“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

Brad Power
September 21, 2022

“When you go from DNA all the way to protein, we know there is a loss of information… All your drugs are actually acting against proteins, so the question becomes, should I measure the levels of protein to see what's actually expressed, whether through mass spectrometry or through immunohistochemistry…? That would be super helpful.” – Sheeno Thyparambil

Meeting Summary

Most cancer patients these days get a genomic analysis (DNA sequencing) of their tumor tissue. Some get transcription analysis (RNA sequencing), even though few oncologists may know how to interpret the RNA results. Few patients get proteomic analysis, so most are missing this opportunity for additional treatment guidance. There are biomarkers that can be identified through proteomic analysis, such as HER2, that can point to targeted drugs with better patient outcomes than standard treatments.

Depending on genomics analysis alone to guide treatment can introduce errors. When you go from DNA to RNA to protein, how much information is lost? When you go from DNA to RNA, let's say 50% of your information is transmitted. And then from RNA to protein, maybe 30% of the information is translated. So, when you go from DNA to proteins (gene expression), we know there is a loss of information. Yet all drugs are acting against proteins, so the question becomes, should I measure the levels of proteins to see what's actually being expressed, either through mass spectrometry or immunohistochemistry, to know if the proteins are being expressed? This can be super helpful.

Dr. Sheeno Thyparambil is the Senior Director (R&D) of the mProbe Precision Oncology division. He has extensive experience in developing and deploying clinical diagnostics products, especially the use of mass spectrometry for clinical tests. He describes how mass spectrometry-based clinical proteomics can guide treatment decisions, providing arguments advanced cancer patients can use with their oncologists to liberate some of their tissue (FFPE) for this test. It’s important to be able to distinguish the additional information that is gained from proteomics, especially beyond genomic testing.

A main reason why a lot of oncologists and patients are interested in mass spectrometry-derived proteomic tests is helping with chemotherapy decisions. Many tests help inform targeted therapy decisions, or whether you need chemo or not, but few help decide what type of chemo regimen to choose. For example, mass spectrometry-derived protein biomarker reports will say, “This patient is likely to respond to some chemotherapies, like epirubicin or doxorubicin, or they will have a resistance to other drugs, such as a cisplatin- or oxaliplatin-based drug.”

On the targeted therapy side, the most popular protein biomarkers are HER2 (human epidermal growth factor receptor 2, an important protein in breast and gastric cancer) and PDL1 (programmed death ligand-1, a protein that helps keep immune cells from attacking non-harmful

cells in the body), but there are others, especially for prostate cancer, such as AR TROP2 (androgen receptor trophoblast cell-surface antigen-2, an important target for antibody-drug conjugates), which point to new drugs. Another example: 15% of all glioblastomas tend to have very high levels of the biomarker TOPO1, which you can treat with a drug (irinotecan). Brian McCloskey has a high expression of B7-H3, for which there are targeted treatments. Knowing his TOPO1 levels could be useful. For patients with metastatic castrate resistant prostate cancer, you should find out what your TROP2 levels are, and if they are high enough, you should consider enrolling in one of two clinical trials of drugs that bind to TROP2.

Proteomic identification of biomarkers can also steer treatment to a clinical trial. For example, a patient showed a very high level of a biomarker (MET, mesenchymal epithelial transition factor receptor), then after a round of chemotherapy, the biomarker jumped. The oncologist decided to switch the patient’s treatment to a phase one clinical trial targeted on the biomarker, which was very successful.

HER2 levels can point to treatments outside of gastric and breast cancers. A patient with pancreatic cancer usually has less than a year of survival. In one case, a pancreatic cancer patient with unusually high HER2 was given an anti-HER2 drug, and this patient is 180 weeks out and still doing very well. HER2 can also be relevant for prostate cancer. In 71 prostate cancer samples, about three to five patients had high levels of HER2. They would want to enroll in an anti-HER2 clinical trial. Even with low HER2, there is a clinical trial that is going on in prostate cancer, which is a combination of a drug for low HER2 – Enhertu or trastuzumab deruxtecan – and a PARP inhibitor.

Mass spectrometry from FFPE tissue can also predict the overall survival of patients. For example, outcomes were accurately predicted in a study of breast cancer patients with high HER2 expression.

The inputs for the test process are relatively straightforward, and the results arrive relatively fast. Once mProbe receives two slides of tumor tissue (FFPE), five days later a clinical report goes to the oncologist with the levels of 72 biomarkers.

What’s next for proteomics? Having examined a few targets in prostate cancer to see what the distribution is, it is surprising to see the number of treatment opportunities, especially for TROP2 and HER2. We can go back at some point and examine the 72 biomarkers to see what else we can find. Not all patients are unique. For every patient, what works? What are the options if something is not working?

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Meeting Notes

SUMMARY KEYWORDS

patient, drug, protein, samples, cancer, biomarkers, levels, clinical trial, proteomics, tissue, adc, clinical, prostate cancer, oncology, gastric cancer, mass spec, slide, express, presenter mode, chemotherapy

SPEAKERS

Sheeno Thyparambil (80%), Brian McCloskey (16%), Saed Sayad (2%), Richard Anders (2%), Emma Shtivelman (<1%)

Brian McCloskey
0:28
Sheeno Thyparambil is going to talk about mass spectrometry-based clinical proteomics for oncology in regulated environments. Sheeno is the Senior Director of R&D at the mProbe Precision Oncology Division. He has extensive experience in developing and deploying clinical diagnostics products. He is the co-inventor on 29 US-issued patents related to the use of mass spectrometry for the development of clinical assays. His specialization is in the integration of molecular oncology, multiomics analysis (analysis of multiple "omes", such as the genome, proteome, transcriptome, epigenome, metabolome, and microbiome), and biomarker data for drug development programs. He's the site head for mProbe’s Rockville unit, which is a CLIA (Clinical Laboratory Improvement Amendments)-certified, CAP (College of American Pathologists)-accredited lab, where he manages a team of scientists, medical doctors, and clinical and R&D staff that is responsible for delivering clinical reports to oncologists for informed decision-making. I am particularly excited about this discussion because, as some of you know, I just had a biopsy about a month ago. I have five cores. I've used one of them for whole exome sequencing, but I have four more at my disposal to shed light on my disease. I'm hopeful that I can have some breakthroughs and be able to leverage Sheeno’s technology.

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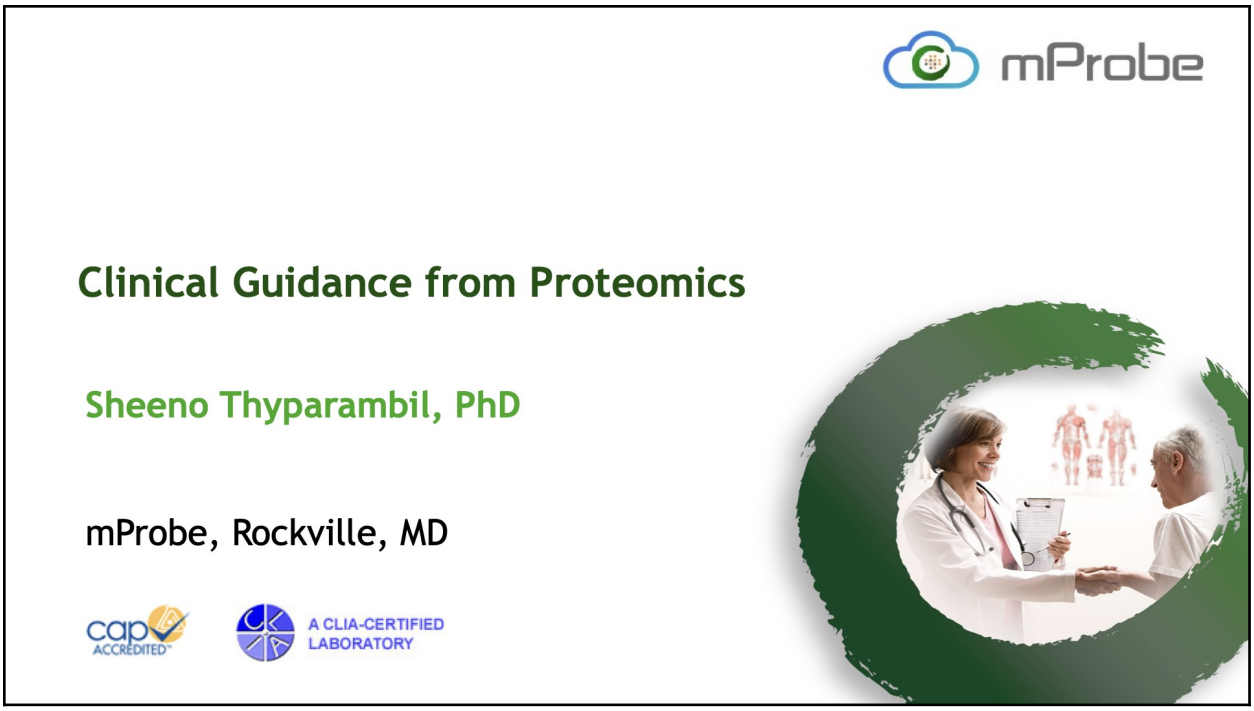

Sheeno Thyparambil
3:42
I'll be talking about the clinical guidance that we have derived in our proteomics reports from 1000s of patients samples.
I am asked a lot: “What are the lessons that you have learned in different cancer types, and in the few prostate samples that you have run over the years? Who has improved? What have you done? And how did you come into this position in the oncology space?”

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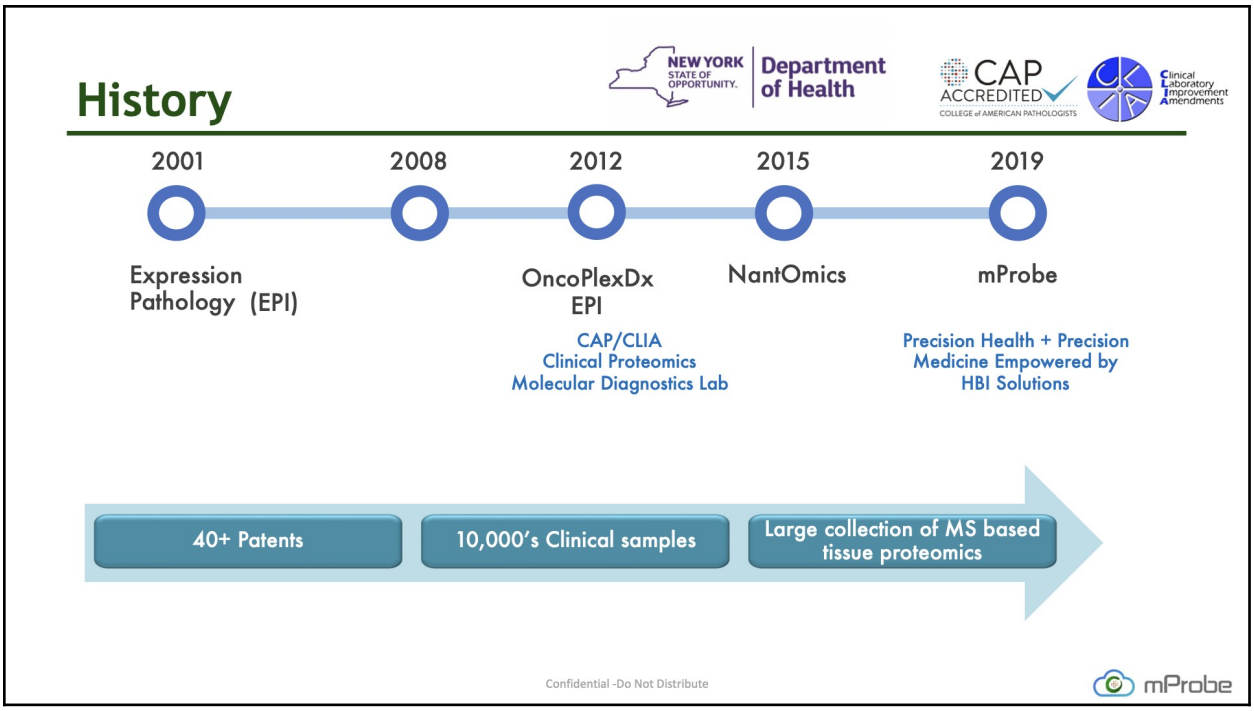

mProbe is my new overlord, but the company really started in 2001 as Expression Pathology. A scientist named Dave Chrisman stepped out from the National Cancer Institute and developed this technology of extracting peptides from formalin fixed paraffin embedded (FFPE) blocks and tissue. Our patent got issued in 2008. I joined in 2009. In 2012 we became a CLIA-certified and CAP-accredited lab. We've been running clinical samples since 2012. We were acquired in 2015 by NantOmics, and we were able to integrate both genomics and proteomics for patients and doctors. mProbe, which is based in San Francisco, was able to get the proteomics lab from NantOmics. What does this all mean? Over the years we have run possibly 10,000 clinical samples. Half of them are actually the CLIA reports that went out, the other was for R&D, mostly for Pharma. We have a large collection of mass spec-based tissue proteomics data.

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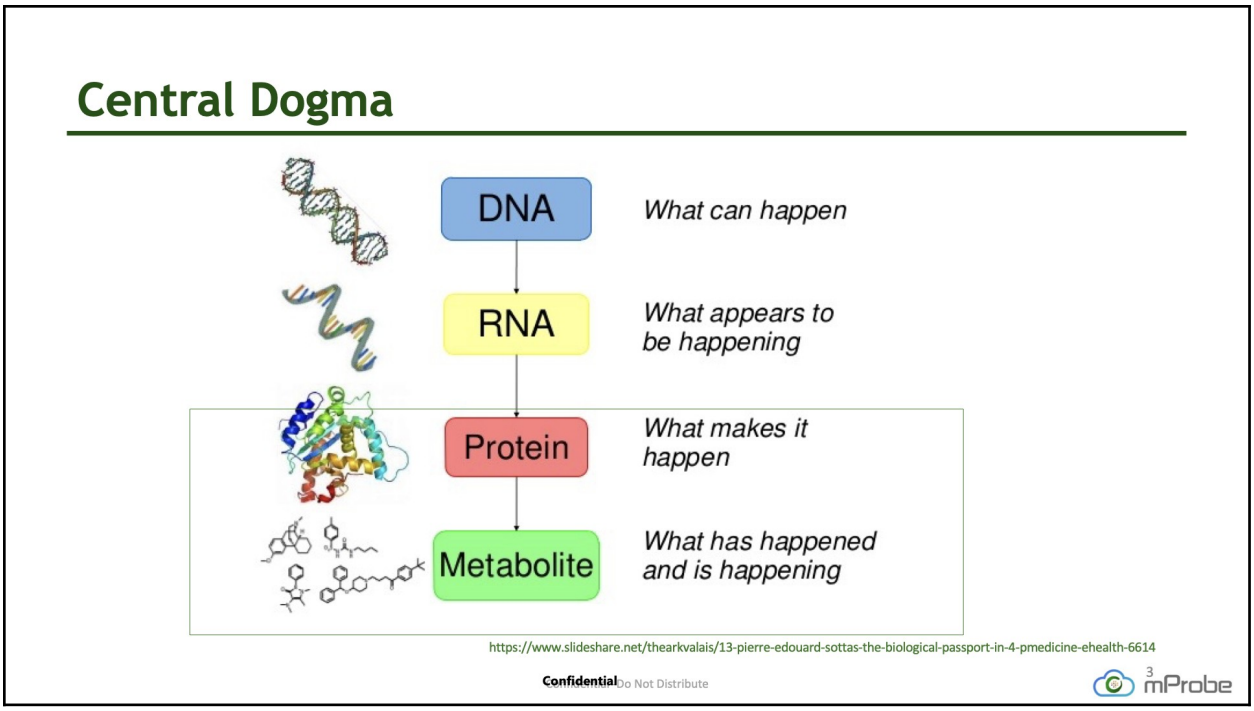
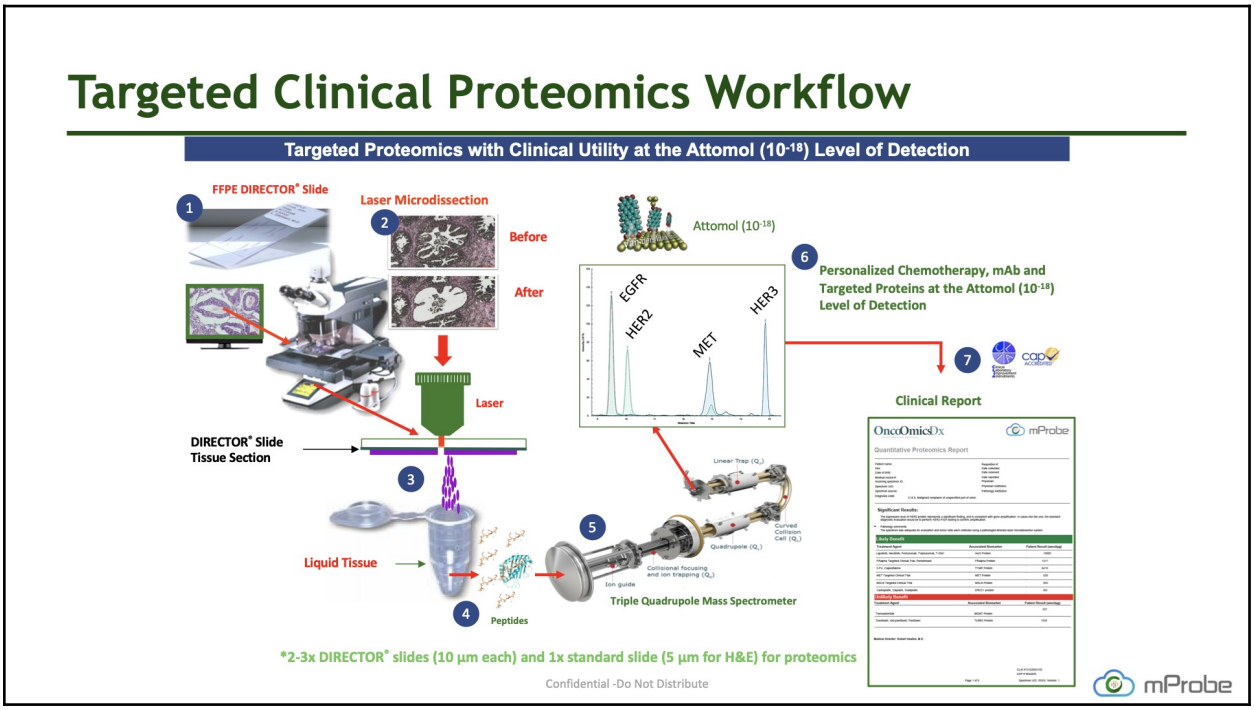


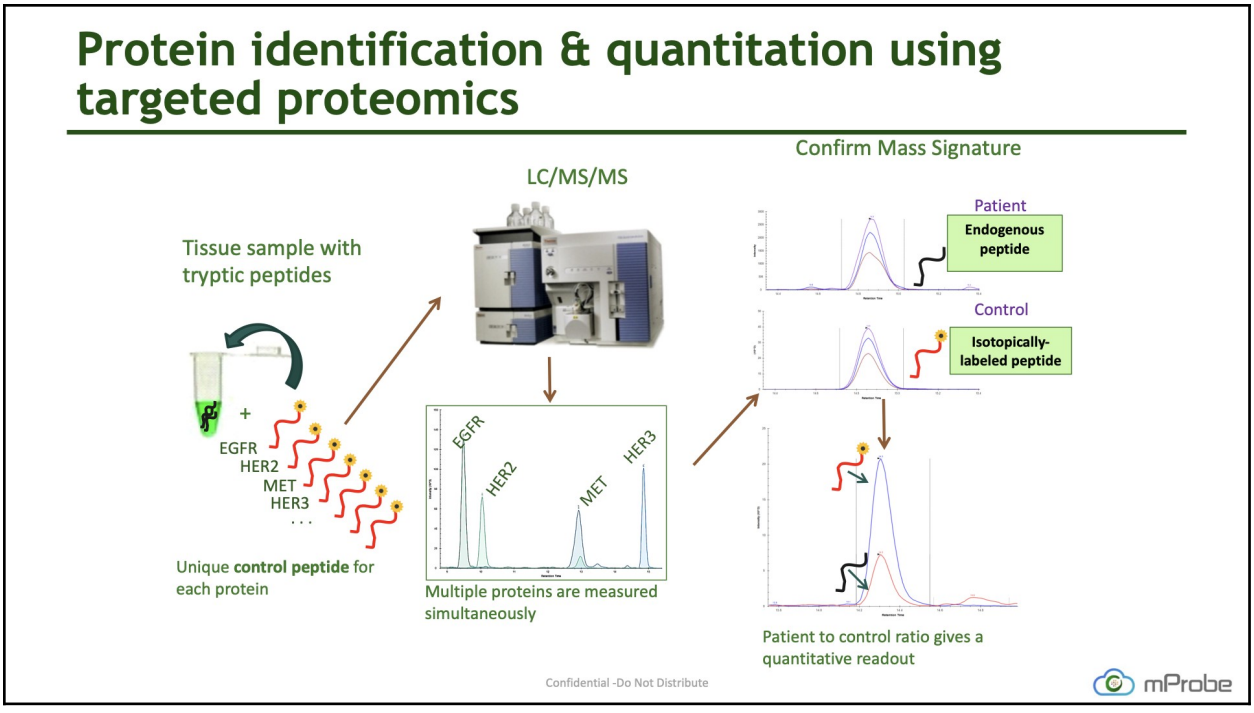
I'm preaching to the choir here, but in the process of DNA to RNA to protein to metabolite, our focus at mProbe is on the protein and metabolites side of things. I'll focus today on the protein side of things.



Sheeno Thyparambil
6:04

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

I'll dive directly into the workflow. This is how it all happens. We have a desk requisition form that is signed off by the oncologist. Our pathology client services team works with the pathology department to get that FFPE tissue block. More often than not, we don't get the block, especially if it is with university systems. So we send our kit to them, which contains a slide that's known as the “DIRECTOR slide”. This is a laser microdissection compatible slide. It's a regular glass slide coated with a proprietary coating so that you can stick it into a laser. A pathologist marks off the tumor areas. Nothing else needs to be added; no coating, nothing on the tumor sample. Once it is in this format, the key invention that happened about a decade plus ago was how to extract peptides from this fixed tissue that you could shoot straight into the mass spec. There are no additional steps other than spinning them down and adding certain buffers. Then it'll go into the mass spec. The beauty of mass spectrometry is the fact that it has been used for a lot of testing in the past. For example, for a vitamin D test that you would get from your physician's office, that is a mass spec-based test. Or a drug abuse test is a mass spec-based test. This has been in the clinical world; it’s just that we had not known much about it. What we essentially did was marry this side of the world with the mass spectrometry side of things. Mass spectrometry comes in two flavors. One is “discovery proteomics”, where you're asking, “What's in my tube? I do not know what's in my tube.” What we are doing here is “targeted proteomics", where we're asking, “How much of a protein of interest, let's say HER2, is there in this tube?” In our case we are looking for 72 biomarkers and quantifying the levels of these biomarkers. Five days later, there is a clinical report that is issued. From start to finish, this is a five-day process. It takes two slides. We're looking at sorts of 10 micron thickness. We're really looking at two to three slides, 20 to 30 micron thickness of slide. Sometimes they ask for a tumor heterogeneity slide. Sometimes we use an H&E (Hematoxylin and Eosin stain) image.



Sheeno Thyparambil

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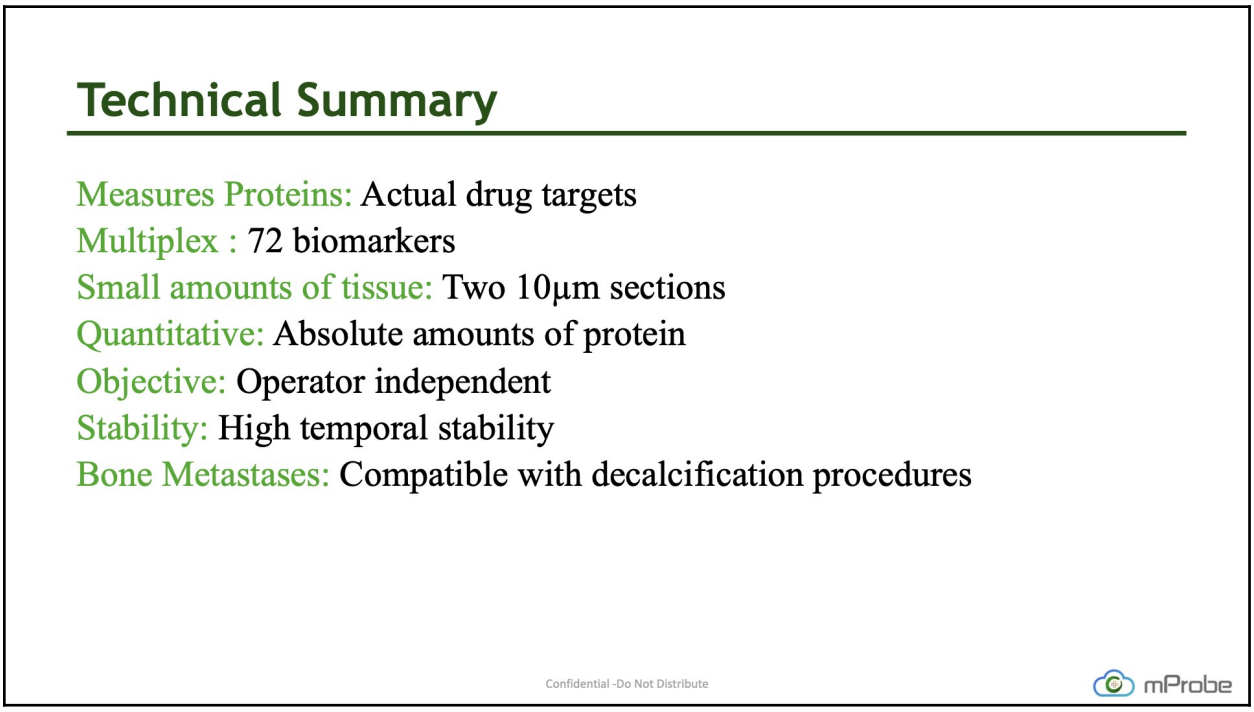
9:56
This is the process, from start to finish. This is what happens behind the scenes. A lot of our time is honestly spent sometimes wrangling the tissue from the pathology labs. That's not a pretty process.



But once it is in house, five days later a clinical report goes to the oncologist. We connect with the oncologist and say, “This is what we saw.” They can get more depth in the clinical report. We are adding heavy peptides, and then we can quantify exactly the levels of 72 biomarkers in that sample.
One thing I want to point out is that for bone mets, this process is compatible. When you have bone tissue, people take an acid, and then it is calcified. If we take an acid decalcification protocol, we end up destroying the DNA, or we end up destroying the shape of the protein. This makes it incompatible for genomic or immunohistochemistry testing. But with mass spectrometry, we don't have that problem, because we are really looking at the linear sequence of the protein. That's technical speak.

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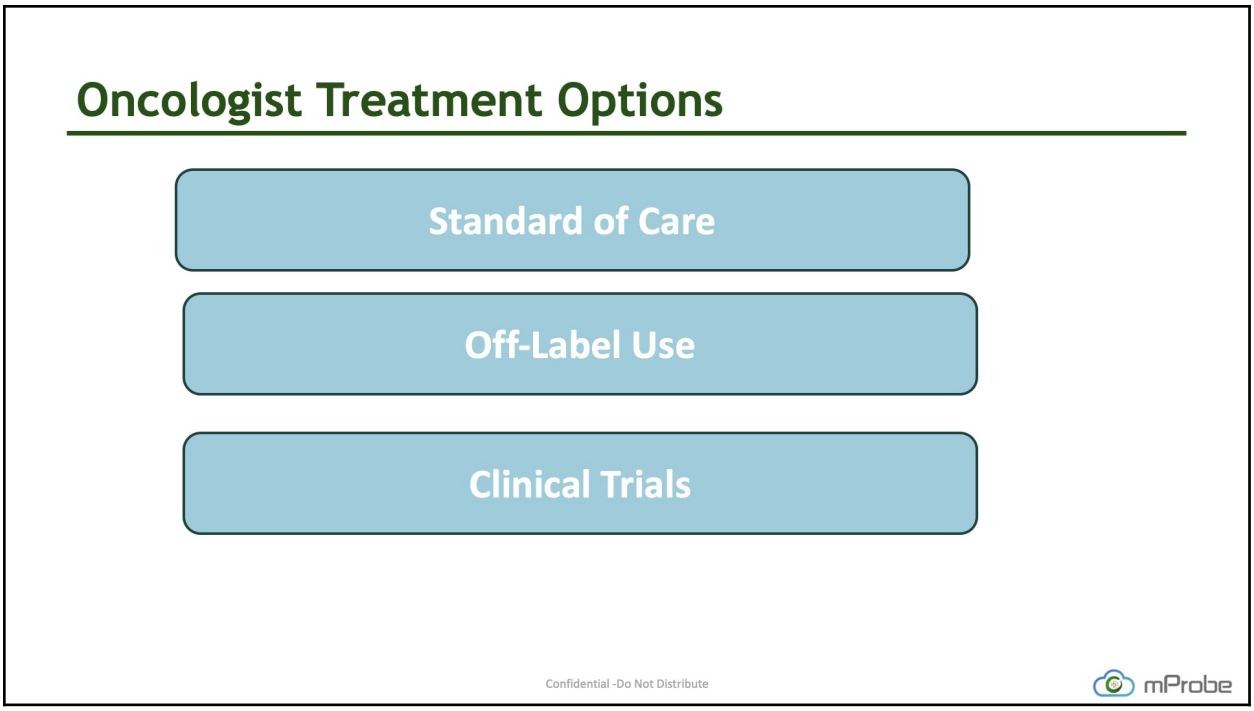

To put ourselves in the shoes of an oncologist, “If a patient is presenting to me, what options do I have?” Stepping back, there are three major ways that an oncologist will treat a patient:

- **Standard of Care**: I can go with the standard of care options. There are a lot of standard of care guidelines out there, and associated treatment options.
- **Off-Label**: We've seen oncologists deploy, especially in the metastatic or late stage setting, off-label use. It's not uncommon to look at the previous JCO reports. 10 years ago, they said 1/3 of all drugs that are given in oncology are off label, i.e., not indicated on FDA prescribing information. There's an awful lot of off label use going on.
- **Clinical Trials**: The other option is I can put this patient into a clinical trial.

How do I decide?
That's what's in my diagnostic report.

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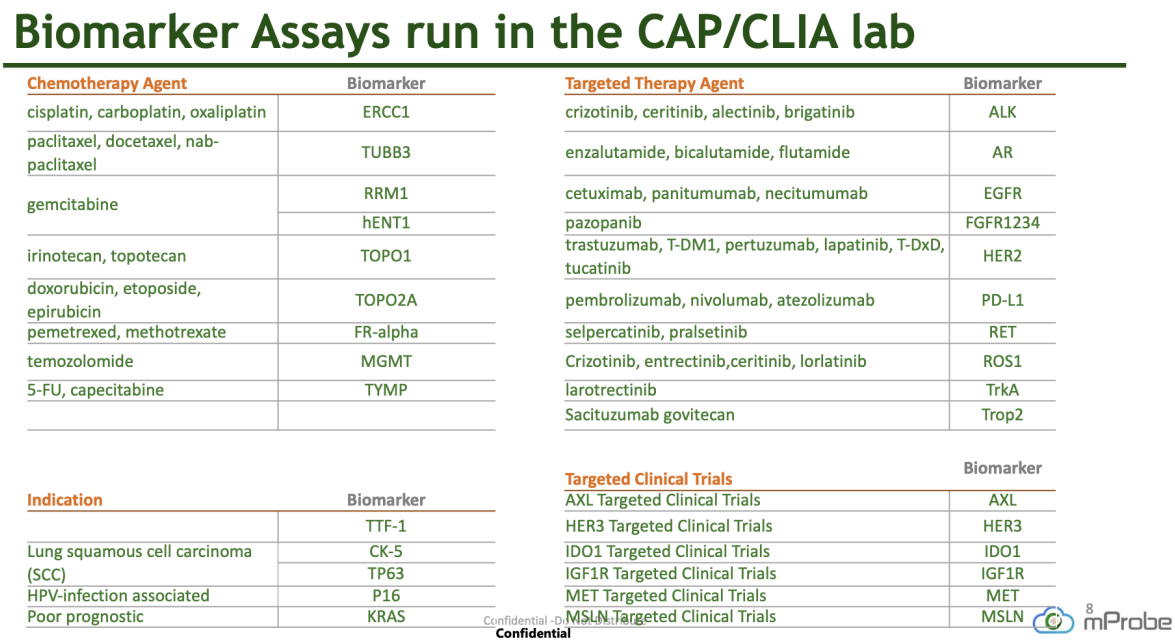

Biomarker Assays run in the CAP/CLIA lab

Chemotherapy Agent	Biomarker
cisplatin, carboplatin, oxaliplatin	ERCC1
paclitaxel, docetaxel, nab-paclitaxel	TUBB3
gemcitabine	RRM1
	hENT1
irinotecan, topotecan	TOPO1
doxorubicin, etoposide, epirubicin	TOPO2A
pemetrexed, methotrexate	FR-alpha
temozolomide	MGMT
5-FU, capecitabine	TYMP

Targeted Therapy Agent	Biomarker
crizotinib, ceritinib, alectinib, brigatinib	ALK
enzalutamide, bicalutamide, flutamide	AR
cetuximab, panitumumab, necitumumab	EGFR
pazopanib	FGFR1234
trastuzumab, T-DM1, pertuzumab, lapatinib, T-DxD, tucatinib	HER2
pembrolizumab, nivolumab, atezolizumab	PD-L1
selpercatinib, pralsetinib	RET
Crizotinib, entrectinib,ceritinib, lorlatinib	ROS1
larotrectinib	TrkA
Sacituzumab govitecan	Trop2

Indication	Biomarker
	TTF-1
Lung squamous cell carcinoma (SCC)	CK-5
	TP63
HPV-infection associated	P16
Poor prognostic	KRAS

Targeted Clinical Trials	Biomarker
AXL Targeted Clinical Trials	AXL
HER3 Targeted Clinical Trials	HER3
IDO1 Targeted Clinical Trials	IDO1
IGF1R Targeted Clinical Trials	IGF1R
MET Targeted Clinical Trials	MET
MSLN Targeted Clinical Trials	MSLN

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We have 72 biomarkers. Broadly speaking, these biomarkers are either meant for chemotherapy, targeted therapy, or for clinical trials. The chemotherapy angle is why a lot of oncologists are interested in this particular test. There are a lot of tests out there that will inform you more on the targeted therapy side of things, but less so on the chemotherapy side of things. What type of chemo agent should I give? There are tests that will tell you whether you need chemo or not. But those tests won't necessarily dwell on what type of chemotherapy I should give. You probably recognize some of the most common ones, especially for prostate cancer, you're thinking about the taxane-based regimens: paclitaxel, docetaxel, abraxane (albumin-bound or nab-paclitaxel), which is a taxane that is coated with albumin. There are a lot of these markers indicating whether you should or should not receive platinum-based therapy. One of the targets that has been used most recently in antibody drug conjugates. The payload on that is basically going to attack the particular molecule topoisomerase I. This is an old drug. How do you deliver potent chemotherapy into a patient's tumor? Some of these biomarkers that we have are what the physicians are using to decide what type of chemo regimen they should go for.

Sheeno Thyparambil
14:44
On the targeted therapy side, the most popular ones are HER2 (human epidermal growth factor receptor 2, a protein in breast cancer) and PDL1 (programmed death ligand-1, a protein that helps keep immune cells from attacking non-harmful cells in the body), but there are others, especially for prostate cancer, such as AR Trop2 (androgen receptor trophoblast cell-surface antigen-2, an important target for antibody-drug conjugates), which point to new drugs. And there are several clinical trial biomarkers as well.
Why the emphasis on chemotherapy?

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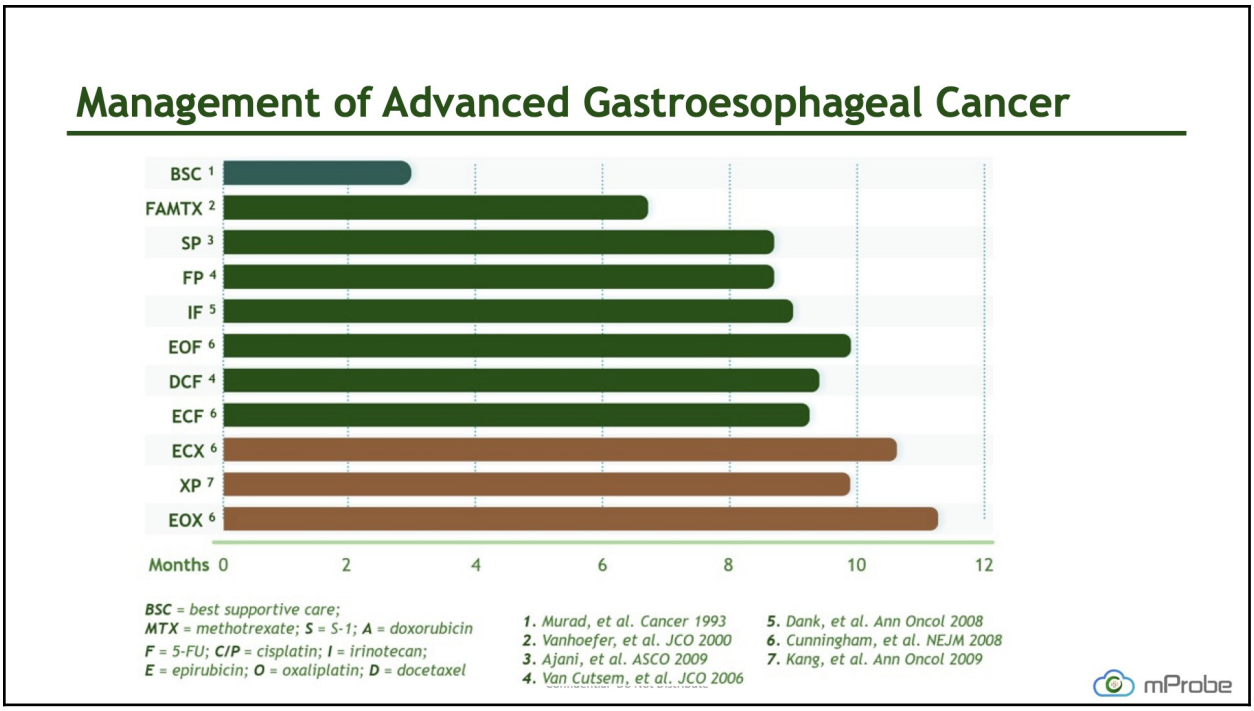

This is a slide of gastric cancer from Dr. Katherine Archie. If you look into advanced gastric cancer, the best standard of care is where you're doing nothing. You're essentially seeing about three months overall survival. But if you look at the different chemo regimens that you can choose, whether you're using for example epirubicin, or doxorubicin, all these combinations, you can see that there is a significant difference. There's a population-wide study of patient outcomes based on the chemotherapy regimen. The question becomes, “How do you choose what kind of chemotherapy regimen to give?” To my knowledge, there are not many tests out there. This is where our reports say, “This patient is going to be likely to respond to epirubicin or doxorubicin or has a resistance to a cisplatin- or oxaliplatin-based drug.”

Sheeno Thyparambil
16:35
Chemo continues to be the treatment backbone for most cancer types. That's one focus area that we have. Over the years, we have run multiple types of cancer. Unfortunately, our prostate cancer database is very small, because for some strange reason, we never focused on prostate cancer. But we have some prostate cancer samples there.

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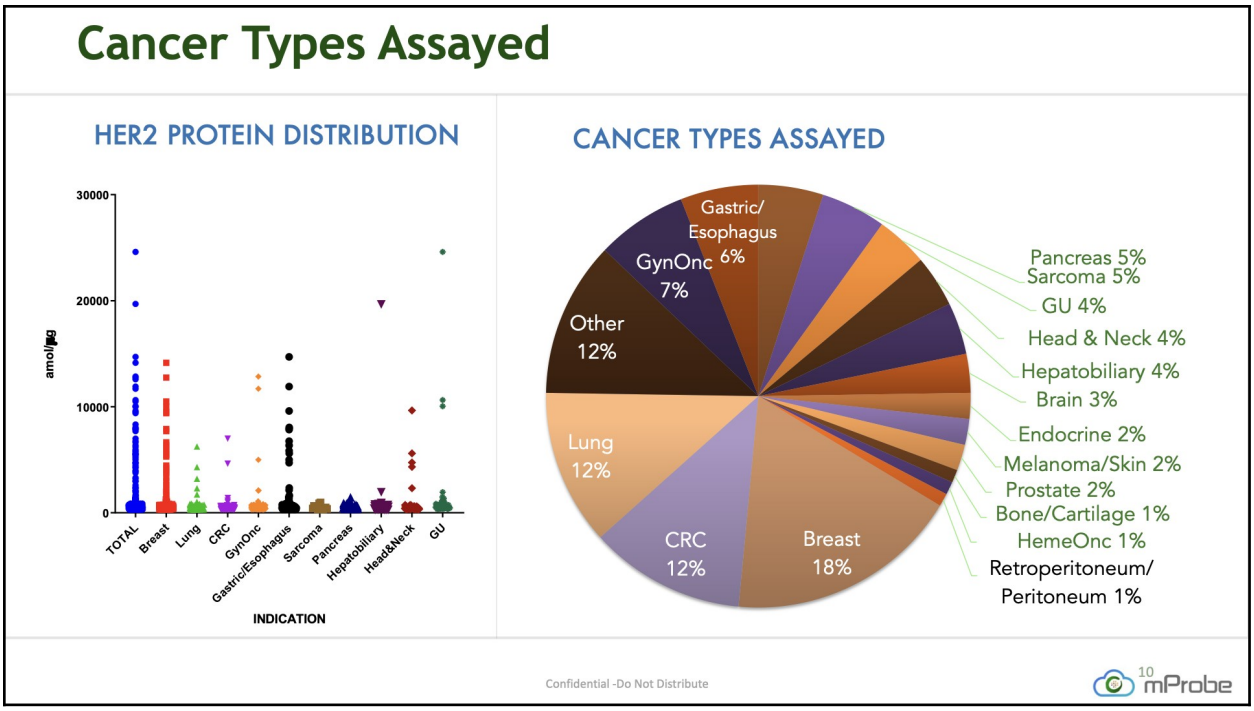
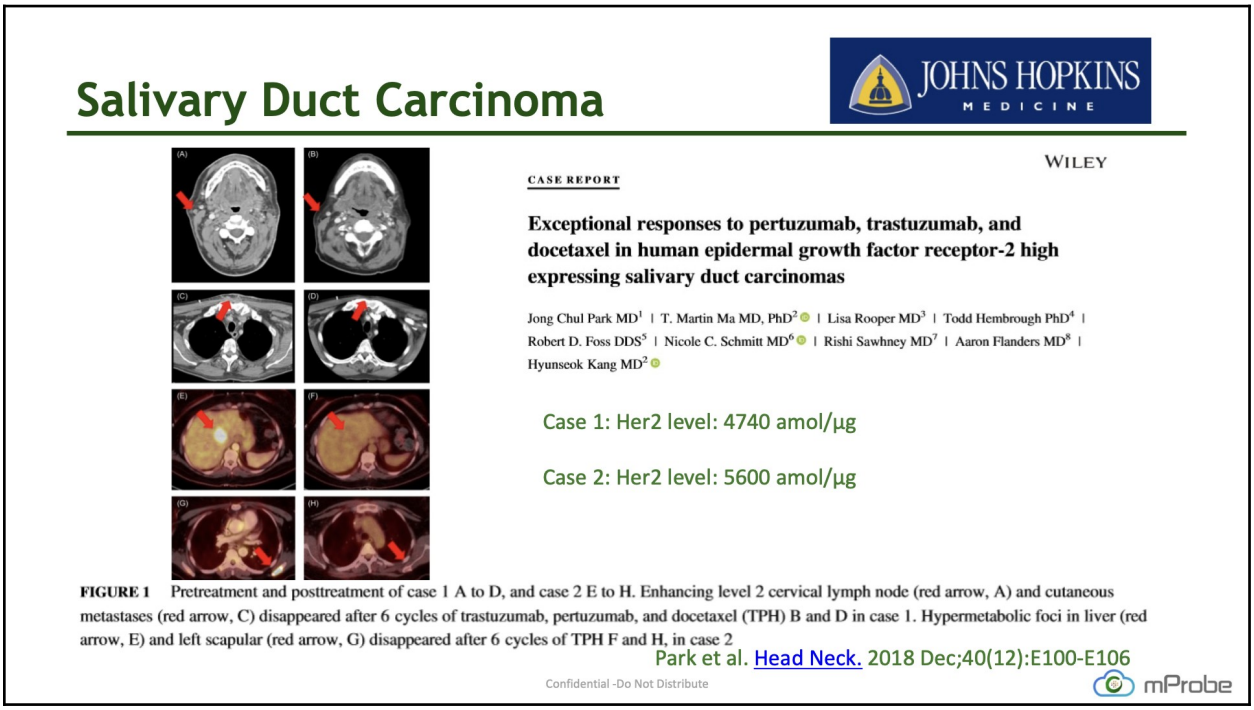


This is a graph showing the HER2 protein distribution. We know that HER2 is really important in breast and gastric cancers, so it is not unsurprising for us to see those levels. We occasionally see outliers of HER2 in other cancer types.



Here's an example of one cancer type, head and neck cancer, in which salivary gland carcinoma came in.

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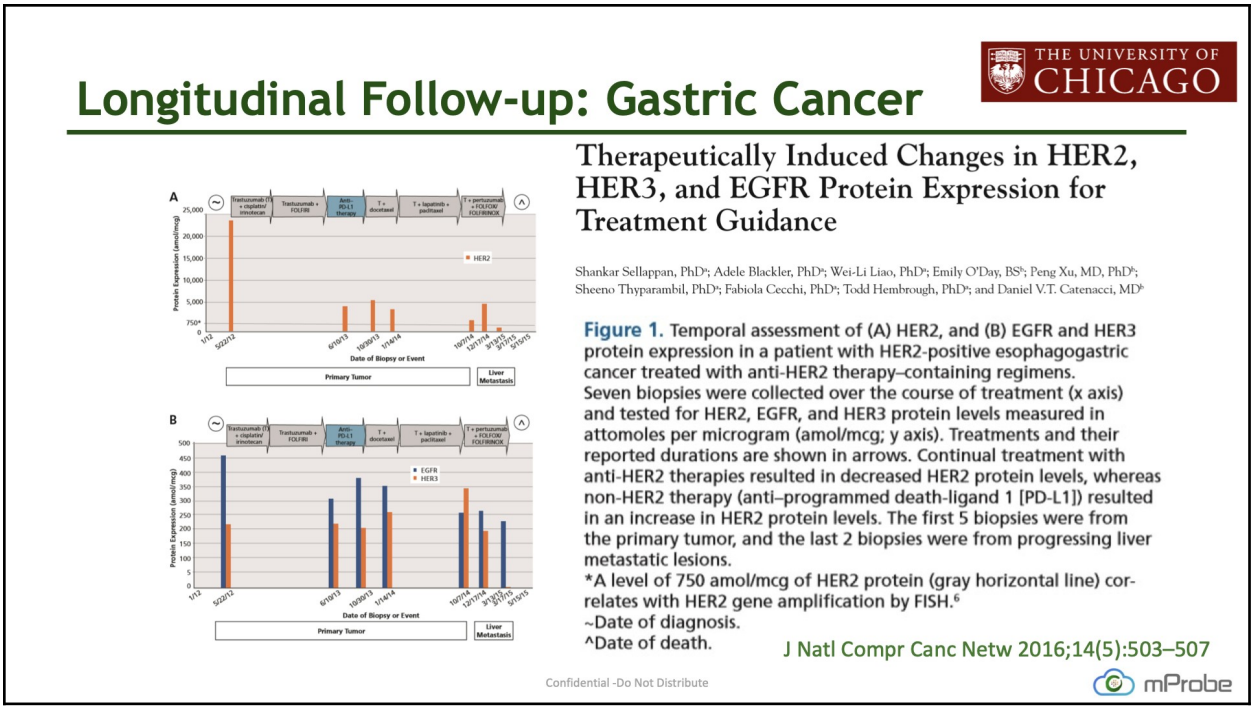
I'll show you some case reports. We had measured the levels of HER2 as a point of reference. Anytime you're more than 750 amol per microgram, you are over-expressing HER2.



Here's a patient from Johns Hopkins that was referred to us. This patient had really high levels of HER2. They treated the patient with a dual combination of two different anti-HER2 therapies at the same time, pertuzumab and trastuzumab, and the patient saw vast improvement in their outcome. We were chasing one particular patient in their cancer episode, where we were looking at targets for HER2, and the physician gave a trastuzumab-based drug. But importantly, when you give a trastuzumab-based drug, the cancer will find a way to evade that, and it will tend to upregulate HER3 levels. We saw that happening, and then he switched and gave a combination of trastuzumab and pertuzumab. And then immediately, we are also seeing another mechanism of resistance go up. So they added more drugs to this patient. This patient had a pretty long survival for an advanced gastric cancer patient. I think it was 39 months compared to less than 12 months.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

Gastric linitis plastica

GUSTAVE ROUSSY CANCER CAMPUS GRAND PARIS

Case report

TJ Tumori Journal

Personalized therapy based on sequential molecular analysis leads to 30 months of survival in a patient with diffuse unresectable gastric linitis plastica

Tumori Journal
2018, Vol. 104(6) NP38–NP41
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DOI: 10.1177/0300891618763215
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SAGE

Linda Mahjoubi[1], Fabiola Cecchi[2], Christophe Massard[1], Fabio Calabro[3], Anas Gazzah[1], Rastislav Bahleda[1], Philippe Jamme[1], Maximiliano Gelli[4], Diane Goere[4], Ludovic Lacroix[5,6], Julien Adam[6], Lukas Heukamp[7], Patrizia Trenta[3], Todd Hembrough[2], Jean-Charles Soria[1,8], Cora Sternberg[3], Michel Ducreux[9]

Based on these proteomic and genomic findings, weekly paclitaxel was selected (Figure 1) and targeted therapy with pertuzumab and trastuzumab was maintained. The patient has been stable for 1 year.

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These are more examples of where our work helped for some of these patients, especially in the gastric cancer space. That's where the drug is in the market.

Gastric/gastroesophogeal junction

Patient results

THE UNIVERSITY OF CHICAGO

Patient

Male, age 59
- Moderately differentiated invasive esophageal adenocarcinoma

Multi-protein expression analysis by SRM-Mass Spectrometry

Assay	Protein marker	Patient Result (amol/µg)	Implication
MET targeted clinical trial	MET	1480	Potential benefit
Cetuximab	EGFR	437	Potential benefit
Targeted therapies	IGF1R, HER2, HER3, KRAS	ND	Reduced likelihood of benefit

Initially 1480 amol/ug (D105C) at diagnosis;
then **2090** amol/ug (D484C) after chemotherapy.

MET inhibitor TKI phase I trial

mProbe

Here's an example of a drug that's not in the market. It is in clinical trials. For this patient we saw very high levels of MET (mesenchymal epithelial transition factor receptor) at 1480. The physician continued to go with the standard of care, then with chemotherapy, and then it

bumped up because that seemed to be a favorite pathway. The patient had a 2090 the next time he was examined. He decided to participate in a phase one clinical trial.

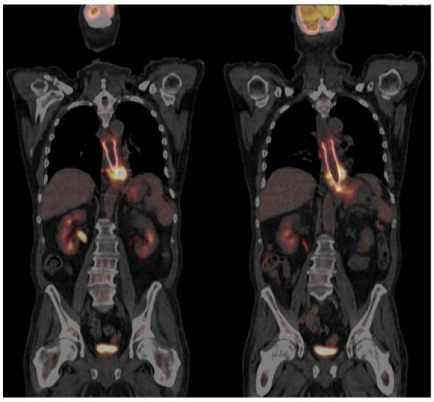
Gastric/gastroesophogeal junction

Patient results

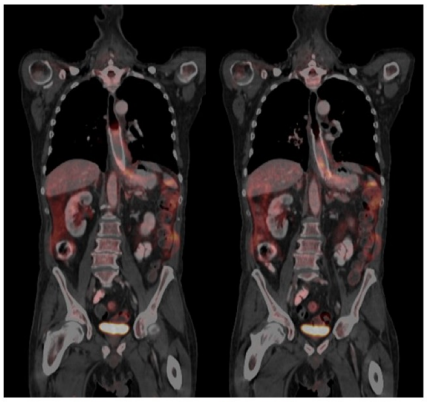
THE UNIVERSITY OF CHICAGO

Pre/Post MET TKI x 6 weeks

Baseline PET
7-22-2014



Post-MET TKI
9-10-14



Catenacci et al. 2014

mProbe

You can see in the scans from July to September that that patient got their cancer resolved. We were able to help a physician make a decision on moving the patient into a clinical trial.

Pancreatic Cancer

Complete remission in a patient with widely metastatic HER2-amplified pancreatic adenocarcinoma following multimodal therapy informed by tumor sequencing and organoid profiling

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20. Independent Clinician, Saratoga, CA

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https://www.medrxiv.org/content/10.1101/2021.12.16.21267326v1.full.pdf

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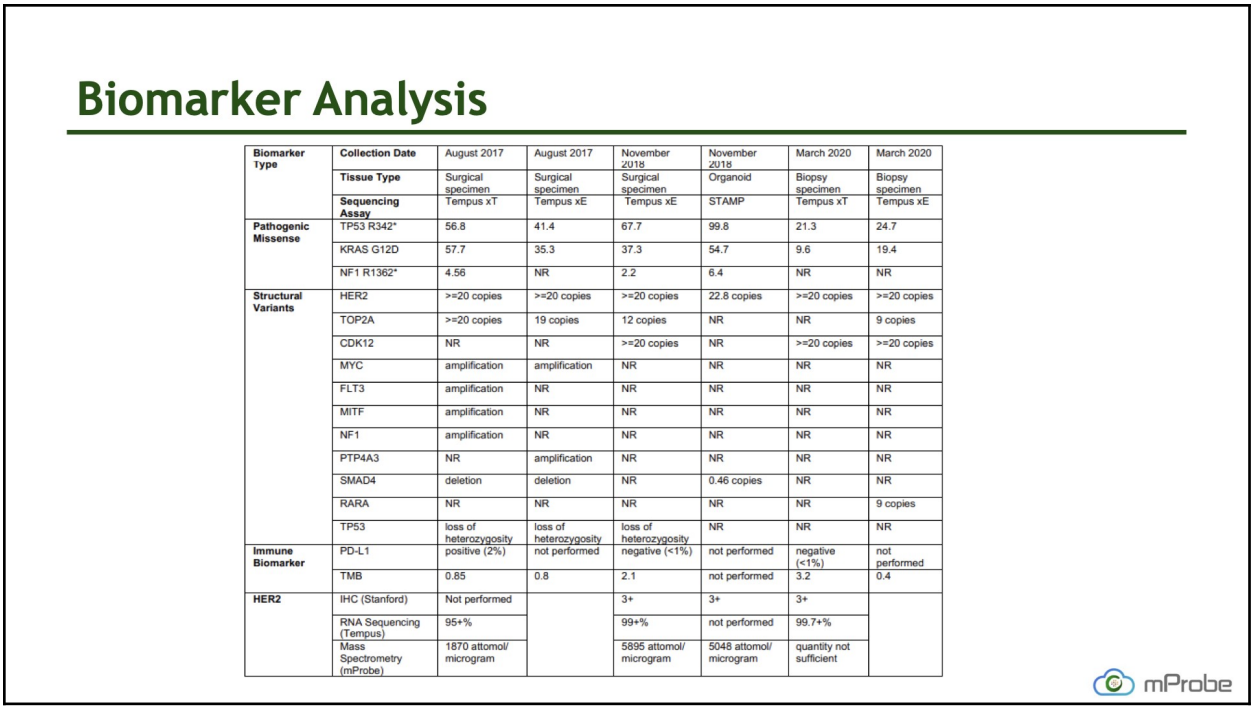
This paper is yet to come out; it's out online. This is one thing that we most recently did in pancreatic cancer. Pancreatic cancer is one of those horrible cancers. This particular patient had complete remission in HER2 pancreatic cancer. There's a whole slew of people in here. And you probably recognize some of some of these companies as well.

Brian McCloskey
20:50
One of them is xCures. Emma might have been involved.

Emma Shtivelman
21:00
No, sorry. I am not involved in xCures trials.

Sheeno Thyparambil
21:09
Dan King was a fellow at Stanford when we did this work. But now he's moved on to Northwell. He's continuing to work with us in pancreatic cancer.



Biomarker Type	Collection Date	August 2017	August 2017	November 2018	November 2018	March 2020	March 2020
	Tissue Type	Surgical specimen	Surgical specimen	Surgical specimen	Organoid	Biopsy specimen	Biopsy specimen
	Sequencing Assay	Tempus xT	Tempus xE	Tempus xE	STAMP	Tempus xT	Tempus xE
Pathogenic Missense	TP53 R342*	56.8	41.4	67.7	99.8	21.3	24.7
	KRAS G12D	57.7	35.3	37.3	54.7	9.6	19.4
	NF1 R1362*	4.56	NR	2.2	6.4	NR	NR
Structural Variants	HER2	>=20 copies	>=20 copies	>=20 copies	22.8 copies	>=20 copies	>=20 copies
	TOP2A	>=20 copies	19 copies	12 copies	NR	NR	9 copies
	CDK12	NR	NR	>=20 copies	NR	>=20 copies	>=20 copies
	MYC	amplification	amplification	NR	NR	NR	NR
	FLT3	amplification	NR	NR	NR	NR	NR
	MITF	amplification	NR	NR	NR	NR	NR
	NF1	amplification	NR	NR	NR	NR	NR
	PTP4A3	NR	amplification	NR	NR	NR	NR
	SMAD4	deletion	deletion	NR	0.46 copies	NR	NR
	RARA	NR	NR	NR	NR	NR	9 copies
	TP53	loss of heterozygosity	loss of heterozygosity	loss of heterozygosity	NR	NR	NR
Immune Biomarker	PD-L1	positive (2%)	not performed	negative (<1%)	not performed	negative (<1%)	not performed
	TMB	0.85	0.8	2.1	not performed	3.2	0.4
HER2	IHC (Stanford)	Not performed		3+	3+	3+	
	RNA Sequencing (Tempus)	95+%		99+%	not performed	99.7+%	
	Mass Spectrometry (mProbe)	1870 attomol/ microgram		5895 attomol/ microgram	5048 attomol/ microgram	quantity not sufficient	

This was one of those beautiful examples. Pancreatic cancers generally don't tend to have that much high HER2. You can see our mass spectrometry results. Remember, 750 is the cutoff for high expression. It is 1870 in 2017. In 2018 look at that, it just bumped up from 1870 to almost 6000. This is the time to put this patient on an anti-HER2 therapy.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

Treatment Protocol and Outcome

Figure 1

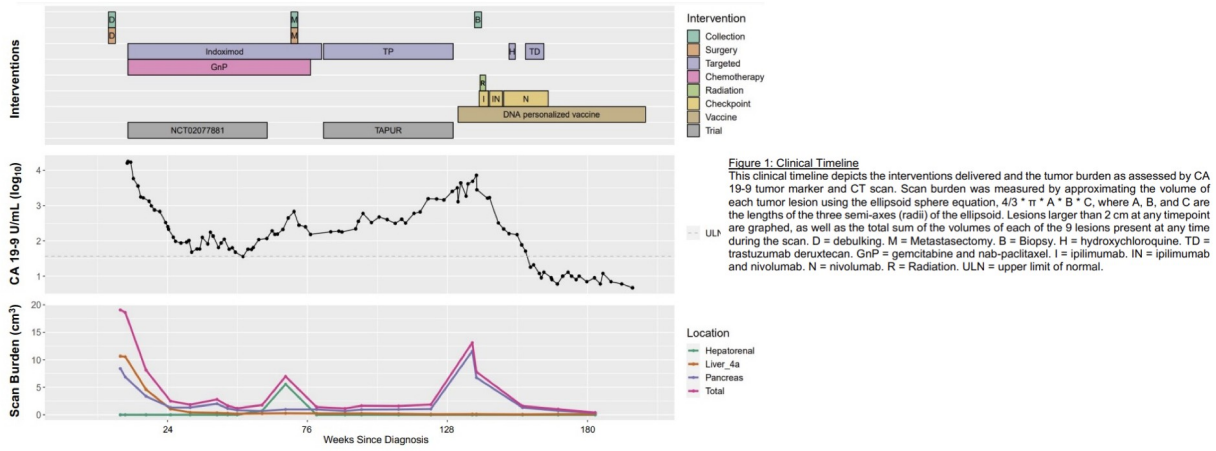


Figure 1: Clinical Timeline
This clinical timeline depicts the interventions delivered and the tumor burden as assessed by CA 19-9 tumor marker and CT scan. Scan burden was measured by approximating the volume of each tumor lesion using the ellipsoid sphere equation, 4/3 * π * A * B * C, where A, B, and C are the lengths of the three semi-axes (radii) of the ellipsoid. Lesions larger than 2 cm at any timepoint are graphed, as well as the total sum of the volumes of each of the 9 lesions present at any time during the scan. D = debulking. M = Metastasectomy. B = Biopsy. H = hydroxychloroquine. TD = trastuzumab deruxtecan. GnP = gemcitabine and nab-paclitaxel. I = ipilimumab. IN = ipilimumab and nivolumab. N = nivolumab. R = Radiation. ULN = upper limit of normal.

mProbe

If you know pancreatic cancer, it's usually less than a year of survival. This patient is at this point 180 weeks out. And she's still doing very well.

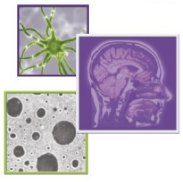
Saint Luke's

Case Report: Glioblastoma

Research Article

For reprint orders, please contact: reprints@futuremedicine.com

CNS Oncology



Comparative proteogenomic characterization of glioblastoma

Samia Asif*,[1], Rawish Fatima[1], Rebecca Krc[1], Joseph Bennett[1] & Shahzad Raza[1]
[1]Saint Luke's Cancer Institute, University of Missouri, Kansas City, MO 64111, USA
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Proteomic analysis in one patient in our study group revealed the presence of TOPO1 and hENT1. TOPO1 is a potential targetable protein for treatment with irinotecan and topotecan. hENT1 protein is a possible target for treatment with gemcitabine [37]. After this patient relapsed within 13 months of standard therapy with TMZ, she was treated with irinotecan and demonstrated good response with PFS at 12 months after its initiation.

Asif et al. CNS Oncol. 2019 Jul 10:CNS37

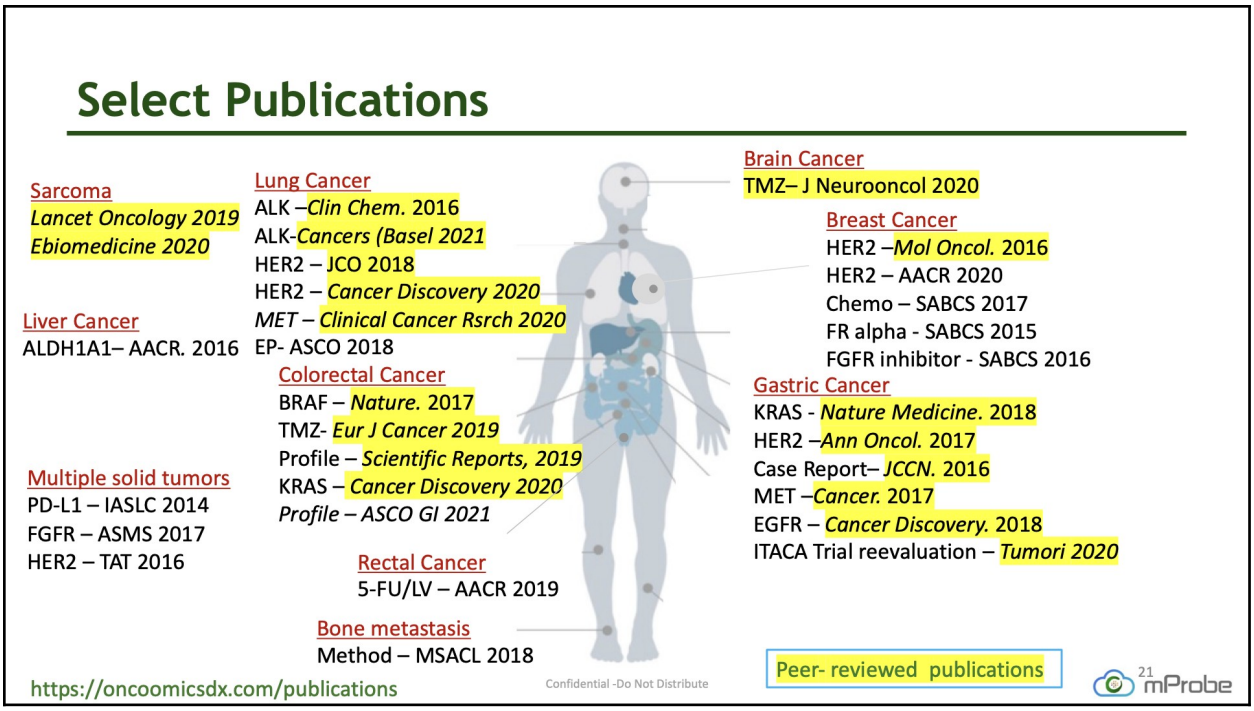
mProbe

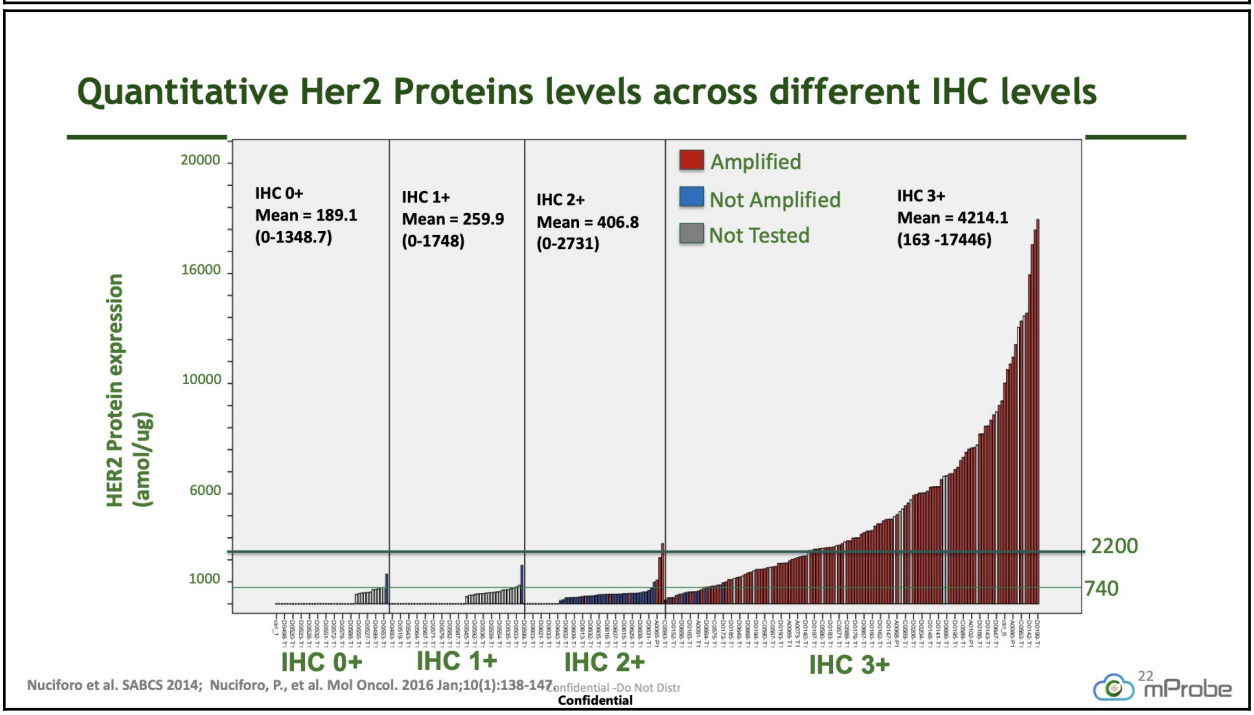
Sheeno Thyparambil
22:17

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

This is a case report of glioblastoma, where a physician said, “I'm going to use your proteomics report.” I'm going to put this patient on irinotecan, which is a cheap drug compared to everything else. The patient had recurrent glioblastoma and PFS of 12 months. Something else that we are putting out in brain cancer shows that 15% of all glioblastomas tend to have very high levels of TOPO1, which you can treat with irinotecan. If you ask a “normal” oncologist, they will say that they don’t want to use irinotecan because it has so many toxicity issues, people are vomiting and all that stuff. Agreed. But did you really look into whether your particular patient had high levels of TOPO1? If so, you might want to include a patient in an irinotecan study.
We've done a lot more work in a lot of different cancer types. Unfortunately, we still haven't done anything in prostate cancer. Most of our work has been focused on gastric and colorectal cancers, understanding the mechanisms of resistance, either big enough, and several case reports, and all that stuff.

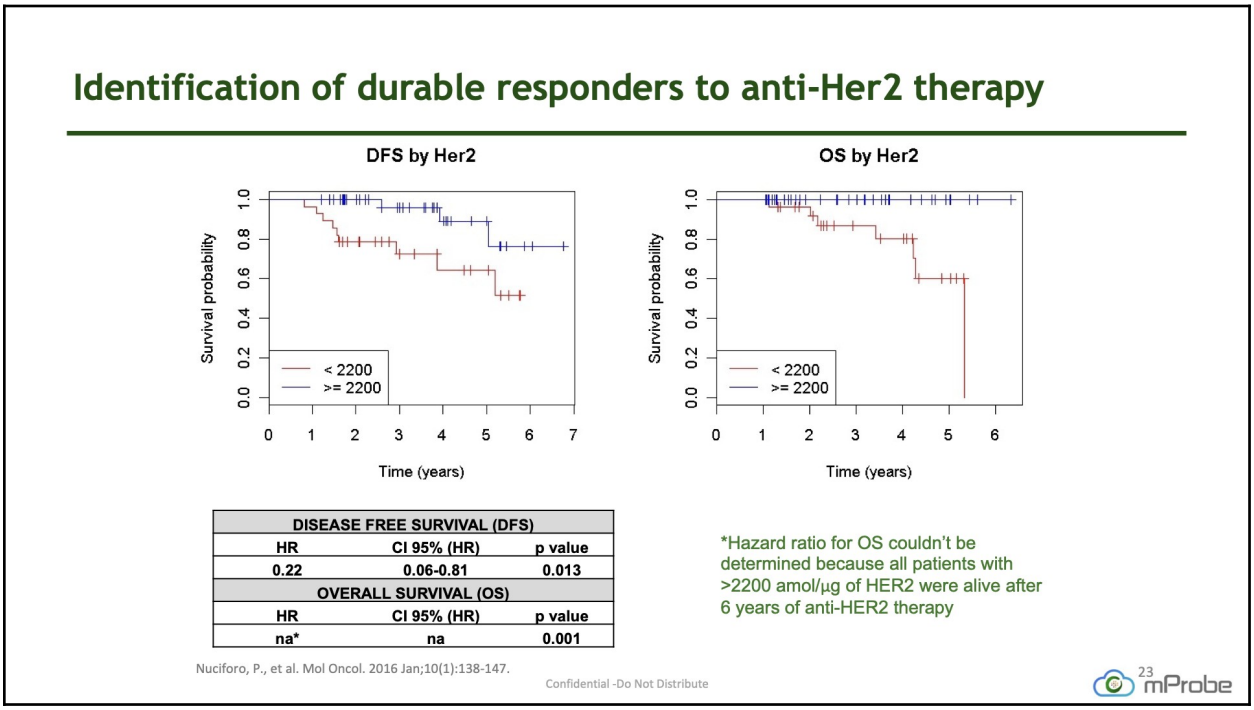
“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]



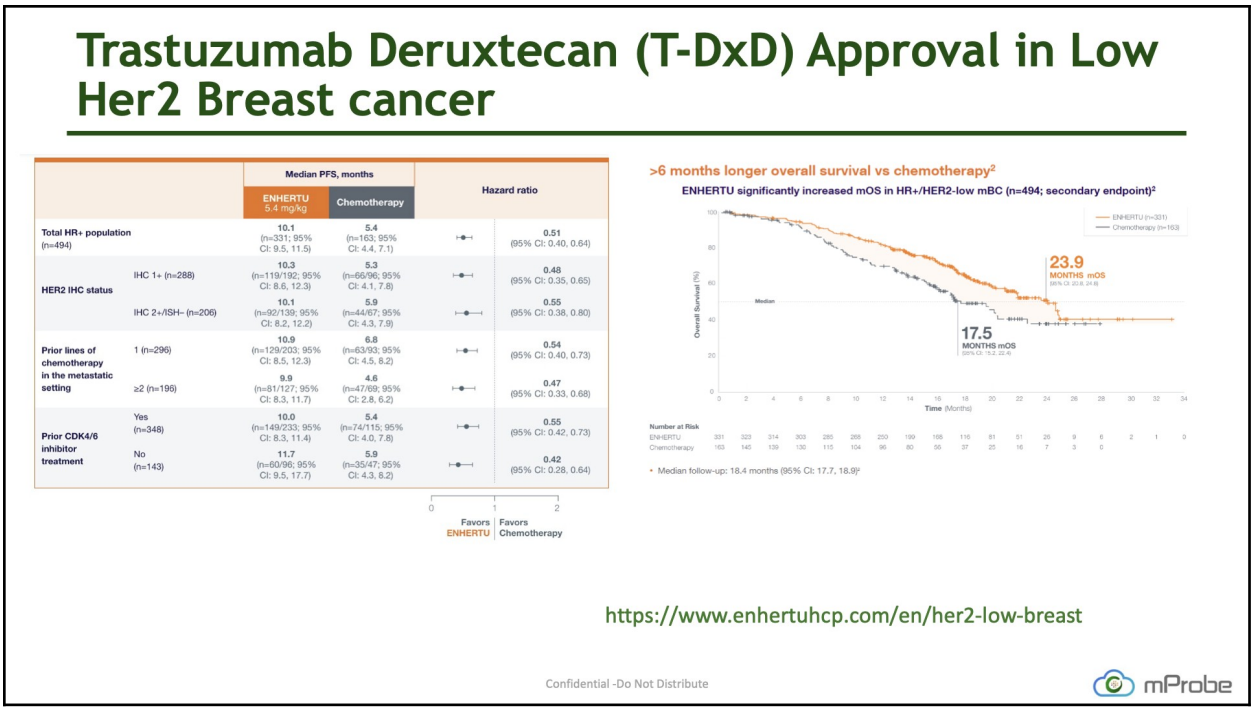


Let me switch gears a little bit here. I need to talk to you about something that is becoming important in HER2. In immunohistochemistry we have zeros, ones, twos, and threes. We got 277 breast cancer patient samples in work that we did with Walter Brown and MSK. We didn't see what we expected in the four different buckets, based on the brown of the stain. It was not unsurprising that we saw HER2 levels overexpression in IHC 3+. But we didn't expect the difference between the highest and the lowest ones. What was surprising to us was the IHC zeros and ones which were historically not treated, or even some that were not amplified, like

50% or 25% of them, had some levels of HER2 in them. If you had asked this question about two to three years ago, people would have said, “Yeah, forget it. Let's ignore the zeros, ones, and twos because they don't really have enough HER2 in them to be actionable.” We had this paper out in 2015-2016 in which we examined some very high expression HER2 levels to see how the overall survival was, again with 740 or 750 being the cutoff for high overexpression of HER2. Long story short, those patients who had 2200 amol per microgram, or in other words, very high expression, had no events at all. You're looking at six or seven years out, when they were treated with an anti-HER2 therapy. Great. So this was a first proof that mass spectrometry from FFPE tissue can predict the overall survival in these patients. This paper opened the doors for us to do a lot more investigations with many, many institutions, such as MSK, University of Chicago, you name it. But going back to this particular figure of IHC 3+ in breast cancer. If you step back and look at all the breast cancer folks, they're roughly 15% to 20%. The rest, the 80%, is really the zeros, ones, and twos. Let's say the zeros are triple negative for this particular argument, and they are usually 15%. The vast majority of the folks are really lying in this ones and twos.

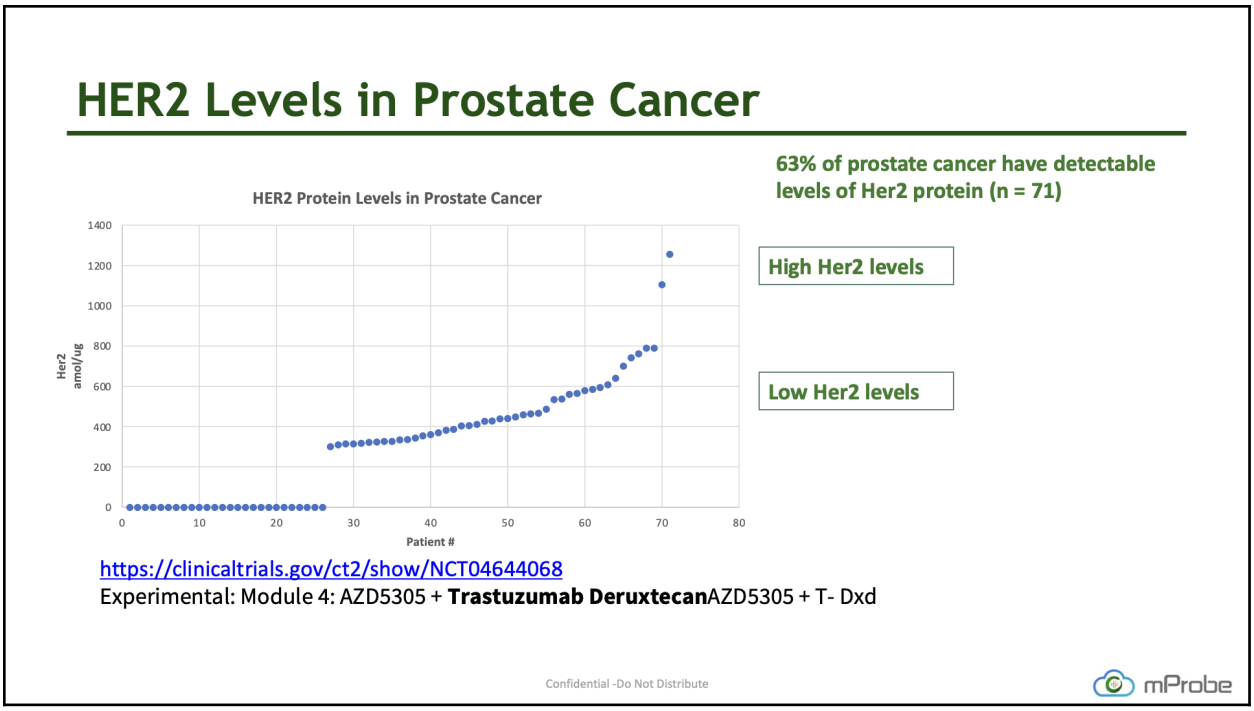


“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]



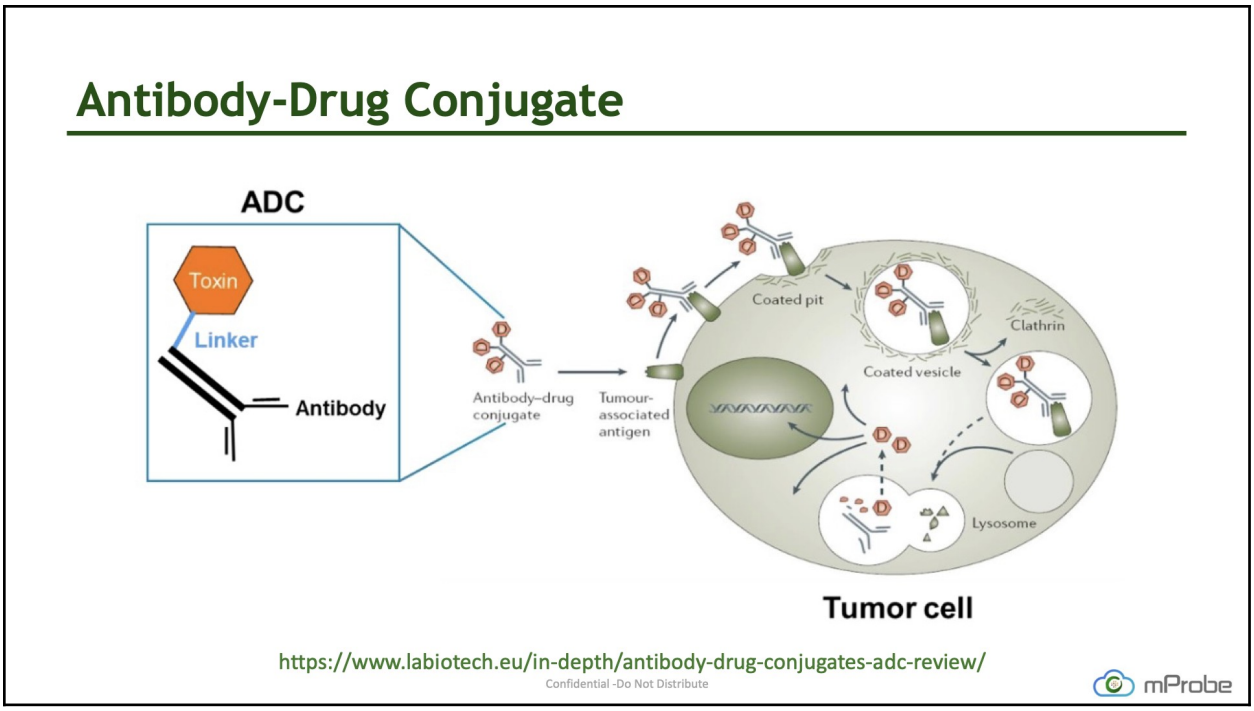
So fast forward two years, there is a drug by the name, trastuzumab deruxtecan (sold under the brand name Enhertu, an antibody-drug conjugate consisting of the humanized monoclonal antibody trastuzumab covalently linked to the topoisomerase I inhibitor deruxtecan. It is licensed for the treatment of breast cancer or gastric or gastroesophageal adenocarcinoma.), an anti-HER2 drug that is from Daiichi Sankyo and AstraZeneca. They enrolled the 1+ and 2+ (low HER2) patients and compared their response to chemotherapy. The overall survival in this particular group of HER2 low was 24 months, compared to 17 months for those taking chemotherapy. This has opened the floodgates for the HER2 low population, and the FDA recently approved this drug.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]



Now, why is this important? Especially in the context of prostate cancer? We had 71 prostate cancer samples in our stock. I went back and said, “Let's examine what the HER2 levels in prostate cancer were?” Something like 40% of them had no HER2 background, or we couldn’t detect it, and about three to five patients had high levels of HER2. These are the patients that you would want to enroll into an anti-HER2 clinical trial, and hopefully they would do well. With low HER2 opening up in breast cancer, this is a population that could potentially get this new drug. There is a clinical trial that is going on in prostate cancer, which is a combination of this particular drug – Enhertu or trastuzumab deruxtecan – and a PARP inhibitor. I would encourage folks to look to know what your HER2 protein levels are. If it is high enough, there are a lot more options. If it is low, there is at least one option that is open. Let's open it up.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]



Let me switch gears a little bit here. I don't know if anybody has talked about antibody-drug conjugates. The antibody-drug conjugate is an antibody that is linked to a toxin with a linker. That's the basic format of the drug. The antibody binds to let's say HER2, and then it gets internalized, and then the toxin gets released. Based on what toxin you use, for example if this toxin is towards tubulin inhibitors, you will go and bind to tubulin. The newer drugs are having the TOPO1 inhibitor, so they go and bind to TOPO1 and prevent cell division. And some of these drugs also have a “bystander activity”; in other words, they can “get out of the cell” and also kill the neighboring cells. The format is an antibody with a toxin.

Brian McCloskey
30:26
A comment about the ADCs that have come up in my treatment recommendations. I expressed very highly for B7-H3 (CD276), and there's an ADC that targets B7-H3. I don't know if B7-H3 has come up in your research, but there's actually a BiTE from Xencor, which is in development, which targets B7-H3. (BiTE = bispecific T-cell engager. BiTE antibodies are distinguished by the linked agent being a T cell rather than a radioactive isotope or chemotherapy. Early results suggest T cell agents are non–cross-resistant to commonly used chemotherapy and can be highly effective, even in chemotherapy-refractory patients.)

Sheeno Thyparambil
31:01
Is it a BiTE or an ADC?

Brian McCloskey
31:05

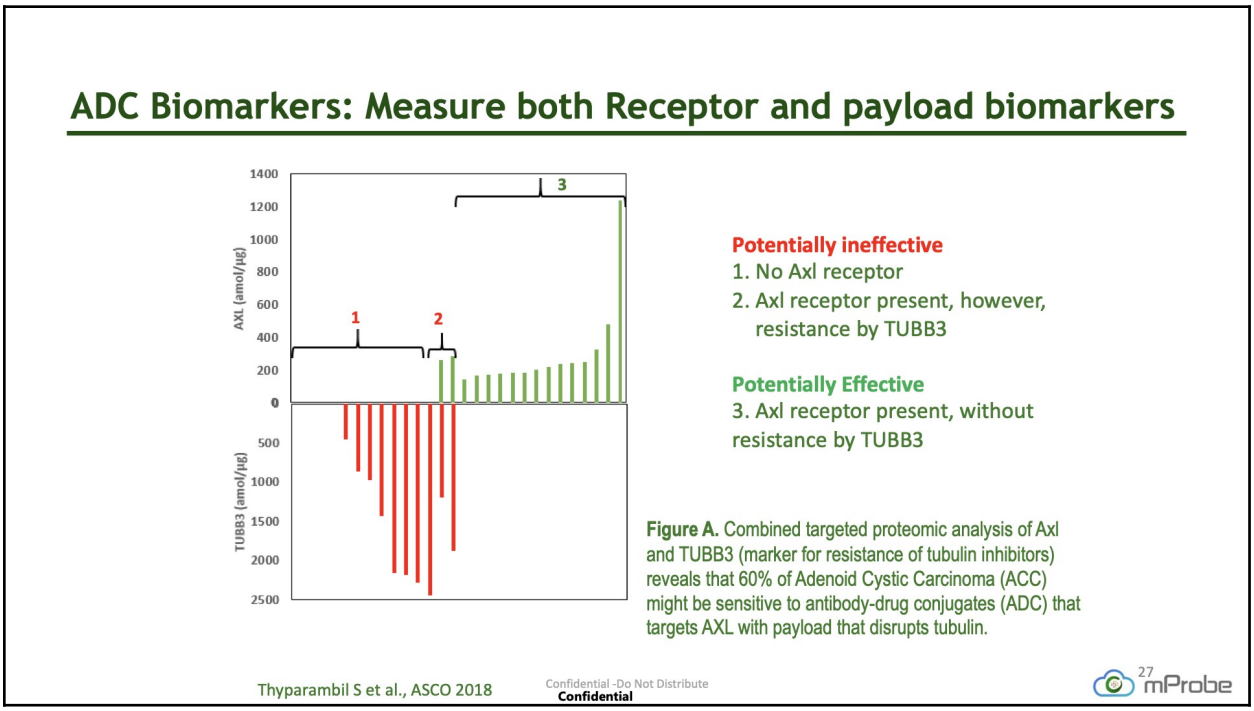
“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

There are two. Daiichi has an ADC that targets B7-H3. And Xencor is developing a BITE that targets B7-H3.

Sheeno Thyparambil
31:18
It is a bispecific, so one arm is binding to the B7-H3, and the other arm is binding to a T cell and essentially getting the immune system to come and recognize that

Brian McCloskey
31:32
Correct. That's the Xencor product.

Sheeno Thyparambil
31:36
And if it is Daiichi, the B7-H3 is your receptor and your payload – the deruxtecan – is going after TOPO1. Knowing your TOPO1 levels is definitely good, but it's not not mandatory.



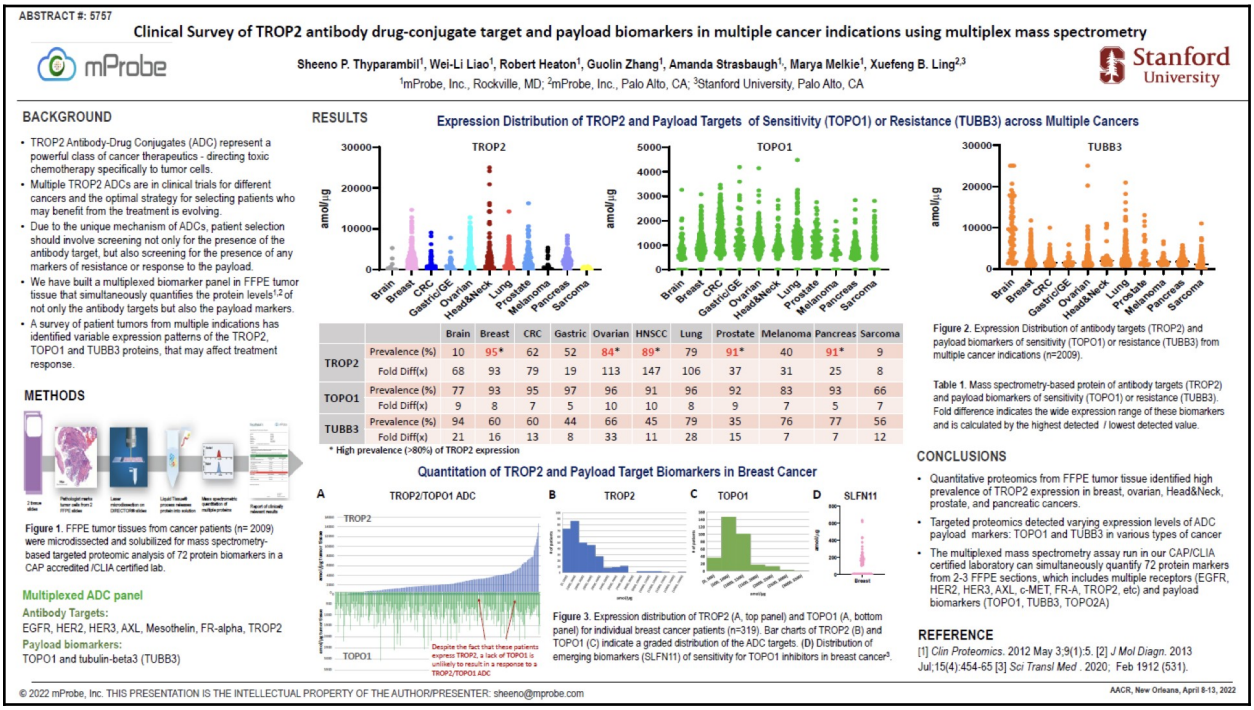
We just set the stage for this ADC biomarker case. We were examining – ignoring the cancer types – an adenoid cystic carcinoma (a very rare form of cancer) and hypokalemic cancer. (Hypokalemia is a potentially life-threatening electrolyte complication that occurs in many patients with cancer.) There are three possible ways an ADC could bind to AXL:

1. The receptor is not present, in which case forget it, the ADC cannot bind to it.
2. The ADC can bind to it, gets internalized, but once it is inside the cell, it has tubulin beta 3, which is a resistance mechanism for many anti-tubulin inhibitors. So that's not one that'll be that effective.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

3. It binds to it, gets inside the cell, and there's no resistance mechanism, and it should work.

These are the three ways an ADC could be successful, or not.



ABSTRACT #: 5757

Clinical Survey of TROP2 antibody drug-conjugate target and payload biomarkers in multiple cancer indications using multiplex mass spectrometry

Sheeno P. Thyparambil[1], Wei-Li Liao[1], Robert Heaton[1], Guolin Zhang[1], Amanda Strasbaugh[1], Marya Melkie[1], Xuefeng B. Ling[2,3]
[1]mProbe, Inc., Rockville, MD; [2]mProbe, Inc., Palo Alto, CA; [3]Stanford University, Palo Alto, CA

BACKGROUND

- TROP2 Antibody-Drug Conjugates (ADC) represent a powerful class of cancer therapeutics - directing toxic chemotherapy specifically to tumor cells.
- Multiple TROP2 ADCs are in clinical trials for different cancers and the optimal strategy for selecting patients who may benefit from the treatment is evolving.
- Due to the unique mechanism of ADCs, patient selection should involve screening not only for the presence of the antibody target, but also screening for the presence of any markers of resistance or response to the payload.
- We have built a multiplexed biomarker panel in FFPE tumor tissue that simultaneously quantifies the protein levels[1,2] of not only the antibody targets but also the payload markers.
- A survey of patient tumors from multiple indications has identified variable expression patterns of the TROP2, TOPO1 and TUBB3 proteins, that may affect treatment response.

METHODS

Figure 1. FFPE tumor tissues from cancer patients (n= 2009) were microdissected and solubilized for mass spectrometry-based targeted proteomic analysis of 72 protein biomarkers in a CAP accredited /CLIA certified lab.

Multiplexed ADC panel

Antibody Targets:
EGFR, HER2, HER3, AXL, Mesothelin, FR-alpha, TROP2

Payload biomarkers:
TOPO1 and tubulin-beta3 (TUBB3)

RESULTS

Expression Distribution of TROP2 and Payload Targets of Sensitivity (TOPO1) or Resistance (TUBB3) across Multiple Cancers

		Brain	Breast	CRC	Gastric	Ovarian	HNSCC	Lung	Prostate	Melanoma	Pancreas	Sarcoma
TROP2	Prevalence (%)	10	95*	62	52	84*	89*	79	91*	40	91*	9
	Fold Diff(x)	68	93	79	19	113	147	106	37	31	25	8
TOPO1	Prevalence (%)	77	93	95	97	96	91	96	92	83	93	66
	Fold Diff(x)	9	8	7	5	10	10	8	9	7	5	7
TUBB3	Prevalence (%)	94	60	60	44	66	45	79	35	76	77	56
	Fold Diff(x)	21	16	13	8	33	11	28	15	7	7	12

* High prevalence (>80%) of TROP2 expression

Quantitation of TROP2 and Payload Target Biomarkers in Breast Cancer

Figure 3. Expression distribution of TROP2 (A, top panel) and TOPO1 (A, bottom panel) for individual breast cancer patients (n=319). Bar charts of TROP2 (B) and TOPO1 (C) indicate a graded distribution of the ADC targets. (D) Distribution of emerging biomarkers (SLFN11) of sensitivity for TOPO1 inhibitors in breast cancer[3].

Figure 2. Expression Distribution of antibody targets (TROP2) and payload biomarkers of sensitivity (TOPO1) or resistance (TUBB3) from multiple cancer indications (n=2009).

Table 1. Mass spectrometry-based protein of antibody targets (TROP2) and payload biomarkers of sensitivity (TOPO1) or resistance (TUBB3). Fold difference indicates the wide expression range of these biomarkers and is calculated by the highest detected / lowest detected value.

CONCLUSIONS

- Quantitative proteomics from FFPE tumor tissue identified high prevalence of TROP2 expression in breast, ovarian, Head&Neck, prostate, and pancreatic cancers.
- Targeted proteomics detected varying expression levels of ADC payload markers: TOPO1 and TUBB3 in various types of cancer
- The multiplexed mass spectrometry assay run in our CAP/CLIA certified laboratory can simultaneously quantify 72 protein markers from 2-3 FFPE sections, which includes multiple receptors (EGFR, HER2, HER3, AXL, c-MET, FR-A, TROP2, etc) and payload biomarkers (TOPO1, TUBB3, TOPO2A)

REFERENCE

[1] *Clin Proteomics*. 2012 May 3;9(1):5. [2] *J Mol Diagn*. 2013 Jul;15(4):454-65 [3] *Sci Transl Med* . 2020; Feb 1912 (531).

AACR, New Orleans, April 8-13, 2022

This is an extremely busy poster which was presented at AACR. There is a new receptor on the cell called TROP2, which is conjugated in a Gilead Immunomedics drug to a TOPO1 inhibitor. Daiichi and AstraZeneca have a drug that is in clinical trials that is also doing the same thing with TROP2 conjugated to a TOPO1 payload.

We examined something like 2000 samples across different cancer types. If you look at prostate cancer, 91% of prostate cancers express TROP2 levels. Not all of them express the same. There is anywhere from either very low levels to a very high level. The minimum to the maximum was a 37x difference. Where in this group would somebody's prostate cancer be? The higher you are, the better? We did some work with MSK and looked at a median expression of TROP2 in triple negative breast cancer. We saw it at around 3000 amol. The reason I was looking at triple negative breast cancer is because the Gilead drug is approved in triple negative breast cancer, and it had a TROP2 to TOPO1 combination. I was surprised, to be honest with you, how many prostate cancer samples had TROP2. And the full difference is definitely 37x. Once the drug is internalized, let's look into what impact it has in the TOPO1 levels. Chasing that figure, 92% of prostate cancers have TOPO1, with a tighter regulation of topoisomerase 1 levels. I'm excited to see how this drug will work in prostate cancer.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

Looking at clinical trials, there are two clinical trials that are ongoing. IMMU-132 is Immunomedics, which was bought by Gilead, so it’s known as Gilead. It's definitely for patients with metastatic castrate resistant prostate cancer. I, again, encourage you to let us know your TROP2 levels. If you're high enough, it's an open label clinical trial. You should consider enrolling in one of these two trials. That was the Daiichi AstraZeneca US drug. Same thing stroke drug 2001.

Brian McCloskey
36:17
Are we just looking at RNA expression for TROP2? Is that what's going to tell us whether or not we're a good candidate for these trials?

Sheeno Thyparambil
36:41
These clinical trials are not asking for any kind of expression. They're just saying, if you have prostate cancer, come on it. My recommendation would be, especially if it is an open label clinical trial, figure out whether your TROP2 protein levels are high enough, then enroll in the clinical trial. That way, you're stacking the odds in your favor. You could be a better candidate for TROP2, or maybe a better candidate for an anti-HER2 ADC. ADC clinical trials are making waves. Figuring out which clinical trial to enroll in would be something handy.

Brian McCloskey
37:39
I know my RNA expression, and there are some other patients on this call that may know it as well. I know my expression relative to a few different cancer cohorts. That's been really helpful,

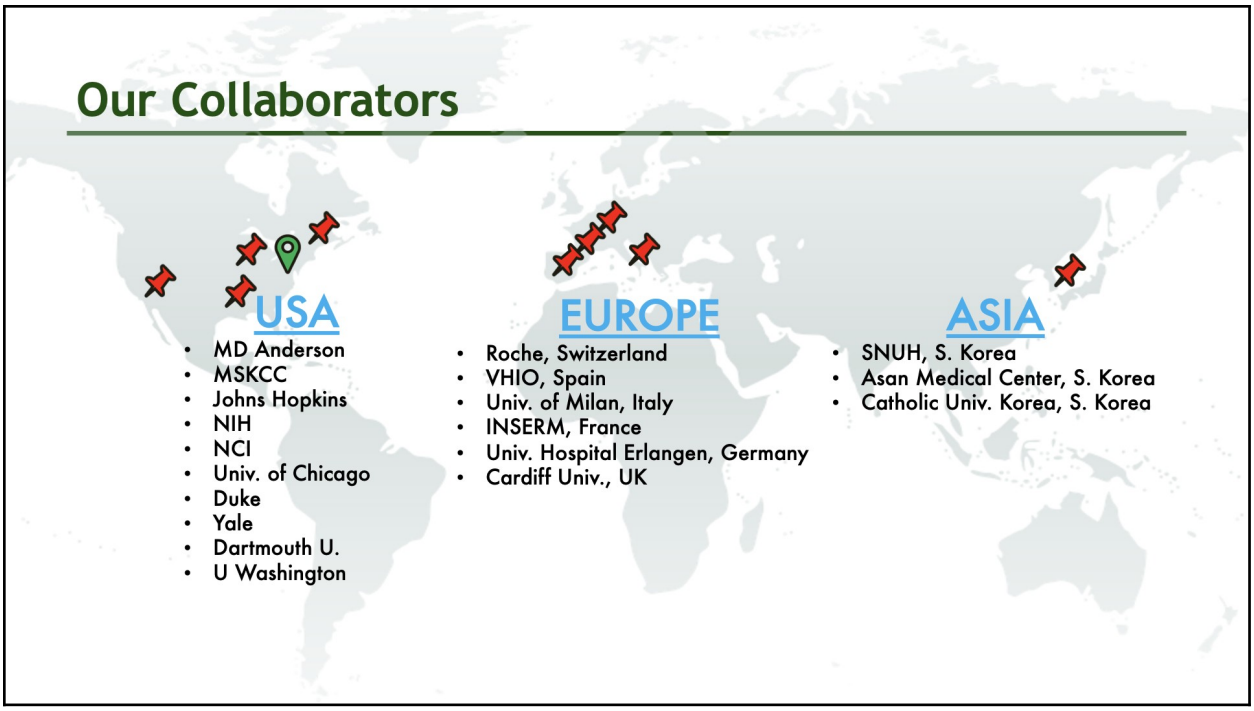
because it identified that I'm a very high expressor for B7-H3 relative to even prostate cancer patients. But when you get into mass spec, are you saying that there can be differences in terms of the biomarker results that might come from RNA expression versus protein expression?

Sheeno Thyparambil
38:20
Not all RNA will express protein. For example, some foundations are sending us samples to see if you're expressing a protein or not. There's a slide I've always liked from Gordon Mills, from back in 2015: when you go from DNA to RNA to protein, how much loss of information really happens? This is what has been replicated in many CPTAC and TCGA cohorts. (The Clinical Proteomic Tumor Analysis Consortium (CPTAC) is a collaborative consortium of institutions and investigators with expertise in proteomics, genomics, cancer biology, oncology, bioinformatics, and clinical chemistry. They perform coordinated research projects to identify the proteins found in cancer specimens whose genomes have already been characterized, such as in NCI’s The Cancer Genome Atlas (TCGA) program. One of the tenets of the consortium is that all the proteomics data is made publicly available in a repository that is accessible by the global research community, similar to the TCGA data portal.) That is why, especially when it comes to cancer vaccines, or some trials, some of the patients are sending us samples to see, “Do I express this protein?” That's an additional comfort for them.

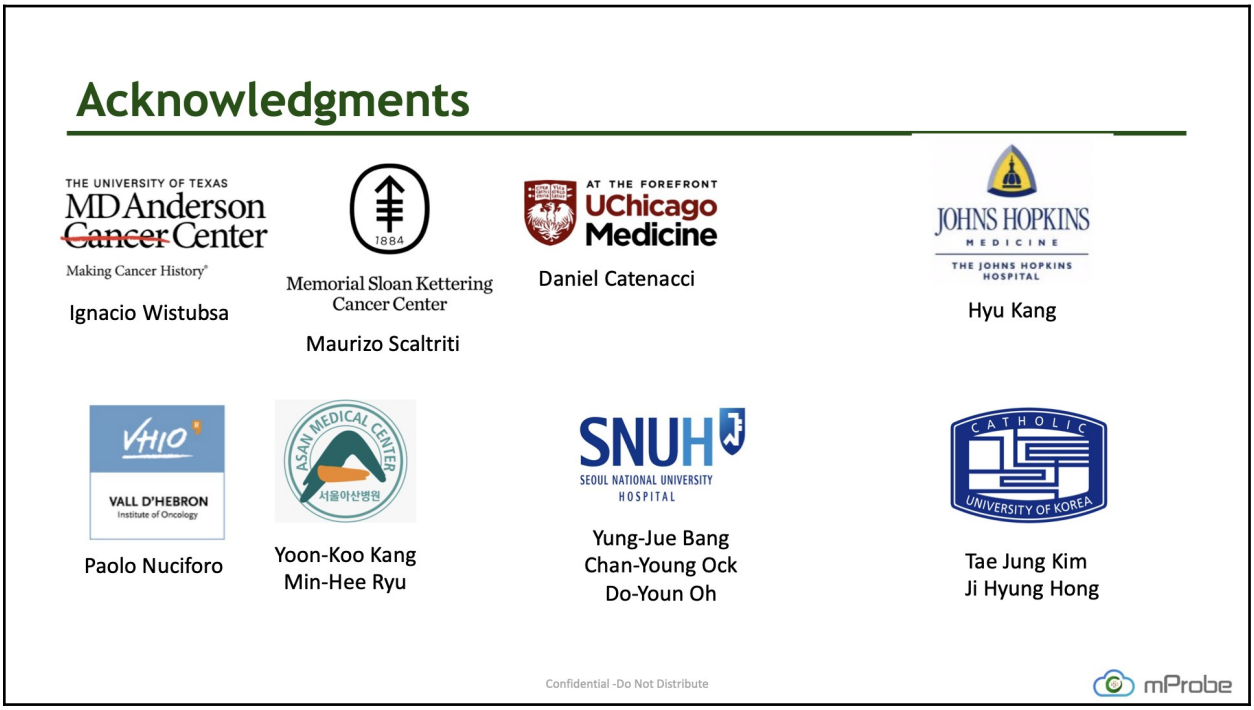
Brian McCloskey
39:25
I've seen a variation on this. So you're saying there is a 35% delta between RNA and protein expression?

Sheeno Thyparambil
39:46
If you go from DNA to RNA, let's say 50% of your information is transmitted. And then from RNA to protein, 30% of the information is translated. It is definitely a variation. This is from a long time ago. There are more and more data that are coming out. The crux of the whole thing is when you go from DNA all the way to protein, we know there is a loss of information. This is a recipe to make this a final product. All your drugs are actually acting against proteins, so the question becomes, should I measure the levels of protein to see what's actually expressed, whether through mass spectrometry or through immunohistochemistry, to know if the proteins are being expressed? That would be super helpful.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]



Sheeno Thyparambil
40:54
We collaborate with some really smart people from different parts of the world.



Some of the smart people are listed here. And from different parts of the world.

“Clinical Guidance from Proteomics” (Sheeno Thyparambil) [#26]

I looked at the very small cohort of prostate cancer and wanted to examine a few targets and see what the distribution is. It surprised me to see the distribution, especially for TROP2 and HER2. We can go back at some point and start examining the 72 biomarkers in there, and see what else we could find out within prostate cancer. Not all patients are unique. For every patient, what works? What are the options if something is not working?

Sheeno Thyparambil
41:55
Here's my contact information. If you plan on ordering any of these tests, there's more information on our website, or you can send an email to orders@mprobe.com. We are migrating information to our main website at oncoomicsdx.com that has a lot of information and papers as well.

Saed Sayad
42:56
I really liked the ADC discussion, and those types of drugs. How about the tissue specificity of those trials? That approach can connect to many different places, especially the receptor.

Sheeno Thyparambil
43:26
One of the risks for an anti-HER2 therapy, which is the one that is most well understood, outside of being an ADC, is a cardiovascular risk. With this particular ADC, where it was conjugated with TOPO1, they saw in 10% of the cases, which is actually now dropping–it's maybe 1% or 2%--there is an interstitial lung disease, or pneumonitis, from the trastuzumab irinotecan ticket. It is a blackbox warning on the Enhertu drug, but the amount of survival difference that you're seeing

in breast cancer and gastric cancer is just staggering for this ADC, so it's definitely a risk benefit analysis.

Saed Sayad
44:35
When you decide about these biomarkers, do you visit the proteomic analysis to detect your biomarkers, or is that a predefined set of biomarkers?

Sheeno Thyparambil
44:53
We are looking at the protein levels using spectrometry. In our clinical lab, it is a predefined set of 72 biomarkers. We will change the composition of the biomarkers at some point, especially with newer drugs coming.

Saed Sayad
45:20
Are you measuring the protein directly? Are you measuring mRNA?

Sheeno Thyparambil
45:28
No, we are measuring the peptide, which is a surrogate readout for the protein.

Brian McCloskey
45:49
You talked about using FFPE? Can you also use fresh frozen tissue?

Sheeno Thyparambil
46:05
We have in the past, but our clinical workflow is FFPE. In some instances, when clinical and folks ask us, we'll send them the protocol to fix it. Then we go from there.

Brian McCloskey
46:26
I want to comment on the speed at which you process: five days is really fast, particularly for mass spec. To give you a benchmark, I'm getting whole exome sequencing from Tempus, and I'm looking at a month turnaround. So five days for mass spec is pretty quick.

Sheeno Thyparambil
46:50
And the chances of failures are kind of high, depending on the tissue type. In pancreatic cancer, we have seen that. I'm not going to name names, but there are companies that will say it has failed because we didn't have enough material. Recently, we have worked with another genomics company, in which we will try to do a laser microdissection of only the tumor areas

and send it to the genomics company – a highly enriched tumor. So now it works. That has rescued a few of the genomic tests.

Brian McCloskey
47:32
I've heard a little bit about that recently. There's definitely some variation in whole exome sequencing. We won't get into companies right now, but I'm aware of what you're referencing. You talked a little bit about the 72 biomarkers. It sounds like you can customize the biomarkers that potentially we would want to target?

Sheeno Thyparambil
47:58
For our clinical workflow, we will keep it the same. It's been 72 biomarkers. In our research panel, we honestly have something like 377 assays. We have had some instances where folks will say, “I would like to see chlorine 18.2. Is it expressed or not expressed?” Or B7-H3.

Brian McCloskey
48:47
So your menu is 377. But you offer 72 of the 377.

Sheeno Thyparambil
48:58
That's, that's, that's the venue, right? 72 is what we offer, but we have a minimum of 377. This is again designed for R&D for Pharma. Sometimes they'll say, “We would like to pick and choose, and let's see what we can do.” But this is mostly for R&D folks. In some instances, some of the patients with oncology say, “I want to see if I express chlorine 18.2.”

Brian McCloskey
49:30
I've had my DNA sequenced a few times. I'm getting whole exome sequencing right now, so I'm going to get my RNA transcription data. Is it fair to say that I can use that as a guide to then determine which of these 377 we would want to focus on? I'm looking at the interplay between whole exome sequencing and mass spec in the proteins that you target.

Sheeno Thyparambil
50:06
Here's the thing with the RNA sequencing: you can see your 20,000 genes, a much bigger picture, a bigger lens. As with proteomics, especially targeted proteomics, your lens is much smaller and its menu is much smaller. Using that data to guide whether you express this particular protein or not is a smart strategy.

Brian McCloskey
50:41
That's the direction that I was thinking of going.

Richard Anders
50:55
I'm an investor in early stage life sciences. I've been talking to Brad for a while. I have had cancer, but not prostate cancer. I'm interested in the hackathon model and ways that people can help themselves.

I have a quick question about your research around the heterogeneity or homogeneity of samples. Do you ever have people who test multiple parts of a sample? I ask because a heterogeneous sample could express a marker in one spot and not another, and if that marker is being targeted by a therapy, it might be under-expressed in the tumor, but not necessarily absent. I just wonder what data you have on that subject?

Sheeno Thyparambil
51:44
This is a theory question. But in general, what we say is, the way we do laser microdissection, we try to get as many spots as possible and average that heterogeneity out. We call it the elevator experiment, and the awning experiment, where we are peeling off different layers of the awning, and we can see that when you're moving in a transverse plane, you do see some heterogeneity. But when you're going down a tissue, at least for one section over the next section, there's not that much difference that we tend to see, at least in the biomarkers that we examine.

Brian McCloskey
52:29
So if there are multiple tumor regions, is it a good idea to sample them? Those might be transverse but that's a really interesting point. But if there are multiple pinpoint regions, do you recommend or is there any data on the advisability of sampling different regions?

Sheeno Thyparambil
52:53
In the experiments that we did, many years ago, we were looking at a very small biomarker set. For that biomarker set, we did not see that much difference. The way we were trying to avoid that heterogeneity problem is we would sample. Let's say we get a section of tissue, and there's tumor spread out there, we would take out one pocket of tumor, and then we would go get another pocket of tumor, combine all of them, because this cancer type is going to be a good representative of the cancer that you have, so that we can say, “These are the biomarkers we saw in this particular man.” Yes, there is definitely a tumor heterogeneity problem, make no mistake about it. But the way we try to avoid it is we try to get an average of the tumor. The other experiment I was describing is, when you slice one section to the next section, if you look at the same area, which is slide one, slide two, slide three, it's actually pretty even. You don't see much difference, at least up until you go to maybe the 20th or 30th section, but until then, you don't really see much difference.