“Hacking the Proteome for Cancer Treatment” (Karin Rodland) [#12]

June 8, 2022
Brad Power

“You have to study the proteins to really understand the state of the biological system.” – Karin Rodland

Meeting Summary

Karin Rodland, Director, Precision Medicine Innovation CoLaboratory at Pacific Northwest National Laboratory, led a discussion about the potential of proteomic analysis to guide treatment decisions in advanced cancer. She is involved in efforts to apply a proteomics-based approach to identify biomarkers for early diagnosis of cancer and other chronic diseases, and in the use of systems biology to identify potential therapeutic targets. Her current research focuses on improving the ability to identify and validate biomarkers of disease by combining expert knowledge of cellular pathways with statistical approaches.

A lot of people have either a genome or transcriptome bias in understanding cancer dynamics. Karin has a protein bias. The way we tend to do precision medicine is on actionable mutations, but it's really the proteins that execute the signals that are in the genome and messaged in the RNA. “You have to study the proteins to really understand the state of the biological system, and to understand what is going on in multiple dimensions within the cell at the same time, because it's usually never a single mutation. We don't know a lot about how multiple mutations in the same tumor interact with each other and change the response to a drug, for example.”

In the field of biomarkers, proteins are the “go to molecule" because they're stable and easy to measure. Protein-based biomarkers, such as PSA for prostate cancer, have become standard reference points, but they are normal function proteins that are specific to the tissue of the prostate – statistically correlated because you have a bigger prostate if you have prostate cancer – but these proteins are not mechanistically driving the cancer, so there are a lot of false negatives. We are still looking for better biomarkers that say something about the state of the cancer, particularly about the immune response to the cancer, that can change the treatment decision.

Examples of Karin’s experience in using protein analysis to guide cancer treatment

- **Prostate cancer**: A prostate cancer diagnostics project found biomarkers that can be used from the first prostate biopsy to accurately predict whether patients will need a prostatectomy without needing a second biopsy sample.
- **Ovarian cancer**: A study of proteins in ovarian cancer patients found that proteins could provide better treatment information than DNA or RNA. The proteomic analysis focused on “phosphorylation”, identifying a distinctive signature from a map of the handoffs of phosphorus in communication pathways from the membrane of the cancer cell to the nucleus, which correlated with patients’ long- or short-term survival. *Karin said that Brian could develop a similar map, including his BRAF overexpression, if he sent his fresh*

frozen tumor tissue to a lab like Olink, and then worked with experts with a systems biology background.

- **Acute myeloid leukemia**: Using protein and phospho protein analysis, they found they could predict drug response better than genomics or mRNA, particularly in understanding resistance in late phase AML, and they developed a drug combination to apply in the early phase to reduce late phase resistance.

Discussion of Testing and Treatment Improvements

- **Drug Combinations**: Drugs are given in sequence. You're given a drug, you respond, you develop resistance, the disease comes back, then they put you on the next drug. Every time you do that, you are selecting new clones with new survival mechanisms. If we knew what combinations to use early on, could we kill every tumor cell, and there would be nothing left to recur? The FDA slows adoption of new approaches because they are very conservative. They do not want to approve something that kills 10,000 people, even if it saves 10 million, because the 10,000 that died will be blasted all over the media.
- **Combination Testing**: Payment is the big issue. As long as payers will only authorize payment for the second test after the first one has failed, you're not going to have physicians ordering multiple tests at the same time. We have a federal agency that makes recommendations against tests, the U.S. Preventive Services Task Force.
- **Proteomics Adoption**: These proteomics tests are available now, or soon, if you find the right academic medical center which is doing the right clinical trial. It's a very hot area of research. To make it standard of care, the most optimistic estimate is three years. You should work with your friendly doctor who is willing to go outside the standard of care to write up a case report on your success.

Requests

- Do you or anyone you know have the skills to take the results of a proteomic analysis to build a pathway map?
- Given the potential of protein analysis to guide clinical decisions for advanced cancer, what can be done to accelerate its use?

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Meeting Notes

Brad Power: We're happy to have with us Karin Rodland, a friend who is based in the Portland, Oregon, area, and southern Washington state, and is an expert in proteomics. I saw Karin speak at an online conference on the potential of proteomics, and since we're very interested in novel testing and how it might inform patients' decisions, Karin graciously agreed to give us a tutorial and introduce us to the potential of proteomics to inform clinical decision-making.

Karin Rodland: A lot of you are in the biotech industry and are familiar with the technology, and all of you are self-educated and have learned a lot about this. Cancer is personal for almost everyone. I used to give talks to encourage community support for Oregon Health Sciences University, and I would ask the audience to raise their hand if anyone in their family had ever had cancer. And every hand in the audience went up routinely. That's my motivation here.

Hacking the Proteome for Cancer Treatment is something that I'm very passionate about. The object of the game – and I know that this is why you have the Prostate Cancer Lab and hackathon – is improving cancer survival, and the way we have tackled the problem at PNNL (the Pacific Northwest National Laboratory) is early detection and targeted therapeutics.
I recognize that this group is past the stage of early detection. Your motivation is that you have been diagnosed with prostate cancer, and it has metastasized or recurred, and you're wondering what your treatment options are, and that's where the targeted therapy comes in. But one of the great strengths of proteomics is in the quest for biomarkers, for early detection and for accurate prognosis and guidance of initial therapy, as well as guidance of later therapy.

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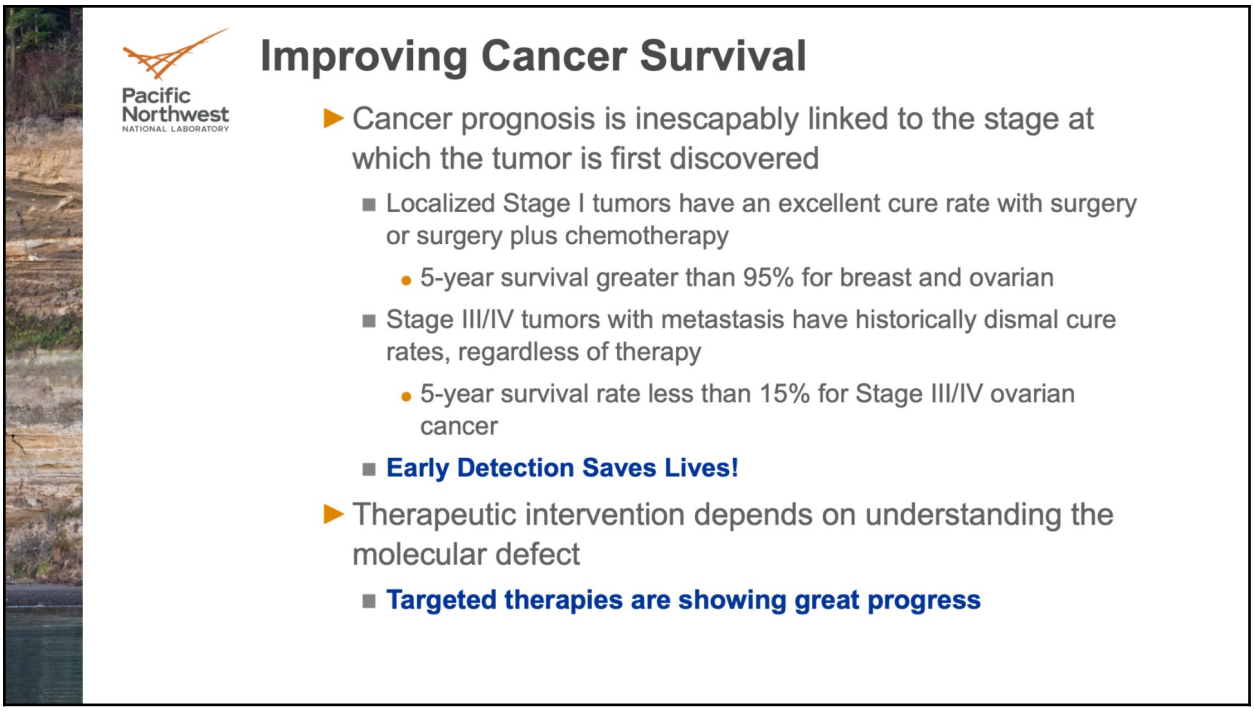
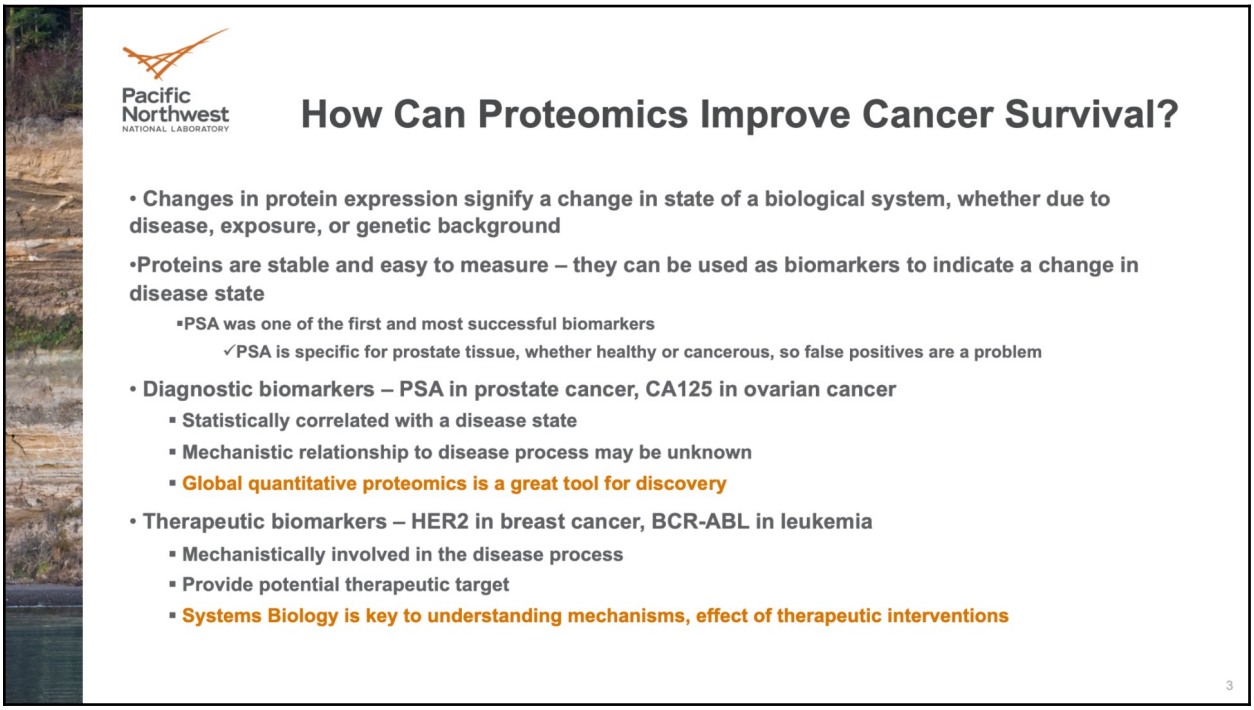


I probably don't have to tell you folks that if you find almost any cancer in stage one, while it is localized and can be removed, surgery is pretty darn curative for almost all cancers. But the later the stage of the cancer, the worse the prognosis.



How can we use proteomics to improve cancer survival?

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I have a protein bias. I know that a lot of the biotech people from San Diego have either a genome or transcriptome bias. There's a plethora of RNA signatures for prognosis of cancer. The way we tend to do precision medicine is on actionable mutations, but it's really the proteins that execute the signals that are in the genome and messaged in the RNA.
You have to study the proteins to really understand the state of the biological system and to understand what is going on in multiple dimensions within the cell at the same time, because it's usually never a single mutation. We don't know a lot about how multiple mutations in the same tumor interact with each other and change the response to a drug, for example.
When it comes to the field of biomarkers, proteins are kind of the “go to molecule" because they're stable, and they're easy to measure. RNA is more labile (likely to change, easily broken down or displaced).

We are now tending towards cell-free DNA or circulating tumor DNA. That's relatively new technology, but over time, protein-based biomarkers have really established themselves.
For example, there are the early detection biomarkers of PSA for prostate cancer and CA125 for ovarian cancer. The big problem with these biomarkers is that they're normal function proteins that are specific to the tissue of the prostate or the ovary. So there are a lot of false negatives. They're statistically correlated because you have a bigger prostate if you have prostate cancer, but they're not mechanistically driving the cancer. So we are still looking for better biomarkers that say something about the state of cancer and particularly about the immune response of the body to the cancer.

There's a lot of potential that I want to focus on in therapeutic biomarkers that actually change the treatment decision. The two that have been around the longest are the HER2 gene in breast cancer and the BCR-ABL chimeric gene in leukemia. Because BCR-ABL activates the ABL kinase, you can use ABL kinase inhibitors to treat Philadelphia chromosome positive leukemias. Because HER2 drives proliferation in breast cancer, you can use anti-HER2 strategies to treat breast cancer. I just saw a new trial that's using HER2-targeted antibodies to deliver doxorubicin specifically to breast cancer cells and allow a higher local dose, another example of a protein-driven therapeutic option that is emerging.

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You guys know this story. You have been through this. The way we treat cancer patients these days is population science. The way it's always been done. The gold standard for physicians is what treatment works the best in the greatest number of patients. And even five years ago, there was a feeling that if you did anything that was outside of the gold standard of treatment, you were letting your patients down.

The interview would go something like this:
“Your PSA is really high. What should we do now? (I know you've been through this.) Well, we'll do a biopsy of your prostate. You're Gleason 7. That's the hard one to call, so we can do a radical prostatectomy, or we can do another seven needle biopsy next year.”
I'm a girl, so I don't know exactly how painful that is, but I can imagine it is not a pleasant experience. It's a pretty coarse decision point.

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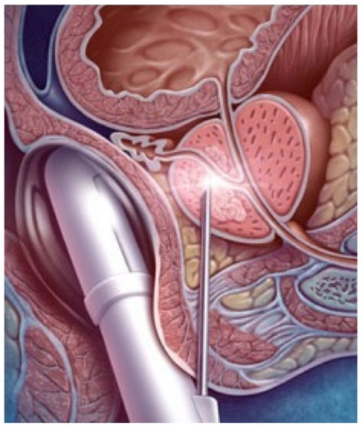
The Early Detection Problem in Prostate Cancer: Discriminating Indolent from Aggressive Disease

Clinical Need:

Ability to identify, at the initial biopsy, low risk prostate cancer patients who are candidates for active surveillance versus aggressive treatment

Approach:

Targeted analysis of protein expression in the prostate



Early Detection Research Network
Biomarkers: the key to early detection

One of the projects that at PNNL has been trying to develop a biomarker that would allow you at the initial biopsy to predict the risk of progressing to aggressive prostate cancer that metastasizes, and therefore have a very objective quantitative, mechanistically-relevant decision tree for determining whether you can just go on active surveillance with repeat biopsies or whether you should have a radical prostatectomy right now.
Because I'm a researcher, I have these slides on how he went about doing that.

Pacific Northwest NATIONAL LABORATORY

Predicting Aggressive Disease

Experimental Design

- ~340 FFPE samples selected and provided by CPDR (blinded analysis)
  - Tissue from radical prostatectomy within 6 months of diagnosis
    - no neoadjuvant hormone therapy
  - Known outcomes
    - no further events after 10 years of follow-up (indolent disease): 171
    - biochemical recurrence (BCR) – intermediate response: 114
    - metastatic disease – highly aggressive prostate cancer: 57
  - 105 in “training” set, 238 in “test” set
- Candidate biomarkers selected from curated literature

CaP prognosis associated genes	Other CaP associated genes	Other cancer related genes
AKT1	AMACR	BRAF
ANXA2	CRISP3	CAMKK2
AR	ERG (pan)	EGFR
AURKA	ERG8	HIF1A
CCND1	ETV1	HPN (TMPRSS1)
CDKN1A	FOLH1 (PSMA)	HSPB1
EZH2	HOXC6	MMP2
FGFR1	KLK2	MMP9
MUC1	KLK3/PSA	PDGFRB
MUC6	KLK11	PIK3CA
MYC	MYO6	PLA2G7
MYCN	NPY	ODC1
NCOA2	PSGR	RAF1
PMP22	SPARC	SERPINI1
SMAD4	TWIST1	STAT3
SPINK1		TGFB1
SPP1 (OPN)		TP53
TFF3		TPM2
		VEGFA

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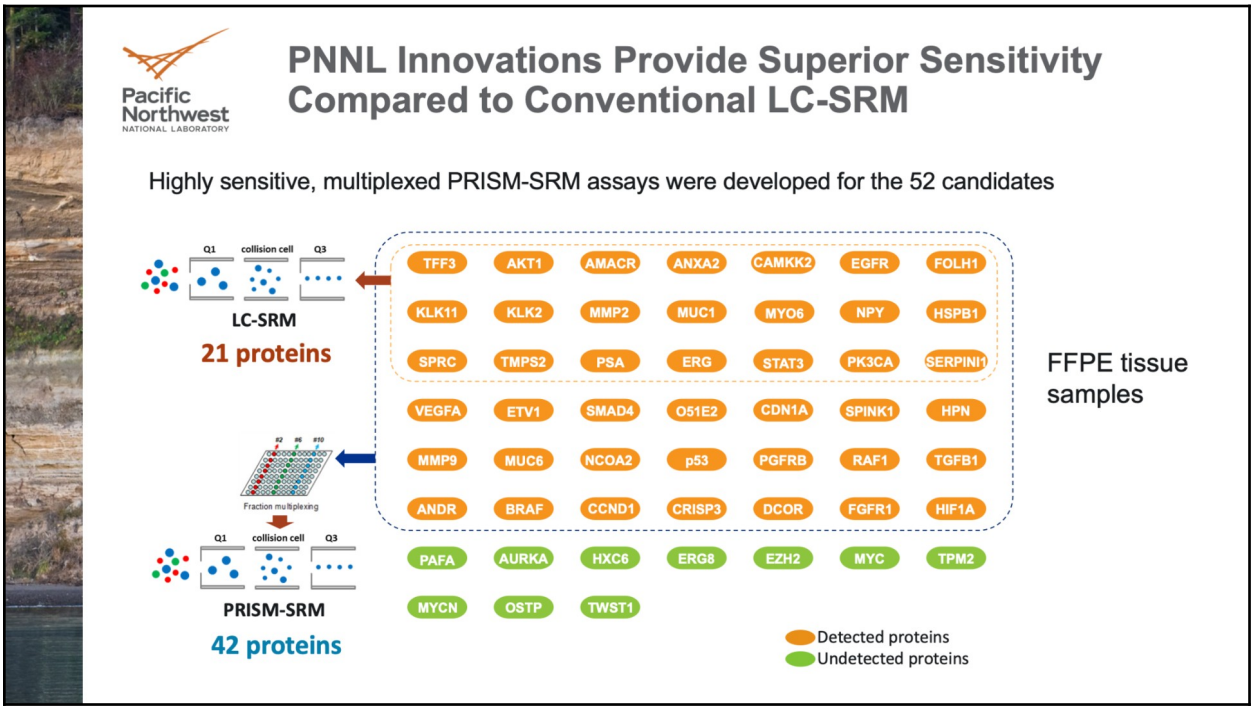
This is an example of how the technology of proteomics can be applied to problems in cancer biology. This type of work is very well funded. I'm sure that you guys in biotech with the startup companies know how this works.

We started with a list of prognosis-associated genes in prostate cancer, and other genes that were based on RNA seq experiments and tissue, and other genes from the curated literature that were either prostate-cancer-specific or just plain cancer-related.

We ended up with about 62 targets. And we had 340 FFPE samples that were provided by the Center for Prostate Disease Research at the Uniformed Services University. The tissue came from a radical prostatectomy that had been performed within six months of diagnosis with no neoadjuvant therapy. Every individual had at least 10 years of follow up. For the “no events”, we had 10 years of follow up with no increase in PSA and no metastasis or recurrence. We had 171 of those patients. We had 114 with rising PSA, and we had 57 with metastatic disease.



We used a technology that we've developed at PNNL which is highly sensitive. It uses fractionation to increase the sensitivity of the mass spectrometry. We call it “PRISM-SRM”. SRM is “selected reaction monitoring”. You have a synthetic peptide for the protein of interest. You spike that into the lysate (disruption of the plasma membrane of a population of cells), and you can get very good quantification and increase the sensitivity because you know exactly what you're looking for.

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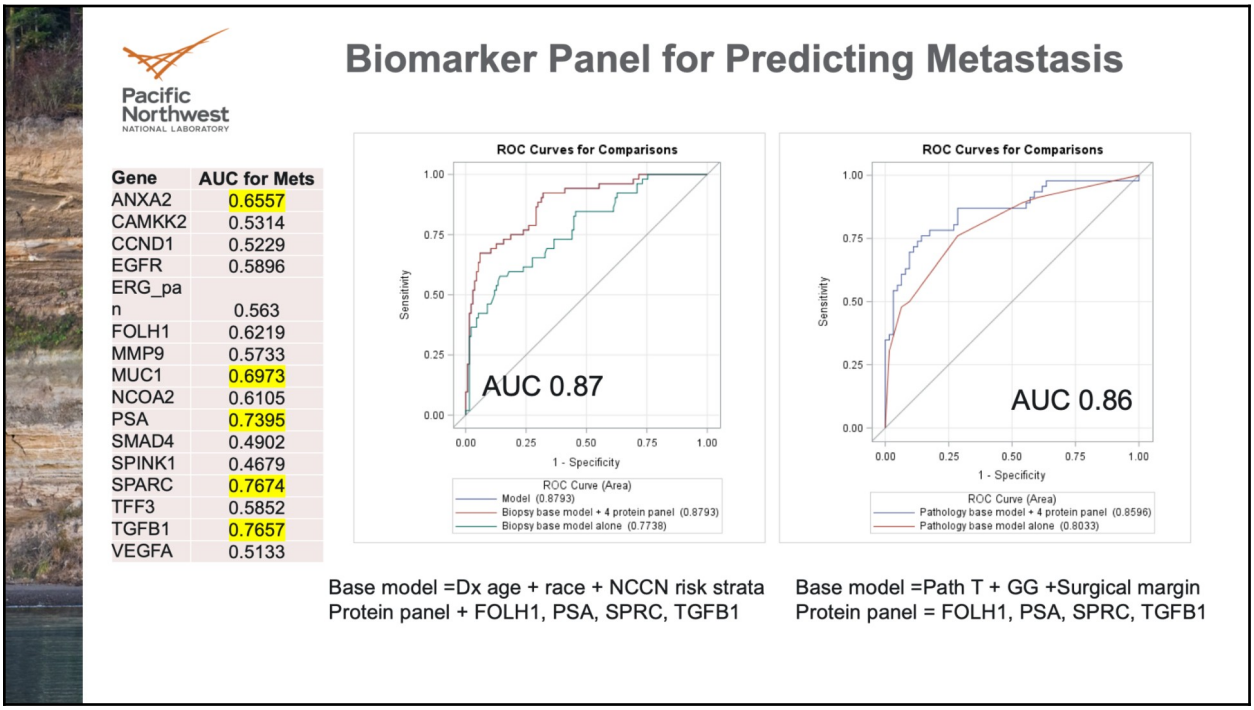
We were able to see 21 proteins with a standard technology for targeted proteomics. We were able to see an additional 42 using our PRISM-SRM technology. There were 10 that we could not see at all.

As always happens using this panel, we were able to not only detect statistically significantly different proteins between metastatic disease and indent disease, but we were able to use machine learning to develop a signature to predict distant metastasis within the next 10 years. We compared it against the biopsy based model, which is the criteria that are used to determine whether you get a biopsy or not.



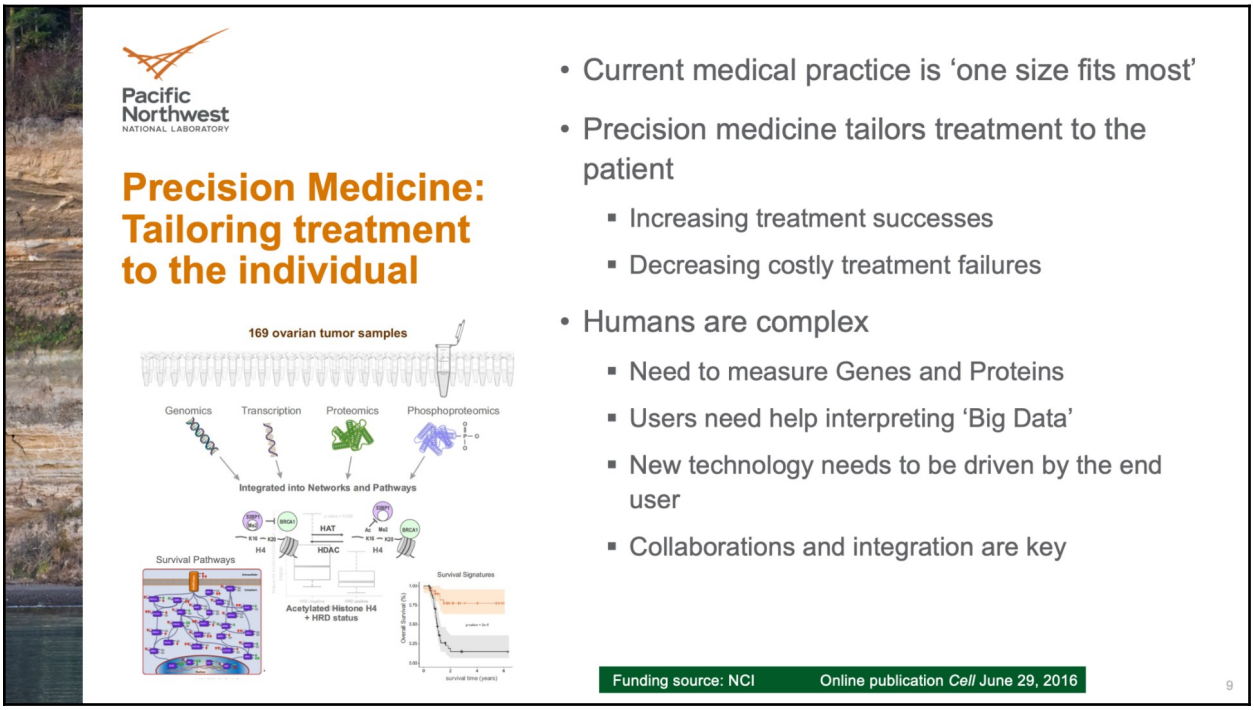
The standard predictive risk stratification is from the NCCN (National Comprehensive Cancer Network standard of care guidelines), which includes your age at diagnosis and your race. We were able to increase the area under the curve from 0.7 to 0.88 almost. (Estimates of the “area under the curve” - AUC - is a means of comparing predictive models. “Receiver Operating Characteristic” - ROC - curves plot the true positive rate against the false positive rate.) Here's the increase using the protein biomarkers.

The other comparison was with the pathology standard, which is the Gleason grade called by the pathologist on that biopsy and the surgical margins.

The increase that we saw was not as great there because the pathology-based model all by itself was 0.80. We got about 0.86 with our model.

Here's the point: The pathology model requires that you have a biopsy. The biopsy model is the criteria that are used to determine whether or not you will have a biopsy. And if we can match

the performance of the biopsy with just the initial diagnostic biopsy – without doing the additional seven-needle or whatever biopsies – we are hoping for significant impacts on the quality of life. That's our prostate cancer project. We're trying to convert this into a less invasive protocol rather than using biopsy samples. We're also working on the sensitivity issues with a single diagnostic biopsy. That's coming along nicely. We have not been able to use urine to replicate our protein signature. We're hoping to be able to use blood, and we're working on that now.



Precision medicine is tailoring treatment to the individual with targeted therapies.

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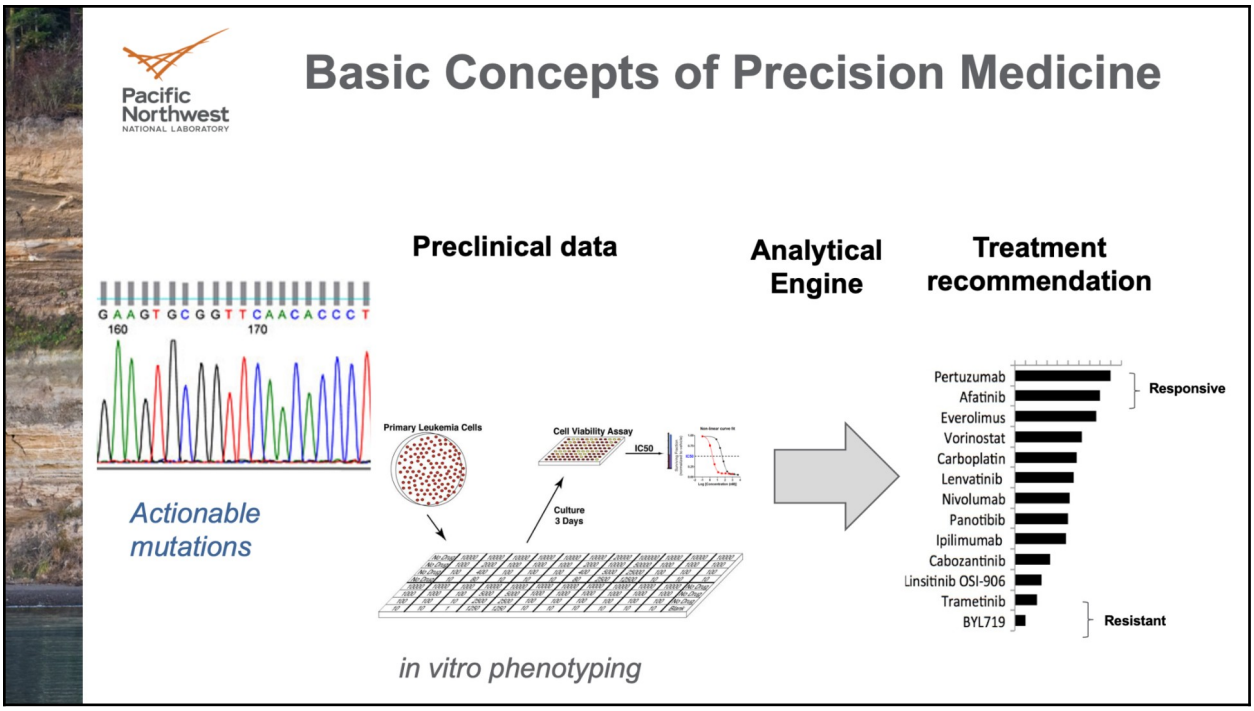

Here's basically how precision medicine is being done right now. You get the patient's tumor DNA or circulating DNA, you look for actionable mutations, and based on actionable mutations, you determine whether it's a druggable mutation or not, and you have a panel of drugs.
You can also add in vitro phenotyping, for example in leukemia. You could take the patient's leukemia cells, plate them in a Petri dish or microtiter plate, and test them against a number of drugs, and determine IC50s. (An IC50 - half maximal inhibitory concentration - is a measure of the potency of a substance in inhibiting a specific biological or biochemical function.) You take all of these data, whether it's RNA seq, a whole genome sequencing, or a whole exome sequencing, and you stick it into an analytical engine, and then you come up with a list of drugs that that particular patient is responsive and resistant to, and a treatment recommendation.
We've been trying to add a layer of proteomics and protein information.

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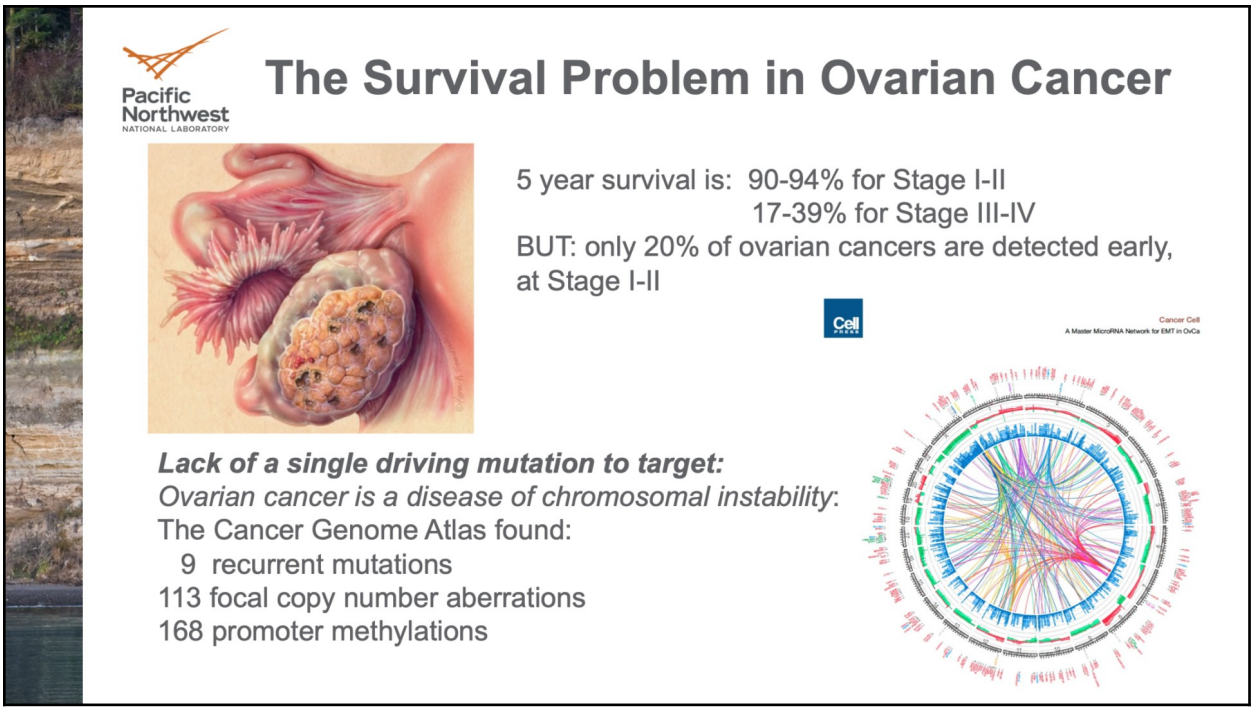

Our first example of adding a protein layer was in ovarian cancer, which is one of the poster children for a bad cancer, right up there with pancreatic cancer. But there's no such thing as a good one. The survival rate for Stage III-IV ovarian cancer is 17-39%. Most women respond well to an initial round of platinum plus Taxol (paclitaxel, a chemotherapy), but almost everyone recurs. And the recurrence is refractory - resistant to further treatment. Eventually the woman succumbs, and one of the problems is that there's no driving mutation other than P53, which is not druggable in ovarian cancer.

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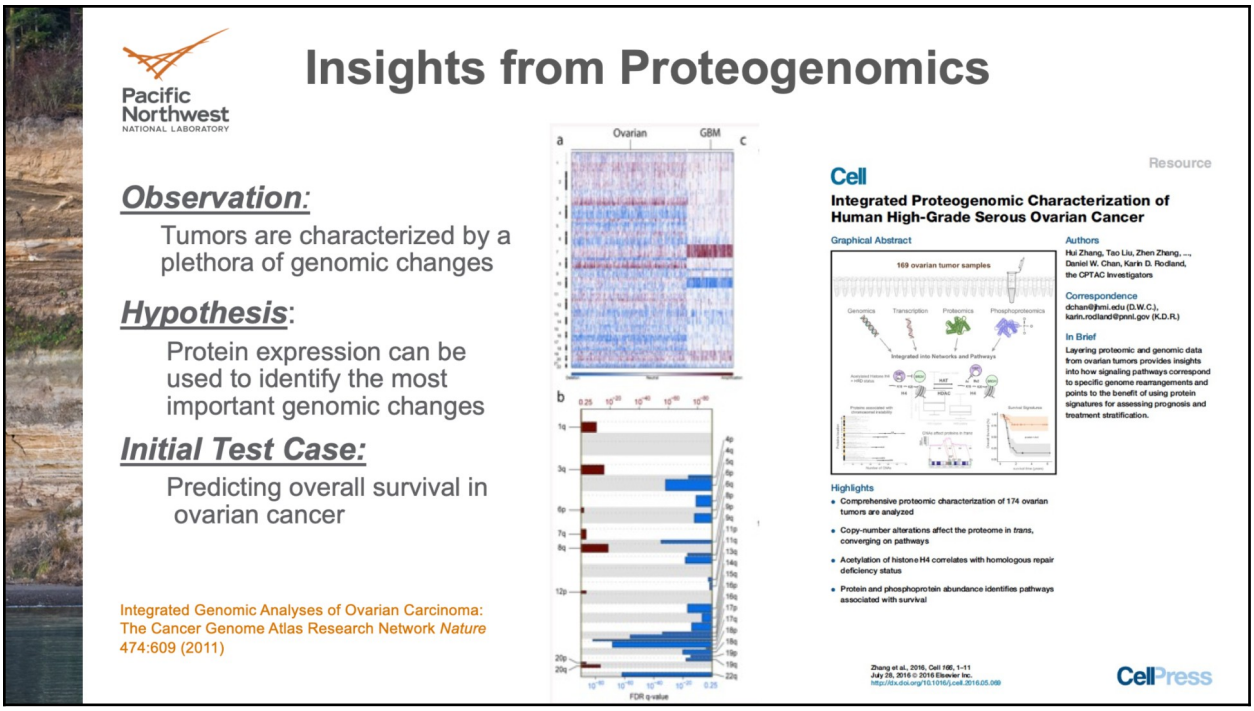
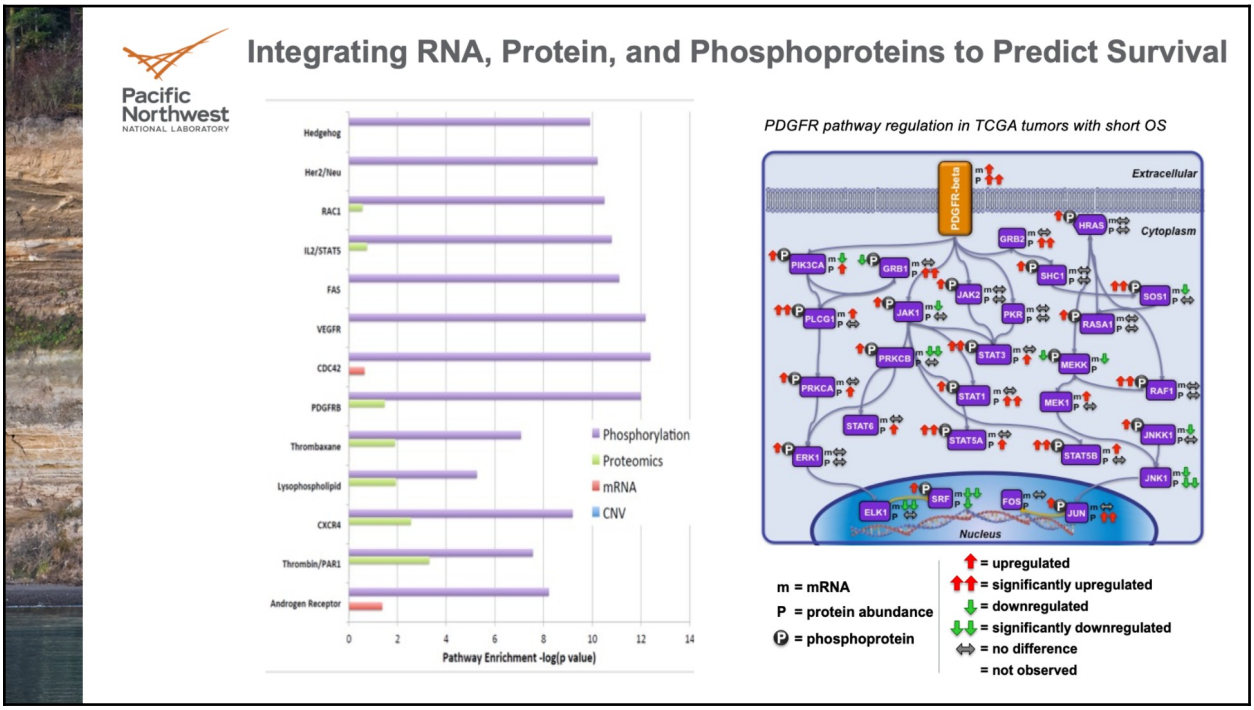


We undertook a proteomic examination of high grade serous (aggressive tumors that usually grow quickly and spread widely before diagnosis) ovarian cancer to determine whether we could get better treatment information out of protein, rather than DNA or RNA.



This is a summary of that work. The panel on the left shows the results that we got from measuring the phosphorylation of proteins.

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The pathway diagram on the right shows the way information flows in a cell – from a receptor on the cell membrane that senses something in the environment like the platelet-derived growth factor, which binds to the platelet-derived growth factor receptor. A series of phosphates are handed off from one protein to the other by kinases (enzymes that attach a phosphate group to a protein), and then removed by phosphatases (an enzyme that removes a phosphate group from a protein). The phosphorylation activates the kinase that gets phosphorylated. (Phosphorylation is the attachment of a phosphate group to a molecule or an ion, which often activates or deactivates many enzymes.) Then it can phosphorylate the protein downstream of it, activate it, and the message gets passed eventually to the nucleus, genes get transcribed, and the cell state changes.

This is an example of what a network looks like. We were able to interrogate every one of these proteins, both for the mRNA levels, with the little m’s for the protein, how much protein there was there with the little p’s, and for whether or not that protein was phosphorylated stoichiometrically (in a ratio of one substances with a quantity of all of the other substances). We were measuring whether there was an increase in the amount of phosphorylation for each kinase molecule for that particular protein. You can see the complexity of the network that we were able to map. You can see the discrepancy between, maybe, the RNA was unchanged, the protein was up, and the phosphorylation was down.

There are many cases of regulation at different levels that we saw. In one case, the mRNA is down, the protein is unchanged, but the phosphorylation is up. It's the phosphorylation that is conveying the information from the cell surface to the nucleus.

The bar graph on the left shows how we tried to predict survival.

In this ovarian cancer patient cohort, we had survival data, and we divided the cohort into short survivors, who died in two years or less, and long survivors, who were still alive at five years or more. That was the data that we had.

We could predict survival. We could distinguish between the short and long survivors very nicely based on pathway analysis using phosphorylation data.

The hedgehog pathway, the HER2 pathway, and the VEGF pathway, when activated, were associated with short survival. Proteins were fairly useful.

There were some pathways that were implicated at the level of protein abundance.
RNA was much less useful in predicting or classifying the patients as short survivors versus long survivors.

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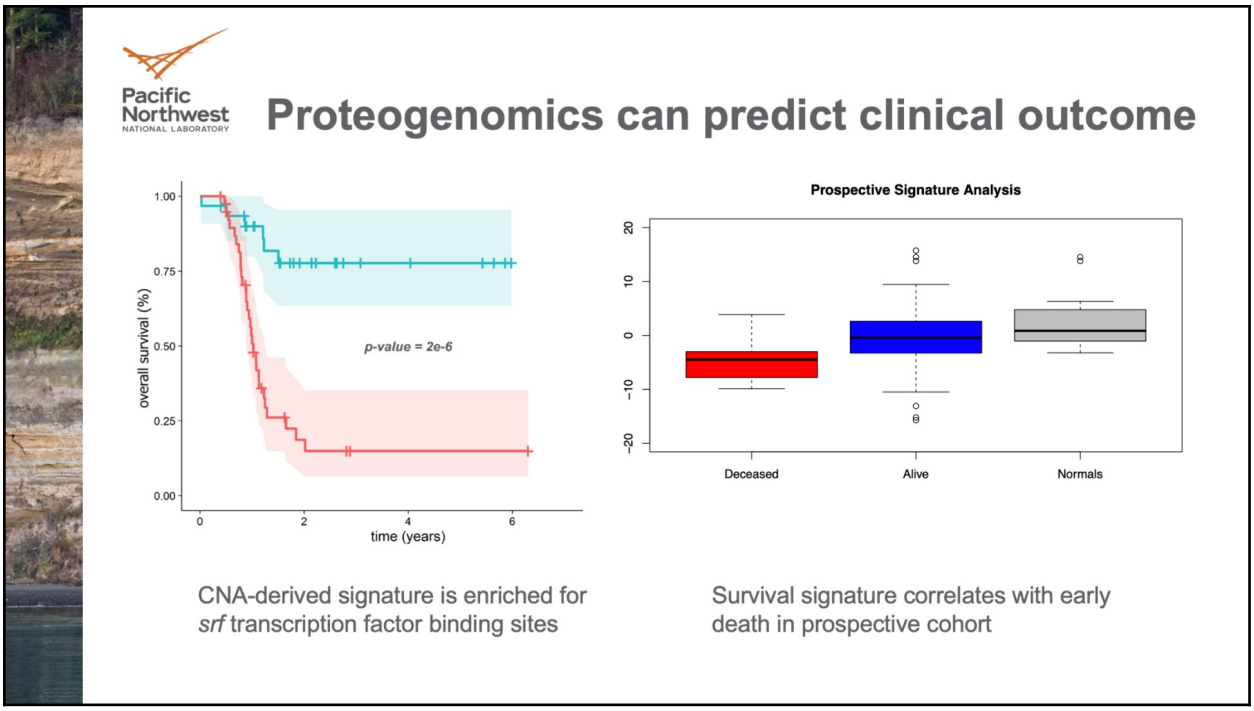


Using the protein data, we were able to develop a signature to predict clinical outcome, to predict short or long survival. You can see that our signature could distinguish between a good outcome of five to six years versus a very poor outcome.

We did a validation experiment in a completely independent cohort. It was a prospective cohort, so we didn't have the 5, 6, 7 years of follow up, but we had two years of follow up, and a significant proportion of the population were dead in two years. They had the signature that was associated with short survival. They had lower levels of the signature that was associated with long survival. Those who were alive had the long survival signature and the normal tissues were even higher.

We thought that that was encouraging for prognosis.

Rick Stanton: Going back to the pathway map you developed: I tried to describe this phosphorylation impact to Brian. From his mRNA analysis, BRAF has been identified as significantly upregulated. I've looked at his transcripts per million against the TCGA prostate cancer cohort of like 550 patients. His BRAF is very high in mRNA expression.
The analogy we made at Amgen was to a soccer game. The phosphorylations are like soccer balls. BRAF is like you have a thousand middle backs. I said, just because you have like a thousand middle backs – where other people have 10 – it doesn't mean they're phosphorylated, but what does it mean? Could you shed some light on the correlation between phosphorylation and mRNA overexpression, or is there anything actionable that Brian can do from this observation that his BRAF is off the charts?

Karin Rodland: That's a very important question that we're tackling in the “Beat AML” clinical study cohort. I didn't put those slides in because we're just submitting the paper, and I only have the figures from the paper. We're trying to determine what source of information is most powerful in predicting the in vitro drug response, and then comparing the in vitro drug response with the patient's actual response to drug treatment. We just got that project renewed for another five years to complete that study. What we're finding again is that phosphoproteomics is one of the best predictors of response. If you do a correlation analysis, there are a number of RNA-protein pairs that are very highly correlated and transcription factors, which we used to call “immediate early genes” (genes which are activated transiently and rapidly in response to a wide variety of cellular stimuli), in the nucleus, you tend to have a very close correlation between the mRNA and the protein. There are other proteins that are more stable in the cell. You make the mRNA, you make the protein, the protein is stable, it sticks around, and the mRNA gets degraded. There is not a good correlation between the mRNA and the protein levels. Things like receptors, the metabolic machinery, ribosomes (minute particles of RNA and associated proteins that bind messenger RNA and transfer RNA to synthesize polypeptides and proteins), and the ribosome biogenesis machinery, tend to be very poorly correlated at the RNA and protein level.

To move the question to Brian, you can look at phosphorylation directly. We do it globally with mass specs. We look at all the proteins all the time. We enrich for phosphorylated peptides on metal columns, or you can do what's called reverse phase protein arrays, where you have a specific target that you're interested in. There are antibodies for phospho-BRAF, and you have the phospho-BRAF antibody and the unphosphorylated BRAF antibody immobilized on a membrane. You hybridize a lysate from the tumor over it, and you can measure phosphorylation that way. We like the global approach because we get to see all this complexity. I have another slide that's not on here that shows what you got with RPPA (a reverse phase protein assay, an antibody-based way of looking at phosphorylation). You got about one quarter of these targets on the RPPA. Because it's antibody-based, you have to know what your target is beforehand. Whereas mass spec is global. It looks at everything all the time.

Rick Stanton: Sensitivity is much less at a global view, isn't it?

Karin Rodland: It tends to be much less unless you do a lot of tricks, which we're all we're very heavily working on to improve the sensitivity problem. Every trick that improves sensitivity either increases the amount of tumor material you need or increases the time it takes to do the experiment. And if you're trying 300 patients, that's significant.

Brian McCloskey: What would be the next test, or series of tests, that I would need to do to get this level of insight?

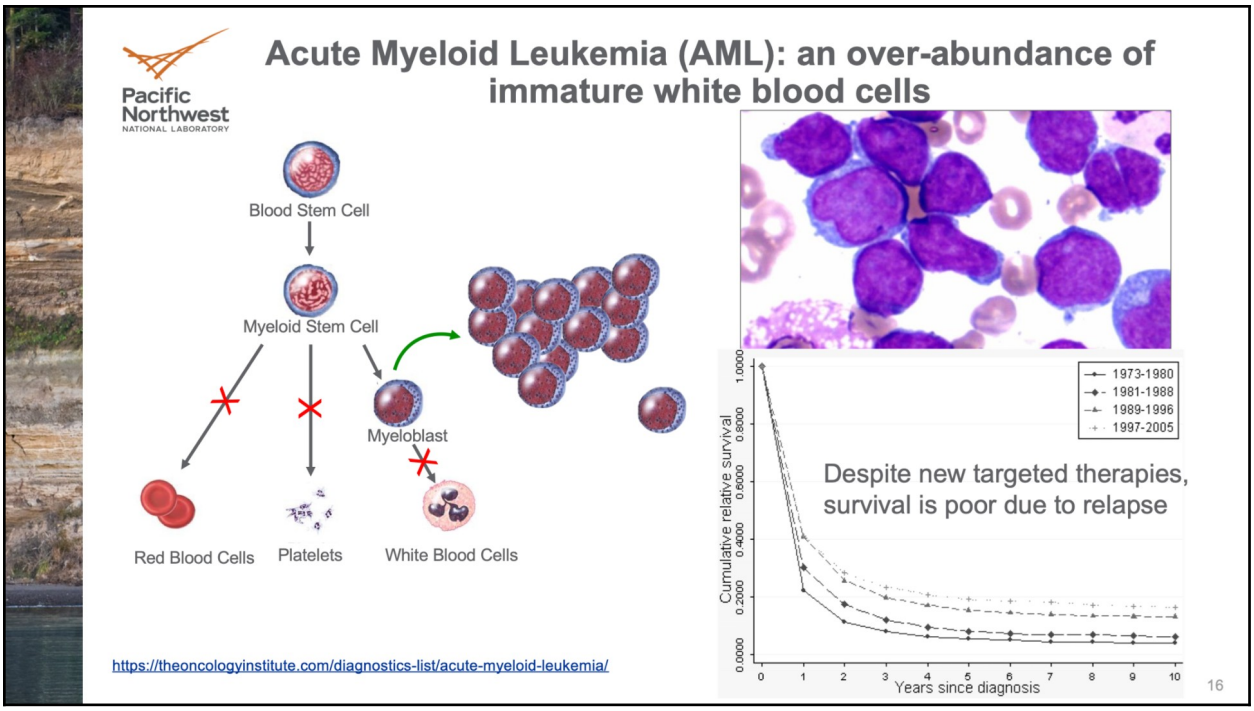
Karin Rodland: For the pipeline that we envision, we have basic science funding to figure out what the relationship is here. I wish we were funded to do this in prostate cancer. It's probably coming up in the next five years that we're going to do prostate cancer at this level. We had been funded to do ovarian cancer, and then we were funded to do leukemia. Once you

understand the biology and identify the driver phosphorylation events, the ones that correlate significantly with survival or progression, then you can go forward to build an antibody-based assay for those specific targets. You can get the answer overnight or in four hours.

I'm trying to think about what is available at the consumer level. There are some companies like Olink and SomaLogic that have very extensive panels that include phosphorylated proteins. They have inhouse service cores where they'll take a sample and run it. I'm sure that it's not cheap. We are contracting with Olink to do the cytokine analysis on 200 serum samples because cytokines in serum are at such low abundance that it's very expensive for us to detect them by mass spec. It's one of those examples of something that takes a lot of effort, and a lot of labor. You could reach out to Olink or SomaLogic. I would slightly recommend Olink. The best thing is if you had frozen tumor samples. FFPE doesn't work well for phosphorylation. That's one of the downsides of it. The formalin process strips the phosphates off, so you need fresh frozen, or OCT (Optimal Cutting Temperature). Fresh frozen is best. Unfortunately, I'm in the process of being retired from my decision making position, so I can't just sneak your sample in. Olink, I'm pretty sure, does it commercially, and I would reach out to them.

There are a lot of people, and I think some of them are members of your group, who have the systems biology background and knowledge to build a map like this. If you got your results, they would go to Cytoscape (an open source software platform for visualizing complex networks and integrating them with any type of attribute data) or another program and build your map like this. What came out of this map is that the PDGF receptor pathway is activated in short survivors. There is an antibody drug that targets the PDGF and VEGF receptors called Bevacizumab. It has been tried in clinical trials with all patients with high grade serous ovarian cancer. It did not have a statistically significant beneficial effect. Our hypothesis is that if you selected out women for that clinical trial who had activation of the PDGFR beta pathway, it may have an effect on that subpopulation.

“Hacking the Proteome for Cancer Treatment” (Karin Rodland) [#12]



There is a precursor to AML (acute myeloid leukemia) called chronic myelogenous leukemia, where two chromosomes, 8 and 11, tangle, break, and rejoin, so that part of 8 is on 11, and part of 11 is on 8. It makes a small chromosome called the Philadelphia chromosome. You can see it when you look at the cells under the microscope if they're in mitosis. That's how chronic myelogenous leukemia is diagnosed – by the presence of the Philadelphia chromosome. That's been known since the 1950s. In the 1980s we discovered that what that did molecularly was put an immune system gene regulatory promoter upstream of the ABL kinase, which is a proliferation-associated kinase. You had an immune cell with an immune-cell-sensitive promoter driving a kinase that drove proliferation. Brian Druker, who was in the next door lab when I was at OHSU, a brilliant guy, decided, “let's find an ABL kinase inhibitor, and only use it on patients with Philadelphia chromosome.” He did that, and it worked. It's Gleevec (imatinib), and the rest is history. It was the first targeted therapy that worked in a clinical trial. My position is that if Brian had been forced to do all leukemias, whether they were Philadelphia-chromosome-positive or not, his targeted therapy would've failed in the way that all preceding targeted therapies failed. There was a difference in a subpopulation, but in the larger clinical trial, it was not statistically significant. How do you know why one sub-population responded and the other didn't?

You have to do the precision medicine side of things. Right now we're focused on the genome. But at PNNL we are focused on adding protein information downstream of the genome. What this slide is saying is that despite targeted therapies for AML, including Gleevec for Philadelphia-chromosome-positive AML, if you take a look at these survival curves from 1973 to 1980, it’s pretty bad. Most people die in two to three years. In 2005, it's improved, but it's not improved a lot – instead of 95% being dead, 80% are dead. For the 15% who survive, it's great. But how about the ones who didn't? There's room for improvement.

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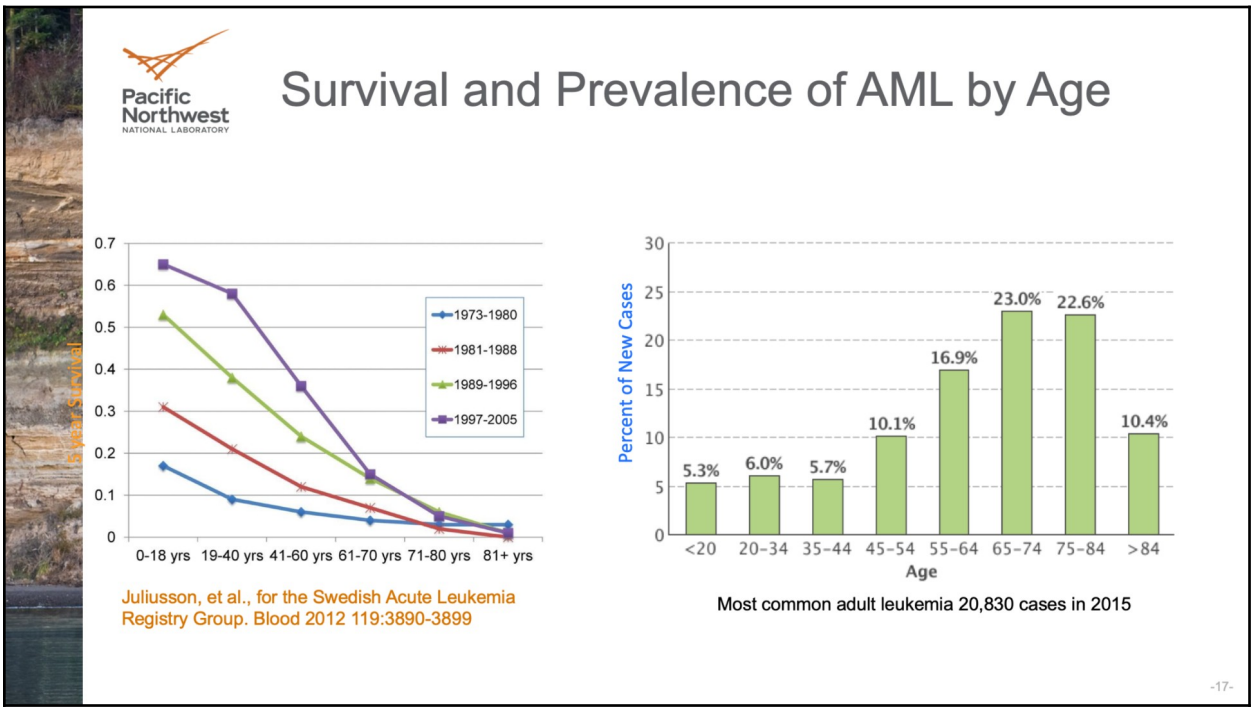
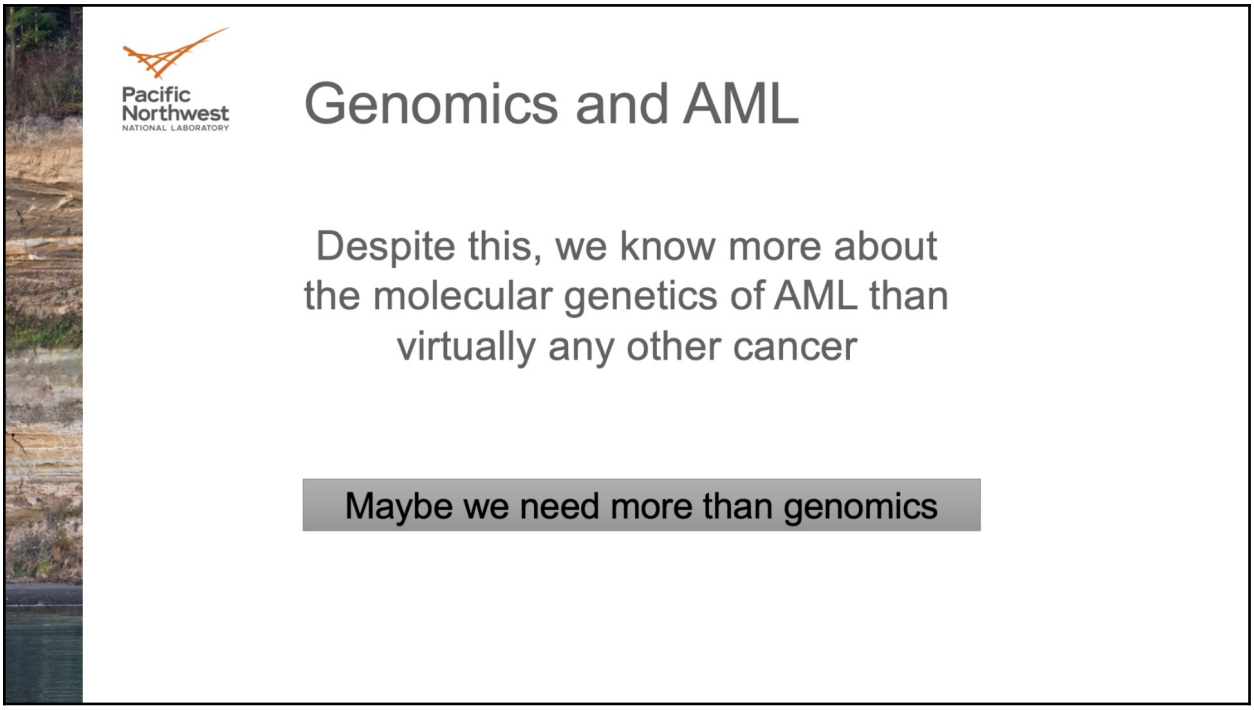
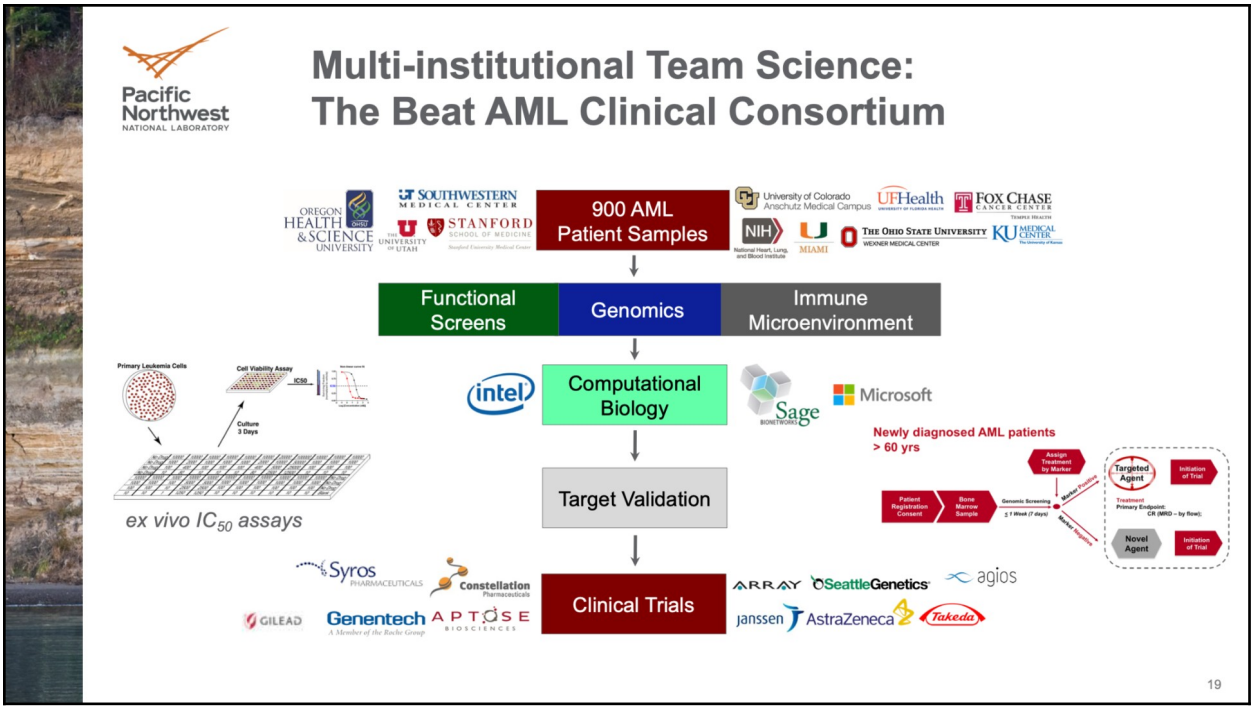


It's particularly a problem with older people who have AML.



The kicker is that despite the failure to improve survival, we know more about the molecular genetics of AML than almost any other cancer because it's a blood cancer, and it's easy to get the samples during recurrence and whatever. So maybe we need more than genomics. That's

my sales pitch. When I talk to other scientists, I argue that we need to add proteomics to genomics to really understand what's going on.



This is the “Beat AML” study, which has many participating academic centers and clinical centers. Sage, Microsoft, and Intel have been involved in the computational biology side of things. One of the key aspects is adding in vitro measurement of drug sensitivity. They take the patient's cells, plate them out, use a hundred or more drugs, and calculate survival curves. We've been trying to use the protein and the phospho protein data to predict the drug response ex vivo, and compare that with the use of the genome or the mRNA to predict the same thing. We are discovering that the protein is better, and integrating.

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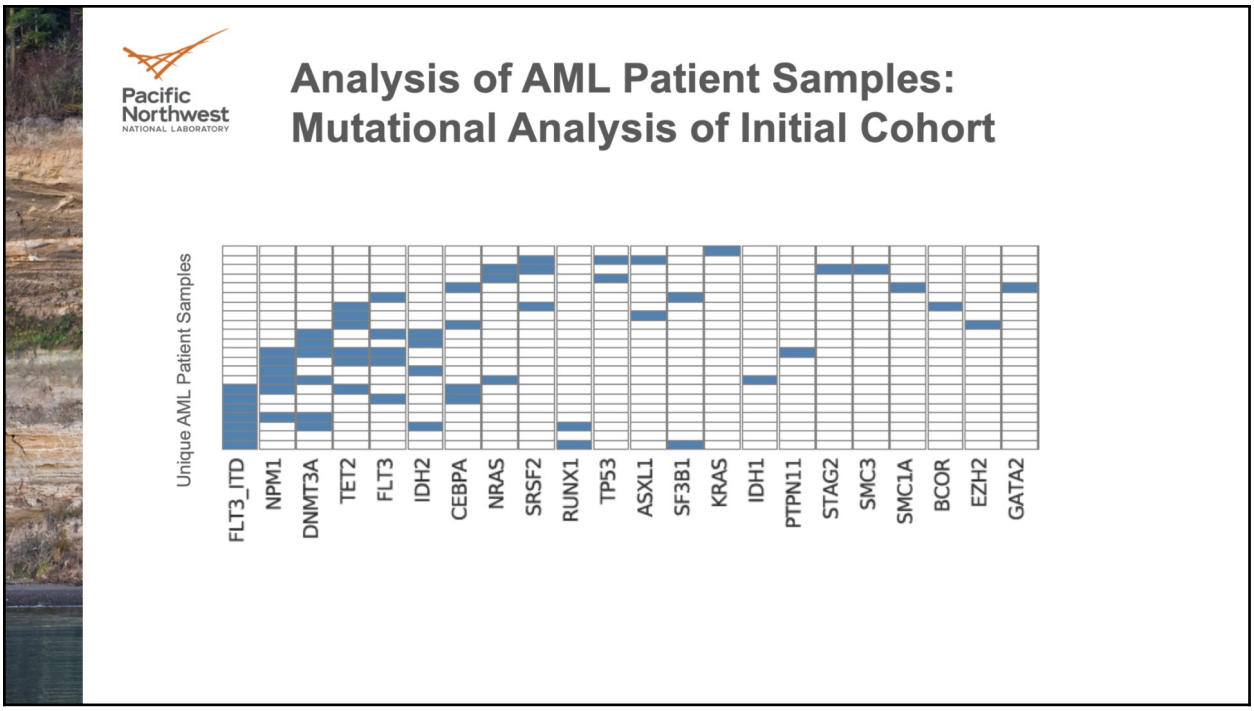
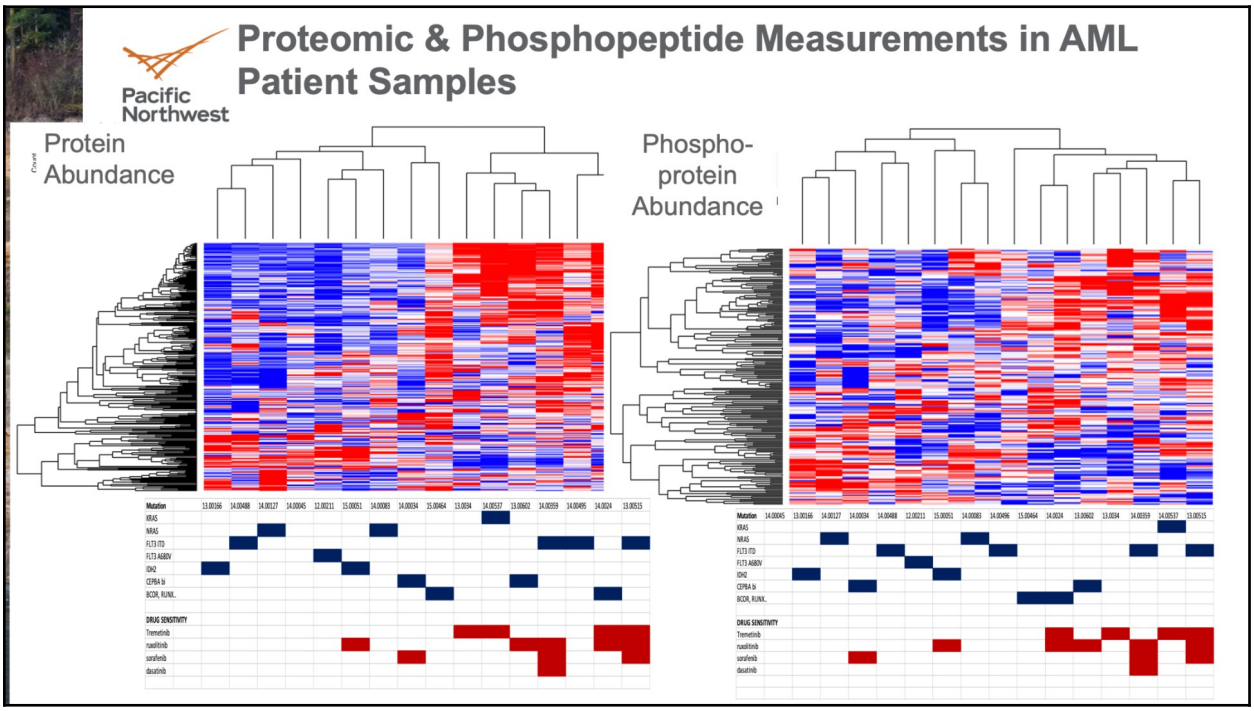


Here are the preliminary results. We had 16 patients that had whole genome sequencing, as well as full RNA seq, and full proteomic analysis. Each row is a patient. Each column is a particular mutation. Almost every single patient has multiple mutations. The FLT3 ITD is the most common mutation, which is well known for this disease.



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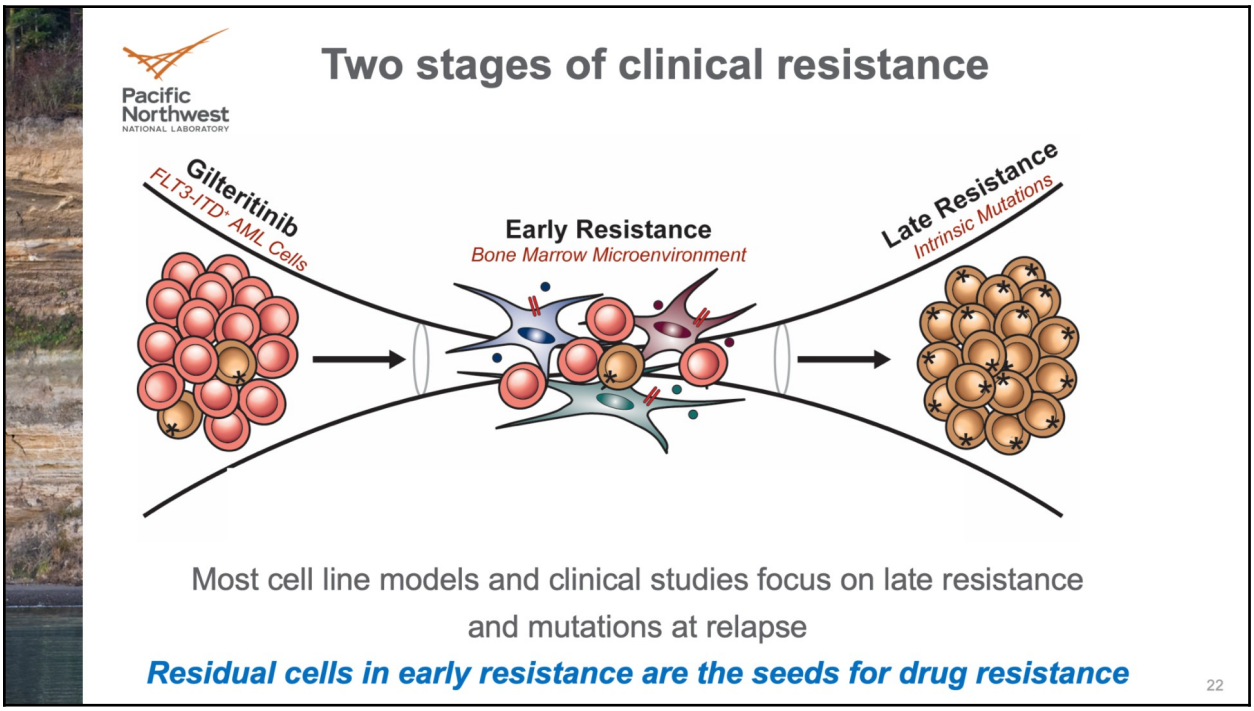
We did protein analysis and phos protein analysis, and we did completely unsupervised clustering. The algorithm does pairwise comparisons and clusters things together that look like each other. There's a cluster for protein abundance, and there's a cluster for phos protein abundance. And the black bars down at the bottom are the mutations. You can see that the mutations are randomly distributed across the clusters of protein and phos protein. In red are the bars for drug sensitivity. You can see that the drug sensitive patients all clustered together. They clustered together with these in upregulated proteins and increased phosphorylation in the same upregulated proteins.

And not surprisingly, these are the proteins that are involved in the proliferation of immune cells and in the immune response, the job that the immune cells are supposed to do. This was our very first preliminary data that indicated that maybe proteins and phos proteins can tell you more about drug sensitivity than the mutations.



Then we got involved in a study of resistance, which I'm sure is of great interest to this group because the definition of recurrence in prostate cancer is the failure of anti-androgen therapies, called “castrate resistant” prostate cancer.

This study benefited from the fact that it was a blood cancer, so we could get multiple samples easily. The concept is that at the bone marrow where the cells mature, it is rich in growth factors that may actually be allowing some of the tumor cells to survive during the drug treatment. They modeled that by growing the cells in the presence of growth factors that are present in the stroma. We did protein and phos protein proteomics. In this model, you have an early phase where the cells only survive because the growth factors are there. But during that phase, when they survive, because growth factors are there, RAS mutations are positively selected.

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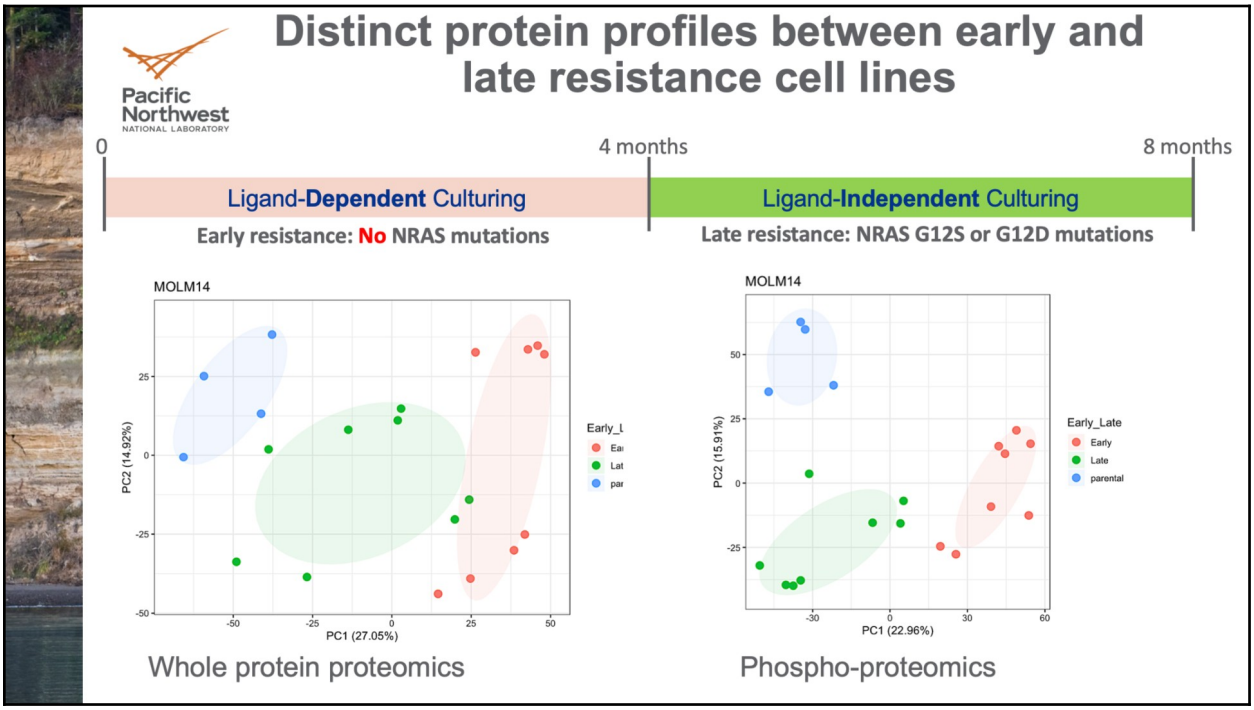
The late resistance does not need the growth factors. The cells will grow in the presence of the drugs, even in the absence of growth factors from the stroma, because they're now RAS-mutant.



With proteomics and phosphoproteomics, we could distinguish between the parental cell lines’ early resistance and late resistance. We went on to develop a signature. We looked at which pathways were enriched during the early resistance, that was growth-factor-dependent, and during the late resistance, that was growth-factor-independent.

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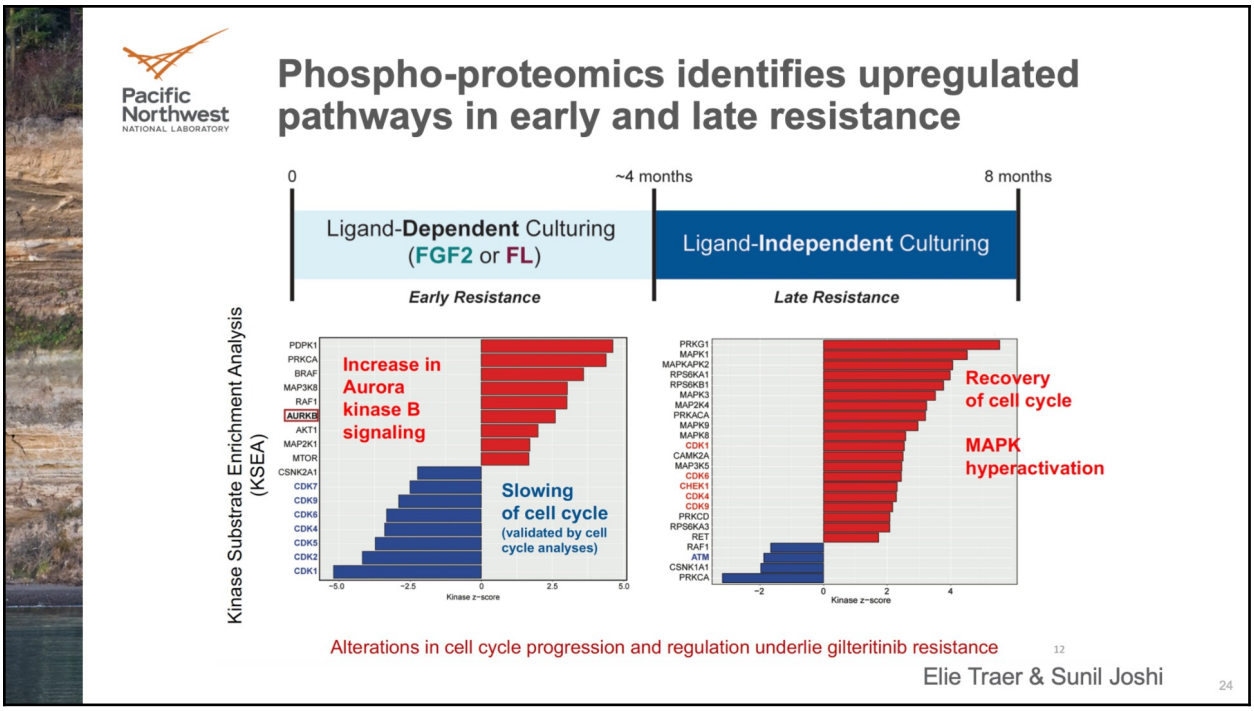
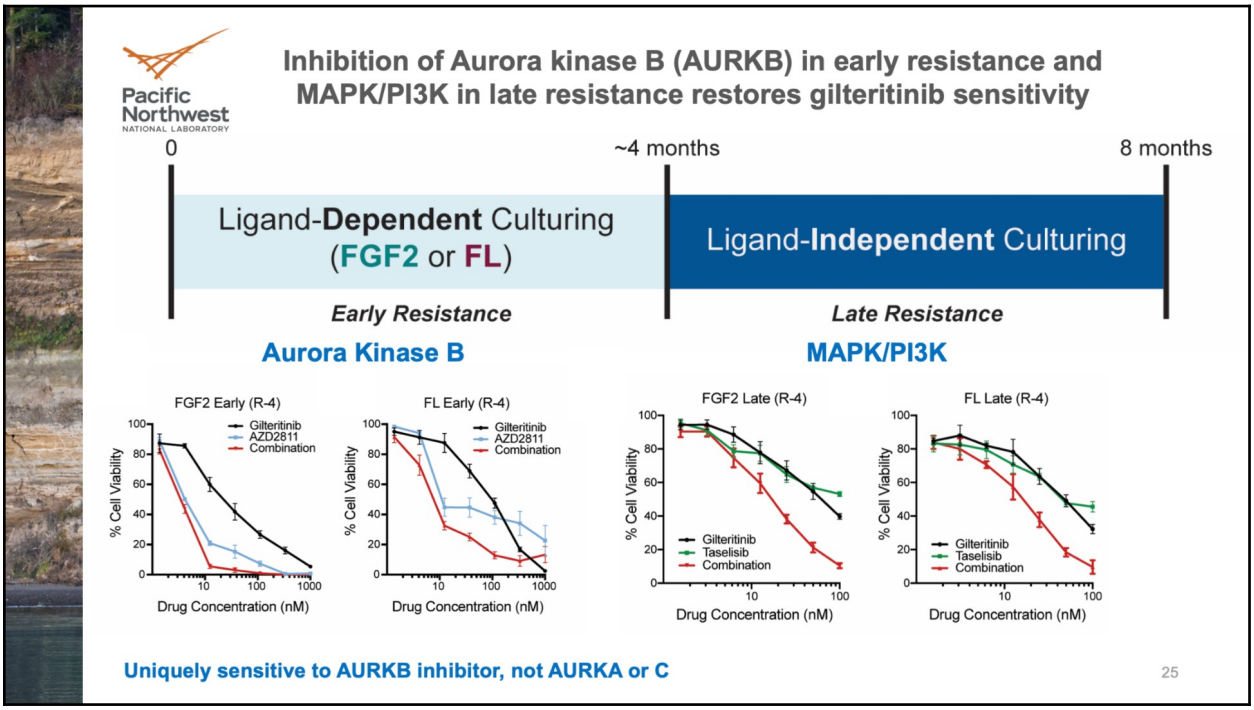
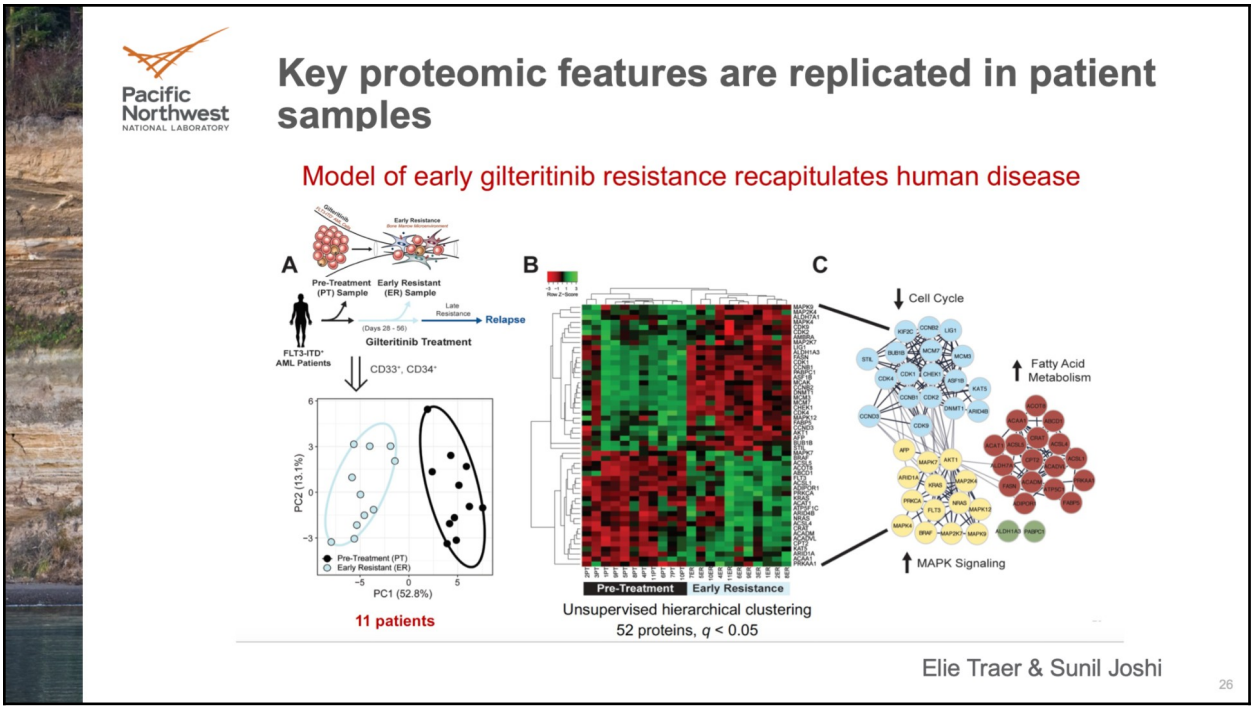


Aurora kinase B turned out to be a very significant protein.
We saw the cell cycle proteins being down-regulated. They came back in late resistance. These cells were on auto throttle. The throttle was stuck in, and they were going no matter what.



We wondered if the Aurora kinase B might be a target to prevent the development of late resistance. They were treated with a drug that inhibits the FLT3 inhibitor, gilteritinib, and

AZD2811, which inhibits Aurora kinase B. They used the two of them together. You can see that whether it was FGF2 or the FLT3, when you used the Aurora kinase B, you killed the cells. When you used Aurora kinase B and the kinase inhibitor, the red line, you killed them even deader, if you want to put it that way. In the late resistance, we had no effect whatsoever of the targeted therapeutic on the FLT3 kinase. But when we combined it with the inhibitor of Aurora kinase B, we did get some cell killing. It was uniquely sensitive to Aurora kinase B, not Aurora kinase A or Aurora kinase C. This is the kind of information that we're after with these studies. I know, you know, it's a research study.



We have cell lines, we have patients, and you're all waiting for this to come around to the point where it will help you specifically. We went on and we did clinical trials. We had samples from patients who were on clinical trials with gilteritinib, and we got samples before treatment during the early resistance phase and during the late resistance phase, and our biomarkers were the same, they were replicated in the actual patient samples. It's not just something that happens in cell culture.

That's my story. That’s translational research. It's still research. We're hoping to be able to take this pipeline and use it to come up with the most common mechanisms by which a particular type of cancer develops resistance to a particular type of therapy, and then inform the clinicians what to do next, or use a combination treatment early in this phase to prevent the outgrowth of the late resistance phase right now.

One of the issues is that drugs are given in sequence. You're given one drug, you have a response, you develop resistance to that drug, the disease comes back, then they put you on a second line therapy. Then if you develop resistance to that, they put you on a third line therapy.

Every time you do that, you select out new clones with new survival mechanisms. If we knew what combinations to use early on, could we actually kill every single solitary tumor cell and there would be nothing left to recur?

Anonymous Caregiver: You triggered a thought for me when you started talking about the serial treatment protocol that most people end up going through. I think about what a relative went through in getting her cancer, ultimately diagnosed. It was a very frustrating process for me to witness as a caregiver, as she was put through one serial test after another, instead of parallelizing it. It took long periods of time to schedule, to get a test, get the results and say, “it's not that,” and in the meantime, she's not being treated for what had developed into very dangerous blood pressure spikes, et cetera. I wonder if you could comment on the need for combination testing early, rather than serial testing, as well.

Karin Rodland: The way we would do it, if you took a look at the “Beat AML” protocol, is when the patient walks in with their initial diagnosis, we would do whole genome sequencing. We would do RNA seq for the RNA seq panels. We would do protein panels, whether they're targeted panels with antibodies, and we would do the ex vivo testing. The “Beat AML” protocol in that particular clinical trial is to do all of the assays at the same time.

The problem – and I've attended about three of these Prostate Cancer Lab meetings – is that there are lots of inherent structural problems in the way we give medical care in this country. Payment is the big issue. As long as payers will only authorize payment for the second test, after the first one has failed, you're not going to have physicians ordering multiple tests at the same time, because they can't get reimbursed for them.

You have an FDA that is very, very conservative. They do not want to approve something that comes back and kills 10,000 people, even if it saved the lives of 10 million, because the 10,000 that died will be blasted all over the media. So they want to only do things one drug at a time because it gets way more complicated when you do two drugs at a time. So they only approve one drug attempt. Now to be fair as a scientist, you can't change two things at once and know which one was important.

You have to change only one thing at a time to know what's important, but we just don't have the investment in the right kinds of clinical trials or preclinical studies to really focus on combination treatments and come up with a rational basis for prescribing combination treatments. You guys have done an end run around the American medical system by taking your care into your own hands and shopping around until you find a doctor or a translational scientist who will do the test you want whether or not it's standard of care.

Maybe if there's enough success with you guys, and you get written up in case reports by that friendly doctor you found who would go outside the standard of care, then maybe we can change the standard of care. My message is to work with your doctor to write up a case report on you.

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Anonymous Caregiver: Why do we consider testing to be qualifying in standard of care? It seems to me, coming from an engineering background, that testing and intervention are two different things.

Karin Rodland: They are. And there's no reason why you can't do every test right off the bat except cost, except for the reimbursement system. I'm going to show my bias. Because we have a for-profit medical system, the number one objective is to hold costs down. You're not going to authorize a test that doesn't have a proven benefit. We have a United States Preventive Services Task Force (an independent, volunteer panel of national experts in disease prevention and evidence-based medicine which works to improve the health of people nationwide by making evidence-based recommendations about clinical preventive services.), who considers that there's a harm in being given a false positive reading. They weigh the harm of a false positive higher than the harm of a false negative. They say we shouldn't have PSA screening. Women should not get mammograms until they're at least 45 years old. They should stop having mammograms after age 72, because you're going to die of something else before you die of breast cancer. We have a federal agency that makes recommendations against tests.

Anonymous Caregiver: There's a harm in not knowing.

Karin Rodland: Exactly. They over evaluate the harm of a false positive. They have some metric about the costs of screening, and how many false positives you're going to have before you have a true positive. Yet every single solitary person I know with a true positive would pay a million bucks.

Anonymous Caregiver: Do you envision a future when patients could write their own lab order, not do their own care or write their own prescription, but just a test?

Karin Rodland: If you're economically privileged, and have the knowledge, you can do that now.

Saed Sayad: I really like this presentation because I believe in 80% of the presentations, there is no clear target question. You mentioned being proactive instead of reactive. Today we do the prostatectomy, and then wait to see if we see recurrence, then we say, “what do we do next?” The majority of the decision tree in the medical field is completely reactive.

What you mention is exactly on target. We need to be proactive. We should see which patient has a high probability of recurrence and decide what we need to do.

I always ask about reproducibility. Based on our analysis of up to 1000 different omics data sets, proteomic data has the lowest level of reproducibility. Compared to mRNA, RNA, and SNP (Single Nucleotide Polymorphism) data and methylation, this is our observation, but it doesn't mean it's true.
How do you deal with this issue? How do you make sure what you are doing on this subset you can extra plate it for the other population.

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Karin Rodland: It's an excellent technical question. We are aware of it. We deal with it. If you were looking at historical data, there were some mass spectrometry technologies at the turn of the century that were inherently irreproducible - SELDI (Surface-Enhanced Laser Desorption/Ionization), and we've gotten rid of it. MALDI (Matrix-Assisted Laser Desorption/Ionization) is not very reproducible. We've determined which mass spectrometry technologies are most reproducible, but there's a stochastic component to detecting anything in the mass spectrometer.

It has to fly. It has to hit the photo detector, and it has to ionize. It's more stochastic than DNA sequencing or RNA sequencing. We know that. The answer is to have large powered studies where you have lots and lots of samples, so that you're averaging out the stochastic component. And there is a lot of human variability. You do not believe anything from mass spectrometry or actually from RNA seq either, unless you have reproduced the finding in a completely independent cohort.

We are doing that for every single discovery experiment. For some of them, we have completed the independent cohort. Some of them are in the process, but that's what you have to do in mass spectrometry. You only believe the things that reproduce in a completely independent patient cohort.

Saed Sayad: I'm not sure if you have access to the public data on the GEO (Gene Expression Omnibus) website or the ArrayExpress (data from high-throughput functional genomics experiments for the research community), because you can find many free public data sets related to biomarkers. I can send you at least 10 different data sets in which you can find tons of biomarkers, but nobody is trying to repeat the same thing and prove it's good, that they are reproducible.

Karin Rodland: There are probably at least three reasons why that is.

1. You get the first publication out and you get your tenure, or your next grant, and there's much less glory in confirming things.
2. You don't get published in the same impact journal. If you did the reproducibility study, and it didn't reproduce, you're not publishing that.
3. It's expensive. You have to go out and find a collaborator with an independent cohort of patients, and go out and get them.

It should be done, but it's not necessarily easy to do.
The pipeline that we are working on with these large programs, like the Early Detection Research Network, is you go from the mass spec to some kind of high throughput, antibody-based measurement, which is more reproducible. Then you can do lots of patients less expensively. You can have a pipeline like that.

Saed Sayad: There are many issues with ROC (receiver operating characteristic) charts (which illustrate the diagnostic ability of a binary classifier system). I've seen many researchers use ROC charts, which are good for assessing the power of the predictability of your model, but

there is no practical use of ROC. There is another option – a decile chart (a data visualization technique that divides a data series into ten equal parts), which I just suggest you look at. You can use a decile chart to answer this question: can we exclude this 50% of patients from this test because they have the very lowest score? There is a cutoff point which we can use in the decile chart, which we don't have in the ROC chart and many tests. We reject them just because there is a bad ROC chart, when in fact, the top 10% of the same group is a very good candidate for testing and you can exclude 90%.

Karin Rodland: A lot of people just use standard R packages, and do whatever has been published. People know what to do with ROC curves. It all depends on the question you want to ask. If the question is screening, then you do have to ask what's the false positive. If the question is surveillance or an additional test, then all you want is a cutoff to cut off the low risk people. There's a problem with a lot of research that people don't actually think through the question they're asking, and what's the most appropriate statistic for the question they want to ask, but that's a whole ‘nother session.

Rick Stanton: Are there any applications for immune modulation?

Karin Rodland: There absolutely are when we do bulk tumor measurements. We are capturing the immune response as well as the epithelial cell response. We are often doing single cell RNA seq, and we're working towards single cell proteomics. We can separate out the immune cell component with a deconvolution program. That's a whole other topic. I am doing so many fun things, I can't talk about them all in one hour.

Brad Power: How long is it before the kinds of tests you've been talking about for AML, ovarian cancer, and prostate cancer would be applicable and available to prostate cancer patients like Brian, Rick, Mike, Ken, and others?

Karin Rodland: They're available now, or soon, if you find the right academic medical center, who's doing the right clinical trial. It's a very hot area of research. How long will it take to make it standard of care? The most optimistic estimate I would say is three years.