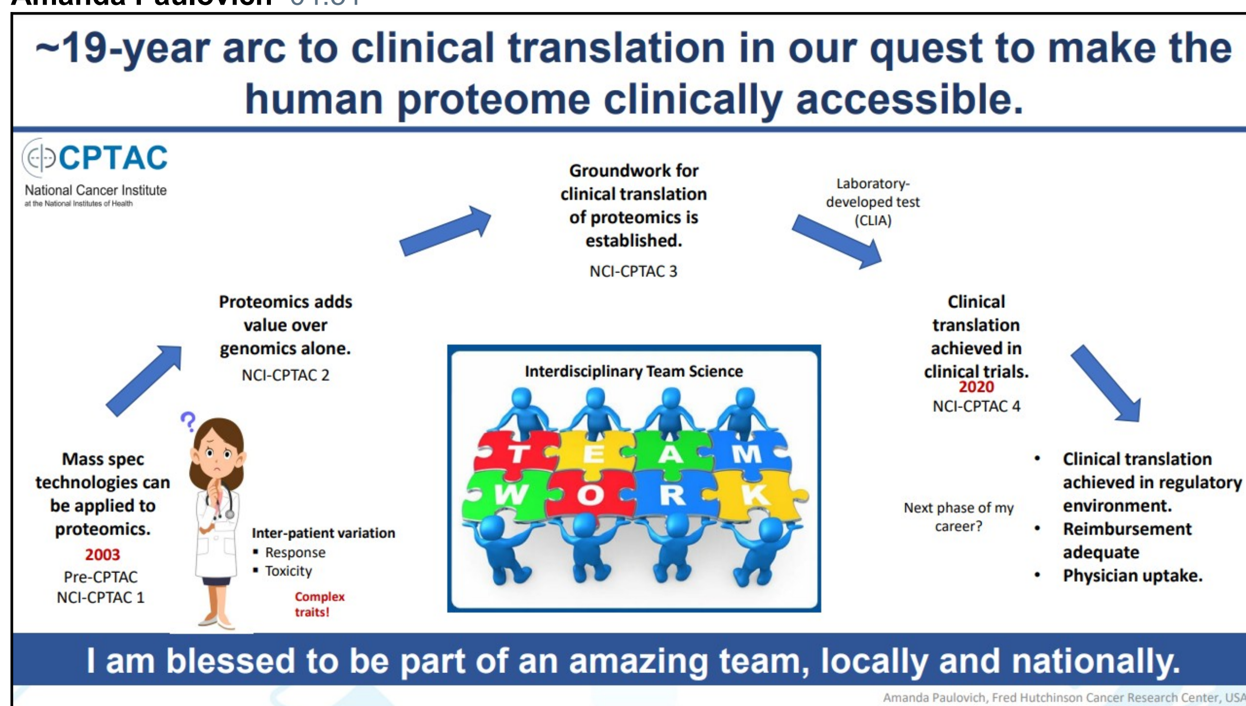
I'm from the Fred Hutch Cancer Center. I'm a clinically trained medical oncologist with a PhD in genetics. Unfortunately, I've seen this disease of cancer from many sides. I've been a cancer patient, having been diagnosed with early-stage breast cancer at the age of 40, which I guess technically makes me a cancer survivor. I'm now a cancer researcher and a clinical oncologist. As an oncologist treating patients, I was very struck by the variability that I saw from patient to patient. I saw patients with what looked like the same tumor would have very different responses to the same therapy, and very different profiles in terms of side effects as well. That led me to yearn for what we now call personalized, or precision oncology, which is what we're trying to achieve. I did a postdoc at one of the major genome centers that completed the human

genome sequence. That was a very critical start on our path to precision oncology, but it's not enough. Genes don't reliably predict response to treatment, and the gene mutations or expression in cells don't reliably predict the expression of proteins in our cancer cells. Since proteins are the targets of therapies, we have to be able to quantify proteins. When I got into this field 19 years ago, and still today, most studies on proteins use conventional technologies that are 50 years old and wholly inadequate to meet the needs of the post genomic community. In fact, there aren't good assays for measuring most human proteins in a clinical setting. This very critical human proteome, as it's called, remains clinically inaccessible. In 2003, I joined Fred Hutch to set up a translational proteomic lab to try and leverage new technologies to solve this gap based on mass spec.

Brad Power 04:33

I know you've been associated with something you've developed in your lab that's become commercially available recently. So I'd like to hear about that as well, please.

Amanda Paulovich 04:51



This is a slide I often use at the end of talks that I give. This is the arc of progress, and Karin Rodland has been here through most of this. We've been colleagues in the National Cancer Institute's clinical proteomic consortium called CPTAC. As I mentioned, in 2003 I came to Fred Hutch. A couple of years later, we launched this NCI CPTAC program and spent five years and probably $30 million convincing the world that these conventional technologies that are 50 years old aren't the only way to measure proteins. We can use newer technology based on mass spec to make reliable measures of proteins. That got us another five years of the program at NCI where we spent five years and another $30+ million proving, in my opinion, common sense, that measuring proteins adds value over just sequencing DNA. That finally proved beyond a shadow of a doubt, even to the genomics-centric folks at the National Cancer Institute who are now on board, that there's value in combining proteomics and genomics - what we now call proteogenomics. Then we spent the last five years and another $40 million, laying the

groundwork for the clinical translation of mass spec-based proteomics. There's a lot of proteogenomic characterization centers looking at the gamut of human tumors trying to characterize them on a molecular level using mass spec-based technologies. For the first time, we also introduced translational research centers to these technologies whose goals are to try and take them and push them into the clinic. In that phase, we saw the development of laboratory-developed tests. In fact, my laboratory is now a CLIA-certified environment, and we have successfully translated this new mass spec-based measurement technology into clinical trials where we run patient samples for correlative studies in our CLIA environment. That's where we're really stuck right now. In my opinion, this is where the field needs to go. We need to achieve clinical translation not just in a CLIA environment, but in a regulatory environment meeting FDA approval, and we need precedent for that before this technology is de-risked enough that pharma will begin to adopt it in its late phase trials, potentially leading to companion diagnostics. Reimbursement for diagnostics in general, and for mass spec specifically, is woefully inadequate. It's very difficult for laboratories to provide the service and stay afloat. Also new technologies always meet the challenge of physician uptake. These are areas that I'm looking strongly at in the next phase of my career - how can we now usher these things a little bit further forward? Brad, you mentioned commercialization as well. My lab, under this NCI program, has developed a large catalog of assays that depend on a valuable and expensive-to-make reagent called a monoclonal antibody. We make all of that publicly available through open-source portals that the National Cancer Institute maintains, and that's for non-commercial research use. Expert labs can run any of those assays that they want. But to fully democratize access to this new technology, we need to make it available also to laboratories and research groups that don't inherently have those technologies in house. It's expensive and complex to use mass spectrometry so we're trying to solve that problem. We recently announced a partnership with CellCarta, which is a global contract research organization based in Canada that does correlative studies for clinical trials where all our assays will be made publicly available. They've licensed Fred Hutch's rights to all those assays to make them available to the community as well to try and get this out there. We really need to take up these new technologies. A critical reason is cancer phenotypes and predicting drug response are far more complicated than a single gene mutation or the measurement of a single protein, which is what we typically do now. If we ever want to achieve precision oncology, we must figure out both on the science side and on the institutional side how to embrace multi-analyte diagnostics that have many proteins, may be coupled with some genes or gene expression, and patient data. This information is going to go into algorithms that are going to output scores that put patients into categories for treatment selection. But our institutions are not set up to deal with any of that. We now have lots of institutional barriers that must be broken down. We need new mass spec technology to enable that part of proteins because the old ones just usually measure one protein at a time.

Brad Power 10:05

You set everything up with the arc with everything we hope to touch on and dive into in more detail.

Kristina Beeler 10:20

I'm Chief Business Officer at Biognosys. My key responsibility is to transform our technological leadership in mass spectrometry into insightful solutions for our biopharma partners. I lead a global commercial team across Europe in the US. I have a scientific background, including a PhD in immunology and oncology. I have been fortunate to see the rise in the use of proteomics and its entry into the clinical space of this technology, especially in the last several years.

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I'll give you a little bit more information about Biognosys as an institution. We have been around for 14 years pioneering the field of mass spectrometry. We are co-inventors of a technology called DA, data independent acquisition mass spectrometry, which has really revolutionized the field of mass spec proteomics. It has become the method of choice for large scale biomarker studies. We have developed a set of proprietary platforms that serve our partners from early R&D/early discovery to the late clinical trial application. We are active across this research pipeline. We have more than 100 customers across the globe that trust our services and products. We are headquartered in Zurich, Switzerland and have recently opened an office in Cambridge, Massachusetts, and are expanding our US footprint.

Brad Power 12:25

You said most of your customers are biopharma. How do you see the insights that you're developing - either the identification of biomarkers or maybe predicting drug response/resistance - how do those translate into clinical use and how does that translation work?

Kristina Beeler 13:08

If we look at the history of the commercial activity of Biognosys, in the early days, we were mostly working with the early adopters of the technology because DA was a novel technology. People had their questions about the approach. The early adopters of the technology were technology-savvy people who were excited about our technologies. The application was in an early R&D setting - basic research, small scale studies answering basic research questions. In the last seven or eight years, we've seen a push from the market for the technology to be adopted much later down the drug discovery pipeline. We've seen the adoption of technology in preclinical settings to understand the mechanism of action in drugs to identify novel drug targets. But also, most recently, we have been applying that technology in clinical trials as pharmacodynamic markers. Now, clients have been waiting for the data to make decisions on the next cohort enrollment. This is something that we could never have imagined a few years ago that our technology would be used for subsequent decision making. This is still a clinical trial setting. Taking that to the next step is still quite a way down the road.

Marlon Ruiz 14:44

I've worked in proteins my entire life. I strongly believe that proteins are where we find a lot of the answers that we're looking for, just like Mandy was saying and the rest of you have said. Olink is a platform provider for proteomics. It's an immunoassay-based technology that utilizes the power of antibodies but also the power of next gen sequencing. Our antibodies are bound to oligos that are complementary. It's two of them that bind to a protein, and they hybridize, are elongated, and amplified via either qPCR or a next gen sequencing that enables us to do up to 3000 proteins in one sample - a very small amount of sample. It's something that's been very complementary to a lot of mass spec methods. It's a very exciting spot to be in.

Brad Power 16:03

A recurring issue on this forum is what we call "the issue of tissue." What is the source material? Is it blood? Is it fresh frozen tissue or FFPE?

Marlon Ruiz 16:23

Generally speaking, we validated our platform on plasma and serum; however, it has been optimized for many different sample types including tissues, cell lysates, supernatants, CSF, aqueous humor and ocular fluids and urine. We've had pretty much all sample matrices on our platform. Although they aren't validated entirely, they are optimized and have shown to work really well.

Michael Förster 16:56

My scientific background is in proteomics. I did a PhD in protein genomics at Karolinska Institute, working on circulating factors and how genes, or polymorphisms, control cytokines in circulation. I took the long way round and did a postdoc but thought complex diseases are too much, so I decided to do something simpler like immunodeficiencies. That's how I stumbled upon cancer because I worked on a gene that was also a susceptibility factor for hemato oncology malignancies. After that, I exited the academic world and went into liquid biopsies. I've worked a little bit on liquid biopsies in prostate cancer for prostate cancer diagnosis. Later, I got involved in genomics diagnosis for all kinds of cancers. More recently, I joined Olink where I oversee most scientific studies that involve targeted oncology, different solid cancers, hematological cancers as well, and oversee the implementation of proteomics. I’m always asking "what can proteomics add to all the questions that genomics cannot answer?" That's my mandate in a nutshell and that's why I'm here today.

Brad Power 18:43

We're going to have Peter Kuhn and his colleague, Diane's Shishido from USC, speak to the PCL community. Peter was a founder of Epic Sciences. We're going to approach this in a future session from the perspective of liquid biopsies. Michael, if you can comment on liquid biopsies that would be appreciated. We've had previous sessions discussing spatial, single cell, proteomics, and liquid biopsies. It seems like they're all the same topic, or maybe it's all integrated. Could you speak to that? There's a lot of research and energy around liquid biopsies because of all the advantages they provide. Can you speak to that and how it intersects with proteomics?

Michael Förster 19:38

Where we see capture proteomics is really based upon our liquid biopsy library which is geared towards serum and plasma.

[“Capture proteomics” are assays that use a capture reagent against preselected proteins to measure them. The capture reagent may be an antibody (for ordinary immunoassays like ELISAs, ELIspots, etc.) or dual capture assays (like Olink’s PEA). You could equally well use aptamers. Mass spectrometry researchers also take advantage of that and sometimes combine antibody capture with a mass spec readout. The main point being that all these assays are not hypothesis-free approaches, as you always use a capture reagent for your target protein.]

Previously, genomics looked at something that's happened in the past. For example, there may have been a cell that underwent oncological transformation because of mutation. The idea was to pick up these mutations and then make predictions for therapy in the future. Where we see proteomics going is more real time in terms of having the ability to monitor proteins and biology in real time. You can monitor cancer-related changes in real time. The biomarker information you derive in real time is closer to the therapeutic intervention. You can gear therapies better to specific patients. If you use capture proteomics or mass spec, we believe both technologies have their advantages and disadvantages and should be seen as complementary. When it

comes to clinical implementation or regulatory approval, people will be more familiar with immunoassays. But that's still to be seen. That's how we see what proteomics and capture proteomics can add.

Brad Power 21:17

I have a background in software. Brian does as well. The old software development methodology was called “waterfall”, which is the Big Bang Theory of software development. You design, then code, then build, then you test and then you implement in big releases over years. The modern software development approach is “agile”. With agile, you do weekly or two-week sprints. You're constantly putting more code and more software out in the marketplace and getting feedback from the market. It seems like that's the same thing you're pointing at with proteomics. For example, genomics is hereditary and that's stable over time. Proteomics is more volatile. It's at the frontline. But if you can then have a more continuous monitoring of it through something like blood, you're getting the live action versus the blueprint that is somewhere in a warehouse.

Michael Förster 22:26

Absolutely. We always think that proteins are the best of both worlds in terms of how they correlate with what you want to measure. But they don't fluctuate too much so that you don't have too much noise and too little signal. Proteins are at that intersection. It has all the good things of being agile, while at the same time being stable enough to hear the diagnostic test.

Brad Power 23:03

Sheeno, could you please introduce yourself and do a quick comment introducing mProbe?

Sheeno Thyparambil 23:17

I'm the Senior Director of R&D and the Site Head for mProbe. Some of you might know this company by the name Oncoplex Diagnostics which was bought out by Nantomics and now in the hands of mProbe. We are a tissue proteomics company that specifically does laser microdissection of the tumor tissue, and then we analyze for 72 different protein biomarkers in a CLIA cap environment. We've been CLIA certified since 2013, and we also have a New York State Department of Health license as well. In fact, an inspection is going on today for recertification. Over the years, we've been fortunate enough to be part of the journey for many cancer patients. We've done analyses on thousands of samples across different cancer types. In some instances, we get to see those outlier biologies and then we communicated with the oncologist who acted on it. The patient has gone from hospice back to the hospital setting. We have seen clinical proteomics in action. Those are the moments that we live for. My training has been in mass spectrometry. I did my PhD under the NCTR FDA with Dr. Edmondson and group, and then my postdoctoral fellowship was in multiple myeloma. I joined this company in 2009, when it was focused on expression pathology. Over the years, we've worked with some of the key opinion leaders in oncology. We've been able to do a lot of retrospective clinical trial analysis and demonstrate the power of mass spectrometry in predicting clinical outcomes. And this is all coming from FFPE tissue proteomics.

Saed Sayad 25:56

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My main question is related to the reproducibility of these technologies. This is the number one issue with all the datasets we are analyzing. We've analyzed more than 500 omics data and the biggest issue with it, compared to the other omics data, is that protein data is the least reproducible.

Karin Rodland 26:46

It depends upon what level of reproducibility you're talking about. If you're talking about technical reproducibility, do I get the same proteins from triplicate aliquots of the same sample? Mass spectrometry is as good as RNAseq or any other analysis technique. If you are talking about biological replicability, if I take three samples of the same cell line grown in three different dishes and measure them by proteomics, again, it's comparable to RNAseq. But because there may be some threshold issues with mass spectrometry, and some variability at the low-end sensitivity range, maybe it's not quite as good as some other methods. But really the CVs are very comparable between proteomics and RNAseq. I'll ask Kristina and Mandy to back me up on that. Where mass spectrometry has gotten a bad rap in your world, is that if you look at human studies across populations, and look at DNA mutations, you can replicate DNA mutations across populations, but you don't necessarily replicate biomarkers, things that have been labeled as a biomarker at the protein level. A lot of that has been due to a couple of factors in the learning curve and experimental design for proteomics experiments over the past 20 years. I think all the bad rap that mass spec has gotten has been from the early 2000s when people didn't really know how to design a serum or plasma proteomic experiment and didn't understand the limitations of mass spectrometry in terms of experimental design. If you take a look at current studies, like the CPTAC studies, they have all done a discovery and a confirmatory set where they have looked at proteomics on 100 tumors and then gotten a separate set of 100 new tumors and every single major biological finding has been validated in the confirmatory cohort.

Saed Sayad 29:09

Say I want to build a risk model using -omics data, all of them have a problem of reproducibility. For example, when you have 1000 patients divided into groups who have cancer and don't, and then we find the biomarkers and build a model. Now, we want to use this model for a new data set that is completely different. The new data set cannot be applied on the old model. We cannot just mix them together because in the future we have completely unknown cases which are not normalized. This is the main problem. We cannot use the same tests based on the existing samples for a new set of the patients. That's the core issue we have with this type of data sets.

Karin Rodland 30:14

I do not think that is a true statement anymore. I'm going to find you a preprint that is just out.

Amanda Paulovich 30:32

To back up Karin, I think what you're talking about isn't reproducibility; it's validation of biomarkers and independent patient cohorts. Reproducibility refers to the analytical precision of a platform over replicates, which with standard operating protocols has been extensively shown in proteomics.

Saed Sayad 31:00

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...The key here is based on the polygenic risk model; it means using the small…

Amanda Paulovich 31:04

That's validation. So all -omics problems have that. You know this better than I do. When you have a relatively small N, compared to the dimensionality of your data, you're going to find signatures that look like they work in one patient cohort and many of those don't replicate in another patient cohort. Those searches show false signals because of the low sample size relative to the complexity of the signature. That's a different issue from reproducibility, and that issue is not specific to mass spectrometry. That issue is true for transcriptional profiling data as well.

Sayed Sayad 31:53

What's the value of this? There are 5 million experiments. We don't have even 2 biomarkers out of this amount of data. How can we resolve this issue? Call it validation on the new dataset, that's okay too. Because right now, when you spend $5 million and test on 1000 cases, generate few or no biomarkers, and then validate on the new dataset? It doesn't work. What's the value?

Karin Rodland 32:29

That's no longer true.

Amanda Paulovich 32:43

I don't think we can speak specifically to your specific 1000 cases example. But you're right in the bigger picture sense that there's been a lot of investment in -omics technologies with disappointing output on the other end. I think that's for a variety of reasons, one of which concerns the technologies, as Karin alluded to, and people's knowledge of how to use them has had to mature. What Karin is saying now is that's the case, and I would agree with her. Two is there's a lot of people doing -omic technologies who don't do it very well. They don't have locked-down platforms. They don't have standard operating protocols; the experimental design is woefully inadequate to have the rigor that you need with a sample size of 1000 patients. To find a statistically sound signature that has a chance of validating an independent patient set, you need highly annotated samples, you need high quality biospecimens, and you need a very specifically defined clinical question with a rigorously defined phenotype to have a hope because these are complex phenotypes. These are not simple, single gene, single protein phenotypes. For example, in the last round of CPTAC, we have a 64-protein signature that predicts about 33% to 50% of platinum refractory ovarian cancers upfront at diagnosis. That signature is robust to validation and two independent patient sets. Now we have a multiplex-targeted mass spec assay to try and validate, and we run up against those problems on the end of my slide. How do we get access to the proper samples and break down these barriers? How do you get reimbursed for stuff like that? How do you get uptake, because it's a 64-protein panel, it's not measuring HER2 in breast cancer, one protein, or EGFR mutations in lung cancer, one gene mutation? That's never going to get at the complexity of the biology nor are the sample sizes that we're all stuck using because the funding agencies have woefully underfunded the studies that have the appropriate statistical power.

Saed Sayad 35:03

Do you know any specific experiments in which they followed robust experiment designs and protocols?

Amanda Paulovich 35:16

I know the work that we've done in this last phase of CPTAC has followed those rules. The question is if we can keep the project going with funding in samples to get to the larger retrospective validation in the setting of some of these clinical trials? That comes with its own challenges.

Saed Sayad 35:41

Did you validate the independent dataset?

Amanda Paulovich 35:44

Three datasets. It works across the discovery and 2 validation datasets with a similar AUC?

Saed Sayad 35:51

And what was the outcome of that result? What was the predictable power of that?

Amanda Paulovich 36:01

It picks up with near 100% specificity - between 35% and 50% of the patients with refractory disease.

Saed Sayad 36:09

Sorry for asking technical questions. How about the positive predictive value? Anything on that? Sensitivity by itself is a good one. But what recall precision. For example, Grail has a $1,000 test for cancer. It has 99.5% specificity but 44% positive predictive. 60% of patients that it labels as a positive will be wrong.

Amanda Paulovich 36:52

Yes, we were required that the test be accurate nearly 100% of the time because for this test to be actionable, you're ultimately potentially denying a patient's standard of care. There's no tolerance for inaccurate calls in this test.

Saed Sayad 37:09

Any number for positive predictive value?

Amanda Paulovich 37:24

We're probably getting into the weeds here, but at the threshold that we set for the ROC curves, we've required that there not be false positive results. I'm assuming this is way more in the weeds than you want to go.

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Brad Power 37:47

I'd like to cue Brian. One of the things we find useful is to go to the patient perspective because that's a point of integration. There's a lot that's going on here, but it comes together at the patient. I'm curious what questions would he have?

Brian McCloskey 38:07

I have my RNAseq information. I know which of 20,000 genes were overexpressed relative to a pan cancer cohort of about 12,000 patients and a prostate cancer cohort of about 375. We know that my RNAseq information is a proxy for the protein expression of my cancer. What is the best way for me to go about integrating proteomics into the definition of my cancer? Also, is it going to be materially different from the RNA expression data that I have already?

Karin Rodland 38:59

What question are you asking with the integration? Are you asking the clinician to come up with a personalized therapeutic regimen for yourself? Are you asking for a prognosis? I mean, those are two separate questions. You are asking for personally guided treatment?

Brian McCloskey 39:21

It's not a prognosis. It's a guided treatment. It could be a personalized approach.

Karin Rodland 39:29

If I was doing an N-of-1 research experiment on you, I would take your RNAseq data and the biological processes that had been implicated in the RNAseq data, and I would use Mandy's targeted assays with her company that she's gone to, and I would verify which kinases in that pathway are actually upregulated and driving the abnormal behavior in the pathway. I would try to find the nodal kinase, the driving kinase in the pathway by a phosphorylation substrate analysis. I'm talking about research level stuff, but it would be possible in a clinical trial, and then I would look for small molecule inhibitors of the kinase that I thought from the phosphor proteomic data was driving your disease.

I've been on several of these discussions, looking at you as a research project and I know exactly what to do. How do we get somebody who has the clinical power and the funding to look at you as a worthwhile research project, and do that very labor-intensive, brain-intensive analysis of your data? And then, how do we turn that into the standard of practice for everybody else? Because obviously, the people who subscribe to Brad's program are intellectually privileged, if not economically privileged. You're working hard to access stuff that's beyond the standard of care. So there's two separate parts of the question. How do we deal with you and make it available to you as a research subject? You just have to find a physician, a clinical trial, that is a precision oncology clinical trial to look at all these different molecular measurements and integrate them and get yourself enrolled. Then the question that Mandy is trying to focus on is how do we turn that into standard of care?

Brian McCloskey 41:35

What about heterogeneity? A year ago, I had three lesions. I was recently scanned using CT and a PSMA PET scan. It's possible that two of the three lesions are no longer there, or at least

they're not showing up on the scans. There's something different about my third lesion - there's heterogeneity. How do we account for heterogeneity using proteomics? Do we use tissue, blood, or a combination?

Karin Rodland 42:29

For heterogeneity, you're going to have to look at the tissue level, and that's where the spatial and single cell analysis comes in. Every single tumor and metastasis is going to be heterogeneous. And every single treatment regimen is going to exert a selective pressure, and we don't understand that process well enough. Mandy, please kick in - you're the MD here. That's the reason why we have not yet cured cancer when it becomes a metastatic disease due to the evolutionary process in each metastasis. We'd have to sample each met. We don't understand the evolutionary process right now. We have a tendency in clinical trials to give drugs serially because we don't know enough about the combination effects when you use drugs simultaneously. I have seen people argue that the current sledgehammer approach we use to chemotherapy to try to kill all the sensitive cells simply provides a desert in which the weeds can grow, and is actually promoting the outgrowth of the resistant cells. Maybe 10 years from now, we may discover we're doing everything wrong. I don't know. I honestly don't know.

Amanda Paulovich 43:44

I agree with most of what Karin said. We don't generally offer treatment if we don't have data that it improves outcomes. There may be better ways to do it, but it's not like we're making people worse in general. This is the issue, right? Brian, you are kind of at the cutting-edge and asking questions that most patients don't ask and that researchers are still grappling with. But it's a real and imminent issue for you. Karin's right. You're stuck advocating for yourself and finding allies on this journey that are willing to go down this path with you outside of standard of care. It becomes a bit of a research endeavor. I don't know if you've seen it, but there's an inspirational story I was just looking for online. It's about how a patient's wife saved his life. He had a life-threatening bacterial infection, and it was resistant to all antibiotics. His organs were shutting down and she read online about a bacteriophage, a virus that could kill these bacteria. She had some high-ranking academic position in the UC system, and was a well-connected, very smart healthcare consumer. She was able to track down partners such as physicians and researchers and rally them around this cause to find a phage that would kill this bacterium that her husband was imminently dying from and was successful. You're kind of in that space, right? You want the next generation of what's going to be offered in medicine. But that, as Karin said, hasn't become the standard of care yet. You really need partners in large academic centers that are willing to go out on a limb, and centers that are legally adventurous enough and willing to go down that path with you. Unfortunately, it's just hard to do. As Karin said, you're pushing the envelope beyond what standard of care is. And I would agree with most of the other stuff that Karin said.

Brian McCloskey 46:14

I'm just going to share with you a little bit about my story. I did RNAseq analysis with Tempus and our other partner, Rick Stanton. Through that analysis, I learned that I over-expressed a few genes relative to these other cohorts. One of them happens to be B7-H3, or CD 276. I had a conversation with my oncologist last September and said, "I found this, and it looks interesting." She was a little bit dismissive about it at first. When I came back three months later, I didn't

bring it up. but she said, "I want to thank you for bringing that data to me. It turns out there actually is an ADC from Daiichi that targets B7-H3." I said, "I know!" :) So now I have 21 different treatment options that have been provided by this amazing, crowdsourced community that we have here. We've whittled that down to eight. This ADC is in the top three, if not number one, right now. Retrospectively, I can apply lessons learned to my future efforts. I've had my DNA sequenced, which wasn't terribly compelling in terms of identifying treatments. It was somewhat helpful in identifying pembrolizumab as a treatment, but DNA just wasn't super helpful. With RNAseq, we found B7-H3 and a corresponding treatment, an ADC. For me, the next step in this journey is proteomics. If I get into proteomics, what are the treatments that we can identify, and how much better would they be than an ADC that targets B7-H3? That's my story. I'm sharing with you in preparation for conversations that I'm going to have with the Prostate Cancer Foundation, and my oncologists obviously, and others. Is that a compelling story?

Karin Rodland 48:49

One of the things that the CPTAC program and others are really working on is integrating the data and using it to make a better predictor. My PTRC (Proteogenomic Translational Research Center) was devoted to acute myeloid leukemia.

“Proteomics and Clinical Decisions” (Panel Discussion) [#19]

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Clinical Proteomics

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Proteomic and phosphoproteomic measurements enhance ability to predict ex vivo drug response in AML

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Abstract

Acute Myeloid Leukemia (AML) affects 20,000 patients in the US annually with a five-year survival rate of approximately 25%. One reason for the low survival rate is the high prevalence of clonal evolution that gives rise to heterogeneous sub-populations of leukemic cells with diverse mutation spectra, which eventually leads to disease relapse. This genetic heterogeneity drives the activation of complex signaling pathways that is reflected at the protein level. This diversity makes it difficult to treat AML with targeted therapy, requiring custom patient treatment protocols tailored to each individual's leukemia. Toward this end, the Beat AML research program prospectively collected genomic and transcriptomic data from over 1000 AML patients and carried out ex vivo drug sensitivity assays to identify genomic signatures that could predict patient-specific drug responses. However, there are inherent weaknesses in using only genetic and transcriptomic measurements as surrogates of drug response, particularly the absence of direct information about phosphorylation-mediated signal transduction. As a member of the Clinical Proteomic Tumor Analysis Consortium, we have extended the molecular characterization of this cohort by collecting proteomic and phosphoproteomic measurements from a subset of these patient samples (38 in total) to evaluate the hypothesis that proteomic signatures can improve the ability to predict response to 26 drugs in AML ex vivo samples. In this work we describe our systematic, multi-omic approach to evaluate proteomic signatures of drug response and compare protein levels to other markers of drug response such as mutational patterns. We explore the nuances of this approach using two drugs that target key pathways activated in AML: quizartinib (FLT3) and trametinib (Ras/MEK), and show how patient-derived signatures can be interpreted biologically and validated in cell lines. In conclusion, this pilot study demonstrates strong promise for proteomics-based patient stratification to assess drug sensitivity in AML.

Background

Acute myeloid leukemia (AML) is characterized by the incomplete maturation of myeloblasts and their expansion in blood and bone marrow, which impacts healthy blood cell formation resulting in decreased numbers of granulocytes, platelets, and red blood cells [1]. Though the number of FDA-approved treatments for AML has increased significantly over the past five years, prognosis remains poor with a 5-year survival rate of 25% for individuals over the age of 20 [2]. Targeted agents have shown promise in mutationally defined subsets of patients, but due to the genetic evolution of this highly heterogenous disease, drug response is often lost and patients relapse. Proper selection of personalized drugs and drug

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"Proteomics and Clinical Decisions" (Panel Discussion) [#19]

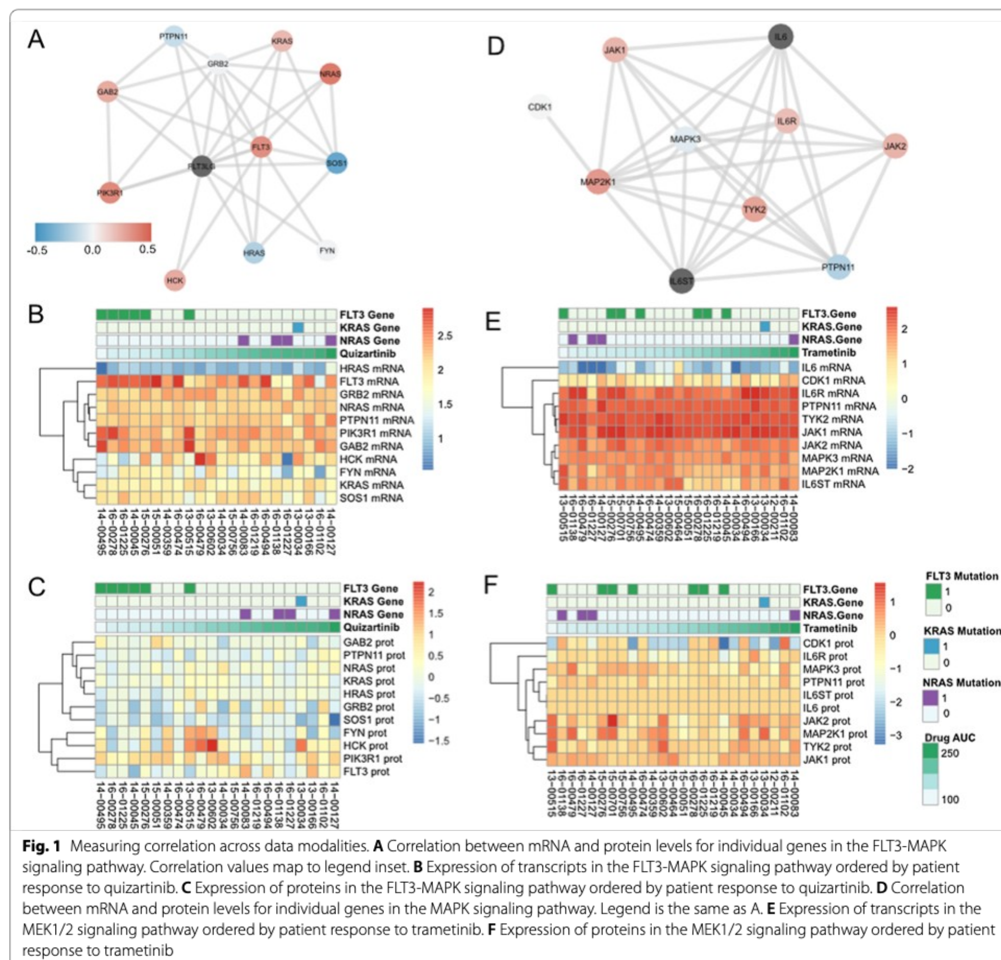

Fig. 1 Measuring correlation across data modalities. **A** Correlation between mRNA and protein levels for individual genes in the FLT3-MAPK signaling pathway. Correlation values map to legend inset. **B** Expression of transcripts in the FLT3-MAPK signaling pathway ordered by patient response to quizartinib. **C** Expression of proteins in the FLT3-MAPK signaling pathway ordered by patient response to quizartinib. **D** Correlation between mRNA and protein levels for individual genes in the MAPK signaling pathway. Legend is the same as A. **E** Expression of transcripts in the MEK1/2 signaling pathway ordered by patient response to trametinib. **F** Expression of proteins in the MEK1/2 signaling pathway ordered by patient response to trametinib

correlated (R = − 0.22, R = − 0.11 respectively). We then compared transcript (Fig. 1B) and protein (Fig. 1C) levels with the AUC for quizartinib by plotting a heatmap of the molecular values ranked by drug response. Lastly, we evaluated the phosphosites identified in our untargeted phosphoproteomics on the 12 proteins in the FLT3 signling pathway in Fig. 1A, also depicted in Additional file 2: Figure S3A. While we were unable to detect any phosphorylation sites on FLT3 itself (most likely due to the undersampling of pTyr in our workflow) we were able to characterize many alterations downstream. However, in some cases, the phosphoproteomic data will correlate with global protein levels (e.g. HCK protein expression correlated with phosphosite occupancy, with a R = 0.62, shown in Additional file 2: Figure S3B). These results suggest that protein abundance can sometimes be an effective surrogate for protein phosphorylation. The results also suggest that focusing on a single specific pathway may not be sufficient, as off-target effects of the drug that can effect sensitivity may be missed, such that integration of data could provide more meaningful results.

We expanded our correlative analysis to study the Ras/MEK pathway, which is downstream of Ras and targeted by trametinib. The correlation of the mRNA

Karin Rodland 49:20

This is from a paper that just showed up on Clinical Proteomics today, and I'm touting my own work. This is using three different bioinformatics methods to predict the response of AML cells to about 25 different small molecule inhibitor drugs. The higher the bar is the lower the average error, and therefore the more value there is to that particular type of molecule as a predictor. You can see in this color that proteomics is the best. But, when you combine the mRNA and the proteomics, which is in this goldenrod color, you can see that's better than mRNA alone, which is in the teal color. There's RNA alone, and there's RNA plus protein. The genes here are better than the RNA, but not as good as the protein. We're developing tools, and that's where we are right now. We're at the process of developing tools as to how we combine these types of data to improve the selection of likely therapeutic drugs that you will respond to. The advantage of this PTRC was that AML is a blood cancer. We have the tumor cells in solution. We have them frozen down and DMSO. We can take them out and plate them into 360 well plates, treat them with dose response curves from 100, or more small molecule inhibitors, some of which are FDA approved, some of which are not. This gives us the density and richness of data that you can use AI methods on and get a valid result. One of the limitations of old-fashioned proteomics with western blots is you could only do, (even say RPPA), you could do 200 things at a time. That's not enough for AI to work on. It's just not. And you were doing 20 patients, 50 patients? CPTAC is now going to 200 patients, that's still relatively small for what you need to do mathematically, but at least it's approaching the level where things do validate in an independent cohort.

Marlon Ruiz 52:14

I'd chime in about specifically the power that's required for the studies to be actionable. They've been alluding to a large number of samples, and we piggyback off that. We'll say that is one of the things that's most detrimental to just one signature. We do find that proteins are where the action happens. You're more likely to find something actionable in the protein side than you would anywhere else. Karin's data definitely showed that.

Michael Förster 52:51

Not looking at cancer per se, but looking at population records, we recently had more studies that use large proteogenomic screens to make diagnosis or predictions for common complex diseases. What will come out of it is a study associated with the UK Biobank, which just released their first big protein genomic screen. One of the researchers there specifically asked a question, "what can genetic risk scores versus protein risk scores add for all kinds of common complex diseases?" In 95% of the cases proteins were superior to doing genetic risk scores. We would hope that the same thing holds true for cancers. The question is always, "what is the value in terms of easy access of tissue versus looking at tissue itself?" That's where the rubber meets the road. Where the future of proteomics lies, maybe for cancer, that is absolutely the case. You must look at the primary tissue, and you won't find a lot of additional information in circulation, but that's still yet to be seen for other common complex diseases. That seems to be the case. It's the other way around that liquid biopsy-based tests on proteins have a good chance of predicting, or giving at least prognosis, and hopefully also predicting response to treatment.

Kristina Beeler 54:53

I'm going to recap from my point of view on the key topics that we've discussed today. Complex biological processes are characterized not by just one level of -omics. They need the multi-omics level and current status. All of us here on the panel are convinced that proteins provide functional information, essential information, to understand the true phenotype. But, combining

all this -omics is key. Combining different proteomics technologies is key to understanding complex diseases. What is more important is really the reproducibility of the technology. Having robust technologies performed in a well-controlled environment, workflows that have technical reproducibility, but also well designed studies are required to bring the answers we're looking for. These are all key aspects.

Amanda Paulovich 56:11

What we really need are better diagnostics. What Brian is asking for is a better diagnosis. What As a patient, and as a doctor treating cancer patients, I was asking for better diagnostics. But we live in a capitalistic society. And there's not a lot of money in making diagnostics. There's a lot of money making the next ADC or blockbuster drug, and it's bankrupting everybody. 80% of healthcare decisions are based on diagnostic tests, but they account for less than 20% of healthcare costs. And if you look at global investment, it's super low. How do we solve this and overcome that and other institutional barriers? I don't know the answer to that. But I do think precision oncology is never moving forward until we do, and in any real way beyond what we're kind of already doing. I've spent months going around talking to congressional lobbying groups and professional societies. I don't see anybody out there lobbying for exactly what we're talking about. I've asked, "Well, how about engaging a patient advocacy group?" What I hear back is, "well, those work, but they take 20 years to get anything done with patient advocacy groups." I don't know if that's true or not, but that's the belief out there. I don't know whether this is something you guys think about, but we must figure this out. Somehow, diagnostics must be valued in the same way that these blockbuster drugs are, and they're way way undervalued right now. I just don't know how it feels moving forward beyond where we are - separate tiny little steps that might have little incremental advantages. We need to leap forward. I don't know if that's something you guys can think about in this group. You need a way to catalyze that to move the field forward for Brian and everybody in that kind of situation.

Brian McCloskey 58:09

That's really helpful. This frames the issue that I need to re-present to the Prostate Cancer Foundation and my oncologist when I see her on Monday. I have been on this diagnostic journey looking at my DNA and RNA and that's been great, but the next step is proteins. We're all saying the same thing. Karin just showed incredible data to prove the value of proteins. The answers are there. We don't have to get all hung up about N-of-1 versus larger cohorts. Let's start. You never know what you're going to find when you start. We have a growing patient network. We have some really amazing people that are supporting me, and you all are part of that. I thank you for that. It's powerful. I think I've got a compelling case. I really believe that, and I won't change that belief. This story is to be continued. I know it's tough. I spent some time in healthcare, and I know how tough it is to move the needle. I'm not naive. But I'm also determined, so this will be continued. I know where I'm going, and you guys have helped me to figure out what the objective is. And it's simple. Thank you.

Karin Rodland 59:50

Brian has said it all, and I said most of what I wanted to in defense of proteomics. Right before we started this, I am going to make one statement. This group is focused on patients who already have late-stage cancer, and they're trying to treat their disease and get better treatment. But wouldn't it be better if we could prevent the disease in the first place, or detect it very, very early when a simple surgery was a complete cure? And that's the other part of liquid biopsies and DNA versus protein. We're doing all we can to help Brian now that he's in this pickle, but we

want to help Brian's son, or brother, or cousin not ever have to have prostate cancer because they detected it very early, and it's cured. Mandy benefited from that, and breast cancer in general did, because that field has progressed to the point where early detection is pretty commonplace, and therefore it's treatable.