“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]

May 11, 2022
Brad Power

Meeting Summary

Advanced prostate cancer patient and bioinformatician Rick Stanton introduced the treatment options he and his medical team are considering, using the standard (NCCN) treatment guidelines as a way to tell the story of how he got here. This built on Rick’s previous session (see the notes from meeting #6 here), in which he walked through his and Brian McCloskey’s medical history. We have discussed potential enhancements to the guidelines, such as:

- Bring testing forward in the guidelines to help people at each decision point.
- Embed more real world evidence in the decisions.
- Add more structure and explicit guidance for decisions in the late stages.
- Make the guidelines more dynamic and predictive.
- Put a treatment strategy with guiding principles on top of it.

To guide his treatment decision, Rick shared his test results (DNA sequencing, immunohistochemistry, and RNA sequencing). The DNA sequencing identified two CDK12 mutations. The RNA sequencing identified three biomarkers which were overexpressed: AR, PSMA, and B7-H3.

Based on the test results and his medical history, Rick and his medical team have identified this list of treatment options:

- Pluvicto (radioligand attacking PSMA, currently unavailable due to production issues)
- Olaparib (targeting CDK12) + CTLA4 inhibitor + PDL1 blockade

- PSMA CAR-T from Poseida (targeting PSMA)
- PSMA bispecific from Calibr (targeting PSMA)
- ARV-766 AR degrader (targeting AR)
- Daiichi antibody drug conjugate to B7-H3 (targeting B7-H3)
- Cabazitaxel (chemotherapy)

Requests

- Do you have any feedback on Rick’s testing and treatment options?

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Meeting Transcript

Brad Power: Today we are going to continue a conversation that we started about the standard of care roadmap for advanced prostate cancer. Rick shared a one-page summary he had done of the NCCN (National Comprehensive Cancer Network) guidelines, and he identified the place beyond the first few lines of therapy (prostate removed, radiation, androgen suppression drugs) where there was a lot of flexibility or discretion in the advanced stages. One of Rick’s initial observations was how that could be more structured and guided by testing. We have also talked about bringing testing forward in the guidelines to help people at each decision point. Saed Sayad suggested making it more dynamic and predictive. We've also discussed putting a strategy on top of it. We spoke with Ryon Graf about embedding more real world evidence in the decisions so that people could understand the outcomes. We have a variety of ideas to enhance the standard of care guidelines. Rick is going to kick it off by sharing where we were before and a couple of ideas he has.

Rick Stanton: A quick update on my situation: on Friday I will have my eighth round of chemo. I am currently stable. My PSA is 2.4, and I have nodal disease. I have four or five lymph nodes in my upper body – neck, middle of my chest, pelvic regions – that lit up on a PSMA scan. The pathologists know where to look, and the interpretation is they aren't growing. My current assessment is stable disease. I will keep going on chemo, for rounds eight, nine, and 10, if possible. And then what happens after round 10 is what I'm going to discuss. How could we bring some molecular evidence to guide the next treatment?

To pick up where we left off, this page is my interpretation of the NCCN guidelines for advanced prostate cancer decisions:

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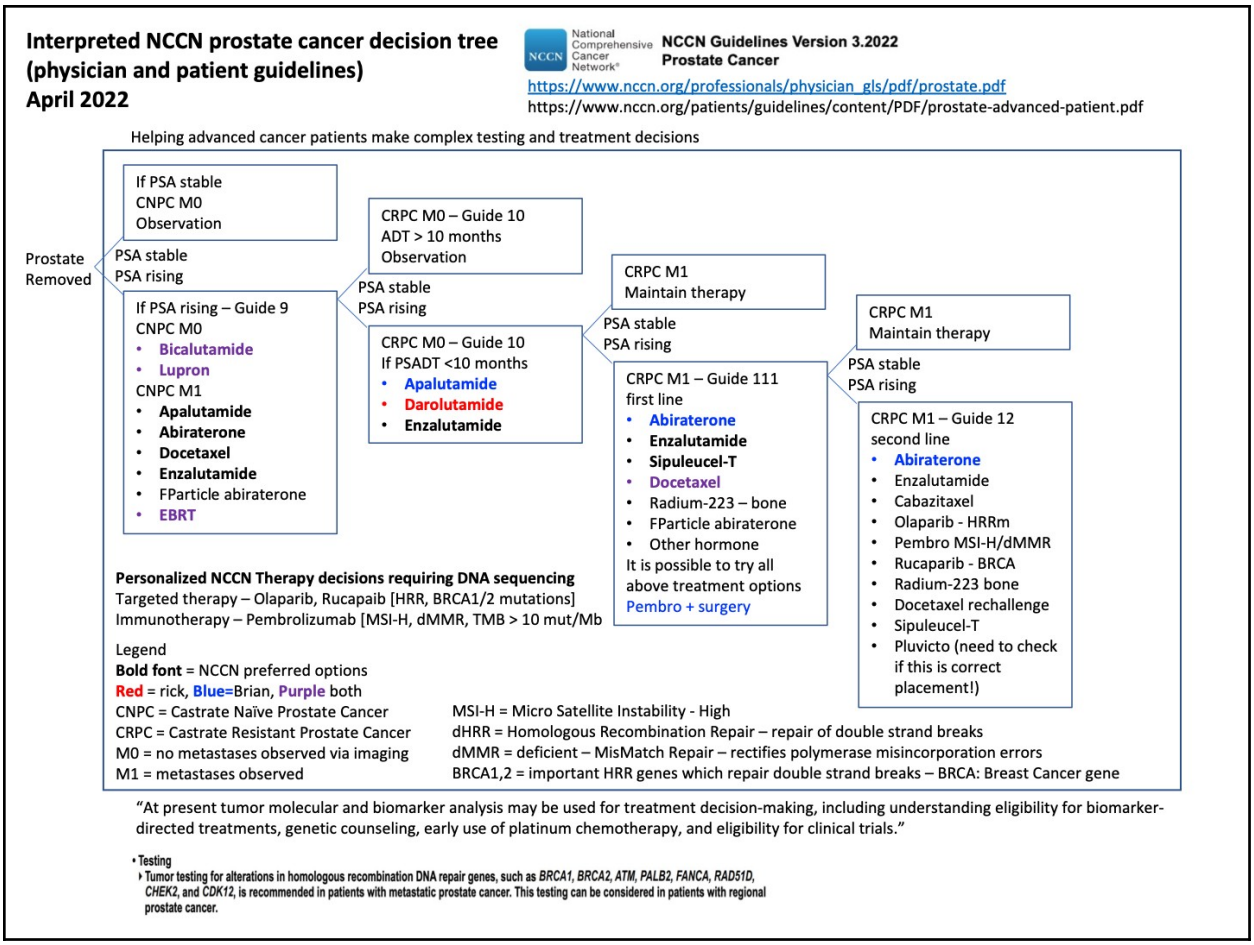

The purple shows where Brian and I have both shared this journey. Where we have diverged, I'm in red, and Brian's in blue. We both had our prostate removed, but our PSA was not zero. We went on hormone therapy, bicalutamide and Lupron, and had radiation therapy (EBRT). Brian is on abiraterone (androgen suppression). I am on docetaxel (chemotherapy), and Brian has had docetaxel as well.

Mike Yancey: When I looked at the NCCN guidelines, I noticed that my situation was hard to find. My prostate was not removed since I was immediately stage four (metastatic). I had to really search to find the starting point in the guidelines that reflected my experience.

Rick Stanton: That’s a good point. I had to pick a place to start. That's the problem with trying to condense 60 pages into one page.
Here is my second slide:

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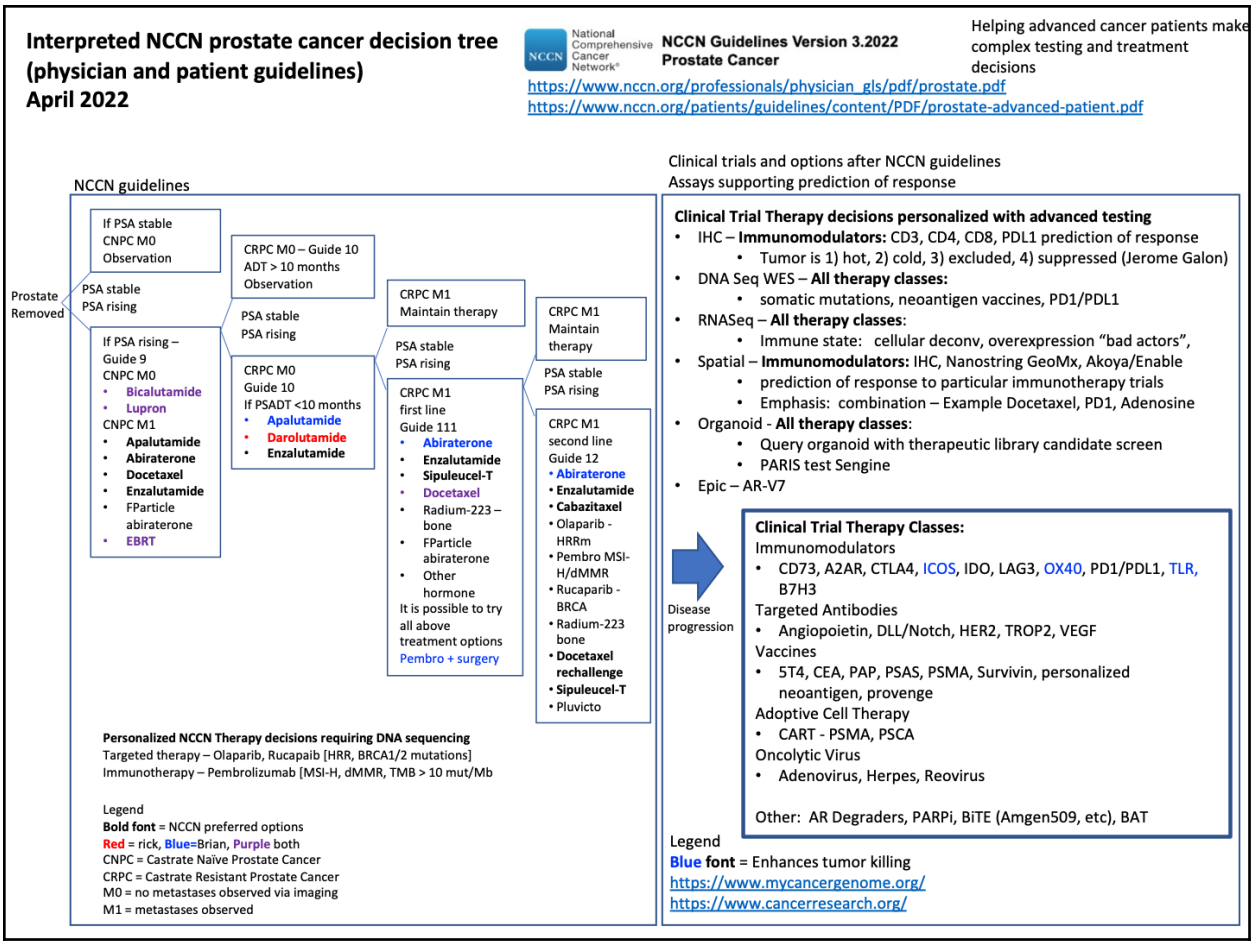

On the left is the same decision tree. And on the right are the guidelines for the steps after that which recommend pursuing clinical trials.

The classes of clinical trials available now include immunomodulators, targeted antibodies, vaccines, and other degraders.

I also list above the therapies some of the tests or assays that would support which clinical trial you would want to go on.

- There's IHC, which stands for immunohistochemistry. This is one type of testing that is pretty typical. You can look at whether you have T-cell infiltration into your tumor. It would inform a decision on whether you might be a candidate for PDL1. If you have T cells in your tumor, they would be able to help kill the tumor if they were not inhibited by the Programmed Death-Ligand 1 (PDL1).
- DNA sequencing will inform decisions on all therapy classes.
- RNA-sequencing can inform decisions on all therapy classes as well.
- Spatial analysis is more cutting edge. You take a slice of the tumor and analyze it using different techniques, such as immunofluorescence. You tag antibodies to these types of immunomodulators so that you can see the presence of different proteins in your tumor. And based upon that, you would be able to choose which of the immunomodulator clinical trials might be a good fit for you.

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- Organoids are cutting edge. You take a little bit of tumor, grow it up, replicate it into, say, 200 little colonies, and query those 200 colonies with different therapeutic candidates to see what kind of response you get.

These more advanced assays that people don't typically get might help inform their decision. We're trying to push the cutting edge.
I'm going to go into the assays that have been done on me next and what they mean to me and my oncology care team.

MOLECULAR TESTING – DNA Sequencing

Rick– WES (Whole Exome Sequencing) on primary tumor in 2020

Result: increased prediction of response to
- **Olaparib**
- **PD1/PDL1 blockade**

NCCN guidelines

“At present tumor molecular and biomarker analysis may be used for treatment decision-making, including understanding eligibility for biomarker-directed treatments, genetic counseling, early use of platinum chemotherapy, and eligibility for clinical trials.”

- Testing
  - Tumor testing for alterations in homologous recombination DNA repair genes, such as *BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2,* and *CDK12,* is recommended in patients with metastatic prostate cancer. This testing can be considered in patients with regional prostate cancer.

GEM ExTra® Report

ASHION
A TGen® Clinical Laboratory

Report Date: 07/20/2020

TUMOR GENOMIC ALTERATIONS[1]				
CDK12				
GENOMIC TARGETS	FDA-APPROVED DRUGS -for patient's cancer	FDA-APPROVED DRUGS -for another cancer	DRUGS PREDICTED NON-BENEFICIAL	POTENTIAL CLINICAL TRIALS
2	1	3	0	Yes
CDK12 (Q115*)	olaparib	niraparib, rucaparib, talazoparib		Yes
CDK12 (Y285fs)	olaparib	niraparib, rucaparib, talazoparib		Yes
TUMOR MUTATION BURDEN (TMB)				
LOW (1 mut/Mb)				No
MICROSATELLITE STATUS (MSI)				
STABLE				No
ADDITIONAL SIGNIFICANT ALTERATIONS				
TMPRSS2/ERG (Fusion)				No

This is a screenshot of my genetic testing and DNA sequencing. I have two CDK12 mutations, and they are targetable by FDA-approved drugs, such as Olaparib, for prostate cancer. I have a low tumor mutation burden. My MSI microsatellite status is stable, and I have a gene fusion. This is the highlight of my DNA sequencing report. I would never have known that I have an FDA-approved drug that might help me, if it wasn't for this DNA sequencing. This is within the NCCN guidelines because they recommend testing for these DNA repair genes and CDK12. My specific mutation is not the same as Brian's. CDK12 mutations are a poor prognostic. They happen in about 5% of prostate cancer patients. This mutation will create a lot of gene fusions, which can be a target for T-cells. Therefore, I would hopefully be responsive to PDL1.

Saed Sayad: Is there any information about using drugs for CDK12?

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Rick Stanton: There's a fair bit of publications about the outcomes of using this drug, Olaparib. It was more hopeful upon approval, and as more data was released, there has been a weaker linkage to improved outcomes as time went on. This is maybe not the first drug that my care team would consider, but it's something. When I talked to Dr. Tanya Dorff, she said, “I see Olaparib in your future. You know, one way or another. You could take it sooner. You could take it later. This is a drug that could help you. And when you exhaust other options, you're probably going to try it.” And that's what I know about it.

Brad Power: Given our recent conversations with Saed Sayad, Bob Gatenby, and John Laird all promoting the notion of drug combinations and lower dosages, are you thinking of this as a monotherapy, or are you thinking this might be in a drug cocktail you could try?

Rick Stanton: That's a great question. It's not just me who will decide. Tanya Dorff, who is one of the authors of the NCCN guidelines, at City of Hope, and a well respected leader, told me of a clinical trial at City of Hope that was a cocktail of Olaparib, a PDL1 inhibitor, and a CTLA4 inhibitor. Those are FDA-approved immunotherapies, but the trial is to have the triplet cocktail. She recommended it should be considered. She felt like Olaparib on its own had a weak response, but in a cocktail triplet it would extend my life.

Brad Power: Building on the discussion we had about the CureMatch report, it would be interesting to see how that cocktail of three would overlap and give coverage to the biomarkers you've identified that are unique to you

Rick Stanton: This is going to get very interesting because I would like to give my biomarker analysis to CureMatch. I gave Ally Perlina at CureMatch my Tempus report, but she told me there were not enough distinctive biomarkers to run their algorithm. She was able to run a report for Brian, but Brian's Tempus report included a section of overexpressed genes which my report didn't include. The bioinformatics team at Tempus said they don't always include it. I'll do my own assessment of overexpression, with help from TGen, and provide that to Ally. And then we can compare CureMatch’s recommendation to what my care team recommends.

Brad Power: Another dimension we've been talking about and you included are organoids. I just listened to a Society for Functional Precision Medicine webinar, where they talked about testing drugs on organoids. It'd be awesome to test your cocktail with organoids. It seems like you have some lead time. It can take months to get the organoids cultured and all set up.
Brian, you've talked to SEngine, which provides organoids, what kind of tissue do they need to culture and organize? Is it fresh tissue, and do you have fresh tissue available or whatever the feedstock is for organoids?

Rick Stanton: I don't know what is required. I have fresh frozen tissue at City of Hope from my primary tumor. I don't know if that's good enough.

Brian McCloskey: They need live, fresh frozen tissue. I don't know the difference here. There's fresh frozen and there's live fresh frozen. I think it has to be shipped within 20 hours.

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Rick Stanton: This first analysis is molecular testing at the DNA level. This was my report from Ashion who subcontracted to TGen. I have a similar report from Tempus, which agreed.
Now I'm going to go to the immunohistochemistry level, then I'm going to go to the RNA seq level. These are the three sets of data that I have.

The immunohistochemistry analysis was done on my primary tumor. You stain a slice of the tumor tissue and you look for something. In this case the pathologist looked for tumor infiltrating lymphocytes, which are T-cells and B-cells. My result was zero to 5% TILs, and that's not as good as we were hoping because my mutation on average does attract these TILs, and the T-cells are your immune response that kills the cancer. Having no T-cells, or zero to 5%, is not a good predictor of a response to immunotherapy on the PD1/ PDL1 blockade.

MOLECULAR TESTING - IHC (ImmunoHistoChemistry)

Rick– IHC (ImmunoHistoChemistry) on primary tumor in 2022

Result: 0 - 5% TILS (Tumor Infiltrating Lymphocytes) (B cells and T cells), no evidence of PDL1

- **Rick’s interpretation = decreased prediction of response to PD1/PDL1 blockade**

Tumor-infiltrating lymphocytes

From Wikipedia, the free encyclopedia

Tumor-infiltrating lymphocytes are white blood cells that have left the bloodstream and migrated towards a tumor. They include T cells and B cells and are part of the larger category of ‘tumor-infiltrating immune cells’ which consist of both mononuclear and polymorphonuclear immune cells, (i.e., T cells, B cells, natural killer cells, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, basophils, etc.) in variable proportions. Their abundance varies with tumor type and stage and in some cases relates to disease prognosis [1][2][3][4][5]

TILs can often be found in the tumor stroma and within the tumor itself. Their functions can dynamically change throughout tumor progression and in response to anticancer therapy[2][3][4][5]

TILs are implicated in killing tumor cells. The presence of lymphocytes in tumors is often associated with better clinical outcomes (after surgery or immunotherapy).[6][7][8][9]

Here is a piece from Wikipedia describing how TILs are implicated in killing tumor cells. Their presence is often associated with better clinical outcomes. I would have hoped for a higher number.
Immunohistochemistry changes between the primary tumor and metastases, especially as metastases are being pushed by therapies and chemotherapies.
One would hope that if a similar IHC stain would be done on one of my metastases we'd see some TILs in there. In case you don't have experience with looking at CD3 staining, here's the slice of tissue, and it's a lymph node biopsy.

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MOLECULAR TESTING - IHC (ImmunoHistoChemistry)

Rick– IHC (ImmunoHistoChemistry) on primary tumor in 2022

Result: 0 - 5% TILS (Tumor Infiltrating Lymphocytes) (B cells and T cells), no evidence of PDL1

- **decreased prediction of response to PD1/PDL1 blockade**

Note – would have preferred a CD3 IHC stain to look like below!

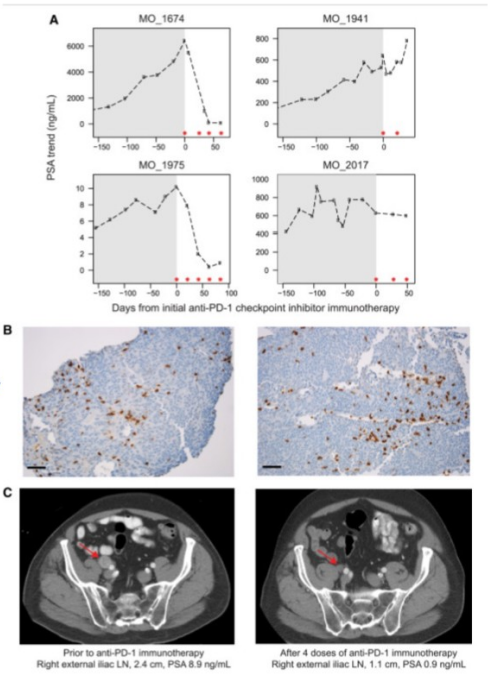
Cell
Volume 173, Issue 7, 14 June 2018, Pages 1770-1782.e14
CellPress

Article

Inactivation of *CDK12* Delineates a Distinct Immunogenic Class of Advanced Prostate Cancer

Yi-Mi Wu [1, 2, 20], Marcin Cieślik [1, 2, 20], Robert J. Lonigro [1], Pankaj Vats [1], Melissa A. Reimers [3], Xuhong Cao [1], Yu Ning [1], Lisha Wang [1], Lakshmi P. Kunju [1, 2, 4], Navonil de Sarkar [5], Elisabeth I. Heath [6, 7], Jonathan Chou [8], Felix Y. Feng [8, 9, 10, 11], Peter S. Nelson [5, 12, 13], Johann S. de Bono [14, 15], Weiping Zou [1, 2, 16], Bruce Montgomery [12, 17], Ajjai Alva [1, 3] ... Arul M. Chinnaiyan [1, 2, 4, 18, 19, 21]

https://www.sciencedirect.com/science/article/pii/S0092867418305658



CD3 IHC staining of lymph node biopsies from MO_1975 prior to anti-PD1 therapy

This is from a paper in Cell on how inactivation of CDK12 (my mutation) delineates a distinct immunogenic class of advanced prostate cancer. So why do we care about this?
If you look at the response of the person in this paper called MO_1975, they were given an anti-PD-1 checkpoint inhibitor immunotherapy, their PSA was at 5, and when they were given the PD1 inhibitor, it basically went to zero. This is a tremendous response. And the rationale was here's the stain for CD3, a protein that is present on T-cells. You can look at this slide and where you see brown, you'd say there are T-cells. If the T-cells are in the tumor, this person is a really good candidate for PDL1 blockade. This is the first easiest look at, will you respond or not to a PDL1 blockade. The City of Hope pathologists did not share the image with me, which I wish they would've. All I got was zero to 5% tumor infiltration, and no evidence of PDL1. My tumor would look like this without the brown stain from CD3. I'm not going to respond to PDL1.

Brad Power: How would we reflect this on the roadmap? What you're saying is, “if I have an IHC stain, it's going to tell me if I'm likely to respond to immunotherapy.” So, we're going to want to go back to the roadmap and build in these “if, then, else,” conditional logic gates.

Rick Stanton: In the roadmap there is some incorporation of molecular testing on whether to include pembrolizumab, a PD1/PDL1 blockade. One is MSI, microsatellite instability high, or mismatch repair genes. From DNA testing, you could pick this, but the roadmap doesn't have a

decision point. Relative to IHC staining, to my awareness, there's nothing in the NCCN guidelines that currently says, “if you do an IHC stain, and you test positive, you should go on a PDL1.” Now this is a little interesting because for lung cancer for a long time, chemo was the first line or one of the first line treatments. And then when this PDL1 blockade came out, if you have lung cancer, you will be stained most likely for tumor infiltrating lymphocytes. And if a pathologist scores you above a threshold, the immunotherapy replaces chemotherapy as the first line therapy. So it's happening in lung cancer. Probably not so much in prostate cancer because most prostate cancer primary tumors are known as cold, meaning not a lot of immune infiltration.

Next I will go into my RNA sequencing results. One of the neat things about RNA seq is that it is pretty cheap. If you send away your tissue to a DNA sequencing lab, they probably run RNA seq. You're getting the relative abundance of gene expression at the RNA level.

MOLECULAR TESTING – RNASeq – preliminary research use only –
Ashion, Tempus (Josh Bell – thank you!), Tgen (Wei Lin - thank you!!)

Rick – preliminary interpretation immune deconvolution Ashion RNASeq data

	cell_type	1
1	B cell	0.030084074
2	Macrophage M1	0.000000000
3	Macrophage M2	0.040975486
4	Monocyte	0.000000000
5	Neutrophil	0.080194891
6	NK cell	0.034393175
7	T cell CD4+ (non-regulatory)	0.018059672
8	T cell CD8+	0.000000000
9	T cell regulatory (Tregs)	0.003094445
10	Myeloid dendritic cell	0.001736494
11	uncharacterized cell	0.791461762

This was one view of what Ashion produced from my RNA seq data, and Tempus also produced RNA seq data on me. I want to thank Josh Bell and Wei LIn at TGen. Full credit, you know, you provided the code.

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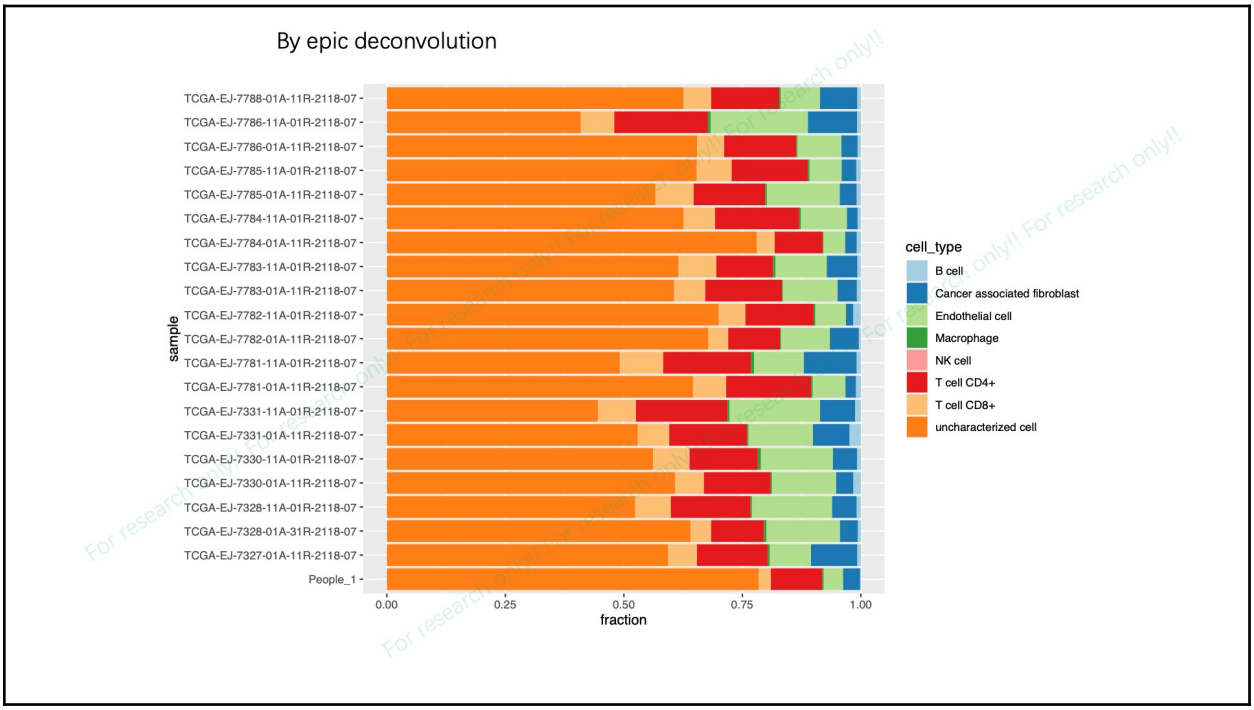
Wei Lin: If you guys are interested in learning how this works, I can show a few slides, which are very simple, just to explain why the molecular profiling helps to deconvolute the cell composition in the tissue.



For RNA seq we can get the gene expression values of the whole transcription because multiple cells contribute to one gene signal. So we need to do deconvolution. There's a tool called the Epic immune deconvolution, which helps us to input the gene expression values and output the fractions of the immune cells and tumor cells. But there's some assumption of what could be in the tumor, but mostly they are these uncharacterized cells. Usually they are tumors because the tumors are not normal cells. They call it uncharacterized, but for other cells, mostly myeloid lineage cells and the lymphoid cells, which including T-cells, B-cells, and something like neutrophil macrophages, that's how we can get the fractions of those cell types. This is just one algorithm because it's based on different assumptions of the algorithms they give for Rick's case. You can see the other patients we pulled from the TCGA (the Cancer Genome Atlas) consortium data repository, and used them as a reference.
And we know for the patient we are interested in what’s at the end.

Rick Stanton: I'm the lowest row in that bar graph (labeled People).

Wei LIn: This is consistent with the imaging data Rick just showed. In his slide he said zero to 5% TILs. It's low end. It's like a cold tumor.

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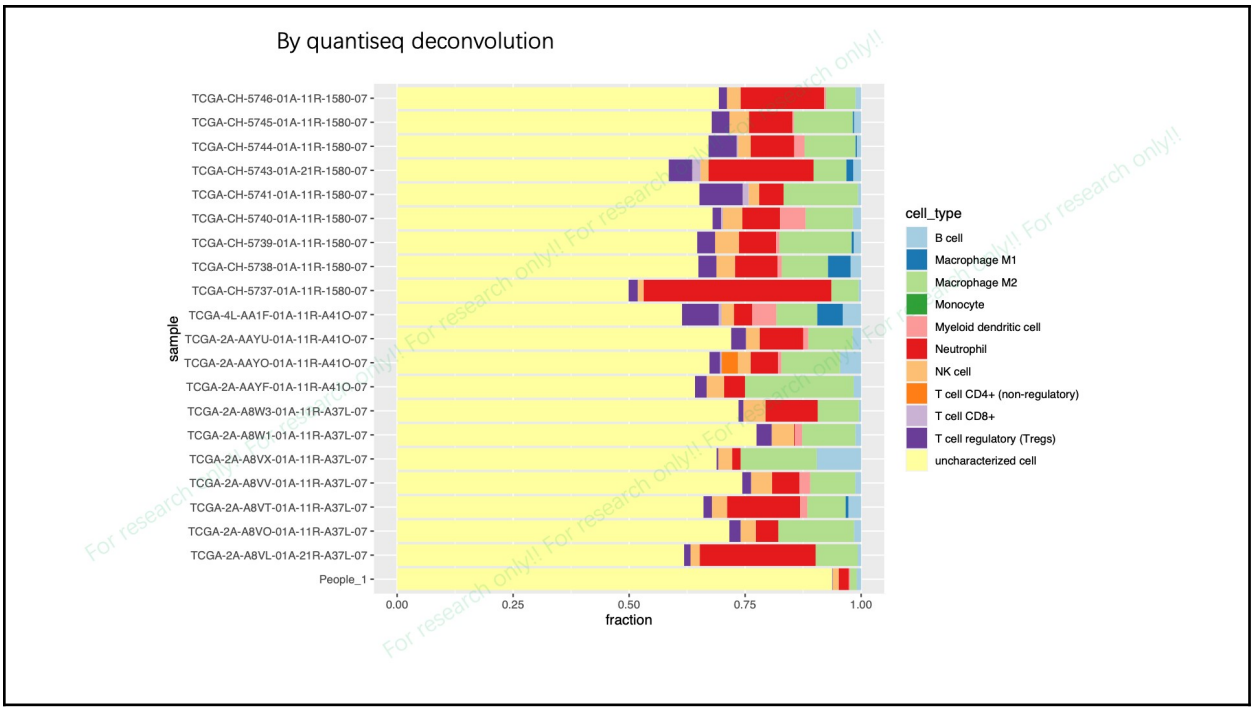

This other algorithm also helps to deconvolute the cell composition even lower. This means that there were no specific patients that showed what end their immune cells are in. I also tried to validate the result because I'm not very familiar with the algorithm.

Expression Quantiles

gene	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	People_1
CD1A	0	0.02039145	0.03695283	0.0577297	0.08821251	0.12222696	0.16750108	0.26437085	0.45143776	3.27836352	0.018271
CD1B	0	0	0	0.01939949	0.0288819	0.04614422	0.06644812	0.09846371	0.17295878	1.21990946	0
CD1C	0.25004541	0.44018748	0.64480553	0.87919256	1.23247506	1.5187584	1.89162222	2.64471572	3.5591246	16.3908357	0.376447
CD2	1.70609275	2.4784434	3.23747197	4.0160252	4.94917342	6.14161627	7.51456963	9.82043278	13.5466603	112.403832	2.61801
CD38	0.59097617	1.73064669	3.65477896	8.37540347	16.0182665	23.1085562	35.9291137	51.0716277	75.9031802	295.108788	7.2821
CD3D	0.65058155	1.05037544	1.45568342	1.79927023	2.21248077	2.79317825	3.47350255	4.72477743	7.05626428	68.6074443	0.591829
CD40	3.70022186	5.75000896	7.45218156	9.1381598	11.4990273	14.1270516	17.4281077	20.8414675	26.2700947	74.2740386	3.66193
CD4	7.17229863	9.96629117	12.466795	15.399779	18.3783015	21.7179796	26.0927371	32.7536614	42.3831355	240.916961	3.09761
CD68	14.1327376	18.5885102	23.1039528	27.0284206	32.3507348	38.9469863	46.2574471	58.5213272	72.0343062	289.730476	5.72483
CD79A	0.30283178	0.45747752	0.65571807	0.91682575	1.26381218	1.8484358	2.78409128	4.19068605	8.58395523	187.569085	0.470484
CD79B	0.84825543	1.19919902	1.6176777	1.91717037	2.2807167	2.70699539	3.43166776	4.74067039	7.006404	86.3635435	0.291797
CD80	0.02949875	0.06495914	0.09786501	0.1303826	0.17173534	0.20700248	0.28173656	0.38797916	0.53874255	4.96011778	0.082032
CD8A	1.71806235	2.44252111	3.49102455	4.32971399	5.16405667	6.31115687	7.67263343	9.64769436	13.41555	105.927527	1.2344
CD8B	0.53768563	0.9773112	1.37805911	1.68966278	1.98743401	2.42709281	2.92901038	3.52210358	4.87081941	30.4567356	0.343915
GZMA	0.60881615	0.91919468	1.18463256	1.46902584	1.81900447	2.27447362	2.99866864	3.77158775	5.8672128	49.2376674	0.658542
GZMB	0.19869964	0.32527799	0.45074663	0.61744139	0.81648364	1.09462071	1.42206328	1.75014153	2.76484052	28.5371465	0.247028
GZMK	0.53363239	0.90428052	1.37432962	1.78505411	2.21316287	2.79631103	3.61064441	4.84031013	7.71875619	40.0875323	0.443751
IFNG	0	0	0.02118927	0.0317241	0.04646725	0.07036457	0.09582872	0.14879381	0.24415003	2.98678738	0.018241
IL1A	0	0	0	0.01569692	0.02234811	0.0294093	0.04530733	0.06668496	0.11866747	1.5180795	0
IL1B	0.23091548	0.41576423	0.59891447	0.83913609	1.13862151	1.51844623	2.17677574	3.13831754	5.50707177	41.1498181	0.369042
IL7R	0.26583692	0.58611271	0.96462715	1.34813018	1.835666	2.50862771	3.5990248	5.50384072	9.52756772	49.143779	9.67141
IL7	0.58685499	1.02156054	1.3213757	1.62135029	1.90534884	2.27053463	2.69574843	3.27838166	4.10758715	11.8448546	1.91799

So I compared the expression quantile for those marker genes, for example, like CD1, CD3, which are the marker genes for specific immune cell types. These are the killer cell markers. These are the inflammatory signals from the neutral field, those kinds of things. And for

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People_1 (Rick’s) expression values, compared to their signals, are mostly at the low end (see the yellow), except this IL7R is at the high end, 90 percentile for others, mostly 10 percentile lower. So we just support the conclusion on these fractions at the low end. That's how it works. It's very simple.

Brad Power: I have a very basic question. I don't know what “deconvolution” means.

Wei Lin: For example, we have a stew. It's made of tomato, beef, and other things. We want to know after we get the stew, which contributes how much. So we try to figure out how much tomato and beef we have put in.

Brad Power: It's percentages of the ingredients in the total stew?

Wei Lin: In the stew you have the molecules. You want to know what part of the beef or what part of tomato contributed those kinds of molecules. Eventually you will know how much tomato you should put to begin with the cook, and how much beef you should begin with the cook. So for this case, different cells contribute different molecules. They share some molecules, but eventually you will know how much cell populations are in that tissue, because you only know the molecules.

Brad Power: It also sounds like you're reverse engineering it, saying, “this must have been how much beef went into it, and this is how much tomato went in.”

Brian McCloskey: It's like the back of a package of food, like how much percentage is carbs, sugars, …

Wei Lin: Exactly. That's the thing, because the main goal is to know how many cells we could work with. For this tumor case, if you want to use immunotherapy, you have to have enough T-cells. From the RNA seq data you only have the molecule abundance, not really the cell abundance. So you want to reverse engineer to get the fraction of the T-cells in that tissue.

Brad Power: To use another word, it sounds to me like “decomposition”. You're breaking it down into the constituent parts.

Rick Stanton: This is the result of some of Wei’s work. I'm going through the different algorithms comparing my data from both Ashion and Tempus. I have two sets of data on my same tissue. How does this make sense so far? I have very low CD8 from this algorithm, even lower than one of Wei’s slides. Basically the conclusion is I have pretty low immune infiltration. So unleashing the immune state with nothing there is probably not a good candidate from this evidence for immunotherapy on the PDL1 axis.

Brian McCloskey: I think this is the first time I've seen this. How does this compare to the work that was done with Tempus?

“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]

Rick Stanton: I still have to look. We were given a similar assessment from Tempus, and I'm now just pulling it together.

Wei Lin: The tumor is kind of dynamic, which means this cell composition could change. For example, with radiation therapy they try to make the cold tumor hot. So another strategy is to increase the immune infiltration, then that could help the downstream immunotherapy treatment.

Rick Stanton: This data is from my naive primary tumor. And so hopefully it's a little better story on my metastases.

I'm going to go a little fast, and give you some glimpses of some slides to think about.

What does RNA seq data even look like? Here are four public examples of melanoma where you have the gene names.

MOLECULAR TESTING – RNASeq – preliminary research use only – Ashion, Tempus, TGen

What does RNASeq data look like?
4 public examples of melanoma data shown below

	LAU125	LAU355	LAU1255	LAU1314
A1BG	0.82	0.58	0.81	0.71
A1CF	0.00	0.01	0.00	0.00
A2M	247.15	24.88	2307.94	20.30
A2M-AS1	1.38	0.20	2.60	0.28
A2ML1	0.03	0.00	0.05	0.02
A3GALT2	0.00	0.00	0.00	0.00
A4GALT	0.17	0.96	0.03	1.70
A4GNT	0.09	0.00	0.05	0.01
AAAS	20.90	15.68	14.09	19.17
AACS	10.14	3.87	3.08	5.39
AACSP1	0.00	0.01	0.05	0.00
AADAC	0.00	0.00	0.00	0.00

....
~22K rows

Then the relative abundance of these four, each column is a patient and you have 22,000 rows roughly. And that's what the data looks like.

“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]

Back in 2019 CIBERSORT was one of the deconvolution algorithms that was published, and it is amazingly accurate. This was the paper, and from the paper you have this RNA profile from these different, this is like the soup that we were talking about. These are the different cell types in the soup, and they have a profile and you put it in this deconvolution matrix. And then we look at Brian and my RNA profiles, and you run this algorithm from this matrix on our data.

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MOLECULAR TESTING – RNASeq – preliminary research use only – Ashion, Tempus, TGen

Rick – preliminary interpretation immune deconvolution

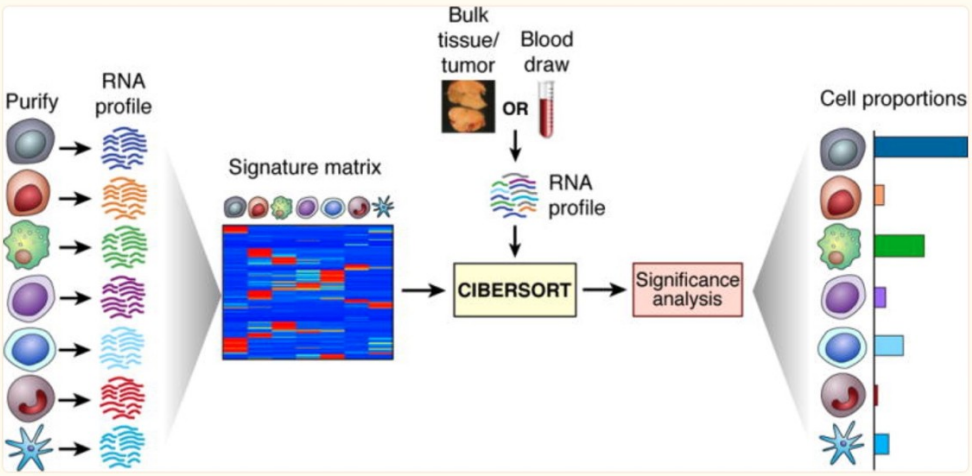

Figure 1

Overview of CIBERSORT. As input, CIBERSORT requires a “signature matrix” comprised of barcode genes that are enriched in each cell type of interest. Once a suitable knowledgebase is created and validated, CIBERSORT can be applied to characterize cell type proportions in bulk tissue expression profiles. Although originally validated using a signature matrix containing 22 functionally defined human immune subsets (LM22) profiled by microarrays, CIBERSORT is a general framework that can be applied to diverse cell phenotypes and genomic data types, including RNA-Seq. To quantitatively capture deconvolution confidence, CIBERSORT calculates several quality control metrics, including a deconvolution p-value.

You look at the significance and you come up with the proportions. So that's the basics of this deconvolution. And then what does this matrix look like?

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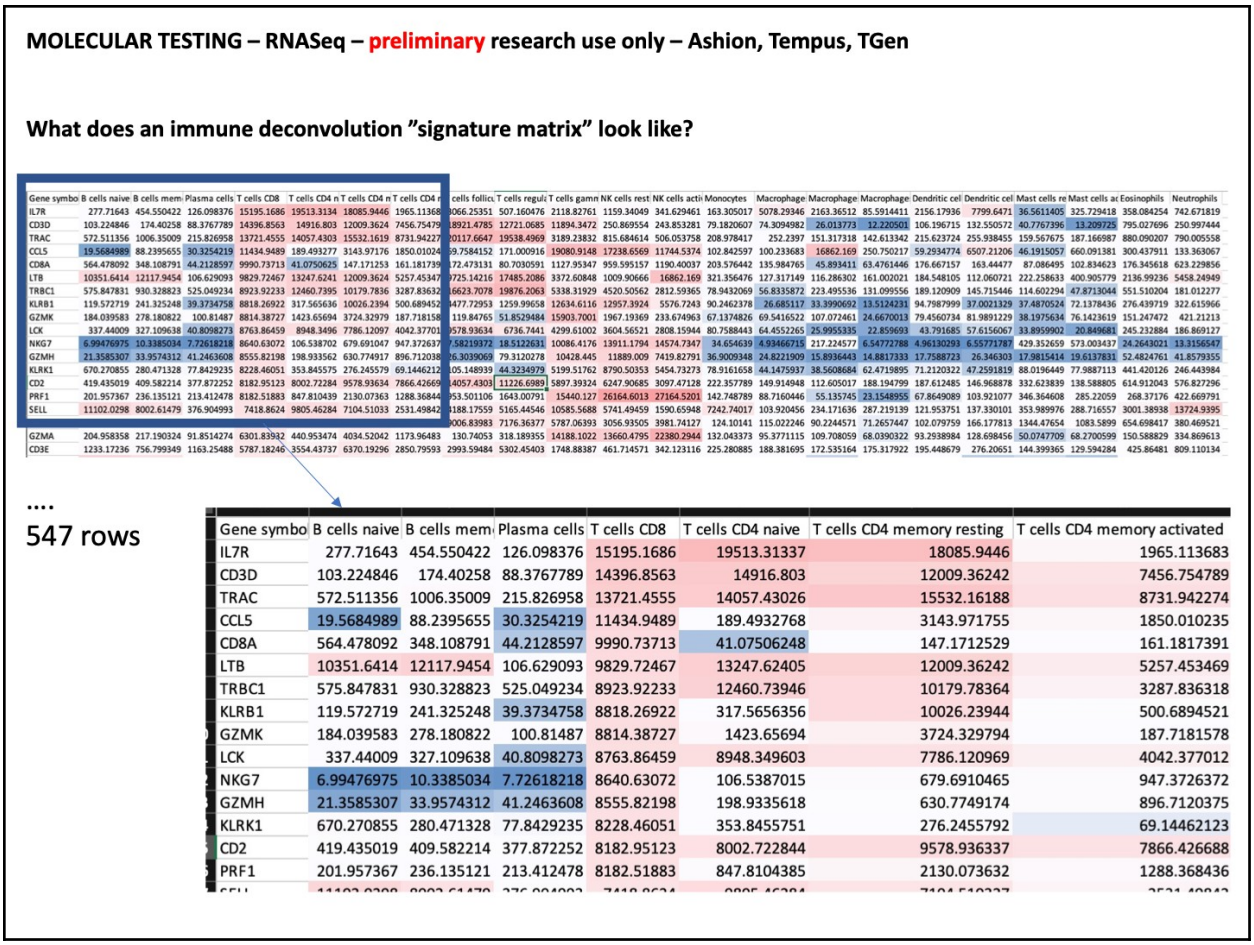


Gene symbo	B cells naive	B cells mem	Plasma cells	T cells CD8	T cells CD4 naive	T cells CD4 memory resting	T cells CD4 memory activated
IL7R	277.71643	454.550422	126.098376	15195.1686	19513.31337	18085.9446	1965.113683
CD3D	103.224846	174.40258	88.3767789	14396.8563	14916.803	12009.36242	7456.754789
TRAC	572.511356	1006.35009	215.826958	13721.4555	14057.43026	15532.16188	8731.942274
CCL5	19.5684989	88.2395655	30.3254219	11434.9489	189.4932768	3143.971755	1850.010235
CD8A	564.478092	348.108791	44.2128597	9990.73713	41.07506248	147.1712529	161.1817391
LTB	10351.6414	12117.9454	106.629093	9829.72467	13247.62405	12009.36242	5257.453469
TRBC1	575.847831	930.328823	525.049234	8923.92233	12460.73946	10179.78364	3287.836318
KLRB1	119.572719	241.325248	39.3734758	8818.26922	317.5656356	10026.23944	500.6894521
GZMK	184.039583	278.180822	100.81487	8814.38727	1423.65694	3724.329794	187.7181578
LCK	337.44009	327.109638	40.8098273	8763.86459	8948.349603	7786.120969	4042.377012
NKG7	6.99476975	10.3385034	7.72618218	8640.63072	106.5387015	679.6910465	947.3726372
GZMH	21.3585307	33.9574312	41.2463608	8555.82198	198.9335618	630.7749174	896.7120375
KLRK1	670.270855	280.471328	77.8429235	8228.46051	353.8455751	276.2455792	69.14462123
CD2	419.435019	409.582214	377.872252	8182.95123	8002.722844	9578.936337	7866.426688
PRF1	201.957367	236.135121	213.412478	8182.51883	847.8104385	2130.073632	1288.368436

Here is LM 22, an example of a signature matrix. We have genes in the rows and the columns are the different cell types. I've sorted this and colored it for basically the algorithm, which is looking for these cell types. CD8 T-cells are what kills the tumor. That's of particular importance. And so you look at this blow up. What is most predictive of CD8 in the soup, and it's these genes in these orders, according to the signature matrix.

Brian McCloskey: What are the numbers? What do they represent? Is it transcription?

Rick Stanton: If this was a linear algebra concept, you would say, this is the influence, these numbers. Let's do it for CD8. This would say, how much IL7 receptor and down to these genes, do I see in your RNA sequence? What is the relative abundance of these genes? And you multiply it by this influence factor. And that will tell you an estimate of the CD8 cells. You look at our gene expression of these genes, and what does our gene expression look like? It looks like any column here. This is the relative abundance. So you take any of these columns, but one at a time, and you multiply the genes times this matrix, and you come up with your assessment.

Wei Lin: This table is the relative abundance of these molecules. So you can see that each column is the cell type and each row is the gene IDs. After the combination of the different cell types, with different percentages, you will get another list of gene versions.

“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]

When you try to get back to the fraction of the cell types, you need to perform deconvolution. It's like reverse engineering because you already know each part, each cell type has a certain molecule abundance. When they combine in different percentages, you will get another. The whole thing is different molecule abundance. Then you use these values to calculate the fractions of those cell types.

Brian McCloskey: He has an overabundance of IL7R in T-cell CD8?

Wei Lin: IL7R is the T-cell marker. That's why it's in T-cells in abundance. For this table, it's like a reference from the conventions, what most people measure about the molecules in that cell type. Then later when we get a real tissue that you can reverse engineer to the cell type population.

Rick Stanton: So what we're doing is kind of comparing IHC staining with RNA seq staining, and we also want to compare spatial, and see if we get concordance of results. And then that would be very good evidence of hope to guide therapy.
There's another aspect to RNA seq interpretation, when you're looking for bad actors or good actors. What do you overexpress? And I have the same overexpression for these three: androgen receptor, PSMA, and B7-H3.

MOLECULAR TESTING – RNASeq – preliminary research use only – Ashion, Tempus, TGen

Rick– RNASeq on primary tumor in 2020

Result: overexpression of

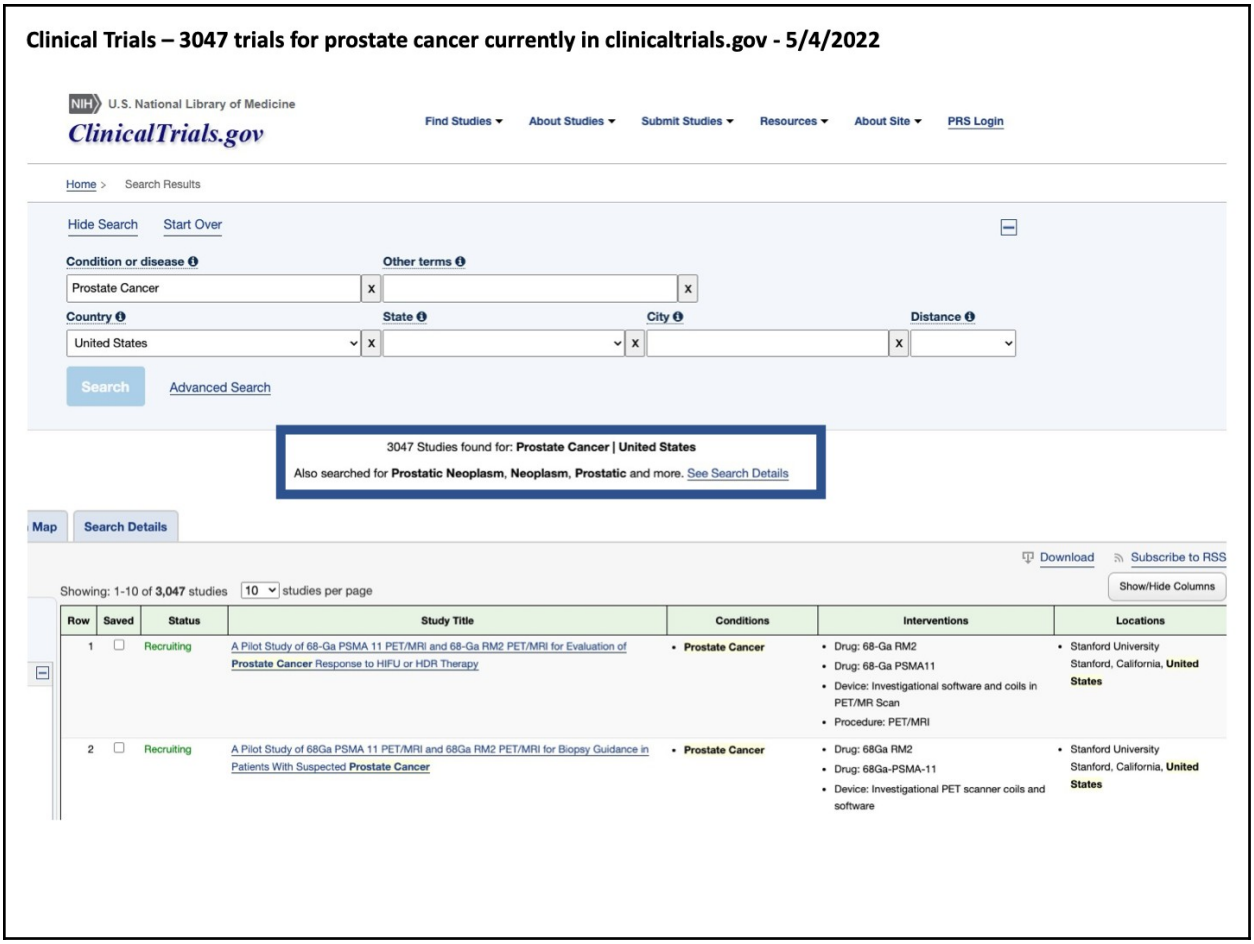
- **AR**
- **PSMA**
- **B7H3**

Relative to cohort of ~500 TCGA prostate cancer patients and ~12K TCGA all cancer patients

Interpretation increased prediction of response to therapies targeting the above

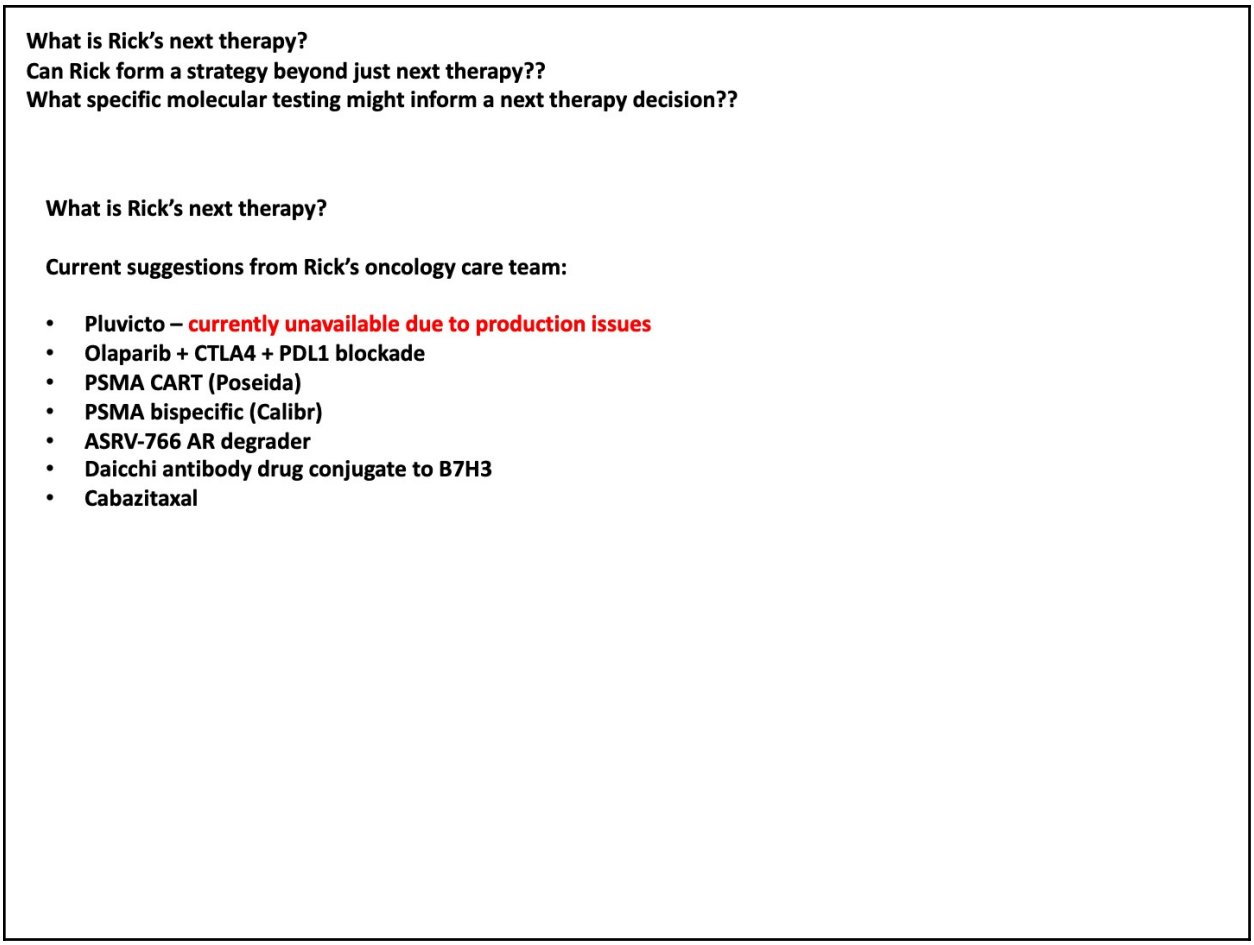
“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]

They'll appear on this list and they'll have high numbers for Brian and me. And so relative to other prostate cancer patients in the public dataset and all cancer patients, this is preliminary, but I have very high expression overexpression of these three genes or proteins.
So why is that important? Because all of these have therapies that we can direct. So this is very encouraging.



Out of the 3047 prostate clinical trials going on, or combinations not in clinical trials, which do I pick as my next therapy?

“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]



Can we form a strategy, and what molecular testing might inform this? You've seen the molecular testing that's been done on me, and these are recommendations from my care team – Tanya Dorff, Rana McKay and John Shen.

- All agree that Pluvicto might be a great next step for me. Why? Because it targets PSMA. And I have a ton of PSMA expression. My PSMA expression was super high at my primary tumor, and after therapy it tends to go higher. So this is number one. Unfortunately, due to production issues, it's not available. According to Rana McKay, it may be available in six to eight weeks. Hopefully I just hang on docetaxel for a while,
- Notice the next one that Tanya Dorff recommended: the Olaparib cocktail, the triplet that we discussed.
- Also because I have high expression of PSMA, the Poseida and Calibr clinical trials.
- And because I have super high androgen receptor, this degrader and a B7-H3 clinical trial.
- And then another chemo.

My care team specifically included the RNA seq data. It's not typical that a prostate cancer patient would get this data, and it's not typical that an oncologist would integrate it into their recommendations for next therapy. We want to do more, but I hope this was a good glimpse at my journey and my next steps, according to my care team.

“Decisions in Advanced Prostate Cancer” (Rick Stanton) [#8]

Brad Power: Chandra Kota mentioned that radiation might be complementary to some of these treatments. And Brian asked if Pluvicto plus immunotherapy might be a useful combination?

Rick Stanton: I think radiation is wonderful if you can get it targeted. There's a lot of evidence that it extends life. And it is synergistic. I hope that'll be a consideration.

Brian McCloskey: I was wondering if you could combine Olaparib with Pluvicto, and get the combined effect of radiation from a radioligand with an immunotherapy like Olaparib?

Rick Stanton: I think that's a great idea because they're both FDA approved.

Brian McCloskey: Maybe we should check to see if there are any clinical trials. Pluvicto is new, but lutetium has been around for a while. It might be worth looking at, whether or not there are any clinical trials.

Chandra Kota: I just want to clarify that the comment I made was related to a locally focused radiation, like you would get with a LINAC (medical linear accelerator). You heard about stereotactic body radiation therapy (SBRT). You might have gotten that already. In lung cancers they're seeing that if you do that with a really high dose, like maybe up to 10 grad per fraction, and then you interleave it with immunotherapies, there seems to be a really significant, synergistic response. They're trying to figure out the timing right now, like whether immunotherapy should go first, or radiation should go first to prime the tumors, the same thing with prostate mets too. If you have a few localized ones, I think that kind of treatment would help, but if it's more diffused, clearly it's not suitable.

Brad Power: These combinations are really interesting. Rick, you should bring your list of drugs up with Bob Gatenby next week.